

Human anti-NGF Neutralizing Antibodies as Selective NGF Pathway Inhibitors

This application is related to and claims priority to U.S. provisional application Serial No. 60/487,431, filed July 15, 2003, the disclosure of which is incorporated by
5 reference herein.

FIELD OF THE INVENTION

The invention relates to human monoclonal antibodies that bind nerve growth factor (NGF). Compositions and methods for treating pain and pain-related disorders are
10 also described.

BACKGROUND OF THE INVENTION

Every day, more than two million people in the United States are incapacitated by chronic pain (Jessell and Kelly, 1991, "Pain and Analgesia" in PRINCIPLES OF
15 NEURAL SCIENCE, 3rd Ed., (Kandel, Schwartz, and Jessell, ed.), Elsevier, New York). Unfortunately, current treatments for pain are only partially effective, and many of these treatments themselves cause debilitating or dangerous side effects. For example, although non-steroidal anti-inflammatory drugs ("NSAIDs") such as aspirin, ibuprofen, and indomethacin are moderately effective against inflammatory pain, they are also renal
20 toxins, and high doses tend to cause gastrointestinal irritation, ulceration, bleeding, and mental confusion. Patients treated with opioids also frequently experience confusion, and long-term opioid use is associated with tolerance and dependence. Local anesthetics such as lidocaine and mexiletine simultaneously inhibit pain and cause loss of normal sensation.

25 Pain is a perception based on signals received from the environment and transmitted and interpreted by the nervous system (for review, see Millan, 1999, *Prog. Neurobiol.* 57:1-164). Noxious stimuli such as heat and touch cause specialized sensory receptors in the skin to send signals to the central nervous system ("CNS"). This process is called nociception, and the peripheral sensory neurons that mediate it are nociceptors.

Depending on the strength of the signal from the nociceptor(s) and the abstraction and elaboration of that signal by the CNS, a person may or may not experience a noxious stimulus as painful. When one's perception of pain is properly calibrated to the intensity of the stimulus, pain serves its intended protective function. However, certain types of tissue damage cause a phenomenon, known as hyperalgesia or pronociception, in which relatively innocuous stimuli are perceived as intensely painful because the person's pain thresholds have been lowered. Both inflammation and nerve damage can induce hyperalgesia. Persons afflicted with inflammatory conditions, such as sunburn, osteoarthritis, colitis, carditis, dermatitis, myositis, neuritis, collagen vascular diseases (which include rheumatoid arthritis and lupus) and the like, often experience enhanced sensations of pain. Similarly, trauma, surgery, amputation, abscess, causalgia, collagen vascular diseases, demyelinating diseases, trigeminal neuralgia, cancer, chronic alcoholism, stroke, thalamic pain syndrome, diabetes, herpes infections, acquired immune deficiency syndrome ("AIDS"), toxins and chemotherapy cause nerve injuries that result in excessive pain.

As the mechanisms by which nociceptors transduce external signals under normal and hyperalgesic conditions become better understood, processes implicated in hyperalgesia can be targeted to inhibit the lowering of the pain threshold and thereby lessen the amount of pain experienced.

Neurotrophic factors have been shown to play significant roles in the transmission of physiologic and pathologic pain. Nerve growth factor (NGF) appears to be particularly important (for review, see McMahon, 1996, *Phil. Trans. R. Soc. Lond.* 351:431-40; and Apfel, 2000, *The Clinical Journal of Pain* 16:S7-S11). Both local and systemic administration of NGF have been shown to elicit hyperalgesia and allodynia (Lewin *et al.*, 1994, *Eur. J. Neurosci.* 6:1903-1912). Intravenous infusion of NGF in humans produces a whole body myalgia while local administration evokes injection site hyperalgesia and allodynia in addition to the systemic effects (Apfel *et al.*, 1998, *Neurology* 51:695-702). There is also a considerable body of evidence implicating endogenous NGF in conditions in which pain is a prominent feature. For example, NGF is upregulated in dorsal root ganglion (DRG) Schwann cells for at least 2 months following peripheral nerve injury and increased levels have been reported in the joints of

animals suffering from a variety of arthritis models (e.g., Aloe *et al.*, 1993, *Growth Factors* 9:149-155). In humans, NGF levels are elevated in synovial fluid from patients with rheumatoid or other types of arthritis (e.g., Aloe *et al.*, 1992, *Arthritis and Rheumatism* 35:351-355). Furthermore, it has been demonstrated that antagonism of
5 NGF function prevents hyperalgesia and allodynia in models of neuropathic and chronic inflammatory pain. For example, in animal models of neuropathic pain (e.g. nerve trunk or spinal nerve ligation) systemic injection of neutralizing antibodies to NGF prevents both allodynia and hyperalgesia (Ramer *et al.*, 1999, *Eur. J. Neurosci.* 11:837-846; and Ro *et al.*, 1999, *Pain* 79:265-274). Examples of anti-NGF antibodies known in the art
10 include, for example, PCT Publication Nos. WO 01/78698, WO 01/64247, WO 02/096458, and WO 2004/032870; US Patent Nos. 5,844,092, 5,877,016, and 6,153,189; Hongo *et al.*, 2000, *Hybridoma* 19:215-227; Hongo *et al.*, 1993, *Cell. Mol. Biol.* 13:559-568; and GenBank Accession Nos. U39608, U39609, L17078, or L17077.

Clearly, there is a need for new safe and effective treatments for pain, particularly
15 by targeting small molecule mediators or exacerbators of pain such as NGF.

SUMMARY OF THE INVENTION

This invention provides novel human monoclonal antibodies that are therapeutically useful for managing pain. Specifically, the invention provides
20 monoclonal antibodies that bind to nerve growth factor (NGF). Preferably, the monoclonal antibodies are human monoclonal antibodies and neutralize biological activities of NGF and are useful for ameliorating the effects of NGF-mediated pain responses. Also provided by the invention are cells that produce, and most preferably, secrete into cell culture media the monoclonal antibodies of the invention. In addition to
25 their use for treating and managing pain, the antibodies of the invention are useful for treating neuropathic and inflammatory pain-related responses.

The invention further provides fusion proteins comprising the sequence of an antibody Fc region and one or more sequences identified as SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID
30 NO: 22, and SEQ ID NOs: 79-130. Such molecules can be prepared using methods as

described, for example, in International Patent Application, Publication No. WO 00/24782, which is incorporated by reference. Such molecules can be expressed, for example, in mammalian cells (e.g. Chinese Hamster Ovary cells) or bacterial cells (e.g. *E. coli* cells).

5 In certain aspects, the invention provides antibodies, preferably monoclonal antibodies, most preferably human antibodies and human monoclonal antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 6, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof and
10 the variable region of the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. Preferably, the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 4.

 In certain aspects, the invention provides antibodies, preferably human antibodies,
15 and more preferably monoclonal antibodies, most preferably human monoclonal antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises an heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE heavy chain constant regions or any allelic variation thereof (as discussed in Kabat *et al.*, 1991, Sequences of Proteins of Immunological
20 Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), included herein by reference, and the variable region of the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. Preferably, an antibody of the invention comprises an amino acid sequence of the IgG2 heavy chain
25 constant region as set forth in SEQ ID NO: 4 or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

 In certain aspects, the invention provides antibodies, preferably human antibodies, and more preferably monoclonal antibodies, most preferably human monoclonal antibodies, comprising a heavy chain and a light chain, wherein the light chain comprises
30 an amino acid sequence as set forth in SEQ ID NO: 8 or an antigen-binding or an

immunologically functional immunoglobulin fragment thereof and the light chain variable region comprises an amino acid sequence as set forth in SEQ ID NO: 12, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

5 In certain aspects, antibodies of the invention comprise a heavy chain and a light chain, wherein the variable region of the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. In other aspects, the light chain variable region comprises an amino acid sequence as set forth in SEQ ID NO: 12, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. In additional
10 aspects, the heavy chain comprises an amino acid sequence as set forth in any of SEQ ID NO: 14, SEQ ID NO: 18, or SEQ ID NO: 20, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. In still further aspects, the light chain comprises an amino acid sequence as set forth in any of SEQ ID NO: 16, 20, 24, or an antigen-binding or an immunologically functional immunoglobulin
15 fragment thereof.

The invention also provides antibodies that bind specifically to NGF, wherein the heavy chain comprises a variable region comprising an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and the light chain comprises a variable region
20 comprising an amino acid sequence as set forth in SEQ ID NO: 12, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

The invention further provides isolated human antibodies that bind specifically to NGF, wherein the antibodies comprise:

(a) a heavy chain having a heavy chain variable region comprising an amino acid
25 sequence as set forth in SEQ ID NO: 79, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 80, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;

(b) a heavy chain having a heavy chain variable region comprising an amino acid
30 sequence as set forth in SEQ ID NO: 81, or an antigen-binding or an immunologically

functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 82, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;

5 (c) a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 83, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 84, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or

10 (d) a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 86, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 87, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

15 In certain aspects, the invention also provides antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region, and wherein the heavy chain variable region comprises a sequence that has at least 75%, preferably 80%, more preferably at least 85%, even more preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and most preferably about 99%, identity to the amino acid sequence as set forth in SEQ ID NO: 10, and wherein the light chain
20 comprises a light chain variable region, and wherein the light chain variable region comprises a sequence that has at least 80%, preferably at least 85%, more preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, and most preferably about 99%, identity to the amino acid sequence as set forth in SEQ ID NO: 12, wherein the antibody binds specifically to NGF.

25 The invention also provides antibodies that bind specifically to NGF, wherein the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 14 or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and the light chain comprises an amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

In certain aspects, the invention provides antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region, and wherein the heavy chain variable region comprises a sequence that has at least 75%, preferably 80%, more preferably at least 85%, even more preferably at least 90%, 91%, 5 92%, 93%, 94%, 95%, 96%, 97%, 98%, and most preferably about 99%, identity to the amino acid sequence as set forth in any of SEQ ID NO: 14, SEQ ID NO: 18, or SEQ ID NO: 22, and wherein the light chain comprises a light chain variable region, and wherein the light chain variable region comprises an amino acid sequence that has at least 80%, preferably at least 85%, more preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 10 97%, 98%, and most preferably about 99%, identity to the amino acid sequence as set forth in SEQ ID NO: 16, wherein the antibody binds specifically to NGF.

The invention also provides single chain antibodies, single chain Fv antibodies, F(ab) antibodies, F(ab)' antibodies and (Fab')₂ antibodies.

In particular aspects, the invention provides a light chain comprising an amino 15 acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

In addition, the invention provides a heavy chain comprising an amino acid sequence as set forth in any of SEQ ID NO: 14, SEQ ID NO: 18, or SEQ ID NO: 22, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

20 The invention also relates to isolated human antibodies that specifically bind NGF, wherein the antibody comprises: (a) human heavy chain framework regions, a human heavy chain CDR1 region, a human heavy chain CDR2 region, and a human heavy chain CDR3 region; and (b) human light chain framework regions, a human light chain CDR1 region, a human light chain CDR2 region, and a human light chain CDR3 25 region. In certain aspects, the human heavy chain CDR1 region can be the heavy chain CDR1 region of the monoclonal antibody (mAb) designated 4D4 as shown in SEQ ID NO:22 and the human light chain CDR1 region can be the light chain CDR1 region of mAb 4D4 as shown in SEQ ID NO:24. In other aspects, the human heavy chain CDR2 region can be the heavy chain CDR2 region of mAb 4D4 as shown in SEQ ID NO:18 and 30 the human light chain CDR2 region can be the light chain CDR2 region of mAb 4D4 as

shown in SEQ ID NO:20. In still other aspects, the human heavy chain CDR3 region is the heavy chain CDR3 region of mAb 4D4 as shown in SEQ ID NO:14, and the human light chain CDR3 region is the light chain CDR3 region of mAb 4D4 as shown in SEQ ID NO:16.

5 The invention also provides isolated human antibodies that specifically bind nerve growth factor, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 10, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, or SEQ ID NO: 87, or an antigen-binding or immunologically functional immunoglobulin
10 fragments thereof.

 The invention further provides isolated human antibodies that specifically bind NGF, comprising a heavy chain and a light chain, wherein the light chain comprises a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 12, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID
15 NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, or SEQ ID NO: 131, or antigen-binding or an immunologically functional immunoglobulin fragments thereof.

 The antibodies of the invention are characterized by the capacity to antagonize at least one *in vitro* and/or *in vivo* activity associated with NGF polypeptides. Preferably, the invention provides isolated anti-human NGF human antibodies with high affinity
20 binding to NGF polypeptides, wherein the antibodies bind to a human NGF polypeptide and dissociates from the human NGF polypeptide with a dissociation constant (K_D) of about 50×10^{-12} M or less, as determined using KinExA, or which inhibit NGF induced survival in an *in vitro* neutralization assay with an IC_{50} of about 1×10^{-8} M or less.

 In a preferred embodiment, the invention provides an isolated anti-human NGF
25 human antibody that has the following characteristics:

 a) inhibits NGF induced survival in an *in vitro* neutralization assay with an IC_{50} of about 1×10^{-9} M or less;

 b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:14; and

c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:16.

The invention also provides isolated human antibodies or an antigen-binding or immunologically functional immunoglobulin fragments thereof that bind specifically to NGF with high affinity, wherein said antibodies or fragments dissociate from a human NGF polypeptide with a K_D of about 1×10^{-9} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-8} M or less, and wherein the antibodies or fragments comprise a heavy chain variable region comprising:

a) a CDR1 region comprising an amino acid sequence of the formula:

10 $a^1 a^2 a^3 a^4 a^5$

wherein:

a^1 is a polar hydrophilic amino acid residue; a^2 is an aromatic amino acid residue; a^3 is a aliphatic, polar hydrophobic, aromatic amino acid residue; a^4 is a neutral hydrophobic or aliphatic amino acid residue; and a^5 is a aliphatic or polar hydrophilic amino acid residue;

b) a CDR2 region comprising an amino acid sequence of the formula:

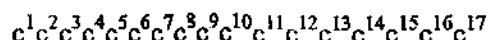
$b^1 b^2 b^3 b^4 b^5 b^6 b^7 b^8 b^9 b^{10} b^{11} b^{12} b^{13} b^{14} b^{15} b^{16} b^{17}$

20 wherein:

b^1 is a aliphatic, polar hydrophobic, or aromatic amino acid residue; b^2 is an aliphatic hydrophobic amino acid residue; b^3 is a polar hydrophilic or aromatic amino acid residue; b^4 is a polar hydrophilic, hydrophobic, or aromatic amino acid residue; b^5 - b^9 are independently polar hydrophilic or aliphatic amino acid residues; b^{10} is a polar hydrophilic, aromatic, or aliphatic amino acid residue; b^{11} is an aromatic or hydrophobic amino acid residue; b^{12} is an aliphatic hydrophobic or polar hydrophilic amino acid residue; b^{13} is an aliphatic, hydrophobic or polar hydrophilic amino acid residue; b^{14} and b^{16} are independently polar hydrophilic amino acid residues; b^{15} is an aliphatic or

aromatic hydrophobic amino acid residue; and b¹⁷ is an aliphatic acidic amino acid residue; and

c) a CDR3 region comprising an amino acid sequence of the formula:



5 wherein:

c¹ is absent or an aliphatic amino acid residue; c² is absent or a polar hydrophilic or an aromatic hydrophobic amino acid residue; c³ and c⁴ are independently absent or a polar hydrophilic, aromatic hydrophobic, or aliphatic amino acid residues; c⁵ is absent or a polar hydrophilic, aliphatic or an aromatic amino acid residue; c⁶ is absent or a polar hydrophilic or aliphatic amino acid residue; c⁷ is a polar hydrophilic or an aliphatic amino acid residue; c⁸ is a polar hydrophilic, hydrophobic or an aromatic amino acid residue; c⁹ is a polar hydrophilic, aliphatic or an aromatic hydrophobic amino acid residue; c¹⁰ a polar hydrophilic, aromatic hydrophobic, or an aliphatic hydrophobic amino acid residue; c¹¹ - c¹³ are independently polar hydrophilic or aromatic hydrophobic amino acid residues; c¹⁴ is an aliphatic or aromatic hydrophobic amino acid residue; c¹⁵ is a polar hydrophilic or neutral hydrophobic amino acid residue; c¹⁶ is absent or a polar hydrophilic amino acid residue; and c¹⁷ is an aromatic hydrophobic or aliphatic hydrophobic amino acid residue.

In one aspect, a¹ is a polar hydrophilic amino acid residue; a² is an aromatic hydrophobic amino acid residue; a³ is an aliphatic hydrophobic amino acid residue; a⁴ is a neutral hydrophobic; a⁵ is a polar hydrophilic amino acid residue; b¹ is a aliphatic or aromatic amino acid residue; b² is Ile; b³ is a polar hydrophilic amino acid residue; b⁴ is a polar hydrophilic or aromatic amino acid residue; b⁵-b⁹ are independently polar hydrophilic or aliphatic amino acid residues; b¹⁰ is an aliphatic amino acid residue; b¹¹ is Tyr; b¹² is an aliphatic hydrophobic amino acid residue; b¹³ is an aliphatic or polar hydrophilic amino acid residue; b¹⁴ and b¹⁶ are independently polar hydrophilic amino acid residues; and b¹⁵ is an aliphatic hydrophobic amino acid residue; b¹⁷ is an aliphatic acidic amino acid residue; c¹ is absent or an aliphatic amino acid residue; c² is absent or a polar hydrophilic or an aromatic hydrophobic amino acid residue; c³ and c⁴ are independently absent or a polar hydrophilic, aromatic hydrophobic, or aliphatic amino

acid residues; c^5 is absent or a polar hydrophilic amino acid residue; c^6 is absent or a polar hydrophilic or aliphatic amino acid residue; c^7 is a polar hydrophilic or an aliphatic amino acid residue; c^8 is a polar hydrophilic, hydrophobic or an aromatic amino acid residue; c^9 is a polar hydrophilic, aliphatic or an aromatic hydrophobic amino acid residue; c^{10} is a polar hydrophilic, aromatic hydrophobic, or an aliphatic hydrophobic amino acid residue; $c^{11} - c^{13}$ are independently polar hydrophilic or aromatic hydrophobic amino acid residues; c^{14} is an aliphatic or aromatic hydrophobic amino acid residue; c^{15} is a polar hydrophilic or neutral hydrophobic amino acid residue; c^{16} is absent or a polar hydrophilic amino acid residue; and c^{17} is an aromatic hydrophobic or aliphatic hydrophobic amino acid residue.

In a particular aspect, a^1 is Ser, Asp, or Thr; a^2 is Tyr; a^3 is Ala, Ser, Trp, or Gly; a^4 is Met or Ile; a^5 is His, Gly, or Asn; b^1 is Tyr, Gly, Ile, or Asp; b^2 is Ile; b^3 is Ser, Thr, Tyr, or Asn; b^4 is Trp, Arg, or Pro; b^5 is Ser, Asn, or Gly; b^6 is Ser, Arg, Asp, or Gly; b^7 is Ser, His, or Gly; b^8 is Ser, Ile, Asp, or Thr; b^9 is Leu, Ile, or Thr; b^{10} is Gly, Lys, or Phe; b^{11} is Tyr; b^{12} is Ala or Ser; b^{13} is Asp, Gly, or Pro; b^{14} is Ser; b^{15} is Val or Phe; b^{16} is Lys or Gln; b^{17} is Gly; c^1 is absent or an aliphatic amino acid residue; c^2 is absent or Tyr; c^3 and c^4 are independently absent, Tyr, Asn, Val, or Glu; c^5 is absent, Ser, Gly, or Trp; c^6 is absent, Ser, Gly, Glu, or Leu; c^7 is Gly, Arg, or Asp; c^8 is Trp, Pro, Ser, or Thr; c^9 is His, Gly, or Tyr; c^{10} is Val, Tyr, or Arg; $c^{11} - c^{13}$ are independently Ser, Phe, Tyr, Asp, or Asn; c^{14} is Phe, Val, or Gly; c^{15} is Met or Asp; c^{16} is absent, Asp, or Asn; and c^{17} is Tyr or Val.

In another particular aspect, a^1 is Ser or Asp; a^2 is Tyr; a^3 is Ala or Ser; a^4 is Met or Ile; a^5 is His or Asn; b^1 is Tyr or Gly; b^2 is Ile; b^3 is Ser, Thr, Tyr, or Asn; b^4 is Trp, Arg, or Pro; b^5 is Ser or Asn; b^6 is Ser or Arg; b^7 is His or Gly; b^8 is Ile or Thr; b^9 is Leu, Ile, or Thr; b^{10} is Gly or Phe; b^{11} is Tyr; b^{12} is Ala or Ser; b^{13} is Asp or Gly; b^{14} is Ser; b^{15} is Val or Phe; b^{16} is Lys or Gln; b^{17} is Gly; c^1 is absent or Gly; c^2 is absent or Tyr; c^3 and c^4 are independently absent, Tyr, Gly, or Val; c^5 is absent or Ser; c^6 is Ser or Gly; c^7 is Gly or Arg; c^8 is Trp or Pro; c^9 is His, Gly, or Tyr; c^{10} is Val or Tyr; $c^{11} - c^{13}$ are independently Ser, Tyr, Phe, or Asp; c^{14} is Phe or Val; c^{15} is Met or Asp; c^{16} is absent or Asp; and c^{17} is Tyr or Val.

In other particular aspects:

5 a) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 22, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 18, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 14;

b) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 92, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 93, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 94;

10 c) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 98, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 99, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 100;

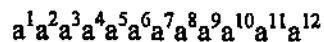
15 d) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 104, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 105, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 106;

20 e) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 110, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 111, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 112; and

25 f) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 116, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 117, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 118.

The invention also provides an isolated human antibody or an antigen-binding or an immunologically functional immunoglobulin fragment thereof that binds specifically to NGF, wherein the antibody or fragment comprises a light chain variable region comprising:

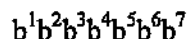
a) a CDR1 region comprising an amino acid sequence of the formula:



wherein:

a^1 is a polar hydrophilic amino acid residue; a^2 , a^{11} and a^{12} are independently aliphatic or hydrophobic amino acid residues; a^3 , a^5 , a^7 and a^8 are independently aliphatic, polar hydrophilic, or hydrophobic amino acid residues; a^4 is a polar hydrophilic amino acid residue; a^6 is an aliphatic or hydrophobic amino acid residue; a^9 is absent, or an aliphatic or polar hydrophilic amino acid residue; and a^{10} is an aliphatic, aromatic, or hydrophobic amino acid residue;

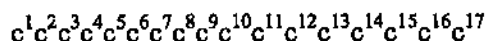
b) a CDR2 region comprising an amino acid sequence of the formula:



wherein:

b^1 is a aliphatic, polar hydrophobic, or hydrophobic amino acid residue; b^2 is an aliphatic or hydrophobic amino acid residue; b^3 and b^4 are independently polar hydrophilic, aliphatic or hydrophobic amino acid residues; b^5 is a polar hydrophilic or aliphatic hydrophobic amino acid residues; b^6 is a polar hydrophilic or aliphatic hydrophobic amino acid residue; and b^7 is a polar hydrophilic amino acid residue; and

c) a CDR3 region comprising an amino acid sequence of the formula:



wherein:

c^1 and c^2 are independently polar hydrophilic amino acid residues; c^3 is a polar hydrophilic, aliphatic or hydrophobic amino acid residue; c^4 , c^5 and c^6 are independently aliphatic, polar hydrophilic, or hydrophobic amino acid residues; c^7 is absent or a polar hydrophilic or an aliphatic hydrophobic amino acid residue; c^8 is a polar hydrophilic or hydrophobic amino acid residue; and c^9 is a polar hydrophilic amino acid residue, and

wherein said antibody or fragment dissociates from a human NGF polypeptide with a K_D of about 1×10^{-9} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-8} M or less.

In one aspect, a^1 , a^3 , a^4 , a^7 and a^8 are independently polar hydrophilic amino acid residues; a^2 , a^6 , a^{11} and a^{12} are independently aliphatic hydrophobic amino acid residues; a^5 is a polar hydrophilic or aliphatic amino acid residue; a^9 is absent, or an aliphatic or polar hydrophilic amino acid residue; a^{10} is an aliphatic or aromatic amino acid residue; b^1 is a aliphatic, polar hydrophobic, or hydrophobic amino acid residue; b^2 is an aliphatic hydrophobic amino acid residue; b^3 , b^4 and b^7 are independently polar hydrophilic amino acid residues; b^5 and b^6 are independently polar hydrophilic or aliphatic hydrophobic amino acid residues; c^1 and c^2 are independently polar hydrophilic amino acid residues; c^3 is a polar hydrophilic, aliphatic or hydrophobic amino acid residue; c^4 , c^5 , and c^6 are independently aliphatic, polar hydrophilic, or hydrophobic amino acid residues; c^7 is absent or an aliphatic hydrophobic amino acid residue; c^8 is a hydrophobic amino acid residue; and c^9 is a polar hydrophilic amino acid residue.

In a particular aspect, a^1 , a^3 , a^4 , and a^7 are Arg, Ser, Gln, and Ser, respectively; a^2 is Ala; a^5 is Gly or Ser; a^8 is Ser or Ile; a^9 is absent, Ser, or Gly; a^{10} is Ala, Tyr, Trp or Phe; b^1 is Asp, Gly, Ala, or Val; b^2 and b^3 are Ala and Ser, respectively; b^4 is Ser or Asn; b^5 is Leu or Arg; b^6 is Glu, Ala, or Gln; b^7 is Ser or Thr; c^1 and c^2 are Gln; c^3 is Phe, Tyr, Arg, or Ala; c^4 is Asn, Gly, or Ser; c^5 is Ser or Asn; c^6 is Tyr, Ser, Trp, or Phe; c^7 is absent, Pro, or His; c^8 is Leu, Trp, Tyr, or Arg; and c^9 is Thr.

In another particular aspect, a^1 , a^2 , a^3 , a^4 , and a^7 are Arg, Ala, Ser, Gln, and Ser, respectively; a^5 is Gly or Ser; a^8 is Ser or Ile; a^9 is absent, Ser, or Gly; a^{10} is Ala or Tyr; b^1 is Asp or Gly; b^2 and b^3 are Ala and Ser, respectively; b^4 is Ser or Asn; b^5 is Leu or Arg; b^6 is Glu, Ala, or Gln; b^7 is Ser or Thr; c^1 and c^2 are Gln; c^3 is Phe, Tyr, Arg, or Ala; c^4 is Asn, Gly, or Ser; c^5 is Ser or Asn; c^6 is Tyr, Ser, Trp, or Phe; c^7 is absent, Pro, or His; c^8 is Leu, Trp, Tyr, or Arg; and c^9 is Thr.

In other particular aspects:

a) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 24, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO:

20, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 16;

5 b) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 95, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 96, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 97;

10 c) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 101, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 102, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 103;

 d) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 107, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 108, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 109;

15 e) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 113, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 114, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 115;

20 f) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 119, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 120, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 121;

25 g) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 122, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 123, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 124;

 h) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 125, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO:

126, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 127;

i) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 128, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 129, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 130; and

j) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 132, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 133, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 134.

Also part of the invention are polynucleotide sequences that encode the novel anti-human NGF human antibodies, vectors comprising the polynucleotide sequences encoding anti-human NGF human antibodies, host cells transformed with vectors incorporating polynucleotides that encode the anti-human NGF human antibodies, formulations comprising anti-human NGF human antibodies and methods of making and using the same.

The invention also provides methods for detecting the level of NGF in a biological sample, comprising the step of contacting the sample with an antibody of the invention or antigen-binding fragment thereof. An anti-NGF antibody of the invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, immunoprecipitation assays and enzyme-linked immunosorbent assays (ELISA) (See, Sola, 1987, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158, CRC Press, Inc.) for the detection and quantitation of NGF. The antibodies can bind NGF with an affinity that is appropriate for the assay method being employed.

In addition, the invention provides methods for treating a disease associated with increased production of NGF, or increased sensitivity to NGF comprising the step of administering a pharmaceutically effective amount of a pharmaceutical composition comprising at least one antibody of the invention or an antigen-binding or an

immunologically functional immunoglobulin fragment thereof to an individual in need thereof.

Specific preferred embodiments of the invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts graphs that demonstrate neutralization of NGF activity in the DRG neuron based neutralization bioassay by 4D4 monoclonal antibodies purified from the hybridoma conditioned media.

