

HIGH-PURITY PRODUCTION OF MULTI-SUBUNIT PROTEINS SUCH AS ANTIBODIES IN TRANSFORMED MICROBES SUCH AS *PICHIA PASTORIS*

RELATED APPLICATION DISCLOSURE

[0001] This application claims the benefit of U.S. Provisional Application Ser. No. 61/525,307 (Atty. Docket No. 67858.730200), filed August 19, 2011 (entitled “MULTI-COPY STRATEGY FOR HIGH-TITER AND HIGH-PURITY PRODUCTION OF MULTI-SUBUNIT PROTEINS SUCH AS ANTIBODIES IN TRANSFORMED MICROBES SUCH AS *PICHIA PASTORIS*,” U.S. Provisional Application Ser. No. 61/488,660 (Atty. Docket No. 67858.730300), filed May 20, 2011, entitled “ANTI-CGRP COMPOSITIONS AND USE THEREOF,” U.S. Provisional Application Ser. No. 61/496,860 (Atty. Docket No. 67858.760000), filed June 14, 2011, entitled “USE OF ANTI-CGRP ANTIBODIES AND ANTIBODY FRAGMENTS TO PREVENT OR INHIBIT PHOTOPHOBIA IN SUBJECTS IN NEED THEREOF, ESPECIALLY MIGRAINE SUFFERERS,” and U.S. Provisional Application Ser. No. 61/496,873 (Atty. Docket No. 67858.770000), filed June 14, 2011, entitled “USE OF ANTI-CGRP ANTIBODIES AND ANTIBODY FRAGMENTS TO TREAT DIARRHEA IN SUBJECTS WITH DISEASES OR TREATMENTS THAT RESULT IN ELEVATED CGRP LEVELS” each of which is incorporated by reference herein in its entirety.

[0002] This application includes a Sequence Listing which is being submitted in ASCII format via EFS-Web, in a file named “67858o711002.txt” created May 8, 2012 and having a size of 315,392 bytes, which is hereby incorporated by reference in its entirety.

FIELD

[0003] The present disclosure generally relates to methods for producing heterologous proteins in transformed cells. In particular, the present disclosure provides improved methods of producing multi-subunit proteins, including antibodies and other multi-subunit proteins, which may or may not be secreted, with decreased production of undesired side-products and/or increased yield. In exemplary embodiments, the transformed cells are a yeast, such as *Pichia pastoris* or *Saccharomyces cerevisiae*.

BACKGROUND

[0004] Conventional antibodies are tetrameric proteins composed of two identical light chains and two identical heavy chains. Pure human antibodies of a specific type can be difficult or impossible to purify from natural sources in sufficient amounts for many purposes. As a consequence, biotechnology and pharmaceutical companies have turned to recombinant DNA-based methods to prepare antibodies on a large scale. The production of functional antibodies generally involves not just the synthesis of the two polypeptides but also a number of post-translational events, including proteolytic processing of the N-terminal secretion signal sequence; proper folding and assembly of the polypeptides into tetramers; formation of disulfide bonds; and typically includes a specific N-linked glycosylation. All of these events take place in the eukaryotic cell secretory pathway, an organelle complex unique to eukaryotic cells.

[0005] Recombinant synthesis of such complex proteins has typically relied on cultures of higher eukaryotic cells to produce biologically active material, with cultured mammalian cells being very commonly used. However, mammalian tissue culture-based production systems incur significant added expense and complication relative to microbial fermentation methods. Additionally, products derived from mammalian cell culture may require additional safety testing to ensure freedom from mammalian pathogens (including viruses) that might be present in the cultured cells or animal-derived products used in culture, such as serum.

[0006] Prior work has help to establish the yeast *Pichia pastoris* as a cost-effective platform for producing functional antibodies that are potentially suitable for research, diagnostic, and therapeutic use. See co-owned U.S. Patents 7,935,340 and 7,927,863, each of which is incorporated by reference herein in its entirety. Methods are also known in the literature for design and optimization of *P. pastoris* fermentations for expression of recombinant proteins, including optimization of the cell density, broth volume, substrate feed rate, and the length of each phase of the reaction. See Zhang et al., "Rational Design and Optimization of Fed-Batch and Continuous Fermentations" in Cregg, J. M., Ed., 2007, *Pichia Protocols* (2nd edition), Methods in Molecular Biology, vol. 389, Humana Press, Totowa, N.J., pgs. 43-63.

[0007] Though recombinant multi-subunit proteins can be produced from cultured cells, undesired side-products may also be produced. For example, the cultured cells may produce the

desired multi-subunit protein along with free monomers, complexes having incorrect stoichiometry, or proteins having undesired or aberrant glycosylation. Purification of the desired multi-subunit protein can increase production cost, and the steps involved in purification may decrease total yield of active complexes. Moreover, even after purification, undesired side-products may be present in amounts that cause concern. For example, glycosylated side-products may be present in amounts that increase the risk of an immune reaction after administration, while aberrant complexes or aggregates may decrease specific activity and may also be potentially immunogenic.

SUMMARY

[0008] Most IgG1 antibody molecules are stabilized by a total of 16 intra-chain and inter-chain disulfide bridges. The intra-chain disulfide bridges stabilize the folding of the IgG domains in both heavy and light chains, while the inter-chain disulfide bridges stabilize the association between heavy and light chains. As a result of these bonds, antibodies form a stable complex containing two heavy chains and two light chains (H2L2). However, due to improper disulfide bond formation, product-associated variants are sometimes found in recombinant antibody preparations, including a complex having one light and one heavy chain (H1L1) and a complex having two heavy chains and one light chain (H2L1). Further, higher order complexes may also form in which additional inter-chain disulfide bonds form, resulting in a greater number of covalently linked subunits.

[0009] As further described below, Applicants have now identified methods of decreasing the production of these complexes containing aberrant disulfide bonds during recombinant production of antibodies from yeast culture. Specifically, the method involves addition of an ethanol bolus to the culture, and resulted in decreased production of the H1L1, H2L1, and H4L4 products-associated variants, and increased purity of the desired H2L2 product. The H1L1 and H2L1 complexes were detected by non-reduced, denaturing SDS-PAGE, and the H4L4 complexes were detected by size exclusion chromatography. Using the subject methods, proper disulfide bond formation was facilitated, resulting in increased antibody purity. This was demonstrated for three different antibodies, all three of which exhibited improved purity when produced with the addition of the ethanol bolus (FIGS. 1-6). These three antibodies are not only are different in sequence but also recognize three different antigens. Moreover, when produced

in the absence of an ethanol bolus, two of the antibodies contained greater amounts of the H1L1 product (FIGS. 1-4), compared with the third antibody (FIG. 5). The two antibodies containing greater amounts of the H1L1 product have a non-canonical or additional disulfide bridge, whereas the third does not. The antibody exemplified in FIGS. 1, 2 and 3 has an additional intra-chain disulfide bridge in the variable light chain domain, while the antibody exemplified in FIG. 4, has an additional intra-chain disulfide bridge in its heavy chain. It has been reported in the literature that the presence of disulfide-bridges in overexpressed proteins increases intracellular stress in the host (see Gasser et al., *Biotechnology and Bioengineering*, Vol. 94, No. 2, pg. 353-61, June 5, 2006; Inan et al., *Biotechnology And Bioengineering*, Vol. 93, No. 4, pg. 771-78, March 5, 2006; Li et al., *Biochem Biophys Res Commun.* 2010 November 19; 402(3): 519-524). This increased stress can also lead to lower viability, as is demonstrated in FIGS. 11, 12, and 13 where both antibodies with the extra intra-chain disulfide bridge have lower viability under the “no-bolus” conditions. The addition of the ethanol bolus, therefore leads to increased viability and increased purity. This may be of use in particular when difficult to express proteins with multiple disulfide bridges are being expressed.

[0010] In one aspect, the present disclosure provides a method of producing a multi-subunit complex, comprising: (a) providing a culture comprising a eukaryotic cells comprising genes that provide for the expression of the subunits of said multi-subunit complex; (b) adding a bolus of ethanol to said culture; and (c) culturing said culture to produce said multi-subunit complex.

[0011] The ethanol bolus may enhance the formation of stable disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

[0012] Said multi-subunit complex may contain one or more polypeptides comprising at least one disulfide bond.

[0013] Said multi-subunit complex may comprise an antibody.

[0014] The method may decrease the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

[0015] The method may decrease the relative abundance of product-associated variants having a higher or lower apparent molecular weight than said desired multi-subunit complex as detected by size exclusion chromatography or gel electrophoresis relative to the same method effected in the absence of the bolus of ethanol.

[0016] The method may decrease the relative abundance of complexes having aberrant stoichiometry relative to the same method effected in the absence of the bolus of ethanol.

[0017] The method may decrease the relative abundance of complexes having aberrant disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

[0018] The method may decrease the relative abundance of complexes having reduced cysteines relative to the same method effected in the absence of the bolus of ethanol.

[0019] The method may decrease the relative abundance of complexes having aberrant glycosylation relative to the same method effected in the absence of the bolus of ethanol.

[0020] The method may modulate the formation or stability of inter-heavy chain disulfide bonds.

[0021] The method may modulate the formation or stability of disulfide bonds linking the light and heavy chains.

[0022] The method may decrease the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

[0023] Said product-associated variants may comprise one or more of the H1L1, H2L1, and H4L4 product-associate variants.

[0024] The method increase the purity of said antibody relative to said method effected in the absence of said bolus of ethanol.

[0025] Step (b) may be effected prior to step (c).

[0026] Step (b) may be effected subsequent to step (c).

[0027] Step (b) may be effected concurrently with step (c).

[0028] Step (b) may result in a concentration of ethanol in said culture of between about 0.01% and about 4% (w/v).

[0029] Step (b) may result in a concentration of ethanol in said culture of between about 0.01% and about 4%, between about 0.02% and about 3.75%, between about 0.04% and about 3.5%, between about 0.08% and about 3.25%, between about 0.1% and about 3%, between about 0.2% and about 2.75%, between about 0.3% and about 2.5%, between about 0.4% and about 2.25%, between about 0.5% and about 1.5%, between about 0.5% and about 2%, between about 0.6% and about 1.75%, between about 0.7% and about 1.5%, or between about 0.8% and about 1.25%.

[0030] Step (b) may result in a concentration of ethanol in said culture that may be at least about 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.2%, 0.3%, 0.4%, 0.6%, 0.6%, 0.7%, 0.8% or 0.9% (w/v).

[0031] Step (b) may result in a concentration of ethanol in said culture that may be at most about 4%, 3.5%, 3%, 2.5%, 2%, 1.8%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, or 0.15% (w/v).