10 Figure 2 depicts graphs that demonstrate VR1 expression stimulated by human NGF activity and neutralization of NGF activity in DRG neuron based neutralization bioassays by an anti-NGF monoclonal antibody (4D4) purified from the hybridoma conditioned media.

15 Figure 3 depicts graphs that demonstrate neutralization of NGF activity in DRG neuron based neutralization bioassays by transiently expressed recombinant anti-NGF 4D4 monoclonal antibodies when expressed as either an IgG1 or IgG2 and in cells grown either in a roller bottle culture (R) or in spinner flasks (S).

20 Figure 4 depicts sequence alignments of neurotrophins. The numbering and secondary structure elements above the sequence refer to mature human NGF. Conserved residues are marked with a star, and regions with low sequence homology are shaded. NGF human is SEQ ID NO: 135; NGF mouse is SEQ ID NO: 136; BDNF is SEQ ID NO: 137; NT3 is SEQ ID NO: 138.

25 Figure 5 shows anti-NGF CDR1 heavy chain alignment and percent identity for the 14D10 (SEQ ID NO: 98), 6H9 (SEQ ID NO: 104), 7H2 (SEQ ID NO: 110), 4G6 (SEQ ID NO: 116), 14D11 (SEQ ID NO: 92), and 4D4 (SEQ ID NO: 22) antibodies.

Figure 6 shows anti-NGF CDR2 heavy chain alignment and percent identity for the 14D10 (SEQ ID NO: 99), 6H9 (SEQ ID NO: 105), 7H2 (SEQ ID NO: 111), 4G6 (SEQ ID NO: 117), 14D11 (SEQ ID NO: 93), and 4D4 (SEQ ID NO: 18) antibodies.

Figure 7 shows anti-NGF CDR3 heavy chain alignment and percent identity for the 14D10 (SEQ ID NO: 100), 6H9 (SEQ ID NO: 106), 7H2 (SEQ ID NO: 112), 4G6 (SEQ ID NO: 118), 14D11 (SEQ ID NO: 94), and 4D4 (SEQ ID NO: 14) antibodies.

Figure 8 shows anti-NGF CDR1 light chain alignment and percent identity for the 14D10 (SEQ ID NO: 95), 6H9 (SEQ ID NO: 107), 7H2 (SEQ ID NO: 113), 4G6a (SEQ ID NO: 119), 4G6b (SEQ ID NO: 122), 4G6c (SEQ ID NO: 125), 4G6d (SEQ ID NO: 128), 4G6e (SEQ ID NO: 132), 14D11 (SEQ ID NO: 95), and 4D4 (SEQ ID NO: 24) antibodies (4G6a is referred to in various Figures as 20031028340; 4G6b is referred to in various Figures as 20031028351; 4G6c is referred to in various Figures as 20031071526; 4G6d is referred to in various Figures as 20031028344; 4G6e is referred to in various Figures as 20031000528).

Figure 9 shows anti-NGF CDR2 light chain alignment and percent identity for the 14D10 (SEQ ID NO: 96), 6H9 (SEQ ID NO: 108), 7H2 (SEQ ID NO: 114), 4G6a (SEQ ID NO: 120), 4G6b (SEQ ID NO: 123), 4G6c (SEQ ID NO: 126), 4G6d (SEQ ID NO: 129), 4G6e (SEQ ID NO: 133), 14D11 (SEQ ID NO: 96), and 4D4 (SEQ ID NO: 20) antibodies (4G6a is referred to in various Figures as 20031028340; 4G6b is referred to in various Figures as 20031028351; 4G6c is referred to in various Figures as 20031071526; 4G6d is referred to in various Figures as 20031028344; 4G6e is referred to in various Figures as 20031000528).

Figure 10 shows anti-NGF CDR3 light chain alignment and percent identity for the 14D10 (SEQ ID NO: 97), 6H9 (SEQ ID NO: 109), 7H2 (SEQ ID NO: 115), 4G6a (SEQ ID NO: 121), 4G6b (SEQ ID NO: 124), 4G6c (SEQ ID NO: 127), 4G6d (SEQ ID NO: 130), 4G6e (SEQ ID NO: 134), 14D11 (SEQ ID NO: 97), and 4D4 (SEQ ID NO: 16) antibodies (4G6a is referred to in various Figures as 20031028340; 4G6b is referred to in various Figures as 20031028351; 4G6c is referred to in various Figures as 20031071526; 4G6d is referred to in various Figures as 20031028344; 4G6e is referred to in various Figures as 20031000528).

Figure 11 shows anti-NGF light chain alignment and percent identity for the 14D10 (SEQ ID NO: 82), 6H9 (SEQ ID NO: 84), 7H2 (SEQ ID NO: 86), 4G6a (SEQ ID NO: 88), 4G6b (SEQ ID NO: 89), 4G6c (SEQ ID NO: 90), 4G6d (SEQ ID NO: 91),

4G6e (SEQ ID NO: 131), 14D11 (SEQ ID NO: 80), and 4D4 (SEQ ID NO: 12) antibodies (4G6a is referred to in various Figures as 20031028340; 4G6b is referred to in various Figures as 20031028351; 4G6c is referred to in various Figures as 20031071526; 4G6d is referred to in various Figures as 20031028344; 4G6e is referred to in various
5 Figures as 20031000528).

Figure 12 shows anti-NGF heavy chain alignment and percent identity for the 4D4 (SEQ ID NO: 10), 4G6 (SEQ ID NO: 87), 14D10 (SEQ ID NO: 81), 14D11 (SEQ ID NO: 79), 7H2 (SEQ ID NO: 85), and 6H9 (SEQ ID NO: 83) antibodies.

10 **DETAILED DESCRIPTION OF CERTAIN PREFERRED EMBODIMENTS**

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited in this application are expressly incorporated by reference herein for any purpose.

15 **Definitions**

Conventional techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described
20 herein. The foregoing techniques and procedures may be generally performed according to methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.*, Sambrook *et al.*, 2001, MOLECULAR CLONING: A LABORATORY MANUAL, 3d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein
25 by reference for any purpose. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Similarly,

conventional techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings: The phrases
5 "biological property", "biological characteristic", and the term "activity" in reference to an antibody of the present invention are used interchangeably herein and include, but are not limited to, epitope affinity and specificity (e.g., anti-human NGF human antibody binding to human NGF), ability to antagonize the activity of the targeted polypeptide (e.g., NGF activity), the *in vivo* stability of the antibody, and the immunogenic properties
10 of the antibody. Other identifiable biological properties or characteristics of an antibody recognized in the art include, for example, cross-reactivity, (i.e., with non-human homologs of the targeted polypeptide, or with other proteins or tissues, generally), and ability to preserve high expression levels of protein in mammalian cells. The aforementioned properties or characteristics can be observed or measured using art-
15 recognized techniques including, but not limited to ELISA, competitive ELISA, surface plasmon resonance analysis, *in vitro* and *in vivo* neutralization assays (e.g., Example 2), and immunohistochemistry with tissue sections from different sources including human, primate, or any other source as the need may be. Particular activities and biological properties of anti-human NGF human antibodies are described in further detail in the
20 Examples below.

The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the isolated polynucleotide (1) is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, (2) is linked to a
25 polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

The term "isolated protein" referred to herein means that a subject protein (1) is free of at least some other proteins with which it would normally be found, (2) is essentially free of other proteins from the same source, e.g., from the same species, (3) is
30 expressed by a cell from a different species, (4) has been separated from at least about 50

percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (5) is not associated (by covalent or noncovalent interaction) with portions of a protein with which the "isolated protein" is associated in nature, (6) is operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (7) does not occur in nature. Such an isolated protein can be encoded by genomic DNA, cDNA, mRNA or other RNA, of synthetic origin, or any combination thereof. Preferably, the isolated protein is substantially free from proteins or polypeptides or other contaminants that are found in its natural environment that would interfere with its use (therapeutic, diagnostic, prophylactic, research or otherwise).

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present.

The terms "polypeptide" or "protein" means molecules having the sequence of native proteins, that is, proteins produced by naturally-occurring and specifically non-recombinant cells, or genetically-engineered or recombinant cells, and comprise molecules having the amino acid sequence of the native protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. The terms "polypeptide" and "protein" specifically encompass anti-NGF antibodies, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of an anti-NGF antibody.

The term "polypeptide fragment" refers to a polypeptide that has an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion. In certain embodiments, fragments are at least 5 to about 500 amino acids long. It will be appreciated that in certain embodiments, fragments are at least 5, 6, 8, 10, 14, 20, 50, 70,
5 100, 110, 150, 200, 250, 300, 350, 400, or 450 amino acids long. Particularly useful polypeptide fragments include functional domains, including binding domains. In the case of an anti-NGF antibody, useful fragments include but are not limited to a CDR region, a variable domain of a heavy or light chain, a portion of an antibody chain or just its variable region including two CDRs, and the like.

10 The term "specific binding agent" refers to a natural or non-natural molecule that specifically binds to a target. Examples of specific binding agents include, but are not limited to, proteins, peptides, nucleic acids, carbohydrates, and lipids. In certain embodiments, a specific binding agent is an antibody.

The term "specific binding agent to NGF" refers to a specific binding agent that
15 specifically binds any portion of NGF. In certain embodiments, a specific binding agent to NGF is an antibody that binds specifically to NGF.

The term "immunologically functional immunoglobulin fragment" as used herein refers to a polypeptide fragment that contains at least the CDRs of the immunoglobulin heavy and light chains. An immunologically functional immunoglobulin fragment of the
20 invention is capable of binding to an antigen. In preferred embodiments, the antigen is a ligand that specifically binds to a receptor. In these embodiments, binding of an immunologically functional immunoglobulin fragment of the invention prevents binding of the ligand to its receptor, interrupting the biological response resulting from ligand binding to the receptor. Preferably, an immunologically functional immunoglobulin
25 fragment of the invention binds specifically to NGF. Most preferably, the fragment binds specifically to human NGF.

The term "naturally-occurring" as used herein and applied to an object refers to the fact that the object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be

isolated from a source in nature and that has not been intentionally modified by man is naturally-occurring.

The term "operably linked" means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions under
5 suitable conditions. For example, a control sequence "operably linked" to a protein coding sequence is ligated thereto so that expression of the protein coding sequence is achieved under conditions compatible with the transcriptional activity of the control sequences.

The term "control sequence" as used herein refers to polynucleotide sequences
10 that can effect expression, processing or intracellular localization of coding sequences to which they are ligated. The nature of such control sequences may depend upon the host organism. In particular embodiments, control sequences for prokaryotes may include a promoter, ribosomal binding site, and transcription termination sequence. In other particular embodiments, control sequences for eukaryotes may include promoters
15 comprising one or a plurality of recognition sites for transcription factors, transcription enhancer sequences, transcription termination sequences and polyadenylation sequences. In certain embodiments, "control sequences" can include leader sequences and/or fusion partner sequences.

The term "polynucleotide" as referred to herein means single-stranded or double-
20 stranded nucleic acid polymers of at least 10 nucleotides in length. In certain embodiments, the nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromuridine, ribose modifications such as arabinoside and 2',3'-dideoxyribose and internucleotide linkage modifications such as
25 phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate and phosphoroamidate. The term "polynucleotide" specifically includes single and double stranded forms of DNA.

The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and/or non-naturally
30 occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset

comprising members that are generally single-stranded and have a length of 200 nucleotides or fewer. In certain embodiments, oligonucleotides are 10 to 60 nucleotides in length. In certain embodiments, oligonucleotides are 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 nucleotides in length. Oligonucleotides may be single stranded or double stranded, e.g. for use in the construction of a genetic mutant. Oligonucleotides of the invention may be sense or antisense oligonucleotides with reference to a protein-coding sequence.

The term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoramidate, and the like. See, e.g., LaPlanche *et al.*, 1986, *Nucl. Acids Res.*, 14:9081; Stec *et al.*, 1984, *J. Am. Chem. Soc.*, 106:6077; Stein *et al.*, 1988, *Nucl. Acids Res.*, 16:3209; Zon *et al.*, 1991, *Anti-Cancer Drug Design*, 6:539; Zon *et al.*, 1991, OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL APPROACH, pp. 87-108 (F. Eckstein, Ed.), Oxford University Press, Oxford England; Stec *et al.*, U.S. Pat. No. 5,151,510; Uhlmann and Peyman, 1990, *Chemical Reviews*, 90:543, the disclosures of which are hereby incorporated by reference for any purpose. An oligonucleotide can include a detectable label to enable detection of the oligonucleotide or hybridization thereof.

The term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e. g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell. and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they

are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The phrase "recombinant host cell" (or simply "host cell") includes a cell into which a recombinant expression vector has been introduced. It will be understood by those of skill in the art that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. A wide variety of host expression systems can be used to express the antibodies of the present invention including bacterial, yeast, baculoviral and mammalian expression systems (as well as phage display expression systems). An example of a suitable bacterial expression vector is pUC19. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook *et al.*, 2001, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Laboratories, Ausubel, F.M. *et al.* (eds.) Current Protocols in Molecular Biology, Greene Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 to Boss *et al.*

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a nucleic acid sequence and then of expressing a

selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

5 The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well
10 known in the art and are disclosed herein. See, e.g., Graham *et al.*, 1973, *Virology* 52:456; Sambrook *et al.*, 2001, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Laboratories; Davis *et al.*, 1986, BASIC METHODS IN MOLECULAR BIOLOGY, Elsevier; and Chu *et al.*, 1981, *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable
15 host cells.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may
20 recombine with that of the cell by physically integrating into a chromosome of the cell, or may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell.

The term "naturally occurring" or "native" when used in connection with
25 biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The term "antigen" refers to a molecule or a portion of a molecule capable of
30 being bound by a selective binding agent, such as an antibody, and additionally capable

of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term "identity," as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences thereof. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or polypeptides, as the case may be, as determined by the match between strings of two or more nucleotide or two or more amino acid sequences. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*i.e.*, "algorithms").

The term "similarity" is used in the art with regard to a related concept, but in contrast to "identity," "similarity" refers to a measure of relatedness, which includes both identical matches and conservative substitution matches. If two polypeptide sequences have, for example, 10/20 identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are five more positions where there are conservative substitutions, then the percent identity remains 50%, but the percent similarity would be 75% (15/20). Therefore, in cases where there are conservative substitutions, the percent similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

Identity and similarity of related nucleic acids and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in COMPUTATIONAL MOLECULAR BIOLOGY, (Lesk, A.M., ed.), 1988, Oxford University Press, New York; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, (Smith, D.W., ed.), 1993, Academic Press, New York; COMPUTER ANALYSIS OF SEQUENCE DATA, Part 1, (Griffin, A.M., and Griffin, H.G., eds.), 1994, Humana Press, New Jersey; von Heinje, G., SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, 1987, Academic Press; SEQUENCE ANALYSIS PRIMER, (Gribskov, M. and Devereux, J., eds.), 1991, M. Stockton Press, New York;

Carillo *et al.*, 1988, *SIAM J. Applied Math.*, 48:1073; and Durbin *et al.*, 1998, BIOLOGICAL SEQUENCE ANALYSIS, Cambridge University Press.

Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are described in publicly available computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, 1984, *Nucl. Acid. Res.*, 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et al.*, 1990, *J. Mol. Biol.*, 215:403-410). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul *et al.* NCB/NLM/NIH Bethesda, MD 20894; Altschul *et al.*, 1990, *supra*). The well-known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, in certain embodiments, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). In certain embodiments, a gap opening penalty (which is calculated as three-times the average diagonal; where the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually one-tenth of the gap opening penalty), as well as a comparison matrix such as PAM250 or BLOSUM 62 are used in conjunction with the algorithm. In certain embodiments, a standard comparison matrix (see Dayhoff *et al.*, 1978, *Atlas of Protein Sequence and Structure*,

5:345-352 for the PAM 250 comparison matrix; Henikoff *et al.*, 1992, *Proc. Natl. Acad. Sci USA*, 89:10915-10919 for the BLOSUM 62 comparison matrix) is also used by the algorithm.

In certain embodiments, the parameters for a polypeptide sequence comparison
5 include the following:

Algorithm: Needleman *et al.*, 1970, *J. Mol. Biol.*, 48:443-453;

Comparison matrix: BLOSUM 62 from Henikoff *et al.*, 1992, *supra*;

Gap Penalty: 12

Gap Length Penalty: 4

10 Threshold of Similarity: 0

The GAP program may be useful with the above parameters. In certain embodiments, the aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

The term "homology" refers to the degree of similarity between protein or nucleic
15 acid sequences. Homology information is useful for the understanding the genetic relatedness of certain protein or nucleic acid species. Homology can be determined by aligning and comparing sequences. Typically, to determine amino acid homology, a protein sequence is compared to a database of known protein sequences. Homologous sequences share common functional identities somewhere along their sequences. A high
20 degree of similarity or identity is usually indicative of homology, although a low degree of similarity or identity does not necessarily indicate lack of homology.

Several approaches can be used to compare amino acids from one sequence to amino acids of another sequence to determine homology. Generally, the approaches fall into two categories: (1) comparison of physical characteristics such as polarity, charge,
25 and Van der Waals volume, to generate a similarity matrix; and (2) comparison of likely substitution of an amino acid in a sequence by any other amino acid, which is based on observation of many protein sequences from known homologous proteins and to generate a Point Accepted Mutation Matrix (PAM).

The percentage of identity may also be calculated by using the program needle (EMBOSS package) or stretcher (EMBOSS package) or the program align X, as a module of the vector NTI suite 9.0.0 software package, using the default parameters (for example, GAP penalty 5, GAP opening penalty 15, GAP extension penalty 6.6).

5 As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See IMMUNOLOGY--A SYNTHESIS, 2nd Edition, (E. S. Golub and D. R. Gren, Eds.), Sinauer Associates: Sunderland, MA, 1991, incorporated herein by reference for any purpose. Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids; unnatural amino acids such as α -, α -disubstituted amino acids,
 10 N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and
 15 imino acids (*e.g.*, 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxyl-terminal direction, in accordance with standard usage and convention.

Naturally occurring residues may be divided into classes based on common side chain properties:

- 20 1) hydrophobic: norleucine (Nor), Met, Ala, Val, Leu, Ile, Phe, Trp, Tyr, Pro;
- 2) polar hydrophilic : Arg, Asn, Asp, Gln, Glu, His, Lys, Ser, Thr ;
- 3) aliphatic : Ala, Gly, Ile, Leu, Val, Pro;
- 4) aliphatic hydrophobic : Ala, Ile, Leu, Val, Pro;
- 25 5) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- 6) acidic: Asp, Glu;
- 7) basic: His, Lys, Arg;
- 8) residues that influence chain orientation: Gly, Pro;

9) aromatic: His, Trp, Tyr, Phe; and

10) aromatic hydrophobic : Phe, Trp, Tyr.

Conservative amino acid substitutions may involve exchange of a member of one of these classes with another member of the same class. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties.

Non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human antibody that are homologous with non-human antibodies, or into the non-homologous regions of the molecule.

In making such changes, according to certain embodiments, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art (see, for example, Kyte et al., 1982, *J. Mol. Biol.* 157:105-131). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, in certain embodiments, the substitution of amino acids whose hydropathic indices are within ± 2 is included. In certain embodiments, those that are within ± 1 are included, and in certain embodiments, those within ± 0.5 are included.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically

functional protein or peptide thereby created is intended for use in immunological embodiments, as disclosed herein. In certain embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.*, with a biological property of the protein.

The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (-3.0 ± 1); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in certain embodiments, the substitution of amino acids whose hydrophilicity values are within ± 2 is included, in certain embodiments, those that are within ± 1 are included, and in certain embodiments, those within ± 0.5 are included. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

Exemplary amino acid substitutions are set forth in Table 1.

Table 1

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu

Original Residues	Exemplary Substitutions	Preferred Substitutions
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth herein using well-known techniques. In certain embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In other
5 embodiments, the skilled artisan can identify residues and portions of the molecules that are conserved among similar polypeptides. In further embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting
10 the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, the skilled artisan can predict the importance of amino acid residues in a protein that correspond to amino acid residues important for activity or
15 structure in similar proteins. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of an
20 antibody with respect to its three dimensional structure. In certain embodiments, one skilled in the art may choose to not make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino
25 acid residue. The variants can then be screened using activity assays known to those

skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change can be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moulton, 1996, *Curr. Opin. in Biotech.* 7:422-427; Chou *et al.*, 1974, *Biochemistry* 13:222-245; Chou *et al.*, 1974, *Biochemistry* 113:211-222; Chou *et al.*, 1978, *Adv. Enzymol. Relat. Areas Mol. Biol.* 47:45-148; Chou *et al.*, 1979, *Ann. Rev. Biochem.* 47:251-276; and Chou *et al.*, 1979, *Biophys. J.* 26:367-384. Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins that have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm *et al.*, 1999, *Nucl. Acid. Res.* 27:244-247. It has been suggested (Brenner *et al.*, 1997, *Curr. Opin. Struct. Biol.* 7:369-376) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.

Additional methods of predicting secondary structure include "threading" (Jones, 1997, *Curr. Opin. Struct. Biol.* 7:377-87; Sippl *et al.*, 1996, *Structure* 4:15-19), "profile analysis" (Bowie *et al.*, 1991, *Science* 253:164-170; Gribskov *et al.*, 1990, *Meth. Enzym.* 183:146-159; Gribskov *et al.*, 1987, *Proc. Nat. Acad. Sci.* 84:4355-4358), and "evolutionary linkage" (See Holm, 1999, *supra*; and Brenner, 1997, *supra*).

In certain embodiments, antibody variants include glycosylation variants wherein the number and/or type of glycosylation site has been altered compared to the amino acid sequences of the parent polypeptide. In certain embodiments, protein variants comprise a

greater or a lesser number of N-linked glycosylation sites than the native protein. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions that eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred antibody variants include cysteine variants wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) compared to the parent amino acid sequence. Cysteine variants may be useful when antibodies must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

In additional embodiments, antibody variants can include antibodies comprising a modified Fc fragment or a modified heavy chain constant region. An Fc fragment, which stands for "fragment that crystallizes," or a heavy chain constant region can be modified by mutation to confer on an antibody altered binding characteristics. See, for example, Burton and Woof, 1992, *Advances in Immunology* 51: 1-84; Ravetch and Bolland, 2001, *Annu. Rev. Immunol.* 19: 275-90; Shields *et al.*, 2001, *Journal of Biol. Chem* 276: 6591-6604; Telleman and Junghans, 2000, *Immunology* 100: 245-251; Medesan *et al.*, 1998, *Eur. J. Immunol.* 28: 2092-2100; all of which are incorporated herein by reference). Such mutations can include substitutions, additions, deletions, or any combination thereof, and are typically produced by site-directed mutagenesis using one or more mutagenic oligonucleotide(s) according to methods described herein, as well as according to methods known in the art (see, for example, Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 3rd Ed., 2001, Cold Spring Harbor, N.Y. and Berger and Kimmel, METHODS IN ENZYMOLOGY, Volume 152, Guide to Molecular

Cloning Techniques, 1987, Academic Press, Inc., San Diego, CA., which are incorporated herein by reference).

According to certain embodiments, amino acid substitutions are those that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (5) confer or modify other physicochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) may be made in the naturally occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In preferred embodiments, a conservative amino acid substitution typically does not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in PROTEINS, STRUCTURES AND MOLECULAR PRINCIPLES, (Creighton, Ed.), 1984, W. H. Freeman and Company, New York; INTRODUCTION TO PROTEIN STRUCTURE (C. Branden and J. Tooze, eds.), 1991, Garland Publishing, New York, N.Y.; and Thornton *et al.*, 1991, *Nature* 354:105, each of which are incorporated herein by reference.

Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics". See Fauchere, 1986, *Adv. Drug Res.* 15:29; Veber & Freidinger, 1985, *TIINS* p.392; and Evans *et al.*, 1987, *J. Med. Chem.* 30:1229, which are incorporated herein by reference for any purpose. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce a similar therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.*, a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from: -CH₂-NH-, -CH₂-S-, -CH₂-CH₂-, -CH=CH- (*cis* and *trans*), -COCH₂-, -CH(OH)CH₂-, and

-CH₂SO-, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used in certain embodiments to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo & Gierasch, 1992, *Ann. Rev. Biochem.* 61:387, incorporated herein by reference for any purpose); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

"Antibody" or "antibody peptide(s)" refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. In certain embodiments, binding fragments are produced by recombinant DNA techniques. In additional embodiments, binding fragments are produced by enzymatic or chemical cleavage of intact antibodies. Binding fragments include, but are not limited to, F(ab), F(ab')₂, Fv, and single-chain antibodies.

The term "heavy chain" includes any immunoglobulin polypeptide having sufficient variable region sequence to confer specificity for NGF. The term "light chain" includes any immunoglobulin polypeptide having sufficient variable region sequence to confer specificity for NGF. A full-length heavy chain includes a variable region domain, V_H, and three constant region domains, C_{H1}, C_{H2}, and C_{H3}. The V_H domain is at the amino-terminus of the polypeptide, and the C_{H3} domain is at the carboxyl-terminus. The term "heavy chain", as used herein, encompasses a full-length heavy chain and fragments thereof. A full-length light chain includes a variable region domain, V_L, and a constant region domain, C_L. Like the heavy chain, the variable region domain of the light chain is at the amino-terminus of the polypeptide. The term "light chain", as used herein, encompasses a full-length light chain and fragments thereof. A F(ab) fragment is comprised of one light chain and the C_{H1} and variable regions of one heavy chain. The heavy chain of a F(ab) molecule cannot form a disulfide bond with another heavy chain molecule. A F(ab') fragment contains one light chain and one heavy chain that contains more of the constant region, between the C_{H1} and C_{H2} domains, such that an interchain disulfide bond can be formed between two heavy chains to form a F(ab')₂ molecule. The Fv region comprises the variable regions from both the heavy and light chains, but lacks

the constant regions. Single-chain antibodies are Fv molecules in which the heavy and light chain variable regions have been connected by a flexible linker to form a single polypeptide chain, which forms an antigen-binding region. Single chain antibodies are discussed in detail in International Patent Application Publication No. WO 88/01649 and
5 U.S. Patent Nos. 4,946,778 and 5,260,203.

A bivalent antibody other than a "multispecific" or "multifunctional" antibody, in certain embodiments, is understood to comprise binding sites having identical antigenic specificity.

In assessing antibody binding and specificity according to the invention, an
10 antibody substantially inhibits adhesion of a ligand to a receptor when an excess of antibody reduces the quantity of ligand bound to receptor by at least about 20%, 40%, 60%, 80%, 85%, or more (as measured, *inter alia*, using an *in vitro* competitive binding assay).

By "neutralizing antibody" is meant an antibody molecule that is able to block or
15 substantially reduce an effector function of a target antigen to which it binds. Accordingly, a "neutralizing" anti-NGF antibody is capable of blocking or substantially reducing an effector function, such as receptor binding and/or elicitation of a cellular response, of NGF. "Substantially reduce" is intended to mean at least about 60%, preferably at least about 70%, more preferably at least about 75%, even more preferably
20 at least about 80%, still more preferably at least about 85%, most preferably at least about 90% reduction of an effector function of the target antigen (e.g., human NGF).

The term "epitope" includes any determinant, preferably a polypeptide determinant, capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings
25 of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a
30 complex mixture of proteins and/or macromolecules. In preferred embodiments, an

antibody is said to specifically bind an antigen when the equilibrium dissociation constant is $\leq 10^{-8}$ M, more preferably when the equilibrium dissociation constant is $\leq 10^{-9}$ M, and most preferably when the dissociation constant is $\leq 10^{-10}$ M.

5 An antibody binds "essentially the same epitope" as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two antibodies bind to identical or sterically overlapping epitopes are competition assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody. Usually, the antigen is immobilized on a substrate, and the ability of unlabeled antibodies to block
10 the binding of labeled antibodies is measured using radioactive or enzyme labels.

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

As used herein, the terms "label" or "labeled" refers to incorporation of a
15 detectable marker, *e.g.*, by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotin moieties that can be detected by labeled avidin (*e.g.*, streptavidin preferably comprising a detectable marker such as a fluorescent marker, a chemiluminescent marker or an enzymatic activity that can be detected by optical or colorimetric methods). In certain embodiments, the label can also be therapeutic.
20 Various methods of labeling polypeptides and glycoproteins are known in the art and may be used advantageously in the methods disclosed herein. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{125}I , ^{131}I), fluorescent labels (*e.g.*, fluorescein isothiocyanate or FITC, rhodamine, or lanthanide phosphors), enzymatic labels (*e.g.*,
25 horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase), chemiluminescent labels, hapten labels such as biotinyl groups, and predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, or epitope tags). In certain embodiments, labels are attached by spacer arms (such as $(\text{CH}_2)_n$, where
30 $n < \text{about } 20$) of various lengths to reduce potential steric hindrance.