[0032] Step (b) may comprise adding ethanol to said culture, adding a carrier comprising ethanol to said culture, adding said cells to a medium or carrier comprising ethanol, or replacing part of the culture medium.

[0033] Said bolus of ethanol may be added to the culture medium over a period of time between 1 and 20 minutes.

[0034] Step (c) may comprise providing oxygen to said cells.

[0035] Said providing oxygen may comprise agitating said culture.

[0036] Said providing oxygen may comprise contacting said culture with a gas mixture comprising oxygen.

[0037] Step (c) may comprise adding a feed comprising a carbon source to said culture.

[0038] Said feed may comprise at least one fermentable carbon source.

[0039] Said feed may comprise one or more of glucose, ethanol, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

[0040] The method may further comprise maintaining the concentration of ethanol between an upper set point and a lower set point during step (c).

[0041] Said lower set point may be about 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.2%, 0.3%, 0.4%, 0.6%, 0.6%, 0.7%, 0.8% or 0.9% (w/v).

[0042] Said upper set point may be about 4%, 3.5%, 3%, 2.5%, 2%, 1.8%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, or 0.15% (w/v).

[0043] Said upper set point may be at most about 1.5%, 1.4%, 1.3, 1.2%, or 1.1% (w/v).

[0044] The method may further comprise maintaining the concentration of ethanol at a set point during step (c).

[0045] Said set point may be about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 0.1%, 0.11%, 0.12%, 0.13%, 0.14%, or 0.15% (w/v).

[0046] Step (c) may comprise maintaining the concentration of ethanol in said culture between about 0.01% and about 4%, between about 0.02% and about 3.75%, between about 0.04% and about 3.5%, between about 0.08% and about 3.25%, between about 0.1% and about 3%, between about 0.2% and about 2.75%, between about 0.3% and about 2.5%, between about 0.4% and about 2.25%, between about 0.5% and about 2%, between about 0.6% and about 1.75%, between about 0.7% and about 1.5%, or between about 0.8% and about 1.25%.

[0047] The concentration of ethanol in said culture may be maintained by controlling production of ethanol by said cells or by addition of ethanol to said culture.

[0048] The step of controlling production of ethanol may comprise controlling one or more of the concentration of glucose, availability of oxygen, intensity of agitation, gas pressure, flow rate of supplied air or other gas mixture, viscosity of the culture, culture density, concentration of oxygen in the supplied air or other gas mixture, and temperature.

[0049] The time between step (a) and step (b) may be less than about 72 hours, less than about 48 hours, less than about 24 hours, less than about 12 hours, less than about 9 hours, less than about 6 hours, less than about 5 hours, less than about 4 hours, less than about 3 hours, less than about 90 minutes, less than about 30 minutes, less than about 5 minutes, or less than about 1 minute.

[0050] The time between step (b) and step (c) may be less than about 10 hours, less than about 9 hours, less than about 8 hours, less than about 7 hours, less than about 6 hours, less than about 5 hours, less than about 4 hours, less than about 3 hours, less than about 2 hours, less than about 90 minutes, less than about 80 minutes, less than about 70 minutes, less than about 60 minutes, less than about 50 minutes, less than about 40 minutes, less than about 30 minutes, less than about 20 minutes, less than about 10 minutes, less than about 5 minutes, or less than about 1 minute.

[0051] The culture of step (a) may be produced by adding a carbon source to said culture, and culturing said culture until the carbon source may be depleted.

[0052] Said carbon source may comprise one or more of: glycerol, glucose, ethanol, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

[0053] The depletion of the carbon source may be determined by detecting a decrease in the metabolic activity of said eukaryotic cells.

[0054] Said decrease in the metabolic activity of said eukaryotic cells may be identified by detecting a decrease in the consumption of oxygen by said eukaryotic cells, by detecting an increase in pH in the culture, by detecting stabilization of the wet cell mass, or by detecting an increase in the concentration of ammonia in the culture.

[0055] Said decrease in the consumption of oxygen by said eukaryotic cells may be identified by detecting an increase in the concentration of dissolved oxygen in said culture.

[0056] Said eukaryotic cells may comprise yeast cells.

[0057] Said yeast cells may comprise methylotrophic yeast.

[0058] Said methylotrophic yeast may be of the genus *Pichia*.

[0059] Said methylotrophic yeast of the genus *Pichia* may be *Pichia pastoris*.

[0060] Said methylotrophic yeast of the genus *Pichia* may be selected from the group consisting of: *Pichia angusta*, *Pichia guillermordii*, *Pichia methanolica*, and *Pichia inositovera*.

[0061] The genes that provide for expression of said multi-subunit complex may be integrated into one or more genomic loci.

[0062] At least one of said genomic loci may be selected from the group consisting of the pGAP locus, 3' AOX TT locus; PpURA5; OCH1; AOX1; HIS4; GAP; pGAP; 3' AOX TT; ARG; and the HIS4 TT locus.

[0063] At least one of the genes encoding said subunits of the multi-subunit complex may be expressed under control of an inducible or constitutive promoter.

[0064] Said inducible promoter may be selected from the group consisting of the AOX1, CUP1, tetracycline inducible, thiamine inducible, and FLD1 promoters.

[0065] At least one of the genes encoding said subunits of the multi-subunit complex may be expressed under control of a promoter selected from the group consisting of: the CUP1, AOX1, ICL1, glyceraldehyde-3-phosphate dehydrogenase (GAP), FLD1, ADH1, alcohol dehydrogenase II, GAL4, PHO3, PHO5, and Pyk promoters, tetracycline inducible promoters, thiamine inducible promoters, chimeric promoters derived therefrom, yeast promoters, mammalian promoters, insect promoters, plant promoters, reptile promoters, amphibian promoters, viral promoters, and avian promoters.

[0066] Said eukaryotic cell may be a diploid, tetraploid cell, or polyploid.

[0067] The method may further comprise purifying said multi-subunit complex from said eukaryotic cells or from the culture medium.

[0068] Said multi-subunit complex may be purified from an intracellular component, cytoplasm, nucleoplasm, or a membrane of said eukaryotic cells.

[0069] Said eukaryotic cells secrete said multi-subunit complex into the culture medium.

[0070] Said multi-subunit complex may be purified from said culture medium.

[0071] Said multi-subunit complex may comprise a monospecific or bispecific antibody.

[0072] Said multi-subunit complex may comprise a human antibody or a humanized antibody or fragment thereof.

[0073] Said humanized antibody may be of mouse, rat, rabbit, goat, sheep, or cow origin.

[0074] Said humanized antibody may be of rabbit origin.

[0075] Said multi-subunit complex may comprise a monovalent, bivalent, or multivalent antibody.

[0076] Said antibody may be purified from said culture by protein A and/or protein G affinity.

[0077] At least one of the genes that provide for expression of a subunit of said multi-subunit complex in at least one of said eukaryotic cells in said panel may be optimized for expression in said eukaryotic cell.

[0078] Said multi-subunit complex may comprise an antibody and the purity of said antibody may be assessed by measuring the fraction of the antibody produced by said eukaryotic cell that may be contained in antibody complexes having the expected apparent hydrodynamic radius, may be contained in antibody complexes having the expected molecular weight, and / or specifically binds a target of said antibody.

[0079] Said multi-subunit complex may comprise an antibody and the yield of said antibody may be assessed by determining the amount of antibody produced by said eukaryotic cell discounting any product-associated variants that may be abnormally glycosylated, contained in antibody complexes other than complexes having the expected apparent hydrodynamic radius, contained in antibody complexes having the expected molecular weight, and / or that fail to specifically bind to the target of said antibody.

[0080] The molecular weight of said antibody complexes may be determined by non-reducing SDS-PAGE.

[0081] Said multi-subunit complex may comprise an antibody, said method may further comprise purifying said antibody.

[0082] Said culture cell may produce a supernatant antibody titer of at least 100 mg / L, at least 150 mg / L, at least 200 mg / L, at least 250 mg / L, at least 300 mg / L, between 100 and 300 mg / L, between 100 and 500 mg / L, between 100 and 1000 mg / L, at least 1000 mg / L, at least 1250 mg/liter, at least 1500 mg/liter, at least about 1750 mg/liter, at least about 2000 mg/liter, at least about 10000 mg/liter, or more.

[0083] One or more subunits of said multi-subunit complex may be expressed from more than one gene copy.

[0084] Said multi-subunit complex may comprise an antibody which may be expressed from between 1-10 copies of a gene encoding the light chain of said antibody and from 1-10 copies of a gene encoding the heavy chain of said antibody.

[0085] The genes that provide for expression of said multi-subunit complex may be integrated into genome of said cells.

[0086] The genes that provide for expression of said multi-subunit complex may be contained on an extrachromosomal element, plasmid, or artificial chromosome.

[0087] Said cells may comprise more copies of the gene that provide for the expression of the light chain of said antibody than copies of the gene that provide for expression of the heavy chain of said antibody.

[0088] The respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells may be: 2 and 2, 2 and 3, 3 and 3, 3 and 4, 3 and 5, 4 and 3, 4 and 4, 4 and 5, 4 and 6, 5 and 4, 5 and 5, 5 and 6, or 5 and 7.

[0089] The respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells may be: 2 and 1, 3 and 1, 4 and 1, 5 and 1, 6 and 1, 7 and 1, 8 and 1, 9 and 1, 10 and 1, 1 and 2, 2 and 2, 3 and 2, 4 and 2, 5 and 2, 6 and 2, 7 and 2, 8 and 2, 9 and 2, 10 and 2, 1 and 3, 2 and 3, 3 and 3, 4 and 3, 5 and 3, 6 and 3, 7 and 3, 8 and 3, 9 and 3, 10 and 3, 1 and 4, 2 and 4, 3 and 4, 4 and 4, 5 and 4, 6 and 4, 7 and 4, 8 and 4, 9 and 4, 10 and 4, 1 and 5, 2 and 5, 3 and 5, 4 and 5, 5 and 5, 6 and 5, 7 and 5, 8 and 5, 9 and 5, 10 and 5, 1 and 6, 2 and 6, 3 and 6, 4 and 6, 5 and 6, 6 and 6, 7 and 6, 8 and 6, 9 and 6, 10 and 6, 1 and 7, 2 and 7, 3 and 7, 4 and 7, 5 and 7, 6 and 7, 7 and 7, 8 and 7, 9 and 7, 10 and 7, 1 and 8, 2 and 8, 3 and 8, 4 and 8, 5 and 8, 6 and 8, 7 and 8, 8 and 8, 9 and 8, 10 and 8, 1 and 9, 2 and 9, 3 and 9, 4 and 9, 5 and 9, 6 and 9, 7 and 9, 8 and 9, 9 and 9, 10 and 9, 1 and 10, 2 and 10, 3 and 10, 4 and 10, 5 and 10, 6 and 10, 7 and 10, 8 and 10, 9 and 10, 10 and 10.

[0090] The culture of step (c) may be grown in a production medium.

[0091] Said production medium may be a minimal medium.

[0092] Said minimal medium lacks selective agents.

[0093] Said minimal medium lacks pre-formed amino acids or other complex biomolecules.

[0094] The production medium may be a complex medium.

[0095] The complex medium may comprise one or more of yeast extract, soy peptones, and other plant peptones.

[0096] The culture of step (c) may be grown to a high cell density.

[0097] Said high cell density may be at least 50 g/L.

[0098] Said high cell density may be at least 100 g/L.

[0099] Said high cell density may be at least 300 g/L.

[0100] Said high cell density may be at least 400 g/L.

[0101] Said high cell density may be at least 500 g/L.

[0102] Said high cell density may be at least 750 g/L.

[0103] The yeast cells may be cultured for at least 20 doublings and maintain high levels of expression of said multi-subunit complex after said at least 20 doublings.

[0104] The cells of step (c) may be cultured for at least 50 doublings and maintain high levels of expression of said multi-subunit complex after said at least 50 doublings.

[0105] The cells of step (c) may be cultured for at least 100 doublings and maintain high levels of expression of said multi-subunit complex after said at least 100 doublings.

[0106] At least one subunit of said multi-subunit complex may comprise a secretion signal.

[0107] Said multi-subunit complex may comprise an antibody.

[0108] The secretion signal may comprise one or more polypeptides selected from the group consisting of: SEQ ID NOS: 414 to 437 and any combination thereof.

[0109] Said multi-subunit complex may be not any of the antibodies disclosed in U.S. Provisional Application No. 61/418,832, filed December 1, 2010, PCT/US11/62963, filed December 1, 2011, U.S. Ser. No. 13/309,295, filed December 1, 2011, U.S. Ser. No. 13/309,153, filed December 1, 2011, U.S. Ser. No. 13/308,665 filed on December 1, 2011, and U.S. Ser. No. 13/308,831, filed December 1, 2011.

[0110] Said multi-subunit complex may not be Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-

NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, and Ab21-NGF, or an Fab2 or Fab1 fragment thereof.

[00111] Said multi-subunit complex may not contain at least one, at least two, at least three, at least four, at least five, or at least all six of the complementarity determining regions (CDRs) contained in any of the following antibodies: Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, or Ab21-NGF and optionally having binding specificity for NGF.

[00112] Said multi-subunit complex may not comprise or consist of the light and heavy chain polypeptide sequences of SEQ ID NOs: 51 and 401, respectively, SEQ ID NOs: 53 and 402, respectively, SEQ ID NOs: 405 and 406, respectively, and SEQ ID NOs: 407 and 408, respectively.

[00113] Said multi-subunit complex may not comprise an antibody containing at least one, at least two, at least three, at least four, at least five, or at least all six of the CDRs of SEQ ID NOs: 55, 56, 57, 58, 59, and 60, and optionally having binding specificity for NGF.

[00114] Said multi-subunit complex may not comprise any of the antibodies or antibody coding sequences disclosed herein in the sections entitled “Anti-NGF Antibodies and Binding Fragments Thereof Having Binding Activity for NGF” and “Polynucleotides Encoding Anti-NGF Antibody Polypeptides.” In one aspect, the present disclosure provides methods of producing a multi-subunit complex, which may comprise: culturing a host cell providing a culture comprising eukaryotic cells that expresses said multi-subunit complex, adding a bolus of ethanol to said culture, and culturing said culture to produce said multi-subunit complex. The multi-subunit complex may comprise one or more disulfide bonds, and may be an antibody.

[00115] The ethanol bolus concentration (expressed as % w/v) may be between about 0.1% and about 5%, such as at least about 0.1%, at least about 0.2%, at least about 0.3%, at least about 0.4%, at least about 0.5%, at least about 0.6%, at least about 0.7%, at least about 0.8%, at least about 0.9%, at least about 1%, up to about 1%, up to about 1.1%, up to about 1.2%, up to about 1.3%, up to about 1.4%, up to about 1.5%, up to about 1.6%, up to about 1.7%, up to about

1.8%, up to about 1.9%, up to about 2%, up to about 3%, up to about 4%, or up to about 5%, such as between about 0.1% and about 1.9%, between about 0.2% and about 1.8%, between about 0.3% and about 1.7%, between about 0.4% and about 1.6%, between about 0.5% and about 1.5%, between about 0.6% and about 1.4%, between about 0.7% and about 1.3%, between about 0.8% and about 1.2%, or between about 0.9% and about 1.1%, such as about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about 2.5%, about 3%, about 4%, or about 5%.

[00116] The method may further comprise purification of said desired multi-subunit complex.

[00117] In exemplary embodiments the ethanol concentration may be controlled subsequent to addition of the ethanol bolus, which may be used to maintain the ethanol concentration at a desired set point or within a desired set point range. The set point (expressed as % w/v) may be between about 0.1% and about 4%, at least about 0.01%, at least about 0.02%, at least about 0.04%, at least about 0.06%, at least about 0.08%, at least about 0.1%, at least about 0.15%, at least about 0.2%, at least about 0.25%, at least about 0.3%, at least about 0.35%, at least about 0.4%, at least about 0.45%, at least about 0.5%, at least about 0.6%, at least about 0.7%, at least about 0.8%, at least about 0.9%, at least about 1%, at least about 1.2%, at least about 1.4%, at least about 1.6%, at least about 1.8%, at least about 2%, up to about 4%, up to about 3.75%, up to about 3.5%, up to about 3.25%, up to about 3%, up to about 2.75%, up to about 2.5%, up to about 2.25%, up to about 2%, up to about 1.75%, up to about 1.5%, up to about 1.25%, up to about 1%, between about 0.01% and about 4%, between about 0.02% and about 3.75%, between about 0.04% and about 3.5%, between about 0.08% and about 3.25%, between about 0.1% and about 3%, between about 0.2% and about 2.75%, between about 0.3% and about 2.5%, between about 0.4% and about 2.25%, between about 0.5% and about 2%, between about 0.6% and about 1.75%, between about 0.7% and about 1.5%, or between about 0.8% and about 1.25%. For example, the set point may be the same as the bolus concentration or within plus or minus 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90% or 100% of the bolus concentration.

[00118] The ethanol concentration set point may be maintained by controlling ethanol production by yeast cells during fermentation. For example, the ethanol concentration may be increased by increasing the concentration of glucose (e.g., increasing the rate of glucose feed), decreasing the availability of oxygen, by decreasing the intensity of agitation (e.g., lowering the fermenter input power), decreasing the gas pressure in the fermenter, decreasing the flow rate of supplied air or other gas mixture, increasing the viscosity of the culture, or decreasing the concentration of oxygen in the supplied air or other gas mixture (e.g., if oxygen supplementation is being used). Ethanol production may also be increased by increasing the fermentation temperature. Likewise, the ethanol concentration may be decreased by decreasing the glucose concentration (e.g., decreasing the rate of glucose feed), decreased by increasing the availability of oxygen, by increasing the intensity of agitation (e.g., increasing the fermenter input power), increasing the gas pressure in the fermenter, increasing the flow rate of supplied air or other gas mixture, decreasing the viscosity of the culture, or increasing the concentration of oxygen in the supplied air or other gas mixture (e.g., if oxygen supplementation is being used). Ethanol production may also be decreased by decreasing the fermentation temperature.

[00119] Using the methods of the present disclosure, the relative abundance of undesired side-product(s) may be decreased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or down to undetectable levels compared to initial abundance levels, relative to conventional methods. Exemplary undesired side-products whose relative abundance may be so decreased may include one or more species having a different apparent molecular weight than the desired multi-subunit complex. For example, apparent molecular weight may be affected by differences in stoichiometry, folding, complex assembly, and/or glycosylation. For example, such undesired side products may be detected using size exclusion chromatography and/or gel electrophoresis, and may have a higher or lower apparent molecular weight than the desired multi-subunit complex. In exemplary embodiments, the undesired side-products may be detected under reducing conditions. In other exemplary embodiments, the undesired side-products may be detected under non-reducing conditions.

[00120] In exemplary embodiments, the present disclosure also provides improved methods and compositions of matter that provide for the recombinant production of antibodies

and other multi-subunit complexes, with a higher yield. In exemplary embodiments, the yield may be increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 100%, or more (relative to conventional methods) using the methods disclosed herein.

[00121] In exemplary embodiments, the host cell in which the multi-subunit proteins may be produced may be a yeast, for example in a *Pichia* species such as *P. pastoris* or another methylotrophic yeast, or in a *Saccharomyces* species such as *S. cerevisiae*, or another yeast such as a *Schizosaccharomyces* (e.g., *S. pombe*). Other examples of methylotrophic yeast which may be utilized in the present invention include *Pichia angusta* (also known in the art as *Hansenula polymorpha*), *Pichia guillermordii*, *Pichia methanolica*, *Pichia inositovera*, *Ogataea nitratoaversa*, and *Candida boidinii*.

[00122] The host cell may be a eukaryotic cell, such as a yeast cell, such as a methylotrophic yeast, such as a yeast of the genus *Pichia*. Exemplary methylotrophic yeasts of the genus *Pichia* include *Pichia pastoris*, *Pichia angusta*, *Pichia guillermordii*, *Pichia methanolica*, and *Pichia inositovera*. The host cell may be produced by mating, e.g., by mating two haploid yeast cells that each contain one or more copies of at least one gene encoding a subunit of the multi-subunit complex.

[00123] In a preferred embodiment, the methylotrophic yeasts of the genus *Pichia* is *Pichia pastoris*. The host cell may be a diploid or tetraploid cell.

[00124] At least one of said genes encoding said subunits of the desired multi-subunit complex, such as said desired antibody light chain and / or heavy chain, may be expressed under control of an inducible or constitutive promoter, such as CUP1 (induced by the level of copper in the medium; see Koller et al., Yeast 2000; 16: 651-656.), tetracycline inducible promoters (see, e.g., Staib et al., Antimicrobial Agents And Chemotherapy, Jan. 2008, p. 146–156), thiamine inducible promoters, AOX1, ICL1, glyceraldehyde-3-phosphate dehydrogenase (GAP), FLD1, ADH1, alcohol dehydrogenase II, GAL4, PHO3, PHO5, and Pyk promoters, chimeric promoters derived therefrom, yeast promoters, mammalian promoters, insect promoters, plant promoters, reptile promoters, amphibian promoters, viral promoters, and avian promoters.

[00125] The host cell may secrete said desired multi-subunit complex into the culture medium. Alternatively or in addition, said desired multi-subunit complex may be retained in said host cell and may be isolated therefrom.