The term "biological sample", as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, mice, monkeys, rats, rabbits, and other animals. Such substances include, but are not limited to, blood, serum, urine, cells, organs, tissues,
5 bone, bone marrow, lymph nodes, and skin.

The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. The expression "pharmaceutically effective amount" in reference to a pharmaceutical composition comprising one or a plurality of the antibodies
10 of the invention is understood to mean, according to the invention, an amount of the said pharmaceutical composition which is capable of abolishing, in the patient considered, the decrease in the sensitivity threshold to external stimuli with a return of this sensitivity threshold to a level comparable to that observed in healthy subjects.

A "disorder" is any condition that would benefit from treatment according to the
15 present invention. "Disorder" and "condition" are used interchangeably herein and include chronic and acute NGF-mediated disorders or NGF-mediated diseases, including those pathological conditions which predispose the mammal to the disorder in question.

The terms "NGF-mediated disease" and "NGF-mediated condition" encompass any medical condition or disorder associated with increased levels of NGF or increased
20 sensitivity to NGF including, but not limited to, acute pain, dental pain, pain from trauma, surgical pain, pain resulting from amputation or abscess, causalgia, demyelinating diseases, trigeminal neuralgia, cancer, chronic alcoholism, stroke, thalamic pain syndrome, diabetes, acquired immune deficiency syndrome ("AIDS"), toxins and chemotherapy, general headache, migraine, cluster headache, mixed-vascular and non-
25 vascular syndromes, tension headache, general inflammation, arthritis, rheumatic diseases, lupus, osteoarthritis, inflammatory bowel disorders, irritable bowel syndrome, inflammatory eye disorders, inflammatory or unstable bladder disorders, psoriasis, skin complaints with inflammatory components, sunburn, carditis, dermatitis, myositis, neuritis, collagen vascular diseases, chronic inflammatory conditions, inflammatory pain
30 and associated hyperalgesia and allodynia, neuropathic pain and associated hyperalgesia

and allodynia, diabetic neuropathy pain, causalgia, sympathetically maintained pain, deafferentation syndromes, asthma, epithelial tissue damage or dysfunction, herpes simplex, disturbances of visceral motility at respiratory, genitourinary, gastrointestinal or vascular regions, wounds, burns, allergic skin reactions, pruritis, vitiligo, general
5 gastrointestinal disorders, colitis, gastric ulceration, duodenal ulcers, vasomotor or allergic rhinitis, or bronchial disorders, dysmenorrhoea, dyspepsia, gastroesophageal reflux, pancreatitis, and visceralgia.

As used herein, the terms "effective amount" and "therapeutically effective amount" when used with reference to a vehicle- or a pharmaceutical composition
10 comprising one or more anti-human NGF human antibody refers to an amount or dosage sufficient to produce a desired result (i.e., where for therapy with the vehicle- or anti-human NGF human antibodies of the present invention the desired result is the desired reduction in inflammation and/or pain, for example) or to support an observable decrease in the level of one or more biological activities of NGF. More specifically, a
15 therapeutically effective amount is an amount of the anti-human NGF human antibody(ies) sufficient to inhibit, for some period of time, one or more of the clinically defined pathological processes associated with the condition at issue, e.g., inflammation or pain, in a subject treated *in vivo* with the agent. In the present invention, an "effective amount" of an anti-NGF antibody may prevent, stop, control, or reduce the perception of
20 pain associated with any painful medical condition. In the methods of the present invention, the term "control" and grammatical variants thereof, are used to refer to the prevention, partial or complete inhibition, reduction, delay or slowing down of an unwanted event, e.g., pain. The effective amount may vary depending on the specific vehicle- or anti-human NGF human antibody(ies) selected, and is also dependent on a
25 variety of factors and conditions related to the subject to be treated and the severity of the disorder. For example, if the vehicle- or anti-human NGF human antibody(ies) is to be administered *in vivo*, factors such as the age, weight and health of the patient as well as dose response curves and toxicity data obtained in preclinical animal work would be among those considered. If the agent is to be contacted with the cells *in vitro*, one would
30 also design a variety of pre-clinical *in vitro* studies to assess such parameters as uptake, half-life, dose, toxicity, etc. The determination of an effective amount or a

therapeutically effective amount for a given agent is well within the ability of those skilled in the art.

As used herein, the terms "nerve growth factor" and "NGF" are defined as all mammalian species of native sequence NGF, including recombinant human NGF 1-120,
5 shown as in SEQ ID NO:30.

As used herein, "substantially pure" or "substantially purified" means a compound or species that is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition). In certain embodiments, a substantially purified fraction is a composition wherein the species
10 comprises at least about 50 percent (on a molar basis) of all macromolecular species present. In certain embodiments, a substantially pure composition will comprise more than about 80%, 85%, 90%, 95%, or 99% of all macromolecular species present in the composition. In certain embodiments, the species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection
15 methods) wherein the composition consists essentially of a single macromolecular species.

The term "patient" includes human and animal subjects.

"Treatment" or "treat" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the
20 disorder as well as those prone to have the disorder or those in which the disorder is to be prevented.

Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

According to certain embodiments of the invention, antibodies directed to NGF
25 may be used to treat neuropathic and inflammatory pain and NGF-mediated diseases, including but not limited to, those mentioned above.

In one aspect of the invention are provided fully human monoclonal antibodies raised against and having biological and immunological specificity for binding to human NGF. In another aspect the invention provides nucleic acids comprising nucleotide

sequences encoding amino acid sequences for heavy and light chain immunoglobulin molecules, particularly sequences corresponding to the variable regions thereof. Particular embodiments of this aspect of the invention are sequences corresponding to complementarity determining regions (CDRs), specifically from CDR1 through CDR3, of the heavy and light chains provided by the invention. In yet another aspect the invention provides hybridoma cells and cell lines that express the immunoglobulin molecules and antibodies, preferably monoclonal antibodies of the invention. The invention also provides biologically and immunologically purified preparations of antibodies, preferably monoclonal antibodies raised against and having biological and immunological specificity for binding to human NGF.

The ability to clone and reconstruct megabase-sized human loci in yeast artificial chromosomes (YACs) and to introduce them into the mouse germline provides an advantageous approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents provides unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy provides a source for production of fully human monoclonal antibodies (MAbs).

The term "human antibody" includes antibodies having variable and constant regions substantially corresponding to human germline immunoglobulin sequences. In certain embodiments, human antibodies are produced in non-human mammals, including, but not limited to, rodents, such as mice and rats, and lagomorphs, such as rabbits. In certain embodiments, human antibodies are produced in hybridoma cells. In certain embodiments, human antibodies are produced recombinantly.

The term "recombinant" in reference to an antibody includes antibodies that are prepared, expressed, created or isolated by recombinant means. Representative examples include antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, 5 antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L.D., *et al.*, *Nucl. Acids Res.* 20:6287-6295,(1992); or antibodies prepared, expressed, created or isolated by any means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from 10 human germline immunoglobulin sequences.

Human antibodies have at least three advantages over non-human and chimeric antibodies for use in human therapy:

1) because the effector portion of the antibody is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more 15 efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC));

2) the human immune system should not recognize the human antibody as foreign, and, therefore the antibody response against such an injected antibody should be less than against a totally foreign non-human antibody or a partially foreign chimeric 20 antibody;

3) injected non-human antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of human antibodies. Injected human antibodies will have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

25 Thus, fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized MAbs, and to thereby increase the efficacy and safety of the administered antibodies. Fully human antibodies of the invention, therefore, can be used in the treatment of chronic and recurring pain, the treatment thereof requiring repeated antibody administration. Thus, one particular 30 advantage of the anti-NGF antibodies of the invention is that the antibodies are fully

human and can be administered to patients in a non-acute manner while minimizing adverse reactions commonly associated with human anti-mouse antibodies or other previously described non-fully human antibodies from non-human species.

One skilled in the art can engineer mouse strains deficient in mouse antibody
5 production with large fragments of the human Ig loci so that such mice produce human antibodies in the absence of mouse antibodies. Large human Ig fragments may preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse cellular machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced
10 human antibody repertoire in these mouse strains yields high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human MAbs with the desired specificity may be produced and selected.

Transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous
15 immunoglobulin production can be employed. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551-2555, (1993); Jakobovits et al., Nature, 362:255-258, (1993; Bruggemann et al., Year in Immun., 7:33 (1993); Nature 148:1547-1553 (1994), Nature
20 Biotechnology 14:826 (1996); Gross, J.A., et al., Nature, 404:995-999 (2000); and U.S. patents nos. 5,877,397, 5,874,299, 5,814,318, 5,789,650, 5,770,429, 5,661,016, 5,633,425, 5,625,126, 5,569,825, and 5,545,806 (each of which is incorporated herein by reference in its entirety for all purposes)). Human antibodies can also be produced in phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1992); Marks
25 et al., J. Mol. Biol., 222:581 (1991)). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therap, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)).

Recombinant human antibodies may also be subjected to *in vitro* mutagenesis (or,
30 when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis)

and, thus, the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from those related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

- 5 In certain embodiments, the skilled artisan can use constant regions from species other than human along with the human variable region(s) in such mice to produce chimeric antibodies.

Naturally Occurring Antibody Structure

- 10 Naturally occurring antibody structural units typically comprise a tetramer. Each such tetramer typically is composed of two identical pairs of polypeptide chains, each pair having one full-length "light" chain (typically having a molecular weight of about 25 kDa) and one full-length "heavy" chain (typically having a molecular weight of about 50-70 kDa). The amino-terminal portion of each light and heavy chain typically includes a
15 variable region of about 100 to 110 or more amino acids that typically is responsible for antigen recognition. The carboxy-terminal portion of each chain typically defines a constant region responsible for effector function. Human light chains are typically classified as kappa and lambda light chains. Heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to, IgG1,
20 IgG2, IgG3, and IgG4. IgM has subclasses including, but not limited to, IgM1 and IgM2. IgA is similarly subdivided into subclasses including, but not limited to, IgA1 and IgA2. Within full-length light and heavy chains, typically, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also
25 including a "D" region of about 10 more amino acids. See, e.g., FUNDAMENTAL IMMUNOLOGY, Ch. 7, 2nd ed., (Paul, W., ed.), 1989, Raven Press, N.Y. (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair typically form the antigen-binding site.

- 30 The variable regions typically exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called

complementarity determining regions or CDRs. The CDRs from the two chains of each pair typically are aligned by the framework regions, which may enable binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4.

- 5 The assignment of amino acids to each domain is typically in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, National Institutes of Health, Bethesda, Md.), or Chothia & Lesk, 1987, *J. Mol. Biol.* 196:901-917; Chothia *et al.*, 1989, *Nature* 342:878-883.

10 **Bispecific or Bifunctional Antibodies**

A bispecific or bifunctional antibody typically is an artificial hybrid antibody having two different heavy chain/light chain pairs and two different binding sites. Bispecific antibodies may be produced by a variety of methods including, but not limited to, fusion of hybridomas or linking of F(ab') fragments. See, e.g., Songsivilai &

- 15 Lachmann, 1990, *Clin. Exp. Immunol.* 79: 315-321; Kostelny *et al.*, 1992, *J. Immunol.* 148:1547-1553.

Preparation of Antibodies

The invention provides antibodies that bind to human NGF. These antibodies can
20 be produced by immunization with full-length NGF or fragments thereof. The antibodies of the invention can be polyclonal or monoclonal, and/or may be recombinant antibodies. In preferred embodiments, antibodies of the invention are human antibodies prepared, for example, by immunization of transgenic animals capable of producing human antibodies (see, for example, International Patent Application, Publication W0 93/12227).

- 25 The complementarity determining regions (CDRs) of the light chain and heavy chain variable regions of anti- NGF antibodies of the invention can be grafted to framework regions (FRs) from the same, or another, species. In certain embodiments, the CDRs of the light chain and heavy chain variable regions of anti-NGF antibody may be grafted to consensus human FRs. To create consensus human FRs, FRs from several

human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. The FRs of the anti-NGF antibody heavy chain or light chain can be replaced with the FRs from a different heavy chain or light chain. Rare amino acids in the FRs of the heavy and light chains of anti-NGF antibody typically are not replaced, while the rest of the FR amino acids can be replaced. Rare amino acids are specific amino acids that are in positions in which they are not usually found in FRs. The grafted variable regions from anti-NGF antibodies of the invention can be used with a constant region that is different from the constant region of anti-NGF antibody. Alternatively, the grafted variable regions are part of a single chain Fv antibody. CDR grafting is described, e.g., in U.S. Patent Nos. 6,180,370, 5,693,762, 5,693,761, 5,585,089, and 5,530,101, which are hereby incorporated by reference for any purpose.

Antibodies of the invention are preferably prepared using transgenic mice that have a substantial portion of the human antibody producing locus inserted in antibody-producing cells of the mice, and that are further engineered to be deficient in producing endogenous, murine, antibodies. Such mice are capable of producing human immunoglobulin molecules and antibodies and do not produce or produce substantially reduced amounts of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving this result are disclosed in the patents, applications, and references disclosed in the specification herein. In preferred embodiments, the skilled worker may employ methods as disclosed in International Patent Application Publication No. WO 98/24893, which is hereby incorporated by reference for any purpose. *See also* Mendez *et al.*, 1997, *Nature Genetics* 15:146-156, which is hereby incorporated by reference for any purpose.

The monoclonal antibodies (mAbs) of the invention 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). Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibodies can be employed, e.g., viral or oncogenic transformation of B-lymphocytes.

The preferred animal system for preparing hybridomas is the mouse. Hybridoma production in the mouse is very well established, and immunization protocols and techniques for isolation of immunized splenocytes for fusion are well known in the art. Fusion partners (e.g., murine myeloma cells) and fusion procedures are also known.

5 In a preferred embodiment, human monoclonal antibodies directed against NGF can be generated using transgenic mice carrying parts of the human immune system rather than the mouse system. These transgenic mice, referred to herein as "HuMab" mice, contain a human immunoglobulin gene minilocus that encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with
10 targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg *et al.*, 1994, *Nature* 368:856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ and in response to immunization, the introduced human heavy chain and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgG κ monoclonal antibodies (Lonberg *et al.*, *supra.*; Lonberg and Huszar, 1995,
15 *Intern. Rev. Immunol.* 13:65-93; Harding and Lonberg, 1995, *Ann. N.Y. Acad. Sci.* 764:536-546). The preparation of HuMab mice is described in detail in Taylor *et al.*, 1992, *Nucleic Acids Res.* 20:6287-6295; Chen *et al.*, 1993, *International Immunology* 5:647-656; Tuailon *et al.*, 1994, *J. Immunol.* 152:2912-2920; Lonberg *et al.*, 1994, *Nature* 368:856-859; Lonberg, 1994, *Handbook of Exp. Pharmacology* 113:49-101;
20 Taylor *et al.*, 1994, *International Immunology* 6:579-591; Lonberg & Huszar, 1995, *Intern. Rev. Immunol.* 13:65-93; Harding & Lonberg, 1995, *Ann. N.Y. Acad. Sci.* 764:536-546; Fishwild *et al.*, 1996, *Nature Biotechnology* 14:845-851, the contents of all of which are hereby incorporated by reference in their entirety. 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;
25 5,874,299; and 5,770,429; all to Lonberg and Kay, as well as U.S. Patent No. 5,545,807 to Surani *et al.*; International Patent Application Publication Nos. WO 93/1227, published June 24, 1993; WO 92/22646, published December 23, 1992; and WO 92/03918, published March 19, 1992, the disclosures of all of which are hereby incorporated by reference in their entirety. Alternatively, the HCo7, HCo12, and KM transgenic mice
30 strains described in the Examples below can be used to generate human anti-NGF antibodies.

The present invention provides human monoclonal antibodies that are specific for and neutralize bioactive human NGF polypeptides. Also provided are antibody heavy and light chain amino acid sequences which are highly specific for and neutralize NGF polypeptides when they are bound to them. This high specificity enables the anti-human
5 NGF human antibodies, and human monoclonal antibodies with like specificity, to be effective immunotherapy for NGF associated diseases.

In one aspect, the invention provides isolated human antibodies that bind the same or essentially the same epitope as the 4D4 antibody provided herein.

In one aspect, the invention provides isolated human antibodies comprising at
10 least one of the amino acid sequences shown in SEQ ID NOS: 10, 12, 14, 16, 18, 20, 22, 24, and 79-130 that binds a NGF polypeptide epitope with high affinity and has the capacity to antagonize NGF polypeptide activity. Preferably, these antibodies binds the same or essentially the same epitope as the 4D4 antibody provided herein.

In preferred embodiments, the isolated human antibodies bind to NGF
15 polypeptide with a dissociation constant (K_D) of 1×10^{-9} M or less and inhibits NGF induced survival in an *in vitro* neutralization assay with an IC_{50} of 1×10^{-7} M or less. In more preferred embodiments, the isolated human antibodies bind to NGF polypeptide with a dissociation constant (K_D) of 1×10^{-10} M or less and inhibits NGF induced survival in an *in vitro* neutralization assay with an IC_{50} of 1×10^{-8} M or less. In an even more
20 preferred embodiment, the isolated anti-NGF human antibodies bind to human NGF polypeptide with a dissociation constant (K_D) of 1×10^{-11} M or less and inhibits NGF induced survival in an *in vitro* assay with an IC_{50} of 1×10^{-9} M or less. Examples of anti-human NGF human antibodies that meet the aforementioned binding and neutralization criteria are provided herein.

25 The most preferred anti-human NGF human antibody of the present invention is referred to herein as 4D4 and has VL and VH polypeptide sequences as shown in SEQ ID NO: 12 and SEQ ID NO: 10, respectively. The polynucleotide sequence encoding the VL and VH of 4D4 is shown in SEQ ID NO: 11 and SEQ ID NO: 9, respectively. The properties of the anti-human NGF human antibodies of the present invention are

specifically disclosed in the Examples. Particularly notable is the high affinity for NGF polypeptide and high capacity to antagonize NGF polypeptide activity demonstrated herein.

The dissociation constant (K_D) of an anti-human NGF human antibody can be
5 determined by surface plasmon resonance as generally described in Example 9. Generally, surface plasmon resonance analysis measures real-time binding interactions between ligand (recombinant NGF polypeptide immobilized on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). Surface plasmon analysis can also be
10 performed by immobilizing the analyte (antibodies on a biosensor matrix) and presenting the ligand (recombinant V in solution). The dissociation constant (K_D) of an anti-human NGF human antibody can also be determined by using KinExA methodology. In certain embodiments of the invention, the antibodies bind to NGF with a K_D of between approximately 10^{-8} M and 10^{-12} M. The term " K_D ", as used herein, is intended to refer to
15 the dissociation constant of a particular antibody-antigen interaction. For purposes of the present invention K_D was determined as shown in Example 9.

In preferred embodiments, the antibodies of the invention are of the IgG1, IgG2, IgG3, or IgG4 isotype. Preferably, the antibodies are of the IgG3 isotype. More preferably, the antibodies are of the IgG1 isotype. Most preferably, the antibodies are of
20 the IgG2 isotype. In other embodiments, the antibodies of the invention are of the IgM, IgA, IgE, or IgD isotype. In preferred embodiments of the invention, the antibodies comprise a human kappa light chain and a human IgG1, IgG2, IgG3, or IgG4 heavy chain. Expression of antibodies of the invention comprising an IgG1 or an IgG2 heavy chain constant region is described in the Examples below. In particular embodiments, the
25 variable regions of the antibodies are ligated to a constant region other than the constant region for the IgG1, IgG2, IgG3, or IgG4 isotype. In certain embodiments, the antibodies of the invention have been cloned for expression in mammalian cells.

In certain embodiments, conservative modifications to the heavy chains and light chains of anti-NGF antibodies (and corresponding modifications to the encoding
30 nucleotides) will produce anti-NGF antibodies having functional and chemical

characteristics similar to those of the anti-NGF antibodies disclosed herein. In contrast, substantial modifications in the functional and/or chemical characteristics of anti-NGF antibodies may be accomplished by selecting substitutions in the amino acid sequence of the heavy and light chains that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis."

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of anti-NGF antibody, or to increase or decrease the affinity of the anti-NGF antibodies described herein.

As it is well known, minor changes in an amino acid sequence such as deletion, addition or substitution of one, a few or even several amino acids may lead to an allelic form of the original protein which has substantially identical properties. Therefore, in addition to the antibodies specifically described herein, other "substantially homologous" antibodies can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis. Therefore, the present invention contemplates "variant" or "mutant" anti-NGF human antibodies having substantially similar characteristics to the anti-NGF human antibodies disclosed herein (See, for example, WO 00/56772, all of which is hereby incorporated herein by reference). Thus, by the term "variant" or "mutant" in reference to an anti-NGF human antibody is meant any binding molecule (molecule X) (i) in which the hypervariable regions CDR1, CDR2, and CDR3 of the heavy chain or the

hypervariable regions CDR1, CDR2, and CDR3 of the light chain taken as a whole are at least 80% homologous, preferably at least 90% homologous, more preferably at least 95% homologous to the hypervariable regions as shown in SEQ ID NOS: 14, 18, and 22 or SEQ ID NOS: 16, 20, and 24, respectively, and (ii) wherein the variant or mutant is
5 capable of inhibiting the activity of human NGF to the same extent as a reference anti-NGF human antibody having framework regions identical to those of molecule X.

Ordinarily, an anti-NGF human antibody variant will have light and/or heavy chain CDRs, when taken as a whole, that are at least about 80% amino acid sequence identity, preferably at least about 85% sequence identity, yet more preferably at least
10 about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity,
15 yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% amino acid sequence identity to the amino acid sequence as shown in SEQ ID NOS: 14, 18, and 22 and/or SEQ ID NOS: 16, 20, and 24, respectively.

More preferably, an anti-NGF human antibody variant will have a light chain variable region, when taken as a whole, that has at least about 80% amino acid sequence
20 identity, yet more preferably at least about 81% sequence identity yet, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at
25 least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence
30 identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence

identity, yet more preferably at least about 99% amino acid sequence identity to the amino acid sequence as shown in SEQ ID NOS: 12, 80, 82, 84, 86, 88, 89, 90, or 91 and/or a heavy chain variable region, when taken as a whole, that has at least about 70% amino acid sequence identity, preferably at least about 75% sequence identity, yet more preferably at least about 80% sequence identity, yet more preferably at least about 81% sequence identity yet, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% amino acid sequence identity to the amino acid sequence as shown in SEQ ID NOS:10, 81, 83, 85, or 87.

20 A "variant" in reference to a polynucleotide is intended to refer to an nucleic acid molecule having at least about 75% nucleic acid sequence identity with a polynucleotide sequence of the present invention. Ordinarily, a polynucleotide variant will have at least about 75% nucleic acid sequence identity, more preferably at least about 80% nucleic acid sequence identity, yet more preferably at least about 81% nucleic acid sequence identity, yet more preferably at least about 82% nucleic acid sequence identity, yet more preferably at least about 83% nucleic acid sequence identity, yet more preferably at least about 84% nucleic acid sequence identity, yet more preferably at least about 85% nucleic acid sequence identity, yet more preferably at least about 86% nucleic acid sequence identity, yet more preferably at least about 87% nucleic acid sequence identity, yet more preferably at least about 88% nucleic acid sequence identity, yet more preferably at least about 89% nucleic acid sequence identity, yet more preferably at least about 90% nucleic

acid sequence identity, yet more preferably at least about 91% nucleic acid sequence identity, yet more preferably at least about 92% nucleic acid sequence identity, yet more preferably at least about 93% nucleic acid sequence identity, yet more preferably at least about 94% nucleic acid sequence identity, yet more preferably at least about 95% nucleic acid sequence identity, yet more preferably at least about 96% nucleic acid sequence identity, yet more preferably at least about 97% nucleic acid sequence identity, yet more preferably at least about 98% nucleic acid sequence identity, yet more preferably at least about 99% nucleic acid sequence identity with a novel nucleic acid sequence disclosed herein.

In particular embodiments, the invention provides antibodies that have a percentage of identity to an antibody of the invention, or an antibody that comprises a heavy chain variable region, a light chain variable region, a CDR1, CDR2, or CDR3 region that has a percentage of identity to a heavy chain variable region, a light chain variable region, a CDR1, CDR2, or CDR3 region of the invention, as shown in Example 10 herein and Figures 5-10.

In certain embodiments, the invention provides an isolated human antibody that specifically binds nerve growth factor and comprises a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising an amino acid sequence that is: at least 70% or 75% identical to the amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 80%, 85%, or 95% homologous to the amino acid sequence as set forth in SEQ ID NO: 81, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 80%, 85%, or 95% identical to the amino acid sequence as set forth in SEQ ID NO: 83, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 75%, 80%, or 85% identical to the amino acid sequence as set forth in SEQ ID NO: 85, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least, 70%, 75%, or 80% identical to the amino acid sequence as set forth in SEQ ID NO: 87, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 56% identical to the amino acid sequence as

set forth in SEQ ID NO: 79, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

In certain embodiments, the invention provides an isolated human antibody that specifically binds nerve growth factor and comprises a heavy chain and a light chain, wherein the light chain comprises a light chain variable region comprising an amino acid sequence that is: at least 70%, 75%, 80%, or 90% identical to the amino acid sequence as set forth in SEQ ID NO: 12 or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 85%, or 90% identical to the amino acid sequence as set forth in SEQ ID NO: 80, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 74%, 90%, or 94% identical to the amino acid sequence as set forth in SEQ ID NO: 88, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 80%, 85%, or 87% identical to the amino acid sequence as set forth in SEQ ID NO: 89, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 85%, 90%, or 94% identical to the amino acid sequence as set forth in SEQ ID NO: 90, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 85%, 90%, 95%, or 99% identical to the amino acid sequence as set forth in SEQ ID NO: 91, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 80%, 90%, 95%, or 96% identical to the amino acid sequence as set forth in SEQ ID NO: 82, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence as set forth in SEQ ID NO: 84, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or at least 70%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence as set forth in SEQ ID NO: 86, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

In certain other embodiments, the invention provides an isolated human antibody that specifically binds nerve growth factor and comprises a human heavy chain CDR1, wherein the heavy chain CDR1 is an amino acid sequence that is at least 40% or 60% identical to the amino acid sequence as set forth in SEQ ID NO: 98, SEQ ID NO: 105,

SEQ ID NO: 110, or SEQ ID NO: 22, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

In other embodiments, the invention provides an isolated human antibody that specifically binds nerve growth factor and comprises a human heavy chain CDR2, wherein the heavy chain CDR2 is an amino acid sequence that is: at least 70%, 82%, or 94% identical to the amino acid sequence as set forth in SEQ ID NO: 99, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% or 76% identical to the amino acid sequence as set forth in SEQ ID NO: 106, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 59% identical to the amino acid sequence as set forth in SEQ ID NO: 18, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% identical to the amino acid sequence as set forth in SEQ ID NO: 117, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or at least 70%, 75% or 80% identical to the amino acid sequence as set forth in SEQ ID NO: 111, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

In yet other embodiments, the invention provides an isolated human antibody that specifically binds nerve growth factor and comprises a human light chain CDR1, wherein the CDR1 is an amino acid sequence that is: at least 70% or 80% identical to the amino acid sequence as set forth in SEQ ID NO: 101, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70%, 75%, 80% or 90% identical to the amino acid sequence as set forth in SEQ ID NO: 95, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 75%, 80%, or 90% identical to the amino acid sequence as set forth in SEQ ID NO: 119, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 75%, 80%, or 90% identical to the amino acid sequence as set forth in SEQ ID NO: 122, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 80% identical to the amino acid sequence as set forth in SEQ ID NO: 125, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 75%, 80%, or 90% identical to the amino acid sequence as set forth in SEQ ID NO: 24, or an antigen-binding or an immunologically

functional immunoglobulin fragment thereof; at least 70% or 80% identical to the amino acid sequence as set forth in SEQ ID NO: 107, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or at least 70% or 80% identical to the amino acid sequence as set forth in SEQ ID NO: 113, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

In additional embodiments, the invention provides an isolated human antibody that specifically binds nerve growth factor and comprises a human light chain CDR2, wherein the CDR2 is an amino acid sequence that is: at least 70% or 85% identical to the amino acid sequence as set forth in SEQ ID NO: 102, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% identical to the amino acid sequence as set forth in SEQ ID NO: 96, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% identical to the amino acid sequence as set forth in SEQ ID NO: 120, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% identical to the amino acid sequence as set forth in SEQ ID NO: 123, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% or 85% identical to the amino acid sequence as set forth in SEQ ID NO: 126, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% or 85% identical to the amino acid sequence as set forth in SEQ ID NO: 129, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% identical to the amino acid sequence as set forth in SEQ ID NO: 20, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% or 85% identical to the amino acid sequence as set forth in SEQ ID NO: 108, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% identical to the amino acid sequence as set forth in SEQ ID NO: 133, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or at least 70% or 85% identical to the amino acid sequence as set forth in SEQ ID NO: 114, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

In other embodiments, the invention provides an isolated human antibody that specifically binds nerve growth factor and comprises a human light chain CDR3, wherein

the CDR3 is an amino acid sequence that is: at least 70% or 85% identical to the amino acid sequence as set forth in SEQ ID NO: 103, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% or 85% identical to the amino acid sequence as set forth in SEQ ID NO: 97, or an antigen-binding
5 or an immunologically functional immunoglobulin fragment thereof; at least 70% or 78% identical to the amino acid sequence as set forth in SEQ ID NO: 121, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% or 78% identical to the amino acid sequence as set forth in SEQ ID NO: 127, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at
10 least 70% or 78% identical to the amino acid sequence as set forth in SEQ ID NO: 130, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% or 78% identical to the amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 70% or 85% identical to the amino acid sequence as set forth in
15 SEQ ID NO: 109, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; at least 78% identical to the amino acid sequence as set forth in SEQ ID NO: 134, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or at least 85% identical to the amino acid sequence as set forth in SEQ ID NO: 115, or an antigen-binding or an immunologically functional
20 immunoglobulin fragment thereof.