[00126] The desired multi-subunit complex may comprise an antibody, such as a monospecific or bispecific antibody. The antibody may be an antibody that specifically binds any antigen.

[00127] The desired multi-subunit complex may be an antibody other than any of the antibodies (e.g., an antibody other than any of the anti-NGF antibodies) disclosed in U.S. Provisional Application No. 61/418,832, filed December 1, 2010, PCT/US11/62963, filed December 1, 2011, U.S. Ser. No. 13/309,295, filed December 1, 2011, U.S. Ser. No. 13/309,153, filed December 1, 2011, U.S. Ser. No. 13/308,665 filed on December 1, 2011, and U.S. Ser. No. 13/308,831, filed December 1, 2011. In an exemplary embodiment, the desired multi-subunit complex may not be any of the following antibodies: Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, and Ab21-NGF. In a further exemplary embodiment, the desired multi-subunit complex may not be an Fab2 fragment of any of the following antibodies: Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, and Ab21-NGF. In a further exemplary embodiment, the desired multi-subunit complex may not be an Fab1 fragment of any of the following antibodies: Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, and Ab21-NGF. In a further exemplary embodiment, the desired multi-subunit complex may not comprise an antibody containing at least one, at least two, at least three, at least four, at least five, or at least all six of the complementarity determining regions (CDRs) contained in any of the following antibodies: Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, or Ab21-NGF and optionally having binding

specificity for NGF. For example, the desired multi-subunit complex may not comprise or may not consist of the light and heavy chain polypeptide sequences SEQ ID NOs: 51 and 401, respectively, and/or SEQ ID NOs: 53 and 402, respectively, and/or SEQ ID NOs: 405 and 406, respectively, and/or SEQ ID NOs: 407 and 408, respectively. As a further example, the desired multi-subunit complex may not comprise an antibody containing at least one, at least two, at least three, at least four, at least five, or at least all six of the CDRs of SEQ ID NOs: 55, 56, 57, 58, 59, and 60, and optionally having binding specificity for NGF.

[00128] The desired multi-subunit complex may comprise an antibody of any type. Exemplary antibody types include antibodies of any mammalian species, e.g., human, mouse, rat, rabbit, goat, sheep, cow, etc. Preferably, the antibody is a human antibody or a humanized antibody that may be of rabbit origin. The desired antibody may be a monovalent, bivalent, or multivalent antibody.

[00129] At least one of said genes that provide for expression of a subunit of the desired multi-subunit complex, such as the light chain and/or heavy chain of a desired antibody, in at least one of said host cells in said panel may be optimized for expression in said host cell (e.g., by selecting preferred codons and/or altering the percentage AT through codon selection).

[00130] The purity of said desired multi-subunit complex, such as a desired antibody, may be assessed by measuring the fraction of the desired multi-subunit complex produced by said host cell that is non-glycosylated, is contained in complexes having the expected apparent hydrodynamic radius and/or apparent molecular weight (e.g., measured by size exclusion chromatography), has the expected electrophoretic mobility (e.g., detected by gel electrophoresis, such as SDS-PAGE, and optionally Western blotting), and / or by measuring the specific activity of the multi-subunit complex (e.g., specific binding a target of a desired antibody).

[00131] The desired multi-subunit complex may be an antibody, and yield of said antibody may be assessed by determining the amount of desired antibody produced by said host cell discounting any product-associated variants that are glycosylated, contained in antibody complexes other than complexes having the expected apparent molecular weight or hydrodynamic radius, and / or that fail to specifically bind to the target of said desired antibody.

[00132] The subject methods may produce a supernatant antibody titer of at least 100 mg / L, at least 150 mg / L, at least 200 mg / L, at least 250 mg / L, at least 300 mg / L, between 100 and 300 mg / L, between 100 and 500 mg / L, between 100 and 1000 mg / L or in excess of 1000 mg/L e.g., as high as 1200 mg/L, as high as 10,000 mg / L, or higher.

[00133] In another aspect, the host cell that produces a desired multi-subunit complex may be a diploid or tetraploid cell of the genus *Pichia*, such as a *Pichia pastoris* cell. The genes that provide for expression of the subunits of said desired multi-subunit complex, such as the light chain and heavy chain of a desired antibody, may be integrated into genome of said host cell, and/or may be contained on an extrachromosomal element, plasmid, or artificial chromosome.

[00134] In another aspect, the host cell that produces a desired multi-subunit complex may be engineered to increase yield and / or purity for example as further described in U.S. Provisional Application Ser. No. 61/525,307 (Atty. Docket No. 67858.730200), filed August 31, 2011, which is incorporated by reference herein in its entirety. As described therein, yield and purity of an antibody or other multi-subunit complex can be greatly improved by altering the number of copies per cell of the genes encoding each subunit. For example, where the desired multi-subunit complex is an antibody, the host cell may comprise more copies of the gene that provide for the expression of the light chain than copies of the gene that provide for expression of the heavy chain. In exemplary embodiments, the host cell may comprise from 1-10 copies of a gene encoding the light chain and from 1-10 copies of a gene encoding the heavy chain. The respective number of copies of the gene encoding the heavy chain and the number of copies of the gene encoding the light chain in said host cell may be: 2 and 2, 2 and 3, 3 and 3, 3 and 4, 3 and 5, 4 and 3, 4 and 4, 4 and 5, 4 and 6, 5 and 4, 5 and 5, 5 and 6, or 5 and 7, respectively. Additional exemplary combinations of heavy and light chain gene copy numbers include any combination of up to ten copies of the heavy and/or light chain gene, such as H2xL1, H3xL1, H4xL1, H5xL1, H6xL1, H7xL1, H8xL1, H9xL1, H10xL1, H1xL2, H2xL2, H3xL2, H4xL2, H5xL2, H6xL2, H7xL2, H8xL2, H9xL2, H10xL2, H1xL3, H2xL3, H3xL3, H4xL3, H5xL3, H6xL3, H7xL3, H8xL3, H9xL3, H10xL3, H1xL4, H2xL4, H3xL4, H4xL4, H5xL4, H6xL4, H7xL4, H8xL4, H9xL4, H10xL4, H1xL5, H2xL5, H3xL5, H4xL5, H5xL5, H6xL5, H7xL5, H8xL5, H9xL5, H10xL5, H1xL6, H2xL6, H3xL6, H4xL6, H5xL6, H6xL6, H7xL6, H8xL6, H9xL6, H10xL6, H1xL7, H2xL7, H3xL7, H4xL7, H5xL7, H6xL7, H7xL7, H8xL7, H9xL7,

H10xL7, H1xL8, H2xL8, H3xL8, H4xL8, H5xL8, H6xL8, H7xL8, H8xL8, H9xL8, H10xL8, H1xL9, H2xL9, H3xL9, H4xL9, H5xL9, H6xL9, H7xL9, H8xL9, H9xL9, H10xL9, H1xL10, H2xL10, H3xL10, H4xL10, H5xL10, H6xL10, H7xL10, H8xL10, H9xL10, H10xL10, where the number following the “H” identifies the number of copies of the heavy chain gene, and the number following the “L” identifies the number of copies of the light chain gene. For example, the specified number of heavy and light chain gene copies may be tandemly integrated into a single locus, or into multiple loci (any or all of which may contain more than one copy). Optionally, each genomic locus may contain no more than three or four tandemly integrated gene copies, thereby promoting copy number stability during propagation and/or antibody production.

[00135] Culturing most typically involves providing cells with an energy source, oxygen, and nutrients. Methods are also known in the literature for design and optimization of *P. pastoris* fermentations for expression of recombinant proteins, including optimization of the cell density, broth volume, substrate feed rate, and the length of each phase of the reaction. See Zhang et al., “Rational Design and Optimization of Fed-Batch and Continuous Fermentations” in Cregg, J. M., Ed., 2007, *Pichia Protocols* (2nd edition), Methods in Molecular Biology, vol. 389, Humana Press, Totowa, N.J., pgs. 43-63. The culture may be provided with a gas mixture comprising oxygen, such as air with or without oxygen supplementation. The yeast culture may be cultured in a culture medium which may be a minimal medium, may lack selective agents, and / or may lack pre-formed amino acids or other complex biomolecules. The culture medium may also be a complex medium (e.g., containing yeast extract and/or plant peptone(s)). The medium may include a nitrogen source (e.g., methylamine chloride, NH_4SO_4 , yeast extract, soy peptone, other plant peptones, etc.). Exemplary minimal media include minimal dextrose medium (MD) (1.34% yeast nitrogen base (YNB) (w/o amino acids), $4 \times 10^{-5}\%$ biotin, and 2% glucose.), buffered minimal glycerol complex medium (BMGY) (1% yeast extract, 2% peptone, 1% glycerol, 1.34% YNB (w/o amino acids), $4 \times 10^{-5}\%$ biotin and 100 mM potassium phosphate (pH 6.0)). Media may include one or more salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as potassium phosphate, Tris, or HEPES), nucleosides (such as adenosine and thymidine), antibiotics (e.g., added to inhibit growth of contaminants and/or for maintenance of a selectable marker), trace elements, and glucose or another energy source. Any supplements and substitutions may also be included at appropriate concentrations that would be known to those skilled in the art.

[00136] The culture may be grown to a high cell density, such as at least 50 g/L, at least 100 g/L, at least 300 g/L, at least 400 g/L, at least 500 g/L, or at least 700 g/L. These culture densities are illustrative rather than limiting, and suitable culture densities may be readily determined by those of ordinary skill in the art.

[00137] The yeast cells may be cultured for at least 20 doublings and maintain high levels of expression of said antibody after said at least 20 doublings.

[00138] The yeast cells may be cultured for at least 50 doublings and maintain high levels of expression of said antibody after said at least 50 doublings.

[00139] The yeast cells may be cultured for at least 100 doublings and maintain high levels of expression of said antibody after said at least 100 doublings.