The sequences of the 4D4 antibody heavy chain and light chain variable regions are shown in SEQ ID NOS: 10 and 12, respectively. However, many of the potential CDR-contact residues are amenable to substitution by other amino acids and still allow the antibody to retain substantial affinity for the antigen. Likewise, many of the
25 framework residues not in contact with the CDRs in the heavy and light chains can accommodate substitutions of amino acids from the corresponding positions from other human antibodies, by human consensus amino acids, or from other mouse antibodies, without significant loss of the affinity or non-immunogenicity of the human antibody. Selection of various alternative amino acids may be used to produce versions of the
30 disclosed anti-NGF antibodies and fragments thereof that have varying combinations of

affinity, specificity, non-immunogenicity, ease of manufacture, and other desirable properties.

In alternative embodiments, antibodies of the invention can be expressed in cell lines other than hybridoma cell lines. In these embodiments, sequences encoding particular antibodies can be used for transformation of a suitable mammalian host cell. According to these embodiments, transformation can be achieved using any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (all of which are hereby incorporated herein by reference for any purpose). Generally, the transformation procedure used may depend upon the host to be transformed. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

A nucleic acid molecule encoding the amino acid sequence of a heavy chain constant region, a heavy chain variable region, a light chain constant region, or a light chain variable region of an NGF antibody of the invention is inserted into an appropriate expression vector using standard ligation techniques. In a preferred embodiment, the anti-NGF antibody heavy chain or light chain constant region is appended to the C-terminus of the appropriate variable region and is ligated into an expression vector. The vector is typically selected to be functional in the particular host cell employed (*i.e.*, the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). For a review of expression vectors, see METH. ENZ. 185 (Goeddel, ed.), 1990, Academic Press.

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a

promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid
5 encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

Optionally, the vector may contain a "tag"-encoding sequence, *i.e.*, an oligonucleotide molecule located at the 5' or 3' end of the anti-NGF antibody polypeptide coding sequence; the oligonucleotide sequence encodes polyHis (such as
10 hexaHis), or another "tag" such as FLAG, HA (hemagglutinin influenza virus), or *myc* for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification or detection of the NGF antibody from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against
15 the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified anti-NGF antibody polypeptide by various means such as using certain peptidases for cleavage.

Flanking sequences may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (*i.e.*, from a species other than the host cell species or
20 strain), hybrid (*i.e.*, a combination of flanking sequences from more than one source), synthetic or native. As such, the source of a flanking sequence may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence is functional in, and can be activated by, the host cell machinery.

25 Flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein will have been previously identified by mapping and/or by restriction endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of a flanking

sequence may be known. Here, the flanking sequence may be synthesized using the methods described herein for nucleic acid synthesis or cloning.

Whether all or only a portion of the flanking sequence is known, it may be obtained using polymerase chain reaction (PCR) and/or by screening a genomic library with a suitable probe such as an oligonucleotide and/or flanking sequence fragment from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, Qiagen® column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (New England Biolabs, Beverly, MA) is suitable for most gram-negative bacteria, and various viral origins (*e.g.*, SV40, polyoma, adenovirus, vesicular stomatitis virus (VSV), or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it also contains the virus early promoter).

A transcription termination sequence is typically located 3' to the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly-T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells; (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex or defined media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. Advantageously, a neomycin resistance gene may also be used for selection in both prokaryotic and eukaryotic host cells.

Other selectable genes may be used to amplify the gene that will be expressed. Amplification is the process wherein genes that are required for production of a protein critical for growth or cell survival are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and promoterless thymidine kinase genes. Mammalian cell transformants are placed under selection pressure wherein only the transformants are uniquely adapted to survive by virtue of the selectable gene present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively increased, thereby leading to the amplification of both the selectable gene and the DNA that encodes another gene, such as an antibody that binds to NGF polypeptide. As a result, increased quantities of a polypeptide such as an anti-NGF antibody are synthesized from the amplified DNA.

A ribosome-binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding sequence of the polypeptide to be expressed.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various pre- or prosequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add pro-sequences, which also may affect glycosylation. The

final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the amino-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired polypeptide, if the enzyme cuts at such area within the mature polypeptide.

Expression and cloning vectors of the invention will typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding the anti-NGF antibody. Promoters are untranscribed sequences located upstream (*i.e.*, 5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control transcription of the structural gene. Promoters are conventionally grouped into one of two classes: inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, uniformly transcribe gene to which they are operably linked, that is, with little or no control over gene expression. A large number of promoters, recognized by a variety of potential host cells, are well known. A suitable promoter is operably linked to the DNA encoding heavy chain or light chain comprising an anti-NGF antibody of the invention by removing the promoter from the source DNA by restriction enzyme digestion and inserting the desired promoter sequence into the vector.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, retroviruses, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, for example, heat-shock promoters and the actin promoter.

Additional promoters which may be of interest include, but are not limited to: SV40 early promoter (Bernoist and Chambon, 1981, *Nature* 290:304-10); CMV promoter (Thomsen *et al.*, 1984, *Proc. Natl. Acad. USA* 81:659-663); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, 1980, *Cell* 22:787-97);
5 herpes thymidine kinase promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1444-45); promoter and regulatory sequences from the metallothioneine gene (Brinster *et al.*, 1982, *Nature* 296:39-42); and prokaryotic promoters such as the beta-lactamase promoter (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. U.S.A.*, 75:3727-31); or the tac promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. U.S.A.*, 80:21-25). Also of
10 interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region that is active in pancreatic acinar cells (Swift *et al.*, 1984, *Cell* 38:639-46; Ornitz *et al.*, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409 (1986); MacDonald, 1987, *Hepatology* 7:425-515); the insulin gene control region that is active in pancreatic beta
15 cells (Hanahan, 1985, *Nature* 315:115-22); the immunoglobulin gene control region that is active in lymphoid cells (Grosschedl *et al.*, 1984, *Cell* 38:647-58; Adames *et al.*, 1985, *Nature* 318:533-38; Alexander *et al.*, 1987, *Mol. Cell. Biol.*, 7:1436-44); the mouse mammary tumor virus control region that is active in testicular, breast, lymphoid and
mast cells (Leder *et al.*, 1986, *Cell* 45:485-95); the albumin gene control region that is
20 active in liver (Pinkert *et al.*, 1987, *Genes and Devel.* 1:268-76); the alpha-feto-protein gene control region that is active in liver (Krumlauf *et al.*, 1985, *Mol. Cell. Biol.*, 5:1639-48; Hammer *et al.*, 1987, *Science* 235:53-58); the alpha 1-antitrypsin gene control region that is active in liver (Kelsey *et al.*, 1987, *Genes and Devel.* 1:161-71); the beta-globin gene control region that is active in myeloid cells (Mogam *et al.*, 1985, *Nature* 315:338-
25 40; Kollias *et al.*, 1986, *Cell* 46:89-94); the myelin basic protein gene control region that is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, *Cell* 48:703-12); the myosin light chain-2 gene control region that is active in skeletal muscle (Sani, 1985, *Nature* 314:283-86); and the gonadotropic releasing hormone gene control region that is active in the hypothalamus (Mason *et al.*, 1986, *Science* 234:1372-78).

30 An enhancer sequence may be inserted into the vector to increase transcription of DNA encoding light chain or heavy chain comprising an anti-NGF antibody of the

invention by higher eukaryotes. Enhancers are *cis*-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent, having been found at positions both 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus is used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers known in the art are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be positioned in the vector either 5' or 3' to a coding sequence, it is typically located at a site 5' from the promoter.

Expression vectors of the invention may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the flanking sequences described herein are not already present in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

After the vector has been constructed and a nucleic acid molecule encoding light chain, a heavy chain, or a light chain and a heavy chain comprising an anti-NGF antibody has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression. The transformation of an expression vector for an anti-NGF antibody into a selected host cell may be accomplished by well known methods including transfection, infection, calcium phosphate co-precipitation, electroporation, microinjection, lipofection, DEAE-dextran mediated transfection, or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

A host cell, when cultured under appropriate conditions, synthesizes an anti-NGF antibody that can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not

secreted). The selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity (such as glycosylation or phosphorylation) and ease of folding into a biologically active molecule

5 Mammalian cell lines available as hosts for expression are well known in the art and include, but are not limited to, immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and a number of other cell lines. In
10 certain embodiments, cell lines may be selected through determining which cell lines have high expression levels and constitutively produce antibodies with NGF binding properties. In another embodiment, a cell line from the B cell lineage that does not make its own antibody but has a capacity to make and secrete a heterologous antibody can be selected.

15 Antibodies of the invention are useful for detecting NGF in biological samples and identification of cells or tissues that produce NGF protein. Antibodies of the invention that specifically bind to NGF may be useful in treatment of NGF mediated diseases. Said antibodies can be used in binding assays to detect NGF and to inhibit NGF from forming a complex with NGF receptors. Said antibodies that bind to NGF and
20 block interaction with other binding compounds may have therapeutic use in modulating NGF mediated diseases. In preferred embodiments, antibodies to NGF may block NGF binding to its receptor, which may result in disruption of the NGF induced signal transduction cascade.

The present invention also relates to the use of one or more of the antibodies of
25 the present invention in the manufacture of a medicament for the treatment of a painful disorder or condition caused by increased expression of NGF or increased sensitivity to NGF in a patient such as any one of disorders or conditions disclosed herein.

In preferred embodiments, the invention provides pharmaceutical compositions comprising a therapeutically effective amount of one or a plurality of the antibodies of
30 the invention together with a pharmaceutically acceptable diluent, carrier, solubilizer,

emulsifier, preservative and/or adjuvant. Preferably, acceptable formulation materials are nontoxic to recipients at the dosages and concentrations employed. In preferred embodiments, pharmaceutical compositions comprising a therapeutically effective amount of anti-NGF antibodies are provided.

- 5 In certain embodiments, acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed.

 In certain embodiments, the pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In such embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. See REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition, (A.R. Gennaro, ed.), 1990, Mack Publishing Company.

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In certain embodiments, the optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. See, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, *supra*. In certain embodiments, such compositions
5 may influence the physical state, stability, rate of *in vivo* release and rate of *in vivo* clearance of the antibodies of the invention.

In certain embodiments, the primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial
10 cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. In preferred embodiments, pharmaceutical compositions of the present invention comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, and may further include sorbitol, sucrose, Tween-20 and/or a
15 suitable substitute therefor. In certain embodiments of the invention, anti-NGF antibody compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (REMINGTON'S PHARMACEUTICAL SCIENCES, *supra*) in the form of a lyophilized cake or an aqueous solution. Further, in certain embodiments, the anti-NGF antibody product may
20 be formulated as a lyophilizate using appropriate excipients such as sucrose.

The pharmaceutical compositions of the invention can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. Preparation of such pharmaceutically acceptable compositions is within the skill of the art.

25 The formulation components are present preferably in concentrations that are acceptable to the site of administration. In certain embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for
30 use in this invention may be provided in the form of a pyrogen-free, parenterally

acceptable aqueous solution comprising the desired anti-NGF antibody in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which the anti-NGF antibody is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation can
5 involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that may provide controlled or sustained release of the product which can be delivered *via* depot injection. In certain embodiments, hyaluronic acid may also be used, having the effect of promoting sustained duration in
10 the circulation. In certain embodiments, implantable drug delivery devices may be used to introduce the desired antibody molecule.

Pharmaceutical compositions of the invention can be formulated for inhalation. In these embodiments, anti-NGF antibodies are advantageously formulated as a dry, inhalable powder. In preferred embodiments, anti-NGF antibody inhalation solutions
15 may also be formulated with a propellant for aerosol delivery. In certain embodiments, solutions may be nebulized. Pulmonary administration and formulation methods therefore are further described in International Patent Application No. PCT/US94/001875, which is incorporated by reference and describes pulmonary delivery of chemically modified proteins.

It is also contemplated that formulations can be administered orally. Anti-NGF
20 antibodies that are administered in this fashion can be formulated with or without carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. In certain embodiments, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized
25 and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the anti-NGF antibody. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

A pharmaceutical composition of the invention is preferably provided to comprise
30 an effective quantity of one or a plurality of anti-NGF antibodies in a mixture with non-

toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions may be prepared in unit-dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving anti-NGF antibodies in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See, for example, International Patent Application No. PCT/US93/00829, which is incorporated by reference and describes controlled release of porous polymeric microparticles for delivery of pharmaceutical compositions. Sustained-release preparations may include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polycesters, hydrogels, polylactides (as disclosed in U.S. Patent No. 3,773,919 and European Patent Application Publication No. EP 058481, each of which is incorporated by reference), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, 1983, *Biopolymers* 22:547-556), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, 1981, *J. Biomed. Mater. Res.* 15:167-277 and Langer, 1982, *Chem. Tech.* 12:98-105), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (European Patent Application Publication No. EP 133,988). Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art. See e.g., Eppstein *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:3688-3692; European Patent Application Publication Nos. EP 036,676; EP 088,046 and EP 143,949, incorporated by reference.

Pharmaceutical compositions used for *in vivo* administration are typically provided as sterile preparations. Sterilization can be accomplished by filtration through sterile filtration membranes. When the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. Compositions for parenteral administration can be stored in lyophilized form or in a

solution. Parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

5 Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) that is reconstituted prior to administration.

The invention also provides kits for producing a single-dose administration unit. The kits of the invention may each contain both a first container having a dried protein
10 and a second container having an aqueous formulation. In certain embodiments of this invention, kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are provided.

The effective amount of an anti-NGF antibody-containing pharmaceutical composition to be employed therapeutically will depend, for example, upon the
15 therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will vary depending, in part, upon the molecule delivered, the indication for which the anti-NGF antibody is being used, the route of administration, and the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient. In certain embodiments, the clinician may
20 titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 $\mu\text{g/kg}$ to up to about 30 mg/kg or more, depending on the factors mentioned above. In preferred embodiments, the dosage may range from 0.1 $\mu\text{g/kg}$ up to about 30 mg/kg ; more preferably from 1 $\mu\text{g/kg}$ up to about 30 mg/kg ; or even more preferably from 5 $\mu\text{g/kg}$ up to about 30 mg/kg .

25 Dosing frequency will depend upon the pharmacokinetic parameters of the particular anti-NGF antibody in the formulation used. Typically, a clinician administers the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as
30 a continuous infusion via an implantation device or catheter. Further refinement of the

appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data. In certain embodiments, the antibodies of the invention can be administered to patients throughout an extended time period.

- 5 Chronic administration of an antibody of the invention minimizes the adverse immune or allergic response commonly associated with antibodies that are raised against a human antigen in a non-human animal, for example, a non-fully human antibody produced in a non-human species.

The route of administration of the pharmaceutical composition is in accord with
10 known methods, e.g. orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, or intralesional routes; by sustained release systems or by implantation devices. In certain embodiments, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

- 15 The composition also may be administered locally via implantation of a membrane, sponge or another appropriate material onto which the desired molecule has been absorbed or encapsulated. In certain embodiments, where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be *via* diffusion, timed-release bolus, or continuous administration.

- 20 It also may be desirable to use anti-NGF antibody pharmaceutical compositions according to the invention *ex vivo*. In such instances, cells, tissues or organs that have been removed from the patient are exposed to anti-NGF antibody pharmaceutical compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

- 25 In particular, anti-NGF antibodies can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptide. In certain embodiments, such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. In certain embodiments, the cells may be immortalized. In other embodiments, in order to decrease
30 the chance of an immunological response, the cells may be encapsulated to avoid

infiltration of surrounding tissues. In further embodiments, the encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

5

EXAMPLES

The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting the invention.

10

Example 1

Generation of human NGF protein from E.coli cells

Cloning of rHu-NGF (1-120)

15 The nucleotide sequence encoding human NGF was amplified from cDNA using the oligonucleotide primers with sequences as shown in SEQ ID NO:27 and SEQ ID NO:28 and standard PCR technology. The 5' primer creates an *NdeI* restriction site and methionine initiation codon immediately preceding codon 1 (serine) of the mature sequence. The 3' primer creates a *BamHI* restriction site immediately following the
20 termination codon. The resulting PCR product was gel purified, digested with restriction endonucleases *NdeI* and *BamHI*, and then ligated into the vector pCFM1656, also digested with *NdeI* and *BamHI*. Ligated DNA was transformed into competent host cells of *E. coli* strain 657. Clones were screened for the ability to produce the recombinant protein product and to possess a plasmid having the correct nucleotide sequence (i.e.,
25 SEQ ID NO:29). The amino acid sequence of the recombinant human NGF 1-120 is shown as SEQ ID NO:30:

The expression vector pCFM1656 (ATCC #69576) was derived from the expression vector system described in US Patent No. 4,710,473. The pCFM1656 plasmid

can be derived from the described pCFM836 plasmid (Patent No. 4,710,473) by: (a) destroying the two endogenous *NdeI* restriction sites by end filling with T4 polymerase enzyme followed by blunt end ligation; (b) replacing the DNA sequence between the unique *AatII* and *ClaI* restriction sites containing the synthetic P_L promoter with a similar fragment obtained from pCFM636 (patent No. 4,710,473) containing the PL promoter and then (c) substituting the small DNA sequence between the unique *ClaI* and *KpnI* restriction sites with oligonucleotide resulting from annealing two probes have nucleotide sequences as shown in SEQ ID NO: 31 and SEQ ID NO:32.

The *E. coli* K12 host strain (Amgen strain 657) is a derivative of *E. coli* W1485 (a K12 strain), obtained from the *E. coli* Genetic Stock Center, Yale University, New Haven, CT (CGSC strain 6159).

Expression of rHu-NGF(1-120)

E. coli cells containing the NGF expression construct (as described above) were fermented in rich medium in fed-batch mode. Cells were grown at 30°C to an OD at 600 nm of 49, and then induced by temperature shift to 42°C. Cells were harvested by centrifugation at four hours post induction. Final OD was 75. Expression yield was determined to be approximately 0.15 g/L.

Refolding and purification of rHu-NGF(1-120)

Cell paste was lysed in a Microfluidizer, centrifuged at 10,000 X g for 30 minutes, the pellet was washed with 1% deoxycholic acid, centrifuged as above, and the resulting pellet was then washed with cold water and re-centrifuged. The resulting pellet (WIBs – washed inclusion bodies) was resuspended in denaturant, 8M guanidine HCl, 50mM Tris pH 8.5, containing 10mM DTT, and solubilized at room temperature for 1 hour, centrifuged at 10,000 x g for 30 minutes, and the supernatant was carefully decanted and then diluted 25-fold into an aqueous buffer containing a redox couple at 4°C, for 5 days. The resulting refold was then titrated to pH 3.0, filtered through a 0.45µm filter. The refold was purified using a Sp-Sepharose fast flow column using a standard NaCl gradient. The pool from the cation exchange column was subsequently

concentrated and aliquots were frozen -80°C. The purity of the protein was assessed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by Coomassie blue stain. The purified protein was greater than 90% main band by this method.

5 **Example 2**

Production of Human Monoclonal Antibodies Against Nerve Growth Factor (NGF)

Transgenic HuMab and KM Mice

Fully human monoclonal antibodies to NGF were prepared using HCo7, HCo12,
10 HCo7+HCo12, and KM strains of transgenic mice, each of which expresses human antibody genes. In each of these mouse strains, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen *et al.* (1993, *EMBO J.* 12:811-820), and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of International Patent Application Publication No.
15 WO 01/09187 (incorporated by reference). Each of these mouse strains carries a human kappa light chain transgene, KCo5, as described in Fishwild *et al.* (1996, *Nature Biotechnology* 14:845-851). The HCo7 strain carries the HCo7 human heavy chain transgene as described in U.S. Patent Nos. 5,545,806, 5,625,825, and 5,545,807 (incorporated by reference). The HCo12 strain carries the HCo12 human heavy chain
20 transgene as described in Example 2 of International Patent Application Publication No. WO 01/09187 (incorporated by reference). The HCo7+HCo12 strain carries both the HCo7 and the HCo12 heavy chain transgenes and is hemizygous for each transgene. The KM mice comprises the SC20 heavy chain transgene as described in Tomizuka *et al.* (1997, *Nature Genet.* 16, 133-143 and 2000, *Proc. Natl. Acad. Sci.* 97, 722-727). This
25 transgene is not integrated into a mouse chromosome, but is instead propagated as an independent chromosome fragment. The fragment includes approximately 15 MB of human chromosome 14. It contains the entire human heavy chain locus including all VH, D and JH gene segments and all heavy chain constant region isotypes. All of these strains are referred to herein as HuMab mice.

HuMab Immunizations:

To generate fully human monoclonal antibodies to NGF, HuMab mice were immunized with purified recombinant NGF derived from *E. coli* cells as antigen (Example 1). General immunization schemes for HuMab mice are described in Lonberg
5 *et al.* (1994, *Nature* 368:856-859; Fishwild *et al.*, *supra.*, and International Patent Application Publication No. WO 98/24884, the teachings of each of which are incorporated by reference). Mice were 6-16 weeks of age upon the first infusion of antigen. A purified recombinant preparation (25-100 μ g) of NGF antigen was used to
10 immunize the HuMab mice intraperitoneally (IP) or subcutaneously (SC).

Immunizations of HuMab transgenic mice were achieved using antigen in complete Freund's adjuvant and two injections, followed by 2-4 weeks IP immunization (up to a total of 9 immunizations) with the antigen in incomplete Freund's adjuvant. Several dozen mice were immunized for each antigen. A total of 118 mice of the HCo7,
15 HCo12, HCo7+HCo12, and KM strains were immunized with NGF antigen. The immune response was monitored by retroorbital bleeds.

To select HuMab mice producing antibodies that bound human NGF, sera from immunized mice was tested by ELISA as described by Fishwild *et al. supra.* Briefly, microtiter plates were coated with purified recombinant NGF from *E. coli* (Example 1) at
20 1-2 μ g/mL in PBS and 50 μ L/well incubated at 4°C overnight, then blocked with 200 μ L/well of 5% chicken serum in PBS/Tween (0.05%). Dilutions of plasma from NGF-immunized mice were added to each well and incubated for 1-2 hours at ambient temperature. The plates were washed with PBS/Tween and then incubated with a goat-anti-human IgG Fc-specific polyclonal reagent conjugated to horseradish peroxidase
25 (HRP) for 1 hour at room temperature. Plates were washed with PBS/Tween and incubated with a goat anti-human IgG Fc-specific polyclonal reagent conjugated to horseradish peroxidase (HRP) for 1 hour at room temperature. After washing, the plates were developed with ABTS substrate (Sigma Chemical Co., St. Louis, MO, Catalog No. A-1888, 0.22 mg/mL) and analyzed spectrophotometrically by determining optical

density (OD) at wavelengths from 415-495 nm. Mice with sufficient titers of anti-NGF human immunoglobulin were used to produce monoclonal antibodies as described below.

Generation of hybridomas producing human monoclonal antibodies to NGF

5 Mice were prepared for monoclonal antibody production by boosting with antigen intravenously 2 days before sacrifice, and spleens were removed thereafter. The mouse splenocytes were isolated from the HuMab mice and fused with PEG to a mouse myeloma cell line using standard protocols. Typically, 10-20 fusions for each antigen were performed.

10 Briefly, single cell suspensions of splenic lymphocytes from immunized mice were fused to one-fourth the number of P3X63-Ag8.653 nonsecreting mouse myeloma cells (ATCC, Accession No. CRL 1580) with 50% PEG (Sigma). Cells were plated at approximately 1×10^5 /well in flat bottom microtiter plates, followed by about a two week incubation in selective medium containing 10% fetal bovine serum, 10% P388D1-
15 (ATCC, Accession No. CRL TIB-63) conditioned medium, 3-5% origen (IGEN) in DMEM (Mediatech, Catalog No. CRL 10013, with high glucose, L-glutamine and sodium pyruvate) plus 5 mM HEPES, 0.055 mM 2-mercaptoethanol, 50 mg/mL gentamycin and 1x IAT (Sigma, Catalog No. CRL P-7185). After 1-2 weeks, cells were cultured in medium in which the HAT was replaced with HT.

20 The resulting hybridomas were screened for the production of antigen-specific antibodies. Individual wells were screened by ELISA (described above) for human anti-NGF monoclonal IgG antibodies. Once extensive hybridoma growth occurred, medium was monitored usually after 10-14 days. Antibody secreting hybridomas were replated, screened again and, if still positive for human IgG, anti-NGF monoclonal antibodies were
25 subcloned at least twice by limiting dilution. The stable subclones were then cultured *in vitro* to generate small amounts of antibody in tissue culture medium for characterization.

Selection of Human Monoclonal Antibodies that Bind to NGF

An ELISA assay as described above was used to screen for hybridomas that showed positive reactivity with NGF immunogen. Hybridomas secreting a monoclonal antibody that bound with high avidity to NGF were subcloned and further characterized. One clone from each hybridoma, which retained the reactivity of parent cells (as determined by ELISA), was chosen for making a 5-10 vial cell bank stored in liquid nitrogen.

An isotype-specific ELISA was performed to determine the isotype of the monoclonal antibodies produced as disclosed herein. In these experiments, microtiter plate wells were coated with 50 μ L/well of a solution of 1 μ g/mL of mouse anti-human kappa light chain in PBS and incubated at 4°C overnight. After blocking with 5% chicken serum, the plates were reacted with supernatant from each tested monoclonal antibody and a purified isotype control. Plates were incubated at ambient temperature for 1-2 hours. The wells were then reacted with various human IgG-specific horseradish peroxidase-conjugated goat anti-human polyclonal antisera and plates were developed and analyzed as described above.

Monoclonal antibodies purified from the hybridoma supernatants that showed significant binding to NGF as detected by ELISA were further tested for biological activity using a variety of bioassays as described below.

Example 3

Selecting and Cloning anti-NGF Antibodies with Potent NGF Neutralizing Activity

The effectiveness of the antibodies initially identified in Example 2 as inhibitors of NGF activity (i.e., NGF “neutralization”) was evaluated by measuring the ability of each modified peptide to block NGF induction of vanilloid receptor-1 (VR1) expression.