[00140] In another aspect, the present disclosure provides a culture medium containing a stable diploid *Pichia* yeast culture produced according to any of the foregoing methods, wherein the culture medium may comprise expression levels of said desired antibody which may be at least about 50 mg/liter, 100 mg/liter, 500 mg/liter, 750 mg/liter, 1000 mg/liter, 1250 mg/liter, 1500 mg/liter, 1750 mg/liter, 2000 mg/liter, or more. These yield values are illustrative rather than limiting. Optionally, yield may be optimized, for example using the methods and general approach described in Zhang et al. (2007), *supra*. For example, yield may be optimized by varying temperature, pH, media composition (e.g., carbon source, carbon source concentration, mixture of two or more carbon sources, nitrogen source and concentration, concentration of salts and nutrients including KH_2PO_4 , K_2HPO_4 , MgSO_4 , potassium sulfate, sodium citrate, potassium sulfate, sodium citrate, trace metals such as cobalt chloride, cupric sulfate, sodium iodide, manganese sulfate, sodium molybdate, boric acid, zinc chloride, ferrous sulfate, vitamins such as biotin, inositol, thiamine, peptone, yeast extract, casamino acids, urea, ammonium phosphate or other ammonium ions, L-arginine-hydrochloride), time, culture density, oxygenation, and other factors that influence yield. For example, yield, expression, and/or purity of the desired multi-subunit complex may in some instances be improved by maintaining the temperature at a desired set point, e.g., a set point between about 15°C and about 30 °C, such as between about 17 °C and about 25 °C). Without intent to be limited by theory, it is hypothesized that controlling the temperature may assist intracellular trafficking through the folding and post-translational

processing pathways, and/or may decrease the activity of cellular proteases. Likewise, yield, expression, and/or purity of the desired multi-subunit complex may in some instances be improved by maintaining the pH of the culture medium at a desired set point, e.g., a set point between pH 3 to pH 8, such as between pH 4 and pH 7.

[00141] In another aspect, the present disclosure provides a culture medium containing a stable diploid *Pichia pastoris* yeast culture produced according to any of the foregoing methods that expresses said desired antibody into a culture medium wherein the cell density of said diploid cells in said culture may be at least about 50 g/L, 100 g/L, 300 g/L, 400 g/L, 500 g/L, 700 g/L or more. These culture densities are illustrative rather than limiting, and suitable culture densities may be readily determined by those of ordinary skill in the art.

[00142] At least one subunit of said antibody or other multi-subunit protein may comprise a secretion signal, such as the *S. chicken* lysozyme (CLY) signal peptide; CLY-L8; *S. cerevisiae* invertase (SUC2) signal peptide; MF-alpha (Prepro); MF-alpha (Pre)-apv; MF-alpha (Pre)-apv-SLEKR; MF-alpha (Prepro)-(EA)3; α F signal peptide; KILM1 signal peptide; repressible acid phosphatase (PHO1) signal peptide; *A. niger* GOX signal peptide; *Schwanniomyces occidentalis* glucoamylase gene (GAM1) signal peptide; human serum albumin (HSA) signal peptide without pro-sequence; human serum albumin (HSA) signal peptide with pro-sequence; ISN signal peptide; IFN signal peptide; HGH signal peptide; phytohaemagglutinin (PHA); Silkworm lysozyme; Human lysozyme (LYZ1); activin receptor type-1; activin type II receptor; *P. pastoris* immunoglobulin binding protein (PpBiP); human antibody 3D6 light chain leader; and any combination thereof.

[00143] The host cell may be produced by mating two haploid yeast cells that each contain one or more copies of a gene encoding one or more subunits of said antibody or other multi-subunit protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[00144] **FIG. 1A-B.** Purity of a recombinantly produced Ab-A was improved by a bolus addition of ethanol prior to the start of a glucose feed in yeast cultures from which the antibodies were produced. Antibodies were harvested after 97 hours of culture and purified by protein-A affinity, then purity was assessed by SDS-PAGE using a non-reduced gel (**FIG. 1A**) to resolve

the desired full antibody (arrow, “Full Ab (H2L2)”) from undesired product-associated variants. Complexes having aberrant stoichiometry were identified based on their molecular weight, affinity for protein A, and additional studies further described below, as a “half antibody” species containing one heavy and one light chain (arrow, “H1L1”) and a complex containing two heavy chains and one light chain (“H2L1”). The relative abundance of the H2L1 and H1L1 complexes was greatly decreased by the bolus addition of ethanol during antibody production. Compare **FIG. 1A**, lanes 2-3 (no bolus) to lane 5 (with bolus). **FIG. 1B** shows the same samples processed under reducing conditions, which separated each of the full antibody, H1L1, and H2L1 complexes into individual heavy and light chains, confirming that the H1L1 and H2L1 complexes are composed of full-length heavy and light chains. Lane order in **FIGS. 1A-B**: Lane 1: molecular weight marker; lanes 2 and 3: control samples prepared from fermentation cultures without a bolus addition of ethanol; lane 4: no sample; lane 5: sample prepared from fermentation cultures with a bolus addition of ethanol.

[00145] **FIGS. 1C-E** show the gel band density plotted along the length of the non-reduced gel (**FIG. 1A**, lanes 2, 3, and 5, respectively); arrows identify the peaks corresponding to the H1L1 species. **FIG. 1F** tabulates the area contained in the H1L1 peaks shown in **FIGS. 1C-E**, demonstrating approximately 90% reduction in the relative abundance of the H1L1 complexes. H2L1 complex abundance was not quantified due to the incomplete resolution from the full antibody peaks.

[00146] **FIGS. 2A-B** and **3A-B** demonstrate reproducibility of the improvement in purity of Ab-A by a bolus addition of ethanol to the yeast cultures. Antibodies were harvested after 87 or 86 hours of culture (**FIGS. 2** and **3**, respectively) and purified by protein-A affinity, then purity was assessed by SDS-PAGE using a non-reduced gel. The abundance of the H1L1 and H2L1 complexes (arrows) were again decreased by the bolus addition of ethanol. Compare **FIG. 2A**, lane 3 (no bolus) to lane 2 (with bolus), and **FIG. 3A**, lanes 4-6 (no bolus) with lanes 2 and 4 (with bolus). **FIG. 2B** shows the same samples as in **FIG. 2A** processed under reducing conditions, again confirming that the observed product-associated variants are composed of full-length heavy and light chains. Lane order in **FIGS. 2A-B**: Lane 1: molecular weight marker; lane 2: sample prepared from a fermentation culture with a bolus addition of ethanol; lane 3: control sample prepared from a fermentation culture with no bolus addition of ethanol. Lane

order in **FIG. 3A**: Lane 1: molecular weight marker; lanes 2 and 4: samples prepared from fermentation cultures with a bolus addition of ethanol; lane 3: no sample; lanes 5-7: control samples prepared from fermentation cultures with no bolus addition of ethanol.

[00147] **FIGS. 2C and 2D** show the gel band density plotted along the length of the non-reduced gel (**FIG. 2A**, lanes 2, and 3, respectively); arrows identify the peaks corresponding to the H1L1 species. **FIGS. 2E and 3B** tabulate the area contained in the H1L1 peaks shown in **FIGS. 2C and FIG. 3A**, demonstrating approximately 85% reduction in the relative abundance of H1L1 complexes in **FIG. 2A** and approximately 87% average reduction in the relative abundance of H1L1 complexes in **FIG. 3A**.

[00148] **FIG. 4A-D**. Purity of a second recombinant antibody ("Ab-B") was also improved by a bolus addition of ethanol prior to the production phase of a fermentation process. Samples of fermentation culture broth were harvested after 67 hours ("T67") or 87 hours ("T87") of culture (**FIGS. 4A-B and 4C-D**, respectively) and antibodies were purified by protein-A affinity. Purity was then assessed by SDS-PAGE using non-reduced gels (**FIGS. 4A and 4C**). At both assessed time points, the abundance of the half-antibody species (H1L1) and the H2L1 complex was greatly decreased in fermentation cultures prepared that received a bolus addition of ethanol, relative to control cultures that did not receive a bolus addition of ethanol. Compare **FIG. 4A**, lanes 2-3 (no bolus) to lanes 6-7 (with bolus), and **FIG. 4C**, lanes 2-3 (no bolus) to lanes 6-7 (with bolus). **FIGS. 4B and 4D** shows the same samples processed under reducing conditions. Lane order in **FIGS. 4A-D**: Lane 1: molecular weight marker; lanes 2-3: control sample prepared from fermentation cultures with no bolus addition of ethanol; lanes 4-5: no sample; lanes 6-7: samples prepared from fermentation cultures with a bolus addition of ethanol.

[00149] **FIGS. 4E and 4F** tabulate the area contained in the H1L1 peaks shown in **FIGS. 4A (T67) and 4C (T87)**, respectively, demonstrating that the bolus addition of ethanol produced about a 73% reduction in the relative abundance of H1L1 complexes at the earlier time point shown **FIG. 4A** and about a 34% average reduction in the relative abundance of H1L1 complexes at the later time point shown in **FIG. 4C**.

[00150] **FIG. 5A-B**. Purity of a third recombinant antibody (Ab-C) was also improved by a bolus addition of ethanol prior to the production phase of fermentation. Antibodies were

harvested after 86 hours of culture and purified by protein-A affinity, then purity was assessed by SDS-PAGE using a non-reduced gel (FIGS. 5A). The H1L1 and H2L1 complexes were less abundant in the Ab-C product even without the addition of a bolus of ethanol, leaving less room for improvement. Nonetheless, the abundance of the half-antibody species (H1L1) and the H2L1 complex was noticeably decreased in fermentation cultures that received a bolus addition of ethanol, relative to control cultures that did not receive a bolus addition of ethanol. Compare FIG. 5A, lanes 5-6 (no bolus) to lane 3 (with bolus). FIG. 5B shows the same samples processed under reducing conditions. Lane order in FIGS. 5A-B: Lane 1: molecular weight marker; lane 2: no sample; lane 3: sample prepared from fermentation cultures that received a bolus addition of ethanol; lane 4: no sample; lanes 5-6: control sample prepared from fermentation cultures that did not receive a bolus addition of ethanol.

[00151] FIG. 5C tabulates the area contained in the H1L1 peaks shown in FIGS. 5A, demonstrating about a 61% average reduction in the relative abundance of H1L1 complexes by the bolus addition of ethanol.

[00152] FIG. 6A-F shows assessment of the relative purity of the Ab-A preparations shown in FIGS. 1-3 by size exclusion chromatography. In each panel, the main peak contains the full antibody containing two heavy and two light chains (H2L2). The H1L1 species was not resolved from the main peak by this method (thought to be due H1L1 dimers forming by non-covalent association which is retained under the conditions used). However, other undesired product-associated variants were detected, including higher molecular weight species (left of main peak) and lower molecular weight species (right of main peak). A prominent peak though to correspond to antibody dimers containing two full antibodies (H4L4) was detected (arrow) and the relative abundance of these was decreased in samples prepared from fermentation cultures that received a bolus addition of ethanol. Compare FIGS. 6A, 6C, and 6E (no bolus) to FIGS. 6B, 6D, and 6F (with bolus).

[00153] FIG. 7 summarizes quantitation of the amount of product-associated variants detected by SEC for the six Ab-A samples shown in FIG. 6 and five additional samples. For each identified sample (col. 1), the run set number (col. 2, identifying fermentation runs that were conducted in parallel), bolus added (either 10g/L or none, col. 3), and elapsed culture time

before culture samples were taken and processed (col. 4) are shown, together with the fraction of protein detected in the main peak ("SEC Main Peak %," col. 5). The bolus addition of ethanol at the end of the growth phase increased the average percentage contained in the main peak, from 80.3% up to 90.6%.