Dorsal Root Ganglion Neuronal Cultures

Dorsal root ganglia (DRG) were dissected one by one under aseptic conditions from all spinal segments of embryonic 19-day old (E19) rats that were surgically removed from the uterus of timed-pregnant, terminally anesthetized Sprague-Dawley rats

(Charles River, Wilmington, MA). DRG were collected in ice-cold L-15 media (GibcoBRL, Grand Island, NY) containing 5% heat inactivated horse serum (GibcoBRL), and any loose connective tissue and blood vessels were removed. The DRG were rinsed twice in Ca^{2+} - and Mg^{2+} -free Dulbecco's phosphate buffered saline (DPBS), pH 7.4 (GibcoBRL). The DRG were then dissociated into single cell suspension using a papain dissociation system (Worthington Biochemical Corp., Freehold, NJ). Briefly, DRG were incubated in a digestion solution containing 20 U/ml of papain in Earle's Balanced Salt Solution (EBSS) at 37°C for fifty minutes. Cells were dissociated by trituration through fire-polished Pasteur pipettes in a dissociation medium consisting of MEM/Ham's F12, 1:1, 1 mg/ml ovomucoid inhibitor and 1 mg/ml ovalbumin, and 0.005% deoxyribonuclease I (DNase).

The dissociated cells were pelleted at 200 x g for five minutes and re-suspended in EBSS containing 1 mg/ml ovomucoid inhibitor, 1 mg/ml ovalbumin and 0.005% DNase. Cell suspension was centrifuged through a gradient solution containing 10 mg/ml ovomucoid inhibitor, 10 mg/ml ovalbumin at 200 x g for six minutes to remove cell debris, and then filtered through a 88- μm nylon mesh (Fisher Scientific, Pittsburgh, PA) to remove any clumps. Cell number was determined with a hemocytometer, and cells were seeded into poly-ornithine 100 $\mu\text{g}/\text{ml}$ (Sigma, St. Louis, MO) and mouse laminin 1 $\mu\text{g}/\text{ml}$ (GibcoBRL)-coated 96-well plates at 10×10^3 cells/well in complete medium. The complete medium consisted of minimal essential medium (MEM) and Ham's F12, 1:1, penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), and 10% heat inactivated horse serum (GibcoBRL). The cultures were kept at 37°C, 5% CO_2 and 100% humidity. For controlling the growth of non-neuronal cells, 5-fluoro-2'-deoxyuridine (75 μM) and uridine (180 μM) were included in the medium.

25

Treatment with NGF and anti-NGF

Two hours after plating, cells were treated with recombinant human β -NGF (Amgen) or recombinant rat β -NGF (R&D Systems, Minneapolis, MN) at a concentration of 10 ng/ml (0.38 nM). Positive controls comprising serial-diluted anti-NGF antibody (R&D Systems) were applied to each culture plate. Test antibodies were

30

added at ten concentrations using 3.16-fold serial dilutions. All of the samples were diluted in complete medium before being added to the cultures. Incubation time was 40 hours prior to measurement of VR1 expression.

5 *Measurement of VR1 Expression in DRG Neurons*

Cultures were fixed with 4% paraformaldehyde in Hanks' balanced salt solution for fifteen minutes, blocked with Superblock (Pierce, Rockford, IL), and permeabilized with 0.25% Nonidet P-40 (Sigma) in Tris-HCl (Sigma)-buffered saline (TBS) for one hour at room temperature. Cultures were rinsed once with TBS containing 0.1% Tween
10 20 (Sigma) and incubated with rabbit anti-VR1 IgG for one and one-half hours at room temperature, followed by incubation of Eu-labeled anti-rabbit second antibody (Wallac Oy, Turku, Finland) for one hour at room temperature. Washes with TBS (3 x five minutes with slow shaking) were applied after each antibody incubation. Enhance solution (150 µl/well, Wallac Oy) was added to the cultures. The fluorescence signal was
15 then measured in a time-resolved fluorometer (Wallac Oy). VR1 expression in samples treated with the modified peptides was determined by comparing to a standard curve of NGF titration from 0–1000 ng/ml. Percent inhibition (compared to maximum possible inhibition) of NGF effect on VR1 expression in DRG neurons was determined by comparing to controls that were not NGF-treated. Results are given in Tables 2 and 5.

20 The cell lines were labeled #110–#129. Antibodies from cell lines #119, #124, and #125 demonstrated extremely potent NGF neutralization activity (Figure 1). The #124 cell line was a parental cell line, also referred to as 4D4. The #119 and #125 cell lines were subclones of the 4D4 parent. An additional sample from the original vial comprising hybridoma #124 (4D4) was grown and labeled #167 (4D4).

25 Antibodies generated by hybridoma #167 (4D4) were subjected to the same DRG neuron based NGF neutralization assay as the previous samples. Antibody #167 (4D4) demonstrated strong anti-NGF activity with an IC_{50} of 0.50 nM (Figure 2), which was consistent with the activity of samples #119, #124, and #125. The activities of the 4 samples are shown in Table 2.

Table 2

Anti-hNGF activity in DRG cells using 0.38 nM hNGF	
Code #	IC50
119 (from 124)	< 1.2 nM
124 (parent)	< 0.57 nM
125 (from 124)	< 0.3 nM
167 (from same sample as 124)*	0.50 nM

N-Terminal Sequencing and Mass Spectrometry

Purified anti-NGF hybridoma antibodies samples were prepared for protein sequencing and LC/MS analysis. Antibodies were purified from conditioned media by concentrating the media using Amicon centrprep-30 until the volume was less than 15 ml. A batch of rProA (Pharmacia) resin was washed 4x with PBS and a 50% slurry made in PBS following the last wash. An appropriate amount of rProA resin (approximately 5ug antibody/ ul resin but use no less than 50 ul resin) was added to the antibody sample and incubated overnight at 4°C. The Ab-resin mixture was centrifuged and the unbound fraction was collected. After addition of 0.5 ml PBS and transfer to a 0.45um Spin-X (CoStar) tube the sample was centrifuged at 10000 rpm for 3 min. The resin was next washed at least 3 times with 0.5 ml PBS and then of 0.1M glycine (pH 2.7) was added at 1.5x volume of resin and incubated for 10 minutes at room temperature followed by another centrifugation for 3 minutes at 10000 rpm, collecting the supernatant. This elution step was repeated two more times and then the combined supernatant was neutralized with 1/25th volume of 1.0 M tris (pH 9.2).

After a final filtering step through a new Spin-x tube (0.2 um) the antibody was quantified using a standard Bradford assay using human IgG as the standard or alternately absorbance at 280 for larger samples. A gel was also run using with 2ug of each sample alongside 2ug of human IgG1,k (Sigma). For mass spectrometry, four micrograms of the samples were deglycosylated, reduced, and loaded onto an HPLC (HP1090) on-line linked to a Finigan LCQ mass spectrometer. The light chain was separated from the heavy chain by reversed phase HPLC. The light chains and heavy chains were also collected for N-terminal protein sequencing analysis.

Both N-terminal sequences of the light chain and heavy chain of the sample of anti-NGF #167 (4D4) antibody matched both N-terminal sequences of the sample of anti-NGF #119 (4D4) antibody. In addition, the measured mass of the antibodies indicated that the isolated antibodies from the #167 and #119 hybridomas were the same. The measured, deconvoluted mass (23096) of the light chain of anti-NGF #167 matched the measured mass (23096) of the light chain of anti NGF Ab #119.

Cloning the anti-NGF Antibody Heavy and Light Chains

The hybridoma expressing the most potent NGF binding monoclonal antibody, 4D4.D7, was used as sources to isolate total RNA using TRIzol[®] reagent (Invitrogen). First strand cDNA was synthesized using a random primer with an extension adapter (5'-GGC CGG ATA GGC CTC CAN NNN NNT-3') (SEQ ID NO: 33) and a 5' RACE (rapid amplification of cDNA ends) preparative assay was performed using the GeneRacer[™] Kit (Invitrogen) according to instructions from the manufacturer. For preparing complete light chain encoding cDNA, the forward primer was the GeneRacer[™] nested primer, and the reverse primer was 5'-GGG GTC AGG CTG GAA CTG AGG-3' (SEQ ID NO: 34). For preparing cDNA encoding the variable region of the heavy chain, the forward primer was the GeneRacer[™] nested primer and the reverse primer was 5'-TGA GGA CGC TGA CCA CAC G-3' (SEQ ID NO 35). RACE products were cloned into pCR4-TOPO (Invitrogen) and the sequences determined. Consensus sequences were used to design primers for full-length antibody chain PCR amplification.

For preparing cDNA encoding anti-NGF 4D4.D7 kappa light chain, the 5' PCR primer encoded the amino terminus of the signal sequence, an *Xba*I restriction enzyme site, and an optimized Kozak sequence (5'-CAG CAG AAG CTT CTA GAC CAC CAT GGA CAT GAG GGT GCC CGC TCA GCT CCT GGG-3'; SEQ ID NO: 36). The 3' primer encoded the carboxyl terminus and termination codon, as well as a *Sall* restriction site (5'-CTT GTC GAC TCA ACA CTC TCC CCT GTT GAA GCT C-3'; SEQ ID NO: 37). The resulting PCR product fragment was purified, digested with *Xba*I and *Sall*, and then gel isolated and ligated into the mammalian expression vector pDSR α 20 (see International Application, Publication No. WO 90/14363, which is herein incorporated by

reference for any purpose. pDSR α 20 was produced by changing nucleotide 2563 in pDSR α 19 from a "Guanosine" to an "Adenosine" by site directed mutagenesis.).

For preparing cDNA encoding anti- NGF 4D4.D7 heavy chain the 5' PCR primer encoded the amino terminus of the signal sequence, an *Xba*I restriction enzyme site, and an optimized Kozak sequence (5'-CAG CAG AAG CTT CTA GAC CAC CAT GGA GTT GGG GCT GTG CTG GGT TTT CCT TGT T-3'; SEQ ID NO: 38). The 3' primer encoded the carboxyl terminus and termination codon, as well as a *Sal*I restriction site (5'-GCA TGT CGA CTC ATT TAC CCG GAG ACA GGG AGA G-3'; SEQ ID NO: 39). The resulting product was purified, digested with *Xba*I and *Sal*I, gel isolated and ligated into the pDSR α 20 vector.

The calculated mass (23099), as determined by translating the nucleotide sequence to predicted amino acids and adding together the molecular weights of the amino acids, of the DNA sequence of the light chain of anti-NGF Ab 4D4 clone matched the measured mass as determined by mass spectrometry. The measured, deconvoluted mass (49479) of the heavy chain of anti-NGF Ab #167 matched the measured mass (49484) of the heavy chain of anti NGF Ab #119 and also matched the theoretical mass (49484) of the DNA sequence of the heavy chain of anti-NGF Ab 4D4 clone (Table 3) within instrumental deviation.

The data of N-terminal protein sequence and LC/MS confirmed that hybridoma #119 expressed the same antibody as hybridoma #167. In addition, the calculated mass of the antibodies based on sequence further confirmed the observation.

Table 3 – Summary of Mass Spectrometry Findings

anti NGF Ab	Measured mass of Ab #167	Measured mass of Ab #119	Theoretical mass derived from DNA sequence of Ab 4D4
light chain	23096	23096	23099
heavy chain	49479	49484	49484

25

Example 4

Expression of Anti-NGF Antibodies in Chinese Hamster Ovary (CHO) Cells

Stable expression of the 4D4 anti-NGF mAb was achieved by co-transfection of 4D4-heavy chain/pDSR α 19 IgG2 or 4D4-heavy chain/pDSR α 19 IgG1 and NGF-kappa/pDSR α 19 plasmids into dihydrofolate reductase deficient (DHFR⁻) serum-free adapted Chinese hamster ovary (CHO) cells using a calcium phosphate method. Transfected cells were selected in medium containing dialyzed serum but not containing hypoxanthine-thymidine to ensure the growth of cells expressing the DHFR enzyme. Transfected clones were screened using assays such as ELISA in order to detect the expression of 4D4 anti-NGF mAb in the conditioned medium. The highest expressing clones were subjected to increasing concentrations of methotrexate (MTX) for DHFR amplification. MTX amplified clones were screened using assays such as ELISA in order to detect higher expression of 4D4 anti-NGF mAb in the conditioned medium. The highest expressing clones were subjected to subcloning to obtain a homogeneous population and creation of cell banks.

Recombinant anti-NGF antibodies of the invention can be generated in Chinese hamster ovary cells deficient in DHFR using the same protocol as described above for the anti-NGF monoclonal antibody. The DNA sequences encoding the complete heavy chain or light chain of each anti-NGF antibody of the invention are cloned into expression vectors. CHO cells are co-transfected with an expression vector capable of expressing a complete heavy chain and an expression vector expressing the complete light chain of the appropriate anti-NGF antibody. For example, to generate the anti-NGF antibody, cells are co-transfected with a vector capable of expressing a complete heavy chain comprising the amino acid sequence as set forth in SEQ ID NO: 40 and a vector capable of expressing a complete light chain comprising the amino acid sequence set forth in SEQ ID NO: 44. Table 4 summarizes complete heavy and complete light chains for the 4D4 antibodies having various IgG heavy chain constant regions.

Table 4

Antibody	Heavy Chain Variable Region + Heavy Chain Constant Region	Complete Heavy Chain
4D4(IgG2)	SEQ ID NO: 10 + SEQ ID NO: 4	SEQ ID NO: 40
4D4(IgG1)	SEQ ID NO: 10 + SEQ ID NO: 2	SEQ ID NO: 41

Antibody	Heavy Chain Variable Region + Heavy Chain Constant Region	Complete Heavy Chain
4D4(IgG4)	SEQ ID NO: 10 + SEQ ID NO: 6	SEQ ID NO: 42
4D4(IgG3)	SEQ ID NO: 10 + SEQ ID NO: 26	SEQ ID NO: 43
Antibody	Light Chain Variable Region + Light Chain Constant Region	Complete Light Chain
4D4	SEQ ID NO: 12 + SEQ ID NO: 8	SEQ ID NO: 44

Example 5

Characterizing the Activity of anti-NGF 4D4 Antibodies

Transiently expressed anti-NGF 4D4 antibodies, generated in cells grown under spinner (S) or roller (R) conditions were tested to confirm their ability to neutralize NGF in a DRG neuron based NGF neutralization bioassay, performed as described above (Example 3).

The NGF antibodies were expressed transiently in serum-free suspension adapted 293T cells. Transfections were performed as either 500 mL or 1L cultures. Briefly, the cell inoculum (5.0×10^5 cells/mL X culture volume) was centrifuged at 2,500 RPM for 10 minutes at 4°C to remove the conditioned medium. The cells were resuspended in serum-free DMEM and centrifuged again at 2,500 RPM for 10 minutes at 4°C. After aspirating the wash solution, the cells were resuspended in growth medium [DMEM/F12 (3:1) + 1X Insulin-Transferrin-Selenium Supplement + 1X Pen Strep Glut + 2mM L-Glutamine + 20 mM HEPES + 0.01% Pluronic F68] in a 1L or 3L spinner flask culture. The spinner flask culture was maintained on magnetic stir plate at 125 RPM which was placed in a humidified incubator maintained at 37°C and 5% CO₂. The plasmid DNA was complexed to the transfection reagent in a 50 mL conical tube. The DNA-transfection reagent complex was prepared in 5% of the final culture volume in serum-free DMEM. 1 µg plasmid DNA/mL culture was first added to serum-free DMEM, followed by 1 µl X-TremeGene RO-1539/mL culture. The complexes were incubated at room temperature for approximately 30 minutes and then added to the cells in the spinner flask. The transfection/expression was performed for 7 days, after which the conditioned medium was harvested by centrifugation at 4,000 RPM for 60 minutes at 4°C.

For roller bottle transient transfections, we used 293T adherent cells grown and maintained in DMEM supplemented with 5% FBS + 1X Non-Essential Amino Acids + 1X Pen Strep Glut + 1X Sodium Pyruvate. Approximately, 4.5×10^7 293T cells were seeded in a 850 cm² roller bottles overnight. The previously seeded cells were then transfected the following day using FuGene6 transfection reagent. The DNA – transfection reagent mixture was prepared in approximately in 6.75 mL serum-free DMEM. 675 µl FuGene6 transfection reagent was first added, followed by 112.5 µg plasmid DNA. The complex was incubated at room temperature for 30 minutes. The entire mixture was then added to a roller bottle. The roller bottle was gassed with a 5% CO₂ gas mixture, capped tightly and placed in a 37°C incubator on a roller rack rotating at 0.35 RPM. The transfection was performed for 24 hours after which the medium was replaced with 100 mL DMEM + 1X Insulin-Transferrin-Selenium Supplement + 1X Pen Strep Glu + 1X Non-Essential Amino Acids + 1X Sodium Pyruvate. Typically, two 100ml 48 hour harvests were obtained from each roller bottle. The harvested serum-free conditioned medium was pooled together and centrifuged at 4,000 RPM for 30 minutes at 4°C.

Both 4D4.IgG1 and 4D4.IgG2 showed potent activity with IC₅₀ values of about 0.14 nM to about 0.2 nM against human NGF (Figure 2). The results of the activity assay are summarized in Table 5. The antibodies showed little activity against rat NGF (Figure 3). The results resemble the activity of the antibodies tested directly from hybridomas described above.

TABLE 5

Ab	IC ₅₀ @ hNGF (nM)	IC ₅₀ @ rNGF (nM)
4D4.IgG1.R	0.1488	> 34 nM
4D4.IgG1.S	0.1587	> 45 nM
4D4.IgG2.R	0.2047	> 59 nM
4D4.IgG2.S	0.2063	> 37 nM

hNGF = human NGF, rNGF = rat NGF, R = Roller culture, S =

Spinner culture

Example 6

Production of anti-NGF Antibody

Anti-NGF antibody is produced by expression in a clonal line of CHO cells. For each production run, cells from a single vial are thawed into serum-free cell culture media. The cells are grown initially in a T-flask followed by spinner flasks and then grown in stainless steel reactors of increasing scale up to a 2000L bioreactor. Production is carried out in a 2000L bioreactor using a fed batch culture, in which a nutrient feed containing concentrated media components is added to maintain cell growth and culture viability. Production lasts for approximately two weeks during which time anti-NGF antibody is constitutively produced by the cells and secreted into the cell culture medium.

The production reactor is controlled at a predetermined pH, temperature, and dissolved oxygen level: pH is controlled by carbon dioxide gas and sodium carbonate addition; dissolved oxygen is controlled by air, nitrogen, and oxygen gas flows.

At the end of production, the cell broth is fed into a disk stack centrifuge and the culture supernatant is separated from the cells. The concentrate is further clarified through a depth filter followed by a 0.2 μ m filter. The clarified conditioned media is then concentrated by tangential flow ultrafiltration. The conditioned media is concentrated 15- to 30- fold. The resulting concentrated conditioned medium is then either processed through purification or frozen for purification at a later date.

20

Example 7

Cross-Reactivity with Other Neurotrophins

The 4D4 antibodies were tested for their cross-reactivity against human NT3 or human BDNF in different bioassays, including the DRG neuron survival assay for human NT3 and the assay of DA uptake in cultured DA neurons for human BDNF.

25

Treatment of DRG cultures with NT3, anti-NT3 and anti-NGF antibodies

- Two hours after plating, DRG cells (isolation procedure described above in Example 3) were treated with recombinant hNT-3 100 ng/ml (3.8 nM). Serial-diluted anti-hNT3 antibody (R&D) was used as a positive control. Unknowns (anti-NGF Ab samples) were added at various concentrations with 10 point, 3.16 fold serial dilutions.
- 5 All the samples were diluted in complete medium before being added to the cultures.

Measurement of MAP2 expression in DRG Neurons

- Cultures were fixed with 4% paraformaldehyde in Hanks' balanced salt solution for 15 min, blocked with Superblock (Pierce) for 1 hour and permeabilized with 0.25% Nonidet P-40 (Sigma) in Tris-HCl (Sigma)-buffered saline (TBS) for 1 hour in room temperature (RT). Cultures were rinsed once with TBS containing 0.1% Tween20 (Sigma) and incubated with mouse anti-MAP2 IgG (Chemicon, Temecula, CA) for 1.5 hour at room temperature, followed by incubation of Eu-labeled anti-mouse secondary antibody (Wallac Oy, Turku, Finland) for 1 hour at room temperature. Washes with TBS
- 10 (3 x 5min with gentle shaking) were applied after each antibody incubation. Enhance solution (150 ml/well, Wallac Oy) was added to the cultures and fluorescence signal was then measured in a time-resolved fluorometer (Wallac Oy).

Embryonic Mesencephalic Culture

- Embryonic 19 day old (E19) Sprague-Dawley rats (Jackson Labs) were used. Ventral midbrain tissue enriched for dopaminergic neurons was removed and transferred to cold, Dulbecco's phosphate buffered saline (DPBS), pH 7.4, without Ca^{++} and Mg^{++} (Gibco). The tissue fragments were dissociated into single cell suspension using a papain dissociation system (Worthington Biochemical Corp., Freehold, NJ). Briefly,
- 20 tissue fragments were incubated in a digestion solution containing 20 unit/ml papain in Earle's Balanced Salt Solution (EBSS) at 37°C for 50 min. Cells were dissociated by trituration through fire-polished Pasteur pipettes in a dissociation medium consisting MEM/Ham's F12 1:1, 1 mg/ml ovomucoid inhibitor and 1 mg/ml ovalbumin and 0.005% deoxyribonuclease I (DNase). The dissociated cells were pelleted at 200 x g for 5 min

and resuspended in EBSS containing 1 mg/ml ovomucoid inhibitor, 1 mg/ml ovalbumin and 0.005% DNase. Cell suspension was centrifuged through a gradient solution containing 10 mg/ml ovomucoid inhibitor, 10 mg/ml ovalbumin at 200 x g for 6 min to remove the cell debris; and filtered through a 25 µg Nitex nylon mesh (Tetko, Inc.) to
5 remove the clumps. The dissociated cells were plated in tissue culture plates at a density of 100,000/cm². The plates were pre-coated with poly-ornithine 100 µg/ml (Sigma) and mouse laminin 1 µg/ml (Gibco BRL) as previously described (Louis JC *et al.*, *J. Pharmacol. Exp. Ther.* 1992; 262:1274-1283.). The culture medium consisted of minimal essential medium (MEM)/Ham's F12, 1:1, 12% horse serum (Gibco), 100 µg/ml
10 transferrin and 2.5 µg/ml insulin (Sigma). The cultures were kept at 37°C, 5% CO₂ and 100% humidity for 6 days.

Treatment of Mesencephalic Cultures with BDNF and anti-BDNF or anti-NGF

BDNF at 10 ng/ml was added to the cells 2 hours after plating, followed by serial
15 concentrations of anti-NGF Ab samples. Anti-BDNF antibody (generated at Amgen) was used as a positive control.

DA Uptake in Mesencephalic Neurons

Dopamine uptake assay were carried out as described previously (Friedman, L.
20 and Mytilineou, C., *Neuroscience Letters* 1987; 79:65-72). At day 6, cultures were washed once with pre-warmed Krebs-Ringer's phosphate buffer (pH 7.4) containing 5.6 mM glucose, 1.3 mM EDTA and 0.5 mM pargylin, a monoamine oxidase inhibitor. The cultures were incubated in uptake buffer containing 50 nM [³H]DA (NEN) for 60 minutes at 37°C. Uptake was stopped by removing the uptake buffer, and the cultures were
25 washed three times with Krebs-Ringer's phosphate buffer. Cells were lysed to release [³H]DA by adding a liquid scintillation cocktail, optiphase supermix (Wallac), directly to the cultures. The cell lysates were then counted for radioactivity in a microbeta-plus liquid scintillation counter (Wallac, Inc.). Low affinity DA uptake was assessed by adding 0.5 mM GBR12909, a specific inhibitor of the high affinity DA uptake sites

(Heikkila RE and Mazino L, *European Journal of Pharmacology* 1984; 103:241-8), to the uptake buffer, and subtracted from the total uptake amount to obtained the high affinity DA uptake value.

Table 6

Antibody	IC50 @ hNT-3 (nM)	IC50 @ hBDNF (nM)
4D4 (IgG2)	> 13.75	> 13.75

5

Example 8

Identification of an Epitope for anti-NGF Antibodies

Epitope Mapping by Limited Proteolysis

Five micrograms (μ g) of NGF were incubated with 4D4 (11 μ g) for 30 minutes at 10 4°C in 0.1M Tris buffer, pH 7.5. The complex was then digested with protease (subtilisin) 1 μ g at 37°C for 1 and 2 hours. HPLC peptide maps were compared to each other to find the peptides that were protected by the 4D4 antibodies. Limited proteolysis of NGF indicated that several major peptides were initially released from NGF. Of particular interest, peptides S18.3, S18.5, and S34.4 were generated and protected with 15 antibody from the proteolysis. Other peaks were not significantly formed or protected. The protected peptides from two experiments (1 hour and 2 hour digestion) are shown in Table 7.

Table 7

			% protection	
			1 hour digestion	2 hour digestion
S16.1	QAA (96-98)	C-terminal	--	57
S18.3	FFETK (53-57) (SEQ ID NO: 45)	Loop region	40	45
S18.5	SSSHPIFHR (1-9) (SEQ ID NO: 46) (HWNSY)* (SEQ ID NO: 47)	N-terminal	40	50

S34.4	NSVEKQYFFE1K (46-57) (SEQ ID NO: 48)	Loop region	69	38
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The percentage of protection was calculated from the peptide peak height. S18.5 contained two peptides, but only one peptide (SSSIPIFHR; SEQ ID NO: 46) was protected with the 4D4 antibody, since the other peptide peak (HWNSY; SEQ ID NO: 47) was unchanged by the addition of 4D4 antibodies, as detected at 280 nm absorbance. Peptide S18.3 was a C-terminal part of S34.4, both from the same loop region. N-terminal and central loop regions were also possible epitopes.

Microcon Separation of Digested Peptides

The subtilisin-digested material (3 µg each) was incubated with active 4D4 antibodies and an inactive monoclonal antibody (#162) (8 µg) for 30 minutes at 4°C in 0.1 M Tris buffer, pH 7.5. The bound/unbound peptides were separated by Microcon 10 (Millipore Corp., Bedford, Mass) and both fractions (bound and unbound) were analyzed by HPLC to find peptides bound to antibodies. Two depleted peaks identified by HPLC comparison of the unbound fractions after treatment with 4D4 antibodies and #162 and Microcon separation were recovered, indicating antibody bound peptides. The 4D4 bound peptides were:

S1 (4.4) ----SRKAVRR (113-119) (SEQ ID NO: 49), C-terminal; and

S2 (28.3) ----EVMVL (35-39) (SEQ ID NO: 50), Loop region.

An NGF sample was alternatively digested with Lys-C (K) for 24 hours. Cysteine residues were reduced and carboxymethylated without denaturant. The sample was incubated with monoclonal antibodies 4D4 and AMG162, followed by Microcon 100 separation. Bound and unbound fractions were analyzed by reversed phase HPLC. Only two peptides were identified as antibody binding K-peptides as indicated below. The calculated mass for the peptides determined by sequence analysis and the mass spectrometry of the peptides were consistent. The peptides, as indicated below, mapped to the N-terminal and C-terminal region.

K1(37.6) ---SSSHPIFIIRGEFSVCDSVSVWVGDK (SEQ ID NO: 51)

Calculated mass = 2821; Observed mass = 2828.2; N-terminal

K2(39.5) ----QAAWRFIRJDTACVCVLSRK (SEQ ID NO: 52)

Calculated mass = 2452; Observed mass = 2459.5; C-terminal

5 The preceding epitope mapping experiments indicated that at least three regions were possible epitopes for the 4D4 antibodies, including N-terminus (1-9), internal (46-57), and C-terminal (96-98) regions. In addition, an AspN digestion revealed that a peptide fragment consisting of ---SSHPIFIIRGEFSVC--- (SEQ ID NO: 53) was protected by the 4D4 antibody, whereas a trypsin digestion showed that a peptide
10 fragment consisting of ---SSHPIFIHR---- (SEQ ID NO: 54) was not protected by the 4D4 antibody. Thus, in the N-terminus, the sequence of GEFSVC (SEQ ID NO: 55) is most important for binding to 4D4 antibodies.

15 In order to more clearly define the epitope for the anti-NGF antibody 4D4.IgG1, a total of 23 peptides were generated synthetically using standard techniques based on the entire human mature NGF (hNGF) sequence (Table 8). The peptides were 15 amino acids long, overlapping by 10 amino acids, and cysteine-tailed at the C-termini to allow for conjugation to a matrix. The human anti-hNGF Ab 4D4.IgG1 described above was used for the mapping experiment.

Table 8

20

Peptide #	Sequence	SEQ ID NO
33582-27-01	SSSHPIFIIRGEFSVC (1-15)	56
33582-27-02	IFHRGEFSVADSVSVC (6-20)	57
33582-27-03	EFVADSVSVWVGDKC (11-25)	58
33582-27-04	DSVSVWVGDKTTATDC (16-30)	59
33582-27-05	WVGDKTTATDIKGKEC (21-35)	60
33582-27-06	TTATDIKGKEVMVLGC (26-40)	61
33582-27-07	IKGKEVMVLGEVNTN (31-45)	62

33582-27-08	VMVLGEVNINNSVFKC (36-50)	63
33582-27-09	EVNINNSVFKQYFFEC (41-55)	64
33582-27-10	NSVFKQYFFETKARDC (46-60)	65
33582-27-11	QYFFETKARDPNPVDC (51-65)	66
33582-27-12	TKARDPNPVDSGARDC (56-70)	67
33582-27-13	PNPVDSGARDIDSKHC (61-75)	68
33582-27-14	SGARDIDSKHWNNSYC (66-80)	69
33582-27-15	IDSKHWNNSYATITHTC (71-85)	70
33582-27-16	WNSYATTTHTFVKALC (76-90)	71
33582-27-17	TTHTFVKALTMDGKC (81-95)	72
33582-27-18	FVKALTMDGKQAAWRC (86-100)	73
33582-27-19	TMDGKQAAWRFIRIDC (91-105)	74
33582-27-20	QAAWRFIRIDTAAVC (96-110)	75
33582-27-21	FIRIDTAAVAVLSRKC (101-115)	76
33582-27-22	TAAVAVLSRKAVRRAC (106-120)	77
33582-27-23	CAAVAVLSRKAVRRA (107-120)	78

The human NGF peptide fragments were diluted in PBS with 5% DMSO, 1 mM EDTA, pH 6.23. The final peptide concentration was normalized to the same molar concentration at 55 μ M (about 100 μ g/ml). Peptides were incubated in Reacti-Bind Maleimide activated 96 well microtiter plates (Pierce Cat # 15150), 100 μ l/well, at room temperature for 2 hours and then at 4°C overnight with agitation. Human NGF (100 μ g/ml) was used as positive control. The plates were washed with wash buffer (KPL) and blocked with 0.2% non-fat dry milk (in PBS-EDTA buffer, pH 6.23) for 2 hours at room temperature and then further blocked with 5% BSA for 1 hour. Plates were then incubated with the human anti-NGF antibody at various concentrations (0, 3, 10, 30 μ g/ml), followed by goat anti-hFc Ab-HRP (KPL) for 2 hours. Signal was developed with TMB substrate and read at 450 nm after addition of stop solution (KPL).

Across the 23 human NGF peptides, at least 4 major peaks were observed, indicating 4D4 binding. These peaks corresponded to the following peptides: Peptide # 1 (SEQ ID NO: 56), SSSHPFHRGEFSVC (1-15); Peptide # 10 (SEQ ID NO: 65), NSVFKQYFFETKARD (46-60); Peptides # 16 – 17 (SEQ ID NO: 71-SEQ ID NO: 72), WNSYATTTHTFVKAL— (76-95); and Peptides # 18 – 21 (SEQ ID NO: 73 - SEQ ID NO: 76), TTTHT— LSRKC (100-115).

The four binding peaks of 4D4 mapped to the N-terminus, C-terminus, internal domains, as well as loops L2 and L4 in NGF as described in Weismann *et al.* (1999, *Nature* 401:184-8). These results are summarized in Table 9.

Table 9

hNGF epitopes	N-terminus	L2	Internal	L4	Internal	C-terminus
Peptide #	peptide # 1 (SEQ ID NO: 56), SSSHPF—, 1-15	peptide # 10 (SEQ ID NO: 65), NSVFKQ—, 46-60	peptide # 16 (SEQ ID NO: 71), WNSYA—, 76-90	peptide # 17 (SEQ ID NO: 72), TMDGKQ—, 81-95	peptide # 19 (SEQ ID NO: 74), TMDGK—, 91-105	peptides # 20-21 (SEQ ID NO: 75 - SEQ ID NO:76), QAAWR—, BB-115
Ab binding signal	+++	+	++	++	+++	++

Wiesmann *et al.* solved the crystal structure of hNGF bound to the trkA receptor, showing that the N-terminus (residues 2–9) was important for receptor binding (Wiesmann *et al.*, 1999, *Nature* 401:184-8). The residues of this segment in NGF are also important for specificity for trkA over trkB or trkC receptors. Antibody 4D4 is selective for human NGF over mouse/rat NGF, as well as BDNF and NT-3 most likely because N-terminal differences between human NGF and other neurotrophins.

Antibody 4D4 binds to peptide #10 (SEQ ID NO: 65) (NSVFK—, 46-60) and peptide #17 (SEQ ID NO: 72) (TTTTHTFVKAL—TMDGKC, 81-95), corresponding respectively to loops L2 and L4, which represent two of seven distinct regions with higher than average sequence diversity among the neurotrophins. Swapping experiments between NGF and BDNF of these seven regions showed that L2 and L4 were important for the biological activity of NGF. Furthermore, substitution of five NT3 residues in loops L2 and L4 with those of NGF introduced NGF-like activity while maintaining NT3

activity. Thus, L2 and L4 are likely regions where antibody 4D4 bind selectively to NGF rather than to BDNF or NT-3.

Antibody 4D4 also binds to peptide #16 (SEQ ID NO: 71) (WNSYATTTHTFVKAL, 76-90), matching an internal domain of the NGF crystal structure. This region is 100% homologous between human NGF and mouse NGF, but distinct from other neurotrophins. 4D4 showed much weaker activity against rat/mouse NGF when compared to its activity against human NGF. Thus, binding to this part of NGF is most likely not critical for species specificity but is important for selectivity amongst neurotrophins.

Antibody 4D4 also binds to the C-terminal region of NGF (peptides # 19-21 (SEQ ID NO: 74 – SEQ ID NO: 76) IMDGK---LSRKC, 91-115), which is one of the regions of human NGF that distinguishes NGF from other neurotrophins (BDNF and NT3). Binding to this region helps to explain why 4D4 is not active against other neurotrophins. Furthermore, there is a single amino acid difference between human NGF and mouse NGF in the C-terminus, suggesting that this single amino acid may be one of the reasons 4D4 is selective for human NGF over rat/mouse NGF, similar to the N-terminus where species differences are observed.

Lastly, 4D4 also interacts with an internal domain described by peptide #10 (SEQ ID NO: 65) (---KARDC, 50-60) of human NGF, which is an important region for NGF binding preferentially to trkA, rather than trkB or trkC, further explaining its selective neutralization activity against human NGF.

Example 9

Affinity Measurement of Monoclonal Antibodies by KinExA

Binding of Ab 4D4 (38859-80) to huNGF (29714-91) was tested on KinExA. Briefly, Reacti-Gel 6x (Pierce) were pre-coated with huNGF and blocked with BSA. 10 pM and 30 pM of Ab 4D4 samples were incubated with various concentrations of huNGF (Amgen) at room temperature for 8 hours before run through the huNGF-coated beads. The amount of the bead-bound antibody was quantified by fluorescent (Cy5) labeled goat

anti-human-IgG antibody (Jackson Immuno Research). The binding signal was proportional to the concentration of free antibody at equilibrium. Dissociation equilibrium constant (K_D) was obtained from nonlinear regression of the competition curves using a dual-curve one-site homogeneous binding model (KinEx™ software).

- 5 The K_D was about 4 pM for Ab 4D4 binding to huNGF.

Example 10

Identification of additional anti-NGF antibodies

- Additional anti-NGF antibodies (designated 14D10, 6G9, 7H2, 14F11, and 4G6),
 10 generated and identified as described in Examples 2 and 3 above, were selected for further study. Briefly, conditioned media was tested for binding activity. Antibodies from the media were purified and sequenced. The predicted mass was compared with mass spectrometry data of antibodies from the conditioned media. The antibodies were cloned. Two of the clones were expressed in CHO cells and tested for activity as
 15 described above. The results are shown in Table 10.

Table 10

clone	IC50 @ hNGF (nM)	IC50 @ rNGF (nM)	Notes	Molecular Clone	IC50 @ hNGF (nM)	IC50 @ rNGF (nM)
7H2	3.294	1.748	cloned	7H2-rFc	0.963	0.792
6H9	3.172	1.699	cloned	6H9-rFc	13.93	0.653
14D10	0.3918	> 13	cloned			
14D11	0.2803	> 20	cloned			
4G6	0.414	> 10	cloned			

- The sequences of the light and heavy chain variable regions of these antibodies were then compared to the 4D4 antibody sequence, as well as to each other (Figures 5
 20 and 6). The percent homologies of the heavy chain variable regions as identified from these comparisons are shown in Table 11. The percent homologies of the light chain variable regions are shown in Table 12. In addition, the percent homologies of the CDR regions of the various antibodies are shown in Figures 5-10.

Table 11

	4D4 VH	14D10 VH	6H9 VH	7H2 VH	14D11 VH	4G6 VH
4D4 VH	100%	70.9%	70.1%	75.6%	47.2%	73.4%
14D10 VH		100%	95.3%	85%	54.3%	81.1%
6H9 VH			100%	86.6%	54.3%	81.1%
7H2 VH				100%	51.2%	79.8%
14D11 VH					100%	56.8%
4G6 VH						100%

Table 12

	V4D4 VK	14D11 LC	4G6a LC	4G6b LC	4G6c LC	14D10 LC	6H9 LC	4G6d LC	7H2 LC	4G6e
V4D4 VK	100%	89%	91%	72%	74%	69%	71%	71%	70%	73%
14D11 LC		100%	94%	68%	71%	67%	68%	68%	68%	70%
4G6a LC			100%	69%	74%	68%	70%	70%	69%	71%
4G6b LC				100%	87%	83%	86%	86%	86%	96%
4G6c LC					100%	91%	94%	94%	94%	91%
14D10 LC						100%	91%	94%	94%	86%
6H9 LC							100%	99%	98%	89%
4G6d LC								100%	99%	89%
7H2 LC									100%	
4G6e										100%

- 5 It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

We claim:

1. An isolated human antibody that binds specifically to NGF, wherein the antibody comprises a heavy chain having a heavy chain variable region and a light chain variable region wherein the heavy chain variable region comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
2. The antibody of claim 1, wherein the light chain variable region comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 12, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
3. The antibody of claim 1, wherein the light chain comprises a human light chain CDR1 that has an amino acid sequence that is at least 85% identical to the amino acid sequence as set forth in SEQ ID NO: 24, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
4. The antibody of claim 1, wherein the light chain comprises a human light chain CDR3 that has an amino acid sequence that is at least 85% identical to the amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
5. The antibody of claim 1, wherein the heavy chain comprises a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to the amino acid sequence as set forth in any of SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
6. The antibody of claim 1 wherein the heavy chain comprises a human heavy chain CDR2 that has an amino acid sequence that is at least 80% identical to the amino acid sequence as set forth in SEQ ID NO: 18, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
7. The antibody of claim 1 which comprises:

- 5 a. a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 10, an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 12, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
- 10 b. a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 14, an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
- 15 c. a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 18, an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or
- 20 d. a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 22, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
- 25 8. The antibody of claim 1, wherein the heavy chain comprises a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and the light chain comprises a light chain variable region having an amino acid sequence as set forth in SEQ ID NO: 12, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
- 30

9. The antibody of claim 1, wherein the heavy chain comprises a variable region and a constant region, wherein the variable region comprises an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or immunogenic fragment thereof.
- 5 10. The antibody of claim 9, wherein the heavy chain comprises an amino acid sequence as set forth in any of SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 22, or an antigen-binding or immunogenic fragment thereof.
11. The antibody of claim 1, wherein the light chain comprises a variable region and a constant region, wherein the variable region comprises an amino acid
10 sequence as set forth in SEQ ID NO: 12, or an antigen-binding or immunogenic fragment thereof.
12. The antibody of claim 11, wherein the light chain comprises an amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or immunogenic fragment thereof.
- 15 13. The antibody of claim 1, comprising:
 - a. human heavy chain framework regions, a human heavy chain CDR1 region, a human heavy chain CDR2 region, and a human heavy chain CDR3 region, wherein the human heavy chain CDR3 region is the heavy chain CDR3 region shown in SEQ ID NO:14; and
 - 20 b. human light chain framework regions, a human light chain CDR1 region, a human light chain CDR2 region, and a human light chain CDR3 region, wherein the human light chain CDR3 region is the light chain CDR3 region shown in SEQ ID NO:16.
14. The isolated human antibody of claim 13, wherein the human heavy chain
25 CDR2 region is the heavy chain CDR2 region as shown in SEQ ID NO: 18 and the human light chain CDR2 region is the light chain CDR2 region shown in SEQ ID NO: 20.
15. The isolated human antibody of claim 13, wherein the human heavy chain CDR1 region is the heavy chain CDR1 region of NGF as shown in SEQ ID

NO: 22 and the human light chain CDR1 region is the light chain CDR1 region shown in SEQ ID NO: 24.

16. The antibody of claim 13, wherein the antibody dissociates from a human NGF polypeptide with a KD of about 1×10^{-9} or less and neutralizes human NGF bioactivity in a standard in vitro assay with an IC₅₀ of about 1×10^{-8} M or less.
17. The antibody of claim 16, wherein the antibody dissociates from a human NGF polypeptide with a KD of about 1×10^{-10} or less and neutralizes human NGF bioactivity in a standard in vitro assay with an IC₅₀ of about 1×10^{-9} M or less.
18. The antibody of claim 17, wherein the antibody dissociates from a human NGF polypeptide with a KD of about 1×10^{-11} or less and neutralizes human NGF bioactivity in a standard in vitro assay with an IC₅₀ of about 0.2×10^{-9} M or less.
19. The antibody of claim 1, wherein the heavy chain and light chain are connected by a flexible linker to form a single-chain antibody.
20. The antibody of claim 19, which is a single-chain Fv antibody.
21. The antibody of claim 1, which is a Fab antibody.
22. The antibody of claim 1, which is Fab' antibody.
23. The antibody of claim 1, which is a (Fab')₂ antibody.
24. The antibody of claim 1, wherein the antibody is a fully human antibody.
25. The antibody of claim 1, wherein the antibody inhibits NGF signaling.
26. The use of the antibody of claim 25 in the manufacture of a medicament for treating a condition caused by increased expression of NGF or increased sensitivity to NGF in a patient.
27. The use of claim 26, wherein the condition is acute pain, dental pain, pain from trauma, surgical pain, pain resulting from amputation or abscess, causalgia, demyelinating

diseases, trigeminal neuralgia, cancer, chronic alcoholism, stroke, thalamic pain syndrome, diabetes, acquired immune deficiency syndrome ("AIDS"), toxins, chemotherapy, general headache, migraine, cluster headache, mixed-vascular or non-vascular syndromes, tension headache, general inflammation, arthritis, rheumatic diseases, lupus, osteoarthritis, fibromyalgia, inflammatory bowel disorders, irritable bowel syndrome, inflammatory eye disorders, inflammatory or unstable bladder disorders, psoriasis, skin complaints with inflammatory components, sunburn, carditis, dermatitis, myositis, neuritis, collagen vascular diseases, chronic inflammatory conditions, inflammatory pain and associated hyperalgesia and allodynia, neuropathic pain and associated hyperalgesia or allodynia, diabetic neuropathy pain, causalgia, sympathetically maintained pain, deafferentation syndromes, asthma, epithelial tissue damage or dysfunction, herpes simplex, disturbances of visceral motility at respiratory, genitourinary, gastrointestinal or vascular regions, wounds, burns, allergic skin reactions, pruritis, vitiligo, general gastrointestinal disorders, colitis, gastric ulceration, duodenal ulcers, vasomotor or allergic rhinitis, or bronchial disorders, dysmenorrhoea, dyspepsia, gastroesophageal reflux, pancreatitis, or visceralgia.

28. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the antibody of claim 25.
29. The use of the pharmaceutical composition of claim 28 in the manufacture of a medicament for treating a condition caused by increased expression of NGF or increased sensitivity to NGF in patient.
30. A method for detecting NGF in a biological sample comprising:
 - a. contacting the sample with the antibody of claim 1, under conditions that allow for binding of the antibody to NGF; and
 - b. measuring the level of bound antibody in the sample.
31. An isolated human antibody that specifically binds nerve growth factor, comprising a heavy chain and a light chain, wherein the heavy chain comprises a heavy chain variable region comprising an amino acid sequence

as set forth in SEQ ID NO: 10, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, or SEQ ID NO: 87, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

- 5 32. The antibody of claim 31, wherein the heavy chain comprises a heavy chain variable region comprising an amino acid sequence that is at least 90% identical to the amino acid sequence as set forth in any of SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, or SEQ ID NO: 79, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
- 10 33. An isolated human antibody that specifically binds NGF, comprising a heavy chain and a light chain, wherein the light chain comprises a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 12, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, or SEQ ID NO: 131, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
- 15 34. The isolated human antibody of claim 33, wherein the light chain comprises a light chain variable region comprising an amino acid sequence that is at least 85% identical to the amino acid sequence as set forth in any of SEQ ID NO: 12, SEQ ID NO: 80, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, or SEQ ID NO: 131, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
- 20 35. The antibody of claim 33, wherein the light chain comprises a light chain variable region comprising an amino acid sequence that is:
- 25 a. at least 89%, or 94% identical to the amino acid sequence as set forth in SEQ ID NO: 80, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;

- b. at least 91%, or 94% identical to the amino acid sequence as set forth in SEQ ID NO: 88, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
 - c. at least 86%, or 87% identical to the amino acid sequence as set forth in SEQ ID NO: 89, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
 - d. at least 87%, 91%, or 94% identical to the amino acid sequence as set forth in SEQ ID NO: 90, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
 - e. at least 86%, 94%, 96%, or 99% identical to the amino acid sequence as set forth in SEQ ID NO: 91, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
 - f. at least 91%, 95%, or 96% identical to the amino acid sequence as set forth in SEQ ID NO: 82, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
 - g. at least 86%, 94%, 95%, 98%, or 99% identical to the amino acid sequence as set forth in SEQ ID NO: 84, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
 - h. at least 86%, 94%, 95%, 98%, or 99% identical to the amino acid sequence as set forth in SEQ ID NO: 86, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or
 - i. at least 86%, 89%, 91%, or 96% identical to the amino acid sequence as set forth in SEQ ID NO: 131, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
36. An isolated human antibody that specifically binds NGF, comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence as set forth in any of SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID

NO: 106, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

- 5 37. The isolated human antibody of claim 36, wherein the heavy chain comprises a human heavy chain CDR2, wherein the heavy chain CDR2 is an amino acid sequence that is at least 80% identical to the amino acid sequence as set forth in SEQ ID NO: 99, SEQ ID NO: 106, SEQ ID NO: 117, SEQ ID NO: 111, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
- 10 38. An isolated human antibody that specifically binds NGF, comprising a heavy chain and a light chain, wherein the light chain comprises an amino acid sequence as set forth in any of SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 24, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 107, SEQ ID NO: 108, 15 SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, or any of SEQ ID NO: 119-134, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
- 20 39. The isolated human antibody of claim 38, comprising a human light chain CDR1, wherein the CDR1 is an amino acid sequence that is at least 85% identical to the amino acid sequence as set forth in SEQ ID NO: 101, SEQ ID NO: 95, SEQ ID NO: 119, SEQ ID NO: 122, SEQ ID NO: 125, SEQ ID NO: 107, SEQ ID NO: 113, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
- 25 40. The isolated human antibody of claim 38, comprising a human light chain CDR3, wherein the CDR3 is an amino acid sequence that is at least 85% identical to the amino acid sequence as set forth in SEQ ID NO: 103, SEQ ID NO: 97, SEQ ID NO: 121, SEQ ID NO: 127, SEQ ID NO: 130, SEQ ID NO: 109, SEQ ID NO: 115, SEQ ID NO: 134, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

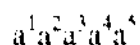
41. An isolated human antibody that specifically binds NGF comprising a heavy chain or a fragment of a heavy chain comprising a CDR3 comprising an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 94, SEQ ID NO: 97, SEQ ID NO: 100, SEQ ID NO: 103, SEQ ID NO: 106, SEQ ID NO: 109, SEQ ID NO: 112, SEQ ID NO: 115, SEQ ID NO: 118, SEQ ID NO: 121, SEQ ID NO: 124, SEQ ID NO: 127, SEQ ID NO: 130, SEQ ID NO: 134 and a variant thereof wherein the variant comprises no more than one amino acid substitution, insertion, or deletion.
42. An isolated human antibody that specifically binds NGF comprising an amino acid sequence at least 90% identical to a sequence selected from the group of sequences consisting of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, and SEQ ID NO: 22, and a variant thereof wherein the variant comprises no more than one amino acid substitution, insertion, or deletion.
43. The antibody of claim 31, 33, 36, 38, 41, or 42, wherein the heavy chain and light chain are connected by a flexible linker to form a single-chain antibody.
44. The antibody of claim 43, which is a single-chain Fv antibody.
45. The antibody of claim 31, 33, 36, 38, 41, or 42, which is a Fab antibody.
46. The antibody of claim 31, 33, 36, 38, 41, or 42, which is Fab' antibody.
47. The antibody of claim 31, 33, 36, 38, 41, or 42, which is a (Fab')₂ antibody.
48. The antibody of claim 31, 33, 36, 38, 41, or 42, wherein the antibody is a fully human antibody.
49. The antibody of claim 31, 33, 36, 38, 41, or 42, wherein the antibody inhibits NGF signaling.
50. The use of an antibody of claim 49 in the manufacture of a medicament for treating a condition caused by increased expression of NGF or increased sensitivity to NGF in a patient.

51. The use of claim 50, wherein the condition is acute pain, dental pain, pain from trauma, surgical pain, pain resulting from amputation or abscess, causalgia, demyelinating diseases, trigeminal neuralgia, cancer, chronic alcoholism, stroke, thalamic pain syndrome, diabetes, acquired immune deficiency syndrome ("AIDS"), toxins, chemotherapy, general headache, migraine, cluster headache, mixed-vascular or non-vascular syndromes, tension headache, general inflammation, arthritis, rheumatic diseases, lupus, osteoarthritis, fibromyalgia, inflammatory bowel disorders, irritable bowel syndrome, inflammatory eye disorders, inflammatory or unstable bladder disorders, psoriasis, skin complaints with inflammatory components, sunburn, carditis, dermatitis, myositis, neuritis, collagen vascular diseases, chronic inflammatory conditions, inflammatory pain and associated hyperalgesia and allodynia, neuropathic pain and associated hyperalgesia or allodynia, diabetic neuropathy pain, causalgia, sympathetically maintained pain, deafferentation syndromes, asthma, epithelial tissue damage or dysfunction, herpes simplex, disturbances of visceral motility at respiratory, genitourinary, gastrointestinal or vascular regions, wounds, burns, allergic skin reactions, pruritis, vitiligo, general gastrointestinal disorders, colitis, gastric ulceration, duodenal ulcers, vasomotor or allergic rhinitis, or bronchial disorders, dysmenorrhoea, dyspepsia, gastroesophageal reflux, pancreatitis, or visceralgia.
52. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the antibody of claim 49.
53. The use of the pharmaceutical composition of claim 52 in the manufacture of a medicament for treating a condition caused by increased expression of NGF or increased sensitivity to NGF in a patient.
54. A method for detecting NGF in a biological sample comprising:
- a. contacting the sample with the antibody of claim 31, 33, 36, 38, 41, or 42, under conditions that allow for binding of the antibody to NGF;
 - b. and measuring the level of bound antibody in the sample.

55. A nucleic acid molecule that encodes the antibody of claim 31, 33, 36, 38, 41, or 42.
56. A host cell comprising the nucleic acid of claim 55.
57. An isolated cell line that produces an antibody according to any of claims 31, 33, 36, 38, 41, or 42.
58. A NGF specific binding agent comprising at least one amino acid sequence selected from SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, and any of SEQ ID NOS: 79-130, and wherein the binding agent can bind to NGF.
59. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the binding agent of claim 58.
60. The use of the pharmaceutical composition of claim 58 in the manufacture of a medicament for treating a condition caused by increased expression of NGF or increased sensitivity to NGF in a patient.
61. The binding agent of claim 58 that is a protein.
62. A nucleic acid molecule that encodes the binding agent of claim 61.
63. A host cell comprising the nucleic acid of claim 62.
64. An isolated cell line that produces the binding agent of claim 63.
65. The use of the binding agent of claim 58 in the manufacture of a medicament for treating a condition caused by increased expression of NGF or increased sensitivity to NGF in a patient.
66. The use of claim 65, wherein the condition is acute pain, dental pain, pain from trauma, surgical pain, pain resulting from amputation or abscess, causalgia, demyelinating diseases, trigeminal neuralgia, cancer, chronic alcoholism, stroke,

thalamic pain syndrome, diabetes, acquired immune deficiency syndrome ("AIDS"), toxins, chemotherapy, general headache, migraine, cluster headache, mixed-vascular or non-vascular syndromes, tension headache, general inflammation, arthritis, rheumatic diseases, lupus, osteoarthritis, fibromyalgia, inflammatory bowel disorders, irritable bowel syndrome, inflammatory eye disorders, inflammatory or unstable bladder disorders, psoriasis, skin complaints with inflammatory components, sunburn, carditis, dermatitis, myositis, neuritis, collagen vascular diseases, chronic inflammatory conditions, inflammatory pain and associated hyperalgesia and allodynia, neuropathic pain and associated hyperalgesia or allodynia, diabetic neuropathy pain, causalgia, sympathetically maintained pain, deafferentation syndromes, asthma, epithelial tissue damage or dysfunction, herpes simplex, disturbances of visceral motility at respiratory, genitourinary, gastrointestinal or vascular regions, wounds, burns, allergic skin reactions, pruritis, vitiligo, general gastrointestinal disorders, colitis, gastric ulceration, duodenal ulcers, vasomotor or allergic rhinitis, or bronchial disorders, dysmenorrhoea, dyspepsia, gastroesophageal reflux, pancreatitis, or visceralgia.

67. A method for detecting NGF in a biological sample comprising:
 - a. contacting the sample with the binding agent of claim 58, under conditions that allow for binding of the binding agent to NGF; and
 - b. measuring the level of bound antibody in the sample.
68. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid as set forth in any of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42 SEQ ID NO: 43, SEQ ID NO: 44, or any of SEQ ID NOS 79-134.
69. An isolated human antibody or an antigen-binding or an immunologically functional immunoglobulin fragment thereof that binds specifically to NGF, wherein the antibody or fragment comprises a heavy chain variable region comprising:
 - a. a CDR1 region comprising an amino acid sequence of the formula:



wherein:

a^1 is a polar hydrophilic amino acid residue;

a^2 is an aromatic amino acid residue;

a^3 is a aliphatic, polar hydrophobic, aromatic amino acid residue;

5 a^4 is a neutral hydrophobic or aliphatic amino acid residue; and

a^5 is a aliphatic or polar hydrophilic amino acid residue;

b. a CDR2 region comprising an amino acid sequence of the formula:

10 $b^1b^2b^3b^4b^5b^6b^7b^8b^9b^{10}b^{11}b^{12}b^{13}b^{14}b^{15}b^{16}b^{17}$

wherein:

b^1 is a aliphatic, polar hydrophobic, or aromatic amino acid residue;

b^2 is an aliphatic hydrophobic amino acid residue;

b^3 is a polar hydrophilic or aromatic amino acid residue;

15 b^4 is a polar hydrophilic, hydrophobic, or aromatic amino acid residue;

b^5 - b^9 are independently polar hydrophilic or aliphatic amino acid residues;

b^{10} is a polar hydrophilic, aromatic, or aliphatic amino acid residue;

b^{11} is an aromatic or hydrophobic amino acid residue;

b^{12} is an aliphatic hydrophobic or polar hydrophilic amino acid residue;

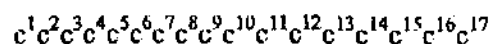
20 b^{13} is an aliphatic, hydrophobic or polar hydrophilic amino acid residue;

b^{14} and b^{16} are independently polar hydrophilic amino acid residues;

b^{15} is an aliphatic or aromatic hydrophobic amino acid residue; and

b^{17} is an aliphatic acidic amino acid residue; and

c. a CDR3 region comprising an amino acid sequence of the formula:



wherein:

c^1 is absent or an aliphatic amino acid residue;

5 c^2 is absent or a polar hydrophilic or an aromatic hydrophobic amino acid residue;

c^3 and c^4 are independently absent or a polar hydrophilic, aromatic hydrophobic, or aliphatic amino acid residues;

10 c^5 is absent or a polar hydrophilic, aliphatic or an aromatic amino acid residue;

c^6 is absent or a polar hydrophilic or aliphatic amino acid residue;

c^7 is a polar hydrophilic or an aliphatic amino acid residue;

c^8 is a polar hydrophilic, hydrophobic or an aromatic amino acid residue;

15 c^9 is a polar hydrophilic, aliphatic or an aromatic hydrophobic amino acid residue;

c^{10} a polar hydrophilic, aromatic hydrophobic, or an aliphatic hydrophobic amino acid residue;

c^{11} - c^{13} are independently polar hydrophilic or aromatic hydrophobic amino acid residues;

20 c^{14} is an aliphatic or aromatic hydrophobic amino acid residue;

c^{15} is a polar hydrophilic or neutral hydrophobic amino acid residue;

c^{16} is absent or a polar hydrophilic amino acid residue; and

25 c^{17} is an aromatic hydrophobic or aliphatic hydrophobic amino acid residue, and wherein said antibody or fragment dissociates from a human NGF polypeptide with a K_D of about 1×10^{-9} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-8} M or less.