[00154] FIG. 8 summarizes quantitation of the amount of product-associated variants detected by SEC for Ab-B antibody samples shown in FIG. 4. For each fermentation run (col. 1), the bolus added at the end of the growth phase (either 10g/L or none, col. 2), and elapsed culture time before culture samples were taken and processed (col. 3) are shown, together with the fraction of protein detected in the main peak ("SEC Main Peak %," col. 4). Overall purity was increased, with the main peak increasing from 76% to 79% at T67 and from 60% to 73% at T87.

[00155] FIG. 9 summarizes quantitation of the amount of product-associated variants detected by SEC for the Ab-C antibody samples shown in FIG. 5. For each identified fermentation run (col. 1), the bolus added (either 10g/L or none, col. 2), and elapsed culture time before the sample was taken and processed (col. 3) are shown, together with the fraction of protein detected in the main peak ("SEC Main Peak %," col. 4). There was little difference in overall purity as detected by this method, with about 89% of product contained in main peak with or without the ethanol bolus. This was apparently due to the high initial purity of the Ab-C antibody even without the bolus addition. Additionally, SEC did not resolve the H1L1 species from the full antibody and accordingly the decreased production of this species due to the ethanol bolus was not reflected in SEC results.

[00156] FIG. 10 summarizes the results of mass spectrometry measurement of the quantity of a free heavy chain (lacking a disulfide bond to a second heavy chain) in Ab-A antibody samples containing high or low amounts of the H1L1 band. As expected, the amount of free heavy chain correlated with the amount of the H1L1 band, confirming the identity thereof as containing one heavy and one light chain and lacking a disulfide bond to a second heavy chain.

[00157] FIGS. 11-13 show the correlation between addition of an ethanol bolus and cell viability. The addition of an ethanol bolus generally improved cell viability and antibody purity for the Ab-A antibody (FIG. 11) and the Ab-B antibody (FIG. 12). These results suggest that the

improvement in cell viability may account for at least part of the improvement in antibody purity from the ethanol bolus addition. Consistent with these results, the Ab-C antibody culture exhibited greater antibody purity and cell viability than the Ab-A and Ab-B cultures (FIG. 13). Apparently because the viability of the Ab-C antibody-producing cultures was already high in these experiments, there was little room for improvement and the cultures exhibited little improvement in viability from the bolus addition of ethanol. In FIGS. 11-13, filled bars indicate no bolus, while open bars indicate a bolus addition of ethanol. Viability was determined from fermentation cultures sampled with 1.5 hours of the time at which samples were collected for purity analyses (as identified in the preceding slides).

[00158] FIG. 14 shows that a wide range of ethanol bolus concentrations can produce the same improvement in antibody purity. Ab-A was produced with a bolus addition of ethanol between 5 g/L (0.5% w/v) and 15 g/L (1.5% w/v) and purified by protein A affinity, then the purity was analyzed by non-reduced SDS-PAGE. Each culture exhibited similarly low levels of the H2L1 and H1L1 complexes at 63 hours (FIG. 14A) and 86 hours (FIG. 14B). Lane order in FIGS. 14A-B: lane 1: molecular weight markers; lanes 2 and 7: 5 g/L bolus; lanes 3 and 5: 10 g/L bolus; lanes 4 and 6: 15 g/L bolus.

[00159] FIG. 15 shows that the time elapsed between the dissolved oxygen spike and the ethanol bolus addition can vary considerably while giving similar improvement in antibody purity. Ab-A was produced with a bolus addition of ethanol of 10 g/L (1% w/v) and purified by protein A affinity, then the purity was analyzed by non-reduced SDS-PAGE (FIG. 15A). The “starvation period,” the time between the dissolved oxygen spike (indicating exhaustion of the carbon source in the culture) and the bolus addition of ethanol, was varied between 0 and 3 hours. Each culture exhibited similarly low levels of the H2L1 and H1L1 complexes irrespective of the duration of the starvation period, indicating that antibody purity is relatively insensitive to absence of a starvation period or a starvation period of at least up to three hours. The same samples were analyzed on a reduced gel (FIG. 15B). Lane order in FIGS. 15A-B: lane 1: molecular weight markers; lanes 2-4: no sample; lane 5: 0 hours starvation period; lane 6: 3 hours starvation period.

[00160] FIG. 16 shows the effect of the equilibration period (the time between ethanol bolus addition and feed start) on antibody purity. The Ab-B antibody was produced with a bolus addition of ethanol of 10 g/L (1% w/v) and purified by protein A affinity, then the purity was analyzed by non-reduced SDS-PAGE (FIG. 16A). The duration of the equilibration period was either 0, 30, or 60 minutes. The 60 minute equilibration period resulted in a lower antibody purity (higher abundance of the H2L1 and H1L1 complexes). Culture viability was also markedly lower with a 60 minute equilibration period, particularly earlier in the culture (at 23 hours, FIG. 16B); viability had improved somewhat by the end of the culture (at 85 hours, FIG. 16C). Lane order in FIG. 16A: lane 1: molecular weight markers; lanes 2 and 4: no sample; lane 3: 30 minutes equilibration; lanes 5 and 6: 60 minutes equilibration time; lanes 7 and 8: 0 minutes equilibration time. In FIGS. 16B-C, filled bars indicate an equilibration period of zero minutes, hatched bars indicate an equilibration period of 30 minutes and open bars indicate an equilibration period of 60 minutes.

DETAILED DESCRIPTION

[00161] Applicants have unexpectedly discovered that purity of multi-subunit complexes expressed from yeast can be greatly improved by addition of a bolus of ethanol to the culture media. A single bolus addition of ethanol was demonstrated to improve purity over a sustained period of production, for up to at least 97 hours.

[00162] The present disclosure provides improved methods and compositions of matter that provide for the recombinant production of antibodies and other multi-subunit complexes with increased purity and decreased production of one or more undesired side-products. In exemplary embodiments, relative to the desired multi-subunit complex the undesired side product(s) may exhibit one or more of: altered stoichiometry, aberrant glycosylation, differences in apparent molecular weight, differences in disulfide bonds, differences in hydrodynamic radius, fragments and/or truncated forms of one or more subunits. Undesired side-products may exhibit one or more additional differences as well. Undesired side-products may also be detected by their effects on a preparation, e.g., alteration in the level of specific activity, immunogenicity, or other effects on physical constitution and/or function of the desired multi-subunit complex.

[00163] For example, when the desired multi-subunit complex is an antibody, the undesired side products may include an H1L1 or “half antibody” species (i.e., containing a heavy chain and a light chain, wherein the heavy chain is not linked by a disulfide bond to another heavy chain), and/or a H2L1 species (i.e., containing two heavy chains and one light chain, but lacking a second light chain).

[00164] Though not intending to be limited by theory, it is hypothesized that a rapid increase in ethanol concentration (which can be brought about by a bolus addition of ethanol) can cause sustained changes in gene expression which confer a lasting improvement in the production of properly folded and assembled multi-subunit complexes and/or increases processing of improperly folded or misassembled multi-subunit complexes, leading to improved purity in the multi-subunit complex. Additionally, it was demonstrated that the improved antibody purity correlated with improved viability of the yeast in the culture, and based thereon Applicants hypothesize that the improved viability may account (at least in part) for the improved purity, though this theory is not intended to be limiting.

[00165] In a preferred embodiment, the heterologous multi-subunit complex is an antibody or antibody fragment, such as a humanized antibody, comprised of two heavy chain subunits and two light chain subunits. Preferred host cells include yeasts, and particularly preferred yeasts include methylotrophic yeast strains, e.g., *Pichia pastoris*, *Hansenula polymorpha* (*Pichia angusta*), *Pichia guillermordii*, *Pichia methanolica*, *Pichia inositovera*, and others (see, e.g., U.S. Patent 4,812,405, 4,818,700, 4,929,555, 5,736,383, 5,955,349, 5,888,768, and 6,258,559 each of which is incorporated by reference in its entirety). The host cell may be produced by methods known in the art, such as transformation, mating, sporulation, etc.

[00166] In a preferred embodiment, the host cell may comprise more than one copy of one or more of the genes encoding the heterologous protein subunits. For example, multiple copies of a subunit gene may be integrated in tandem into one or more chromosomal loci. Tandemly integrated gene copies are preferably retained in a stable number of copies during culture for the production of the multi-subunit complex. For example, in the examples described below, gene copy numbers were generally stable for *P. pastoris* strains containing three to four tandemly integrated copies of light and heavy chain antibody genes.

[00167] One or more of the genes encoding the heterologous protein subunits are preferably integrated into one or more chromosomal loci of a host cell. Any suitable chromosomal locus may be utilized for integration, including intergenic sequences, promoters sequences, coding sequences, termination sequences, regulatory sequences, etc. Exemplary chromosomal loci that may be used in *P. pastoris* include PpURA5; *OCH1*; *AOX1*; *HIS4*; and *GAP*. The encoding genes may also be integrated into one or more random chromosomal loci rather than being targeted. In preferred embodiments, the chromosomal loci are selected from the group consisting of the pGAP locus, 3' AOX TT, and the HIS4 TT locus. In additional exemplary embodiments, the genes encoding the heterologous protein subunits may be contained in one or more extrachromosomal elements, for example one or more plasmids or artificial chromosomes.

[00168] In exemplary embodiments, the multi-subunit protein may comprise two, three, four, five, six, or more non-identical subunits. Additionally, each subunit may be present one or more times in each multi-subunit protein. For example, the multi-subunit protein may be a multi-specific antibody such as a bi-specific antibody comprising two non-identical light chains and two non-identical heavy chains.

[00169] The subunits may be expressed from monocistronic genes, polycistronic genes, or any combination thereof. Each polycistronic gene may comprise multiple copies of the same subunit, or may comprise one or more copies of each different subunit.

[00170] Exemplary methods that may be used for manipulation of *Pichia pastoris* (including methods of culturing, transforming, and mating) are disclosed in Published Applications including U.S. 20080003643, U.S. 20070298500, and U.S. 20060270045, and in Higgins, D. R., and Cregg, J. M., Eds. 1998. *Pichia* Protocols. Methods in Molecular Biology. Humana Press, Totowa, N.J., and Cregg, J. M., Ed., 2007, *Pichia* Protocols (2nd edition), Methods in Molecular Biology. Humana Press, Totowa, N.J., each of which is incorporated by reference in its entirety.

[00171] An exemplary expression cassette that may be utilized is composed of the glyceraldehyde dehydrogenase gene (GAP gene) promoter, fused to sequences encoding a secretion signal, followed by the sequence of the gene to be expressed, followed by sequences

encoding a *P. pastoris* transcriptional termination signal from the *P. pastoris* alcohol oxidase I gene (AOX1). The Zeocin resistance marker gene may provide a means of enrichment for strains that contain multiple integrated copies of an expression vector in a strain by selecting for transformants that are resistant to higher levels of Zeocin. Similarly, G418 or Kanamycin resistance marker genes may be used to provide a means of enrichment for strains that contain multiple integrated copies of an expression vector in a strain by selecting for transformants that are resistant to higher levels of Geneticin or Kanamycin.

[00172] Host strains that may be utilized include auxotrophic *P. pastoris* or other *Pichia* strains, for example, strains having mutations in *met1*, *lys3*, *ura3* and *ade1* or other auxotrophy-associated genes. Preferred mutations are incapable of giving rise to revertants at any appreciable frequency and are preferably partial or even more preferably full deletion mutants. Preferably, prototrophic diploid or tetraploid strains are produced by mating a complementing sets of auxotrophic strains.

[00173] Transformation of haploid *P. pastoris* strains and genetic manipulation of the *P. pastoris* sexual cycle may be performed as described in *Pichia* Protocols (1998, 2007), *supra*.

[00174] Prior to transformation, each expression vector may be linearized by restriction enzyme cleavage within a region homologous to the target genomic locus (e.g., the GAP promoter sequence) to direct the integration of the vectors into the target locus in the host cell. Samples of each vector may then be individually transformed into cultures of the desired strains by electroporation or other methods, and successful transformants may be selected by means of a selectable marker, e.g., antibiotic resistance or complementation of an auxotrophy. Isolates may be picked, streaked for single colonies under selective conditions and then examined to confirm the number of copies of the gene encoding the subunit of the multi-subunit complex (e.g., a desired antibody) by Southern Blot or PCR assay on genomic DNA extracted from each strain. Optionally, expression of the expected subunit gene product may be confirmed, e.g., by FACS, Western Blot, colony lift and immunoblot, and other means known in the art. Optionally, haploid isolates are transformed additional times to introduce additional heterologous genes, e.g., additional copies of the same subunit integrated at a different locus, and / or copies of a different subunit. The haploid strains are then mated to generate diploid strains (or strains of higher

ploidy) able to synthesize the multi-protein complex. Presence of each expected subunit gene may be confirmed by Southern blotting, PCR, and other detection means known in the art.

Where the desired multi-protein complex is an antibody, its expression may also be confirmed by a colony lift/immunoblot method (Wung et al. Biotechniques 21 808-812 (1996) and / or by FACS.

[00175] This transformation protocol is optionally repeated to target a heterologous gene into a second locus, which may be the same gene or a different gene than was targeted into the first locus. When the construct to be integrated into the second locus encodes a protein that is the same as or highly similar to the sequence encoded by the first locus, its sequence may be varied to decrease the likelihood of undesired integration into the first locus. For example, the sequence to be integrated into the second locus may have differences in the promoter sequence, termination sequence, codon usage, and/or other tolerable sequence differences relative to the sequence integrated into the first locus.

[00176] To mate *P. pastoris* haploid strains, each strain to be crossed can be patched together onto mating plates. For example, multiple matings can be conveniently performed at the same time by streaking each strain to be mated across a plate suitable for its growth, and the mating partners may be streaked across a second plate (preferably the plates are rich media such as YPD). Typically, after one or two days incubation at 30° C., cells from the two plates can be replica plated in a crisscross fashion onto a mating plate, resulting in a cross-hatched pattern with each pair of strains being co-plated and having the opportunity to mate at the intersection of a pair of the original streak lines. The mating plate can then be incubated (e.g., at 30° C.) to stimulate the initiation of mating between strains. After about two days, the cells on the mating plates can be streaked, patched, or replica plated onto media selective for the desired diploid strains (e.g., where the mated strains have complementary autotrophies, drop-out or minimal medium plates may be used). These plates can be incubated (e.g., at 30° C.) for a suitable duration (e.g., about three days) to allow for the selective growth of the desired diploid strains. Colonies that arise can be picked and streaked for single colonies to isolate and purify each diploid strain.

[00177] Expression vectors for use in the methods of the invention may further include yeast specific sequences, including a selectable auxotrophic or drug marker for identifying transformed yeast strains. A drug marker may further be used to amplify copy number of the vector in a yeast host cell, e.g., by culturing a population of cells in an elevated concentration of the drug, thereby selecting transformants that express elevated levels of the resistance gene.

[00178] In an exemplary embodiment, one or more of the genes encoding the heterologous protein subunits are coupled to an inducible promoter. Suitable exemplary promoters include the alcohol oxidase 1 gene promoter, formaldehyde dehydrogenase genes (FLD; see U.S. Pub. No. 2007/0298500), and other inducible promoters known in the art. The alcohol oxidase 1 gene promoter, is tightly repressed during growth of the yeast on most common carbon sources, such as glucose, glycerol, or ethanol, but is highly induced during growth on methanol (Tschopp et al., 1987; U.S. Pat. No. 4,855,231 to Stroman, D. W., et al). For production of foreign proteins, strains may be initially grown on a repressing carbon source to generate biomass and then shifted to methanol as the sole (or main) carbon and energy source to induce expression of the foreign gene. One advantage of this regulatory system is that *P. pastoris* strains transformed with foreign genes whose expression products are toxic to the cells can be maintained by growing under repressing conditions.

[00179] In another exemplary embodiment, one or more of the heterologous genes may be coupled to a regulated promoter, whose expression level can be upregulated under appropriate conditions. Exemplary regulated promoters include the CUP1 promoter (induced by the level of copper in the medium), tetracycline inducible promoters, thiamine inducible promoters, the AOX1 promoter, and the FLD1 promoter.

[00180] Though much of the present disclosure describes production of antibodies, the methods described herein are readily adapted to other multi-subunit complexes as well. Without intent to be limited by theory, it is believed that the yield and purity of multi-subunit complexes can be greatly influenced by the concentration and stoichiometry of the subunits, which are in turn influenced by the level of expression of the genes responsible for production of each subunit. The methods disclosed herein may readily be utilized to improve the yield and / or purity of any recombinant multi-subunit complex comprising two or more different subunits.

[00181] Additionally, the present methods are not limited to production of multi-protein complexes but may also be readily adapted for use with ribonucleoprotein (RNP) complexes including telomerase, hnRNPs, Ribosomes, snRNPs, signal recognition particles, prokaryotic and eukaryotic RNase P complexes, and any other complexes that contain multiple distinct protein and / or RNA subunits. The host cell that expresses the multi-subunit complex may be produced by methods known in the art. For example, a panel of diploid or tetraploid yeast cells containing differing combinations of gene copy numbers may be generated by mating cells containing varying numbers of copies of the individual subunit genes (which numbers of copies preferably are known in advance of mating).

Definitions

[00182] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

[00183] *Bolus addition:* In the present disclosure, "bolus addition" generally refers to rapid change in concentration of a substance (such as ethanol) in contact with cultured cells (for example, in a culture medium). For example, the substance may be added to the cultured cells in a single addition, a succession of more than one addition, and/or infused over a period of time (e.g., over about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 90, or 120 minutes). The substance may also be added by replacing the culture medium in part or in full, for example by concentrating the cells (using centrifugation, filtration, settling, or other methods), removing part or all of the medium, and adding the substance, or by adding the cells to a medium containing the

substance. The substance may be admixed with a carrier (e.g., culture media, water, saline, etc.). For example, a bolus addition of ethanol may comprise the addition of pure or concentrated ethanol (e.g., 100%, 95%, 70%, 50%, 60%, 40%, 30%, 20%, etc.) to the culture medium in an amount sufficient to produce the desired concentration. As another example, the cells may be added to a medium containing ethanol, e.g., by adding an inoculum containing the cells to a medium containing ethanol.

[00184] *Bolus concentration:* In the present disclosure, “bolus concentration” generally refers to the concentration that results from a bolus addition of a substance (e.g., ethanol).

[00185] *Mating competent yeast species:* In the present invention this is intended to broadly encompass any diploid or tetraploid yeast which can be grown in culture. Such species of yeast may exist in a haploid, diploid, or other polyploid form. The cells of a given ploidy may, under appropriate conditions, proliferate for an indefinite number of generations in that form. Diploid cells can also sporulate to form haploid cells. Sequential mating can result in tetraploid strains through further mating or fusion of diploid strains. The present invention contemplates the use of haploid yeast, as well as diploid or other polyploid yeast cells produced, for example, by mating or fusion (e.g., spheroplast fusion).

[00186] In one embodiment of the invention, the mating competent yeast is a member of the *Saccharomycetaceae* family, which includes the genera *Arxiozyma*; *Ascobotryozyma*; *Citeromyces*; *Debaryomyces*; *Dekkera*; *Eremothecium*; *Issatchenkia*; *Kazachstania*; *Khuyveromyces*; *Kodamaea*; *Lodderomyces*; *Pachysolen*; *Pichia*; *Saccharomyces*; *Saturnispora*; *Tetrapisispora*; *Torulaspora*; *Williopsis*; and *Zygosaccharomyces*. Other types of yeast potentially useful in the invention include *Yarrowia*; *Rhodospiridium*; *Candida*; *Hansenula*; *Filobasium*; *Sporidiobolus*; *Bullera*; *Leucosporidium* and *Filobasidella*.

[00187] In a preferred embodiment of the invention, the mating competent yeast is a member of the genus *Pichia* or is another methylotroph. In a further preferred embodiment of the invention, the mating competent yeast of the genus *Pichia* is one of the following species: *Pichia pastoris*, *Pichia methanolica*, and *Hansenula polymorpha* (*Pichia angusta*). In a particularly preferred embodiment of the invention, the mating competent yeast of the genus *Pichia* is the species *Pichia pastoris*.

[00188] *Haploid Yeast Cell*: A cell having a single copy of each gene of its normal genomic (chromosomal) complement.

[00189] *Polyploid Yeast Cell*: A cell having more than one copy of its normal genomic (chromosomal) complement.

[00190] *Diploid Yeast Cell*: A cell having two copies (alleles) of essentially every gene of its normal genomic complement, typically formed by the process of fusion (mating) of two haploid cells.