70. The antibody or fragment according to Claim 69 wherein:

a¹ is a polar hydrophilic amino acid residue;

a² is an aromatic hydrophobic amino acid residue;

5 a³ is an aliphatic hydrophobic amino acid residue;

a⁴ is a neutral hydrophobic;

a⁵ is a polar hydrophilic amino acid residue;

b¹ is a aliphatic or aromatic amino acid residue;

b² is Ile;

10 b³ is a polar hydrophilic amino acid residue;

b⁴ is a polar hydrophilic or aromatic amino acid residue;

b⁵-b⁹ are independently polar hydrophilic or aliphatic amino acid residues;

b¹⁰ is an aliphatic amino acid residue;

15 b¹¹ is Tyr;

b¹² is an aliphatic hydrophobic amino acid residue;

b¹³ is an aliphatic or polar hydrophilic amino acid residue;

b¹⁴ and b¹⁶ are independently polar hydrophilic amino acid residues; and

20 b¹⁵ is an aliphatic hydrophobic amino acid residue;

b¹⁷ is an aliphatic acidic amino acid residue;

c¹ is absent or an aliphatic amino acid residue;

c² is absent or a polar hydrophilic or an aromatic hydrophobic amino acid residue;

- c³ and c⁴ are independently absent or a polar hydrophilic, aromatic hydrophobic, or aliphatic amino acid residues;
- c⁵ is absent or a polar hydrophilic amino acid residue;
- c⁶ is absent or a polar hydrophilic or aliphatic amino acid residue;
- 5 c⁷ is a polar hydrophilic or an aliphatic amino acid residue;
- c⁸ is a polar hydrophilic, hydrophobic or an aromatic amino acid residue;
- c⁹ is a polar hydrophilic, aliphatic or an aromatic hydrophobic amino acid residue;
- 10 c¹⁰ is a polar hydrophilic, aromatic hydrophobic, or an aliphatic hydrophobic amino acid residue;
- c¹¹ - c¹³ are independently polar hydrophilic or aromatic hydrophobic amino acid residues;
- c¹⁴ is an aliphatic or aromatic hydrophobic amino acid residue;
- 15 c¹⁵ is a polar hydrophilic or neutral hydrophobic amino acid residue;
- c¹⁶ is absent or a polar hydrophilic amino acid residue; and
- c¹⁷ is an aromatic hydrophobic or aliphatic hydrophobic amino acid residue.
- 20 71. The antibody or fragment according to Claim 69 wherein:
- a¹ is Ser, Asp, or Thr; a² is Tyr; a³ is Ala, Ser, Trp, or Gly; a⁴ is Met or Ile; a⁵ is His, Gly, or Asn; b¹ is Tyr, Gly, Ile, or Asp; b² is Ile; b³ is Ser, Thr, Tyr, or Asn; b⁴ is Trp, Arg, or Pro; b⁵ is Ser, Asn, or Gly; b⁶ is Ser, Arg, Asp, or Gly; b⁷ is Ser, His, or Gly; b⁸ is Ser, Ile, Asp, or Thr; b⁹ is Leu, Ile, or Thr; b¹⁰ is Gly, Lys, or Phe; b¹¹ is Tyr; b¹² is Ala or Ser; b¹³ is Asp, Gly, or Pro; b¹⁴ is Ser; b¹⁵ is Val or Phe; b¹⁶ is Lys or Gln; b¹⁷ is Gly; c¹ is absent or an aliphatic amino acid residue; c² is absent or Tyr; c³ and c⁴ are independently
- 25

absent, Tyr, Asn, Val, or Glu; c⁵ is absent, Ser, Gly, or Trp; c⁶ is absent, Ser, Gly, Glu, or Leu; c⁷ is Gly, Arg, or Asp; c⁸ is Trp, Pro, Ser, or Thr; c⁹ is His, Gly, or Tyr; c¹⁰ is Val, Tyr, or Arg; c¹¹ - c¹³ are independently Ser, Phe, Tyr, Asp, or Asn; c¹⁴ is Phe, Val, or Gly; c¹⁵ is Met or Asp; c¹⁶ is absent, Asp, or Asn; and c¹⁷ is Tyr or Val.

5 72. The antibody or fragment according to Claim 69 wherein:

a¹ is Ser or Asp; a² is Tyr; a³ is Ala or Ser; a⁴ is Met or Ile; a⁵ is His or Asn; b¹ is Tyr or Gly; b² is Ile; b³ is Ser, Thr, Tyr, or Asn; b⁴ is Trp, Arg, or Pro; b⁵ is Ser or Asn; b⁶ is Ser or Arg; b⁷ is His or Gly; b⁸ is Ile or Thr; b⁹ is Leu, Ile, or Thr; b¹⁰ is Gly or Phe; b¹¹ is Tyr; b¹² is Ala or Ser; b¹³ is Asp or Gly; b¹⁴ is Ser; b¹⁵ is Val or Phe; b¹⁶ is Lys or Gln; b¹⁷ is Gly; c¹ is absent or Gly; c² is absent or Tyr; c³ and c⁴ are independently absent, Tyr, Gly, or Val; c⁵ is absent or Ser; c⁶ is Ser or Gly; c⁷ is Gly or Arg; c⁸ is Trp or Pro; c⁹ is His, Gly, or Tyr; c¹⁰ is Val or Tyr; c¹¹ - c¹³ are independently Ser, Tyr, Phe, or Asp; c¹⁴ is Phe or Val; c¹⁵ is Met or Asp; c¹⁶ is absent or Asp; and c¹⁷ is Tyr or Val.

15 73. The antibody of claim 69, wherein:

a) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 22, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 18, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 14;

20 b) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 92, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 93, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 94;

25 c) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 98, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 99, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 100;

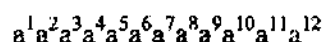
d) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 104, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 105, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 106;

5 e) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 110, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 111, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 112; or

10 f) the heavy chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 116, the heavy chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 117, and the heavy chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 118.

74. An isolated human antibody or an antigen-binding or an immunologically functional immunoglobulin fragment thereof that binds specifically to NGF, wherein the antibody or fragment comprises a light chain variable region comprising:

15 a. a CDR1 region comprising an amino acid sequence of the formula:



wherein:

20 a^1 is a polar hydrophilic amino acid residue;

a^2 , a^{11} and a^{12} are independently aliphatic or hydrophobic amino acid residues;

a^3 , a^4 , a^7 and a^8 are independently aliphatic, polar hydrophilic, or hydrophobic amino acid residues;

25 a^4 is a polar hydrophilic amino acid residue;

a^6 is an aliphatic or hydrophobic amino acid residue;

a^9 is absent, or an aliphatic or polar hydrophilic amino acid residue; and

c⁷ is absent or a polar hydrophilic or an aliphatic hydrophobic amino acid residue;

c⁸ is a polar hydrophilic or hydrophobic amino acid residue; and

c⁹ is a polar hydrophilic amino acid residue,

5 and wherein said antibody or fragment dissociates from a human NGF polypeptide with a K_D of about 1 x 10⁻⁹ or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC₅₀ of about 1 x 10⁻⁸ M or less.

75. The antibody or fragment according to Claim 74 wherein:

10 a¹, a³, a⁴, a⁷ and a⁸ are independently polar hydrophilic amino acid residues;

a², a⁶, a¹¹ and a¹² are independently aliphatic hydrophobic amino acid residues;

a⁵ is a polar hydrophilic or aliphatic amino acid residue;

15 a⁹ is absent, or an aliphatic or polar hydrophilic amino acid residue;

a¹⁰ is an aliphatic or aromatic amino acid residue;

b¹ is a aliphatic, polar hydrophobic, or hydrophobic amino acid residue;

20 b² is an aliphatic hydrophobic amino acid residue;

b³, b⁴ and b⁷ are independently polar hydrophilic amino acid residues;

b⁵ and b⁶ are independently polar hydrophilic or aliphatic hydrophobic amino acid residues;

25 c¹ and c² are independently polar hydrophilic amino acid residues;

c³ is a polar hydrophilic, aliphatic or hydrophobic amino acid residue;

c^4 , c^5 , and c^6 are independently aliphatic, polar hydrophilic, or hydrophobic amino acid residues;

c^7 is absent or an aliphatic hydrophobic amino acid residue;

c^8 is a hydrophobic amino acid residue; and

5 c^9 is a polar hydrophilic amino acid residue.

76. The antibody or fragment according to Claim 75 wherein:

a^1 , a^3 , a^4 , and a^7 are Arg, Ser, Gln, and Ser, respectively;

a^2 is Ala;

10 a^5 is Gly or Ser;

a^8 is Ser or Ile;

a^9 is absent, Ser, or Gly;

a^{10} is Ala, Tyr, Trp or Phe;

b^1 is Asp, Gly, Ala, or Val;

15 b^2 and b^3 are Ala and Ser, respectively;

b^4 is Ser or Asn;

b^5 is Leu or Arg;

b^6 is Glu, Ala, or Gln;

b^7 is Ser or Thr;

20 c^1 and c^2 are Gln;

c^3 is Phe, Tyr, Arg, or Ala;

c^4 is Asn, Gly, or Ser;

c^5 is Ser or Asn;

c^6 is Tyr, Ser, Trp, or Phe;

c⁷ is absent, Pro, or His;
c⁸ is Leu, Trp, Tyr, or Arg; and
c⁹ is Thr.

77. The antibody or fragment according to Claim 75 wherein:

5 a¹, a², a³, a⁴, and a⁷ are Arg, Ala, Ser, Gln, and Ser, respectively;
 a⁵ is Gly or Ser;
 a⁸ is Ser or Ile;
 a⁹ is absent, Ser, or Gly;
 a¹⁰ is Ala or Tyr;
10 b¹ is Asp or Gly;
 b² and b³ are Ala and Ser, respectively;
 b⁴ is Ser or Asn;
 b⁵ is Leu or Arg;
 b⁶ is Glu, Ala, or Gln;
15 b⁷ is Ser or Thr;
 c¹ and c² are Gln;
 c³ is Phe, Tyr, Arg, or Ala;
 c⁴ is Asn, Gly, or Ser;
 c⁵ is Ser or Asn;
20 c⁶ is Tyr, Ser, Trp, or Phe;
 c⁷ is absent, Pro, or His;
 c⁸ is Leu, Trp, Tyr, or Arg; and
 c⁹ is Thr.

78. The antibody of claim 74, wherein:

-

- a) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 24, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 20, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 16;
- 5 b) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 95, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 96, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 97;
- 10 c) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 101, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 102, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 103;
- 15 d) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 107, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 108, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 109;
- 20 e) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 113, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 114, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 115;
- 25 f) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 119, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 120, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 121;
- g) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 122, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 123, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 124;

- h) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 125, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 126, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 127;
- 5 i) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 128, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 129, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 130; or
- 10 j) the light chain CDR1 has an amino acid sequence as set forth in SEQ ID NO: 132, the light chain CDR2 has an amino acid sequence as set forth in SEQ ID NO: 133, and the light chain CDR 3 has an amino acid sequence as set forth in SEQ ID NO: 134.
79. A polynucleotide encoding the antibody or fragment according to any of Claims 69-78.
- 15 80. An expression vector comprising the polynucleotide of Claim 79.
81. A host cell comprising the expression vector of Claim 80.
82. The host cell according to Claim 81 wherein the cell is a eukaryotic cell.
83. The host cell according to Claim 82 wherein the cell is a CHO cell.
- 20 84. A medicament for treating a painful disorder or condition associated with increased expression of NGF or increased sensitivity to NGF, the medicament comprising a pharmaceutically effective amount of a monoclonal antibody or an immunologically functional immunoglobulin fragment thereof, or pharmaceutically acceptable salts of the monoclonal antibody or the fragment, wherein said monoclonal antibody is at least one of said monoclonal antibody
- 25 of Claim 1, and a pharmaceutically acceptable carrier, diluent or excipient.
85. The medicament of claim 84, wherein said monoclonal antibody comprises:
- a) a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an

- immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 12, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;
- 5 b) a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 14, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically
- 10 functional immunoglobulin fragment thereof;
- c) a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 18, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence
- 15 as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or
- d) a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 20, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light
- 20 chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 16, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.
86. A medicament for treating a painful disorder or condition associated with increased expression of NGF or increased sensitivity to NGF, the medicament
- 25 comprising a pharmaceutically effective amount of a monoclonal antibody or an immunologically functional immunoglobulin fragment thereof, comprising an amino acid sequence that has at least 80% sequence identity to the amino acid sequence set forth in SEQ ID NO: 10 or an amino acid sequence that has at least 90% sequence identity to the amino acid sequence as set forth in SEQ
- 30 ID NO: 12, and a pharmaceutically acceptable carrier, diluent or excipient.

87. The medicament of claim 86, wherein said monoclonal antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-9} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-8} M or less.
- 5 88. The medicament of claim 86, wherein the antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-10} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-9} M or less.
- 10 89. The medicament of claim 86, wherein the antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-11} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 0.2×10^{-9} M or less.
- 15 90. A use of a pharmaceutically effective amount of the antibody of claim 1 for the manufacture of a medicament suitable for treating a painful disorder or condition associated with increased expression of NGF or increased sensitivity to NGF.
- 20 91. The use of claim 90, wherein said monoclonal antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-9} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-8} M or less.
92. The use of claim 90, wherein the antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-10} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-9} M or less.
- 25 93. The use of claim 90, wherein the antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-11} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 0.2×10^{-9} M or less.
94. The use of claim 93, wherein said painful disorder or condition is selected from the group consisting the condition is acute pain, dental pain, pain from

5 trauma, surgical pain, pain resulting from amputation or abscess, causalgia,
 demyelinating diseases, trigeminal neuralgia, cancer, chronic alcoholism,
 stroke, thalamic pain syndrome, diabetes, acquired immune deficiency
 syndrome ("AIDS"), toxins, chemotherapy, general headache, migraine,
 10 cluster headache, mixed-vascular or non-vascular syndromes, tension
 headache, general inflammation, arthritis, rheumatic diseases, lupus,
 osteoarthritis, fibromyalgia, inflammatory bowel disorders, irritable bowel
 syndrome, inflammatory eye disorders, inflammatory or unstable bladder
 disorders, psoriasis, skin complaints with inflammatory components, sunburn,
 15 carditis, dermatitis, myositis, neuritis, collagen vascular diseases, chronic
 inflammatory conditions, inflammatory pain and associated hyperalgesia and
 allodynia, neuropathic pain and associated hyperalgesia or allodynia, diabetic
 neuropathy pain, causalgia, sympathetically maintained pain, deafferentation
 syndromes, asthma, epithelial tissue damage or dysfunction, herpes simplex,
 20 disturbances of visceral motility at respiratory, genitourinary, gastrointestinal
 or vascular regions, wounds, burns, allergic skin reactions, pruritis, vitiligo,
 general gastrointestinal disorders, colitis, gastric ulceration, duodenal ulcers,
 vasomotor or allergic rhinitis, or bronchial disorders, dysmenorrhoea,
 dyspepsia, gastroesophageal reflux, pancreatitis, or visceralgia.

20 95. An isolated human antibody that binds specifically to NGF, wherein the
 antibody comprises:

25 (a) a heavy chain having a heavy chain variable region comprising an amino
 acid sequence as set forth in SEQ ID NO: 79, or an antigen-binding or an
 immunologically functional immunoglobulin fragment thereof, and a light
 chain having a light chain variable region comprising an amino acid sequence
 as set forth in SEQ ID NO: 80, or an antigen-binding or an immunologically
 functional immunoglobulin fragment thereof;

30 (b) a heavy chain having a heavy chain variable region comprising an amino
 acid sequence as set forth in SEQ ID NO: 81, or an antigen-binding or an
 immunologically functional immunoglobulin fragment thereof, and a light

chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 82, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof;

(c) a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 83, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 84, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof; or

(d) a heavy chain having a heavy chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 86, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof, and a light chain having a light chain variable region comprising an amino acid sequence as set forth in SEQ ID NO: 87, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

96. An isolated human antibody that binds specifically to nerve growth factor (NGF), wherein the antibody comprises a heavy chain variable region, said heavy chain variable region comprising CDR1 (SEQ ID NO: 22), CDR2 (SEQ ID NO: 18), and CDR3 (SEQ ID NO: 14) of SEQ ID NO: 10, and a light chain variable region, said light chain variable region comprising CDR1 (SEQ ID NO: 24), CDR2 (SEQ ID NO: 20), and CDR3 (SEQ ID NO: 16) of SEQ ID 12.

97. The antibody of claim 96, wherein the heavy chain comprises a heavy chain variable region comprising SEQ ID NO: 10, and the light chain comprises a light chain variable region comprising SEQ ID NO: 12.

98. An isolated human antibody that binds specifically to nerve growth factor (NGF), comprising:

(a) human heavy chain framework regions, a human heavy chain CDR1 region comprising SEQ ID NO: 22, a human heavy chain CDR2 region comprising SEQ ID NO: 18, and a human heavy chain CDR3 region comprising SEQ ID NO: 14; and

- (b) human light chain framework regions, a human light chain CDR1 region comprising SEQ ID NO: 24, a human light chain CDR2 region comprising SEQ ID NO: 20, and a human light chain CDR3 region comprising SEQ ID NO:16.

99. An isolated human antibody that binds specifically to nerve growth factor (NGF), wherein the antibody comprises a heavy chain variable region comprising SEQ ID NO:10.

100. An isolated human antibody that binds specifically to nerve growth factor (NGF), wherein the antibody comprises a light chain variable region comprising SEQ ID NO:12.

101. An isolated human antibody that binds specifically to nerve growth factor (NGF), wherein the antibody comprises a light chain comprising SEQ ID NO:44.

102. An isolated human antibody that binds specifically to nerve growth factor (NGF), wherein the antibody comprises a heavy chain comprising SEQ ID NO:40.

103. An isolated human antibody that binds specifically to nerve growth factor (NGF), wherein the antibody comprises a light chain comprising SEQ ID NO:44 and a heavy chain comprising SEQ ID NO:40.

104. The antibody of any of claims 96-103, wherein the antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-9} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-8} M or less.

105. The antibody of any of claims 96-103, wherein the antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-10} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-9} M or less.

106. The antibody of any of claims 96-103, wherein the antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-11} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 0.2×10^{-9} M or less.

107. The antibody of any of claims 96-103, wherein the antibody comprises a heavy chain and a heavy light chain, wherein the light chain and the heavy chain are connected by a flexible linker to form a single-chain antibody.

108. The antibody of claim 107, which is a single-chain Fv antibody.

109. The antibody of any of claims 96-103, which is a Fab antibody.

110. The antibody of any of claims 96-103, which is a Fab' antibody.

111. The antibody of any of claims 96-103, which is a $(Fab')_2$ antibody.

112. The antibody of any of claims 96-103, wherein the antibody is a fully human antibody.

113. The antibody of any of claims 96-103, wherein the antibody inhibits NGF signaling.

114. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the antibody of any of claims 96-113.

115. The pharmaceutical composition of claim 114, wherein the heavy chain and light chain of the antibody are connected by a flexible linker to form a single chain antibody.

116. The pharmaceutical composition of claim 115, wherein the antibody is a single chain Fv antibody.

117. The pharmaceutical composition of claim 114, wherein the antibody is a Fab' antibody.

118. The pharmaceutical composition of claim 114, wherein the antibody is a (Fab')₂ antibody.

119. The pharmaceutical composition of claim 114, wherein the antibody is fully human.

120. The pharmaceutical composition of claim 114, wherein the antibody inhibits NGF signaling.

121. An isolated nucleic acid molecule comprising a nucleotide sequence of

(a) SEQ ID NO: 9;

(b) SEQ ID NO: 11;

(c) SEQ ID NO: 21, SEQ ID NO: 17, and SEQ ID NO: 13; or

(d) SEQ ID NO: 23, SEQ ID NO: 19, and SEQ ID NO: 15;

or a polynucleotide variant thereof, wherein the variant has at least about 90% nucleic acid sequence identity.

122. A host cell comprising the nucleic acid of claim 121.

123. An isolated cell line comprising the nucleic acid of claim 121.

124. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of:

(a) SEQ ID NO: 10;

(b) SEQ ID NO: 12;

- (c) SEQ ID NO: 22, SEQ ID NO: 18, and SEQ ID NO: 14;
- (d) SEQ ID NO: 24, SEQ ID NO: 20, and SEQ ID NO: 16;
- (e) SEQ ID NO: 40;
- (f) SEQ ID NO: 41;
- (g) SEQ ID NO: 42;
- (h) SEQ ID NO: 43; or
- (i) SEQ ID NO: 44.

125. A host cell comprising the nucleic acid of claim 124.

126. An isolated cell line comprising the nucleic acid of claim 124.

127. An isolated nucleic acid molecule that encodes a NGF specific binding agent, wherein the specific binding agent comprises at least one amino acid sequence selected from:

- (a) SEQ ID NO: 10;
- (b) SEQ ID NO: 12;
- (c) SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18;
- (d) SEQ ID NO: 20, SEQ ID NO: 22, and SEQ ID NO: 24;
- (e) SEQ ID NO: 40;
- (f) SEQ ID NO: 41;
- (g) SEQ ID NO: 42;
- (h) SEQ ID NO: 43; or
- (i) SEQ ID NO: 44;

or an antigen-binding fragment thereof, wherein the antigen-binding fragment can bind to NGF.

128. The isolated nucleic acid molecule of claim 127, wherein the nucleic acid molecule encodes a specific binding agent that is a protein.

129. A host cell comprising the isolated nucleic acid of claim 127.
130. An isolated cell line comprising the isolated nucleic acid of claim 127.
131. The isolated nucleic acid molecule of claim 127, wherein the nucleic acid molecule encodes a specific binding agent that is an antibody.
132. The isolated nucleic acid molecule of claim 131, wherein the nucleic acid molecule encodes an antibody comprising:
- (a) SEQ ID NO: 10;
 - (b) SEQ ID NO: 12;
 - (c) SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18;
 - (d) SEQ ID NO: 20, SEQ ID NO: 22, and SEQ ID NO: 24;
 - (e) SEQ ID NO: 40;
 - (f) SEQ ID NO: 41;
 - (g) SEQ ID NO: 42;
 - (h) SEQ ID NO: 43; or
 - (i) SEQ ID NO: 44,

or a variant thereof wherein the variant comprises no more than one amino acid substitution, insertion, or deletion.

133. A host cell comprising the nucleic acid of claim 132.
134. An isolated cell line comprising the nucleic acid of claim 132.

02-1240.ST25
SEQUENCE LISTING

<110> Kenneth, Wild
Treonor, James
Huang, Haichun
Inoue, Heather
Zhang, Tie J.
Martin, Frank

<120> Human anti-NGF Neutralizing Antibodies as Selective NGF Pathway Inhibitors

<130> 02-1240

<150> US 60/487,431

<151> 2003-07-15

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 Trp Gly Gln Gly Ile Leu Val Thr Val Ser Ser
 115 120

<210> 11

<211> 321

<212> DNA

<213> Homo sapiens

<400> 11

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 gggaaagctc ctaagctcct gatctatgat gccctccagtt tggaaagtgg ggteccatca 180
 aggttcagcg gcagtggatc tgggacagat ttcactctca ccatcagcag cctgcagcct 240
 gaagattttg caacttatta ctgtcaacag ttttaatagtt acccgetcac ttccggcgga 300
 gggaccaagg tggagatcaa a 321

<210> 12
 <211> 107
 <212> PRT
 <213> Homo sapiens

<400> 12

Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
 20 25 30
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45
 Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 13
 <211> 42
 <212> DNA
 <213> Homo sapiens

<400> 13
 gtatatagca gtggtggca cgtctctgat tattttgact ac

42

<210> 14
 <211> 14
 <212> PRT
 <213> Homo sapiens

<400> 14

Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
 1 5 10

<210> 15
 <211> 27

<212> DNA

<213> Homo sapiens

<400> 15

caacagttta atagttaccc gtcact

27

<210> 16

<211> 9

<212> PRT

<213> Homo sapiens

<400> 16

Gln Gln Phe Asn Ser Tyr Pro Leu Thr
1 5

<210> 17

<211> 51

<212> DNA

<213> Homo sapiens

<400> 17

tacattagtc gtagtagtca taccatattc tacgcagact ctgtgaaggg c

51

<210> 18

<211> 17

<212> PRT

<213> Homo sapiens

<400> 18

Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 19

<211> 21

<212> DNA

<213> Homo sapiens

<400> 19

gatgcctcca gtttggaag t

21

<210> 20

<211> 7

<212> PRT

<213> Homo sapiens

<400> 20

Asp Ala Ser Ser Leu Glu Ser
1 5

<210> 21

<211> 15

<212> DNA

<213> Homo sapiens

<400> 21

agttatagca tgaac

15

<210> 22

<211> 5

<212> PRT

<213> Homo sapiens

<400> 22

Ser Tyr Ser Met Asn
1 5

<210> 23

<211> 33

<212> DNA

<213> Homo sapiens

<400> 23

cgggcaagtc agggcattag cagtgttta gcc

33

<210> 24

<211> 11

<212> PRT

<213> Homo sapiens

<400> 24

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
 1 5 10

<210> 25

<211> 1131

<212> DNA

<213> Homo sapiens

<400> 25

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tggaaactcag gcgccctgac cagcggggtg cacacattcc cggctgtcct acagtcctca    180
ggactctact ccttcagcag cgtgggtgac gtgccctcca gcagcttggg caccagac    240
tacacctgca acgtgaatca caagccagc aacaccaagg tggacaagag agttagatc    300
aaaacccac ttgggtgacac aactcacaca tgcacacggt gccagagcc caaatcttgt    360
gacacacctc cccctgtccc acggtgccc gagcccaaat ctgttgacac acctccccg    420
tgcctacggt gccagagcc caaatcttgt gacacacctc ccccatgcc acggtgccc    480
gcacctgaac tcttgggagg acgtcagtc ttcctcttcc ccccaaaccc caggataac    540
cttatgattt cccggacccc tgaggtcagc tgcgtgggtg tggacgtgag ctaggaagac    600
cccgagggtc agticaagtg gtacgtggac ggctgggagg tgcataatgc caagacaaag    660
ccgctgggagg agcagttcaa cagcagttc cgtgtggtca gcgtcctcac cgtcctgccc    720
caggactggc tgaacggcaa ggagtacaag tgaaggtct ccaacaaagc cctccagccc    780
cccatcgaga aaaccatctc caaanccaaa ggacagcccc gagaaccaca ggtgtacacc    840
ctgcccccac cccgggagga gatgaccaag aaccagggtc gccctgacctg cctggtcaca    900
ggctttctac ccagcgacat cgccttggag tgggagagca gcgggcagcc ggagaacaac    960
tacaacacaa cgcctcccat gctggactcc gacggctcct tcttctctta cagcaagctc   1020
accgtggaca agagcaggtg gcagcagggg aacatctctt catgctccgt gatgcattga   1080
gctctgcaca accgttccac gcagaagagc ctctccctgt ctccgggtaa a           1131

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<211> 376

<212> PRT

<213> Homo sapiens

<400> 26

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 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 50 55 60
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 65 70 75 80
 Tyr Thr Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95
 Arg Val Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro
 100 105 110
 Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg
 115 120 125
 Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys
 130 135 140
 Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
 145 150 155 160
 Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
 165 170 175
 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
 180 185 190
 Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr
 195 200 205
 Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 210 215 220
 Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His
 225 230 235 240
 Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
 245 250 255
 Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln
 260 265 270
 Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
 275 280 285
 Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
 290 295 300
 Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn
 305 310 315 320
 Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu
 325 330 335
 Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile
 340 345 350

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Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln
355 360 365

Lys Ser Leu Ser Leu Ser pro Gly
370 375

<210> 27

<211> 29

<212> DNA

<213> Artificial

<220>

<223> PCR primer for human NGF

<400> 27

acaccacata tgtcatcatc ccatcccat

29

<210> 28

<211> 40

<212> DNA

<213> Artificial

 $\langle 220 \rangle$

<223> PCR Primer for human NGF

<400> 28

accacaggaat cctccttata cacgacgcac agcttttaagg

40

<210> 29

<213> 375

<2.12> DNA

<213> Artificial

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<223> Nucleotide sequence encoding recombinant human met-NGF

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agcgtgtggg ttgggggataa gaccaccgcc acagacatca agggcaagga ggtgatggtg 120

ttcggagagg tgaacattaa caacagtgtg ttcaaacagt accttttttga gaccaagtgc 180

cgqgacccaa atcccgattga cagcgggtgc cggggcattg actcaaagca ctggaactca 240

tattgtacca cgactcacac ctttgtcaag gcgctgacca tggatggcaa gcaagctgcc 300

tggcggttta tccggataga tacggcctgt gtgtgtgtgc tcagccgtaa agctgtgcgt 360

cgtgcataag gatcc

375

<210> 30

<211> 121

<212> PRT

<213> Artificial

<220>

<223> amino acid sequence of recombinant human met-NGF (1-120)

<400> 30

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Met Ser Ser Ser His Pro Ile Phe His Arg Gly Glu Phe Ser Val Cys
1           5           10           15
Asp Ser Val Ser Val Trp Val Gly Asp Lys Thr Thr Ala Thr Asp Ile
20           25           30
Lys Gly Lys Glu Val Met Val Leu Gly Glu Val Asn Thr Asn Asn Ser
35           40           45
Val Phe Lys Gln Tyr Phe Phe Glu Thr Lys Cys Arg Asp Pro Asn Pro
50           55           60
Val Asp Ser Gly Cys Arg Gly Ile Asp Ser Lys His Trp Asn Ser Tyr
65           70           75           80
Cys Thr Thr Thr His Thr Phe Val Lys Ala Leu Thr Met Asp Gly Lys
85           90           95
Gln Ala Ala Trp Arg Phe Ile Arg Ile Asp Thr Ala Cys Val Cys Val
100          105          110
Leu Ser Arg Lys Ala Val Arg Arg Ala
115          120

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<210> 31

<211> 55

<212> DNA

<213> Artificial

<220>

<223> 5' primer sequence to generate expression vector pCFM1656 (ATCC # 69576)

<400> 31

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<210> 32

<211> 49

<212> DNA

<213> Artificial

<220>

<223> 3' primer sequence to generate expression vector pCFM1656 (ATCC # 69576

<400> 32
taaactaaga tcttctctct tattgtatac caattgcgca accttaagc 49

<210> 33

<211> 24

<212> DNA

<213> Artificial

<220>

<223> PCR primer

<220>

<221> Unsure

<222> (18)..(23)

<223> n is a, c, t, or g

<400> 33
ggccg gatag gcctccannn nnnt 24

<210> 34

<211> 21

<212> DNA

<213> Artificial

<220>

<223> PCR primer

<400> 34
ggggtcaggc tggaactgag g 21

<210> 35

<211> 19

<212> DNA

<213> Artificial

<220>

<223> PCK primer

<400> 35

tgaggacgt gaccacacg

19

<210> 36

<211> 54

<212> DNA

<213> Artificial

<270>

<223> PCR primer

<400> 36

cagcagaagc ttctagacca ccatggacat gagggtagcc gtcagctcc tggg

54

<210> 37

<211> 34

<212> DNA

<213> Artificial

<270>

<223> PCR primer

<400> 37

cttgctgact caacactctc cctgttgaa gctc

34

<210> 38

<211> 55

<212> DNA

<213> Artificial

<220>

<223> PCR primer

<400> 38

cagcagaagc ttctagacca ccatggagt1 ggggctgtgc tgggttttcc ttgtt

55

<210> 39

<211> 34

<212> DNA

<213> Artificial

<220>

<223> PCR primer

<400> 39

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<210> 40

<211> 449

<212> PRT

<213> Homo sapiens

<400> 40

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
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Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Leu	Arg	Ser	Tyr
			20					25					30		

Ser	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			

Ser	Tyr	Ile	Ser	Arg	Ser	Ser	His	Thr	Ile	Phe	Tyr	Ala	Asp	Ser	Val
	50					55					60				

Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
65					70					75					80

Leu	Gln	Met	Asp	Ser	Leu	Arg	Asp	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys
			85						90					95	

Ala	Arg	Val	Tyr	Ser	Ser	Gly	Trp	His	Val	Ser	Asp	Tyr	Phe	Asp	Tyr
			100					105					110		

Trp	Gly	Gln	Gly	Ile	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly
		115					120					125			

Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser
	130					135					140				

Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val
145					150					155					160

Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe
				165					170					175	

Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val
		180						185					190		

Thr	Val	Pro	Ser	Ser	Asn	Phe	Gly	Thr	Gln	Thr	Tyr	Thr	Cys	Asn	Val
		195					200					205			

Asp	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Thr	Val	Glu	Arg	Lys
	210					215					220				

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Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
 225 230 235 240
 Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 245 250 255
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 260 265 270
 Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 275 280 285
 Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val
 290 300
 Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu
 305 310 315 320
 Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys
 325 330 335
 Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 340 345 350
 Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
 355 360 365
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
 385 390 395 400
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 405 410 415
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 420 425 430
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 435 440 445

Lys

<210> 41

<211> 453

<212> PRT

<213> Homo sapiens

<400> 41

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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 20 25 30
 Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val
 50 55 60

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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asp Ser Leu Arg Asp Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95
 Ala Arg Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
 100 105 110
 Trp Gly Gln Gly Ile Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 115 120 125
 Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
 130 135 140
 Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 145 150 155 160
 Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 165 170 175
 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 180 185 190
 Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
 195 200 205
 Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
 210 215 220
 Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
 225 230 235 240
 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 245 250 255
 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 260 265 270
 Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 275 280 285
 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
 290 295 300
 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 305 310 315 320
 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 325 330 335
 Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 340 345 350
 Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
 355 360 365
 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 370 375 380
 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 385 390 395 400
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 405 410 415
 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser

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420						425						430			
Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser
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Leu	Ser	Pro	Gly	Lys											
		450													
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<213> Homo sapiens															
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Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Leu	Arg	Ser	Tyr
			20					25				30			
Ser	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Ser	Tyr	Ile	Ser	Arg	Ser	Ser	His	Thr	Ile	Phe	Tyr	Ala	Asp	Ser	Val
	50					55				60					
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
65					70					75					80
Leu	Gln	Met	Asp	Ser	Leu	Arg	Asp	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys
				85					90					95	
Ala	Arg	Val	Tyr	Ser	Ser	Gly	Trp	His	Val	Ser	Asp	Tyr	Phe	Asp	Tyr
			100					105				110			
Trp	Gly	Gln	Gly	Ile	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly
		115					120					125			
Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser
						135					140				
Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val
145					150					155					160
Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe
				165					170				175		
Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val
			180					185				190			
Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val
		195					200					205			
Asp	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys
	210					215					220				
Tyr	Gly	Pro	Pro	Cys	Pro	Ser	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly
225					230					235					240
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile
				245					250					255	

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Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
 260 265 270
 Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
 275 280 285
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg
 290 295 300
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
 305 310 315 320
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
 325 330 335
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 340 345 350
 Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
 355 360 365
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 370 375 380
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
 385 390 395 400
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp
 405 410 415
 Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
 420 425 430
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
 435 440 445
 Gly Lys
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<210> 43

<211> 499

<212> PRT

<213> Homo sapiens

<400> 43

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Arg Ser Tyr
 20 25 30
 Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Arg Ser Ser His Thr Ile Phe Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asp Ser Leu Arg Asp Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

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Ala Arg Val Tyr Ser Ser Gly Trp His Val Ser Asp Tyr Phe Asp Tyr
 100 105 110
 Trp Gly Gln Gly Ile Leu Val Trp Val Ser Ser Ala Ser Thr Lys Gly
 115 120 125
 Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Gly Gly
 130 135 140
 Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 145 150 155 160
 Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 165 170 175
 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 180 185 190
 Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Thr Cys Asn Val
 195 200 205
 Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Leu Lys
 210 215 220
 Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro Arg Cys Pro Glu Pro
 225 230 235 240
 Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu Pro Lys
 245 250 255
 Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu Pro Lys Ser
 260 265 270
 Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Ala Pro Glu Leu Leu
 275 280 285
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 290 295 300
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 305 310 315 320
 His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr Val Asp Gly Val Glu
 325 330 335
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
 340 345 350
 Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 355 360 365
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 370 375 380
 Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Glu
 385 390 395 400
 Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
 405 410 415
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 420 425 430
 Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro
 435 440 445
 Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr

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460

450 455
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser Val
465 470 475 480
Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu
485 490 495

Ser Pro Gly

<210> 44

<211> 214

<212> PRT

<213> Homo sapiens

<400> 44

Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
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Phe Phe Glu Thr Lys
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Ser Ser Ser His Pro Ile Phe His Arg
1 5

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His Trp Asn Ser Tyr
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Asn Ser Val Glu Lys Gln Tyr Phe Phe Glu Thr Lys
1 5 10

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Ser Arg Lys Ala Val Arg Arg
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Glu Val Met Val Leu
1 5

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<400> 51

Ser Ser Ser His Pro Ile Phe His Arg Gly Glu Phe Ser Val Cys Asp
1 5 10 15

Ser Val Ser Val Trp Val Gly Asp Lys
20 25

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Gln Ala Ala Trp Arg Phe Ile Arg Ile Asp Thr Ala Cys Val Cys Val
1 5 10 15

Leu Ser Arg Lys
20

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Ser Ser His Pro Ile Phe His Arg Gly Glu Phe Ser Val Cys

1 5

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<400> 54

Ser Ser His Pro Ile Phe His Arg
1 5

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Gly Glu Phe Ser Val Cys
5

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<400> 56

Ser Ser Ser His Pro Ile Phe His Arg Gly Glu Phe Ser Val Cys
1 5 10 15

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<400> 57

Ile Phe His Arg Gly Glu Phe Ser Val Ala Asp Ser Val Ser Val Cys
1 5 10 15

<210> 58

<211> 16

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<213> Homo sapiens

<400> 58

Glu Phe Ser Val Ala Asp Ser Val Ser Val Trp Val Gly Asp Lys Cys
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Asp Ser Val Ser Val Trp Val Gly Asp Lys Thr Thr Ala Thr Asp Cys
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Trp Val Gly Asp Lys Thr Thr Ala Thr Asp Ile Lys Gly Lys Glu Cys
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Thr Thr Ala Thr Asp Ile Lys Gly Lys Glu Val Met Val Leu Gly Cys
 1 5 10 15

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Ile Lys Gly Lys Glu Val Met Val Leu Gly Glu Val Asn Ile Asn
 1 5 10 15

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<400> 63

Val	Met	Val	Leu	Gly	Glu	Val	Asn	Ile	Asn	Asn	Ser	Val	Phe	Lys	Cys
1				5					10					15	

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Glu	Val	Asn	Ile	Asn	Asn	Ser	Val	Phe	Lys	Gln	Tyr	Phe	Phe	Glu	Cys
1				5					10					15	

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<400> 65

Asn	Ser	Val	Phe	Lys	Gln	Tyr	Phe	Phe	Glu	Thr	Lys	Ala	Arg	Asp	Cys
1				5					10					15	

<210> 66

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<213> Homo sapiens

<400> 66

Gln	Tyr	Phe	Phe	Glu	Thr	Lys	Ala	Arg	Asp	Pro	Asn	Pro	Val	Asp	Cys
1				5					10					15	

<210> 67

<211> 16

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<213> Homo sapiens

<400> 67

Thr	Lys	Ala	Arg	Asp	Pro	Asn	Pro	Val	Asp	Ser	Gly	Ala	Arg	Asp	Cys
1				5					10					15	

<210> 68

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<212> PRT

<213> Homo sapiens

<400> 68

Pro	Asn	Pro	Val	Asp	Ser	Gly	Ala	Arg	Asp	Ile	Asp	Ser	Lys	His	Cys
1				5					10					15	

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<400> 69

Ser	Gly	Ala	Arg	Asp	Ile	Asp	Ser	Lys	His	Trp	Asn	Ser	Tyr	Cys
1				5					10					15

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<400> 70

Ile	Asp	Ser	Lys	His	Trp	Asn	Ser	Tyr	Ala	Thr	Thr	Thr	His	Thr	Cys
1				5					10					15	

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<400> 71

Trp	Asn	Ser	Tyr	Ala	Thr	Thr	Thr	His	Thr	Phe	Val	Lys	Ala	Leu	Cys
1				5					10					15	

<210> 72

<211> 16

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<213> Homo sapiens

<400> 72

Thr	Thr	Thr	His	Thr	Phe	Val	Lys	Ala	Leu	Thr	Met	Asp	Gly	Lys	Cys
1				5					10					15	

<210> 73

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<400> 73

Phe	Val	Lys	Ala	Leu	Thr	Met	Asp	Gly	Lys	Gln	Ala	Ala	Trp	Arg	Cys
1				5					10					15	

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<212> PRT

<213> Homo sapiens

<400> 74

Thr	Met	Asp	Gly	Lys	Gln	Ala	Ala	Trp	Arg	Phe	Ile	Arg	Ile	Asp	Cys
1				5					10					15	

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<400> 75

Gln	Ala	Ala	Trp	Arg	Phe	Ile	Arg	Ile	Asp	Thr	Ala	Ala	Val	Cys
1				5					10					15

<210> 76

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<213> Homo sapiens

<400> 76

Phe Ile Arg Ile Asp Thr Ala Ala Val Ala Val Leu Ser Arg Lys Cys
 1 5 10 15

<210> 77

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<400> 77

Thr Ala Ala Val Ala Val Leu Ser Arg Lys Ala Val Arg Arg Ala Cys
 1 5 10 15

<210> 78

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<400> 78

Cys Ala Ala Val Ala Val Leu Ser Arg Lys Ala Val Arg Arg Ala
 1 5 10 15

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<400> 79

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
 1 5 10 15

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Asn Phe Thr Thr Tyr
 20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Lys Tyr Ser Pro Ser Phe
 50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
 65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Ala Arg Asn Tyr Tyr Gly Ser Gly Thr Tyr Tyr Tyr Tyr Tyr Gly Met

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100	105	110
Asn Val Trp	Gly Gln Gly Thr Thr Val Thr Val Ser Ser	
115	120	125

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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ile Trp	
20 25 30	
Leu Ala Trp Tyr Gln His Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile	
35 40 45	
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly	
50 55 60	
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro	
65 70 75 80	
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Trp	
85 90 95	
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	
100 105	

<210> 81
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<400> 81

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg	
1 5 10 15	
Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Glu Asp Tyr	
20 25 30	
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	
Ser Gly Ile Ser Trp Asn Arg Gly Ile Ile Gly Tyr Ala Asp Ser Val	
50 55 60	
Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr	
65 70 75 80	
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys	
85 90 95	

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Ala Lys Glu Gly Tyr Tyr Gly Ser Gly Arg Pro Gly Tyr Phe Tyr Tyr
 100 105 110

Val Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120 125

<210> 82

<211> 108

<212> PRT

<213> homo sapien

<400> 82

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Gly
 20 25 30

Phe Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Ala Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
 85 90 95

Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 83

<211> 127

<212> PRT

<213> homo sapien

<400> 83

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Asp Asp Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Ser Trp Asn Arg Gly Ile Ile Gly Tyr Ala Gly Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

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Val Lys Glu Gly Tyr Tyr Gly Ser Gly Arg Pro Gly Tyr Phe Tyr Tyr
100 105 110

Val Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 84

<211> 108

<212> PRT

<213> homo sapien

<400> 84

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Val Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
85 90 95

Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 85

<211> 121

<212> PRT

<213> homo sapien

<400> 85

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Thr Trp Asn Ser Gly Ile Leu Gly Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys

85

90

95

Ala Lys Glu Glu Gly Ser Gly Arg Tyr Tyr Asn Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 86

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<212> PRT

<213> homo sapien

<400> 86

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Tyr
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 87

<211> 124

<212> PRT

<213> homo sapien

<400> 87

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Asp Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Glu Gln Trp Leu Asp Pro Tyr Tyr Tyr Tyr Tyr Gly Met Asp
100 105 110

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 88

<211> 107

<212> PRT

<213> homo sapien

<400> 88

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Glu Lys Ala Pro Lys Ser Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 89

<211> 107

<212> PRT

<213> homo sapien

<400> 89

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Gly Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Pro Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

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Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp His Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 90

<211> 108

<212> PRT

<213> homo sapien

<400> 90

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Gly Phe Thr Leu Thr Ile Ser Ser Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro
85 90 95

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 91

<211> 107

<212> PRT

<213> homo sapien

<400> 91

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Trp
Page 38

85 02-1240.ST25 95
 90
 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105
 <210> 92
 <211> 5
 <212> PRT
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 <400> 92
 Thr Tyr Trp Ile Gly
 1 5
 <210> 93
 <211> 17
 <212> PRT
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 <400> 93
 Ile Ile Tyr Pro Gly Asp Ser Asp Thr Lys Tyr Ser Pro Ser Phe Gln
 1 5 10 15
 Gly
 <210> 94
 <211> 16
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 <400> 94
 Asn Tyr Tyr Gly Ser Gly Thr Tyr Tyr Tyr Tyr Tyr Gly Met Asn Val
 1 5 10 15
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 <211> 11
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 <213> homo sapien

 <400> 95
 Arg Ala Ser Gln Gly Ile Ser Ile Trp Leu Ala
 1 5 10

<210> 96

<211> 7

<212> PRT

<213> homo sapien

<400> 96

Ala Ala Ser Ser Leu Gln Ser
1 5

<210> 97

<211> 9

<212> PRT

<213> homo sapien

<400> 97

Gln Gln Ala Asn Ser Phe Pro Trp Thr
1 5

<210> 98

<211> 5

<212> PRT

<213> homo sapien

<400> 98

Asp Tyr Ala Met His
1 5

<210> 99

<211> 17

<212> PRT

<213> homo sapien

<400> 99

Gly Ile Ser Trp Asn Arg Gly Ile Ile Gly Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

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<400> 100

Gly Tyr Tyr Gly Ser Gly Arg Pro Gly Tyr Phe Tyr Tyr Val Met Asp
1 5 10 15

Val

<210> 101

<211> 12

<212> PRT

<213> homo sapien

<400> 101

Arg Ala Ser Gln Ser Val Ser Ser Gly Phe Leu Ala
1 5 10

<210> 102

<211> 7

<212> PRT

<213> homo sapien

<400> 102

Gly Ala Ser Ser Arg Ala Thr
1 5

<210> 103

<211> 9

<212> PRT

<213> homo sapien

<400> 103

Gln Gln Tyr Gly Ser Ser Pro Tyr Thr
1 5

<210> 104

<211> 5

<212> PRT

<213> homo sapien

<400> 104

Asp	Tyr	Ala	Met	His
1				5

<210> 105

<211> 17

<212> PRT

<213> homo sapien

<400> 105

Gly	Ile	Ser	Trp	Asn	Arg	Gly	Ile	Ile	Gly	Tyr	Ala	Gly	Ser	Val	Lys
1				5					10					15	

Gly

<210> 106

<211> 17

<212> PRT

<213> homo sapien

<400> 106

Gly	Tyr	Tyr	Gly	Ser	Gly	Arg	Pro	Gly	Tyr	Phe	Tyr	Tyr	Val	Met	Asp
1				5					10					15	

Val

<210> 107

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<212> PRT

<213> homo sapien

<400> 107

Arg	Ala	Ser	Gln	Ser	Val	Ser	Ser	Ser	Tyr	Leu	Ala
1				5					10		

<210> 108

<211> 7

<212> PRT

<213> homo sapien

<400> 108

Val Ala Ser Ser Arg Ala Thr
1 5

<210> 109

<211> 9

<212> PRT

<213> homo sapien

<400> 109

Gln Gln Tyr Gly Ser Ser Pro Tyr Thr
1 5

<210> 110

<211> 5

<212> PRT

<213> homo sapien

<400> 110

Asp Tyr Ala Met His
1 5

<210> 111

<211> 17

<212> PRT

<213> homo sapien

<400> 111

Gly Ile Thr Trp Asn Ser Gly Ile Leu Gly Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 112

<211> 11

<212> PRT

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<400> 112

Glu Gly Ser Gly Arg Tyr Tyr Asn Phe Asp Tyr
1 5 10

<210> 113

<211> 12

<212> PRT

<213> homo sapien

<400> 113

Arg	Ala	Ser	Gln	Ser	Val	Ser	Ser	Ser	Tyr	Leu	Ala
1			5						10		

<210> 114

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<212> PRT

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<400> 114

Gly	Ala	Ser	Ser	Arg	Ala	Thr
1			5			

<210> 115

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<400> 115

Gln	Gln	Tyr	Gly	Ser	Ser	Tyr	Thr
1			5				

<210> 116

<211> 5

<212> PRT

<213> homo sapien

<400> 116

Asp	Tyr	Gly	Met	Asn
1			5	

<210> 117

<211> 17

<212> PRT

<213> homo sapien

<400> 117

Asp Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val Lys
 1 5 10 15

Gly

<210> 118

<211> 15

<212> PRT

<213> homo sapien

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Glu Gln Trp Leu Asp Pro Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val
 1 5 10 15

<210> 119

<211> 11

<212> PRT

<213> homo sapien

<400> 119

Arg Ala Ser Gln Gly Ile Ser Ser Trp Leu Ala
 1 5 10

<210> 120

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<212> PRT

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<400> 120

Ala Ala Ser Ser Leu Gln Ser
 1 5

<210> 121

<211> 9

<212> PRT

<213> homo sapien

<400> 121

Gln Gln Tyr Asn Ser Tyr Pro Trp Thr
 1 5

<210> 122

<211> 11

<212> PRT

<213> homo sapien

<400> 122

Arg	Ala	Ser	Gln	Gly	Val	Ser	Ser	Tyr	Leu	Ala
1				5					10	

<210> 123

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<212> PRT

<213> homo sapien

<400> 123

Asp	Ala	Ser	Asn	Arg	Ala	Thr
1				5		

<210> 124

<211> 9

<212> PRT

<213> homo sapien

<400> 124

Gln	Gln	Arg	Ser	Asn	Trp	His	Arg	Thr
1				5				

<210> 125

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<212> PRT

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<400> 125

Arg	Ala	Ser	Gln	Ser	Val	Ser	Ser	Ser	Tyr	Leu	Ala
1				5					10		

<210> 126

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Gly Ala Ser Ser Arg Ala Thr
1 5

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Gln Gln Tyr Asn Ser Tyr Pro Trp Thr
1 5

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<400> 128

Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala
1 5 10

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<213> homo sapien

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Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 130

<211> 9

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<213> homo sapien

<400> 130

Gln Gln Arg Ser Asn Trp Pro Trp Thr
1 5

<210> 131

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<400> 131

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
 20 25 30
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45
 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
 85 90 95
 Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 132
 <211> 11
 <212> PRT
 <213> homo sapien

<400> 132

Arg Ala Ser Gln Gly Val Ser Ser Tyr Leu Ala
 1 5 10

<210> 133
 <211> 7
 <212> PRT
 <213> homo sapien

<400> 133

Gly Ala Ser Ser Arg Ala Thr
 1 5

<210> 134
 <211> 9
 <212> PRT

<213> homo sapien

<400> 134

Gln Gln Tyr Gly Ser Ser Pro Tyr Thr
 1 5

<210> 135

<211> 120

<212> PRT

<213> homo sapien

<400> 135

Ser Ser Ser His Pro Ile Phe His Arg Gly Glu Phe Ser Val Cys Asp
 5 10 15
 Ser Val Ser Val Trp Val Gly Asp Lys Thr Thr Ala Thr Asp Ile Lys
 20 25 30
 Gly Lys Glu Val Met Val Leu Gly Glu Val Asn Ile Asn Asn Ser Val
 35 40 45
 Phe Lys Gln Tyr Phe Phe Glu Thr Lys Cys Arg Asp Pro Asn Pro Val
 50 55 60
 Asp Ser Gly Cys Arg Gly Ile Asp Ser Lys His Trp Asn Ser Tyr Cys
 65 70 75 80
 Thr Thr Thr His Thr Phe Val Lys Ala Leu Thr Met Asp Gly Lys Gln
 85 90 95
 Ala Ala Trp Arg Phe Ile Arg Ile Asp Thr Ala Cys Val Cys Val Leu
 100 105 110
 Ser Arg Lys Ala Val Arg Arg Ala
 115 120

<210> 136

<211> 120

<212> PRT

<213> mus musculus

<400> 136

Ser Ser Thr His Pro Val Phe His Met Gly Glu Phe Ser Val Cys Asp
 1 5 10 15
 Ser Val Ser Val Trp Val Gly Asp Lys Thr Thr Ala Thr Asp Ile Lys
 20 25 30
 Gly Lys Glu Val Thr Val Leu Ala Glu Val Asn Ile Asn Asn Ser Val
 35 40 45
 Phe Arg Gln Tyr Phe Phe Glu Thr Lys Cys Arg Ala Ser Asn Pro Val
 50 55 60

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Glu Ser Gly Cys Arg Gly Ile Asp Ser Lys His Trp Asn Ser Tyr Cys
 65 70 75 80
 Thr Thr Thr His Thr Phe Val Lys Ala Leu Thr Thr Asp Glu Lys Gln
 85 90 95
 Ala Ala Trp Arg Phe Ile Arg Ile Asp Thr Ala Cys Val Cys Val Leu
 100 105 110
 Ser Arg Lys Ala Thr Arg Arg Ala
 115 120

<210> 137

<211> 126

<212> PRT

<213> homo sapien

<400> 137

His Ser Asp Pro Ala Arg Arg His Ser Asp Pro Ala Arg Arg Gly Glu
 1 5 10 15
 Leu Ser Val Cys Asp Ser Ile Ser Glu Trp Val Thr Ala Ala Asp Lys
 20 25 30
 Lys Thr Ala Val Asp Met Ser Gly Gly Thr Val Thr Val Leu Glu Lys
 35 40 45
 Val Pro Val Ser Lys Gly Gln Leu Lys Gln Tyr Phe Tyr Glu Thr Lys
 50 55 60
 Cys Asn Pro Met Gly Tyr Thr Lys Glu Gly Cys Arg Gly Ile Asp Lys
 65 70 75 80
 Arg His Trp Asn Ser Gln Cys Arg Thr Thr Gln Ser Tyr Val Arg Ala
 85 90 95
 Leu Thr Met Asp Ser Lys Lys Arg Ile Gly Trp Arg Phe Ile Arg Ile
 100 105 110
 Asp Thr Ser Cys Val Cys Thr Leu Thr Ile Lys Arg Gly Arg
 115 120 125

<210> 138

<211> 119

<212> PRT

<213> homo sapien

<400> 138

Tyr Ala Glu His Lys Ser His Arg Gly Glu Tyr Ser Val Cys Asp Ser
 1 5 10 15
 Glu Ser Leu Trp Val Thr Asp Lys Ser Ser Ala Ile Asp Ile Arg Gly
 20 25 30
 His Gln Val Thr Val Leu Gly Glu Ile Lys Thr Gly Asn Ser Pro Val

35 40 45
 Lys Gln Tyr Phe Tyr Glu Thr Arg Cys Lys Glu Ala Arg Pro Val Lys
 50 55 60
 Asn Gly Cys Arg Gly Ile Asp Asp Lys His Trp Asn Ser Gln Cys Lys
 65 70 75 80
 Thr Ser Gln Thr Tyr Val Arg Ala Leu Thr Ser Glu Asn Asn Lys Leu
 85 90 95
 Val Gly Trp Arg Trp Ile Arg Ile Asp Thr Ser Cys Val Cys Ala Leu
 100 105 110
 Ser Arg Lys Ile Gly Arg Thr
 115