[00191] *Tetraploid Yeast Cell*: A cell having four copies (alleles) of essentially every gene of its normal genomic complement, typically formed by the process of fusion (mating) of two diploid cells. Tetraploids may carry two, three, four, or more different expression cassettes. Such tetraploids might be obtained in *S. cerevisiae* by selective mating homozygotic heterothallic a/a and alpha/alpha diploids and in *Pichia* by sequential mating of haploids to obtain auxotrophic diploids. For example, a [met his] haploid can be mated with [ade his] haploid to obtain diploid [his]; and a [met arg] haploid can be mated with [ade arg] haploid to obtain diploid [arg]; then the diploid [his] can be mated with the diploid [arg] to obtain a tetraploid prototroph. It will be understood by those of skill in the art that reference to the benefits and uses of diploid cells may also apply to tetraploid cells.

[00192] *Yeast Mating*: The process by which two yeast cells fuse to form a single yeast cell. The fused cells may be haploid cells or cells of higher ploidy (e.g., mating two diploid cells to produce a tetraploid cell).

[00193] *Meiosis*: The process by which a diploid yeast cell undergoes reductive division to form four haploid spore products. Each spore may then germinate and form a haploid vegetatively growing cell line.

[00194] *Selectable Marker*: A selectable marker is a gene or gene fragment that confers a growth phenotype (physical growth characteristic) on a cell receiving that gene as, for example through a transformation event. The selectable marker allows that cell to survive and grow in a selective growth medium under conditions in which cells that do not receive that selectable marker gene cannot grow. Selectable marker genes generally fall into several types, including

positive selectable marker genes such as a gene that confers on a cell resistance to an antibiotic or other drug, temperature when two temperature sensitive (“ts”) mutants are crossed or a ts mutant is transformed; negative selectable marker genes such as a biosynthetic gene that confers on a cell the ability to grow in a medium without a specific nutrient needed by all cells that do not have that biosynthetic gene, or a mutagenized biosynthetic gene that confers on a cell inability to grow by cells that do not have the wild type gene; and the like. Suitable markers include but are not limited to: ZEO; NEO (G418); LYS3; MET1; MET3a; ADE1; ADE3; URA3; and the like.

[00195] *Integrated:* A genetic element (typically a heterologous genetic element) that are covalently joined into a chromosome of an organism.

[00196] *Tandemly integrated:* Two or more copies of a genetic element that are integrated in adjacent locations in a chromosome. The two or more copies do not necessarily have the orientation; e.g., for transcribed genes, some copies may be transcribed from the Watson strand and others from the Crick strand.

[00197] *Host cell:* In the context of the present disclosure, the term host cell refers to a cell (e.g., a eukaryotic cell, such as a *Pichia* cell) which contains a heterologous gene. For example, the heterologous gene may provide for the expression of a subunit of a desired multi-subunit complex, a gene involved in protein folding (e.g., a chaperone), expression, or secretion, and/or another desired gene. The heterologous gene may be integrated into the genome of the eukaryotic cell or contained in extrachromosomal element such as a plasmid or artificial chromosome.

[00198] *Expression Vector:* These DNA vectors contain elements that facilitate manipulation for the expression of a foreign protein within the target host cell. Conveniently, manipulation of sequences and production of DNA for transformation is first performed in a bacterial host, e.g. *E. coli*, and usually vectors will include sequences to facilitate such manipulations, including a bacterial origin of replication and appropriate bacterial selection marker. Selection markers encode proteins necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes

encode proteins that (a) confer resistance to antibiotics or other toxins, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media. Exemplary vectors and methods for transformation of yeast are described, for example, in Burke, D., Dawson, D., & Stearns, T. (2000). *Methods in yeast genetics: a Cold Spring Harbor Laboratory course manual*. Plainview, N.Y.: Cold Spring Harbor Laboratory Press, which is incorporated by reference herein in its entirety.

[00199] Expression vectors for use in the methods of the invention may further include yeast specific sequences, including a selectable auxotrophic or drug marker for identifying transformed yeast strains. A drug marker may further be used to select for amplification of copy number of the vector in a yeast host cell.

[00200] The polypeptide coding sequence of interest is typically operably linked to transcriptional and translational regulatory sequences that provide for expression of the polypeptide in yeast cells. These vector components may include, but are not limited to, one or more of the following: an enhancer element, a promoter, and a transcription termination sequence. Sequences for the secretion of the polypeptide may also be included, e.g. a signal sequence, and the like. A yeast origin of replication is optional, as expression vectors are often integrated into the yeast genome.

[00201] Though optional, in one embodiment of the invention, one or more subunit of the multi-subunit complex is operably linked, or fused, to a secretion sequence that provides for secretion of the expressed polypeptide into the culture media, which can facilitate harvesting and purification of the heterologous multi-subunit complex. Even more preferably, the secretion sequences provide for optimized secretion of the polypeptide from the host cells (e.g., yeast diploid cells), such as through selecting preferred codons and/or altering the percentage AT through codon selection. It is known in the art that secretion efficiency and / or stability can be affected by the choice of secretion sequence and the optimal secretion sequence can vary between different proteins (*see, e.g.,* Koganesawa et al., *Protein Eng.* 2001 Sep;14(9):705-10, which is incorporated by reference herein in its entirety). Many potentially suitable secretion signals are known in the art and can readily be tested for their effect upon yield and/or purity of a particular heterologous multi-subunit complex. Any secretion sequences may potentially be

used, including those present in secreted proteins of yeasts and other species, as well as engineered secretion sequences. Exemplary secretion sequences that may be utilized include: chicken lysozyme (CLY) signal peptide (MRSLLILVLCFLPLAALG (SEQ ID NO:414)), CLY-L8 (MRLLLLLLLLLPLAALG (SEQ ID NO:415)), *S. cerevisiae* invertase (SUC2) signal peptide (MLLQAFLFLLAGFAAKISA (SEQ ID NO:416)), MF-alpha (Prepro) (MRFPSIFTAVLFAASSALA-APVNTTTE-EGVSLEKR (SEQ ID NO:417)), MF-alpha (Pre)-apv (MRFPSIFTAVLFAASSALA-APV (SEQ ID NO:418)), MF-alpha (Pre)-apv-SLEKR (MRFPSIFTAVLFAASSALA-APVSLEKR (SEQ ID NO:419)), MF-alpha (Prepro)-(EA)3 (MRFPSIFTAVLFAASSALA-APVNTTTE-EGVSLEKR-EAEAEA (SEQ ID NO:420)), α F signal peptide (MRFPSIFTAVLFAASSALA-APVNTTTE-DETAQIPAEAVIGYSDLEGDFDVAVLPFSNSTNNGLLFINTTASIAAKE-EGVSLEKR (SEQ ID NO:421)), KILM1 signal peptide (MTKPTQVLVRSVSILFFITLLHLVVALNDVAGPAETAPVSLLPR (SEQ ID NO:422)), repressible acid phosphatase (PHO1) signal peptide (MFSPILSLEIILALATLQSVFA (SEQ ID NO:423)), *A. niger* GOX signal peptide (MQTLLVSSLVVSLLAAALPHYIR (SEQ ID NO:424)), *Schwanniomyces occidentalis* glucoamylase gene (GAM1) signal peptide (MIFLKLKISIVIGLGLVSAIQA (SEQ ID NO:425)), human serum albumin (HSA) signal peptide with pro-sequence (MKWVTFISLLFLFSSAYSARGVFRR (SEQ ID NO:426)), human serum albumin (HSA) signal peptide without pro-sequence (MKWVTFISLLFLFSSAYS (SEQ ID NO:427)), ISN signal peptide (MALWMRLLPLLALLALWGPDPAAA (SEQ ID NO:428)), IFN signal peptide (MKYTSYILAFQLCIVLGS LGCDLP (SEQ ID NO:429)), HGH signal peptide (MAADSQTPWLLTFSLLCLLPQEPGA (SEQ ID NO:430)), phytohaemagglutinin (PHA) (MKKNRMMMMIWSVGVVWMLLLVGGSYG (SEQ ID NO:431)), Silkworm lysozyme (MQKLIIFALVVL CVGSEA (SEQ ID NO:432)), Human lysozyme (LYZ1) (MKALIVLGLVLLSVTVQG (SEQ ID NO:433)), activin receptor type-1 (MVDGVMILPVLIMIALPSPS (SEQ ID NO:434)), activin type II receptor (MGAAAKLAFVFLISCSSG (SEQ ID NO:435)), *P. pastoris* immunoglobulin binding protein (PpBiP) (MLSLKPSWLTLAALMYAMLLVVVPFAKPVRA (SEQ ID NO:436)), and human antibody 3D6 light chain leader (MDMRVPAQLLGLLLLWLP GAKC (SEQ ID NO:437)). See Hashimoto et al., Protein Engineering vol. 11 no. 2 pp.75–77, 1998; Oka et al., Biosci Biotechnol Biochem. 1999 Nov; 63(11):1977-83; Gellissen et al., FEMS Yeast Research 5 (2005) 1079–

1096; Ma et al., *Hepatology*. 2005 Dec;42(6):1355-63; Raemaekers et al., *Eur J Biochem*. 1999 Oct 1;265(1):394-403; Koganawa et al., *Protein Eng.* (2001) 14 (9): 705-710; Daly et al., *Protein Expr Purif.* 2006 Apr;46(2):456-67 ; Damasceno et al., *Appl Microbiol Biotechnol* (2007) 74:381–389; and Felgenhauer et al., *Nucleic Acids Res.* 1990 Aug 25;18(16):4927, each of which is incorporated by reference herein in its entirety). The multi-subunit complex may also be secreted into the culture media without being operably linked or fused to a secretion signal. For example, it has been demonstrated that some heterologous polypeptides are secreted into the culture media when expressed in *P. pastoris* even without being linked or fused to a secretion signal. Additionally, the multi-subunit complex may be purified from host cells (which, for example, may be preferable if the complex is poorly secreted) using methods known in the art.

[00202] Media or cells comprising a desired multi-subunit complex may be recovered from the culture. Optionally, the secreted proteins may be purified. For example, cells comprising a desired multi-subunit complex may be lysed using mechanical, chemical, enzymatic, and/or osmotic methods (e.g., freezing with liquid nitrogen, using a homogenizer, spheroplasting, sonication, agitation in the presence of glass beads, using detergents, etc.). The desired multi-subunit complex may be concentrated, filtered, dialyzed, etc., using methods known in the art. The desired multi-subunit complex may be purified based on, for example, its molecular mass (e.g., size exclusion chromatography), isoelectric point (e.g., isoelectric focusing), electrophoretic mobility (e.g., gel electrophoresis), hydrophobic interaction chromatography (e.g., HPLC), charge (e.g., ion exchange chromatography), affinity (e.g., in the case of an antibody, binding to protein A, protein G, and/or an epitope to which the desired antibody binds), and/or glycosylation state (e.g., detected by lectin binding affinity). Multiple purification steps may be performed to obtain the desired level of purity. In an exemplary embodiment, the desired multi-subunit complex may comprise an immunoglobulin constant domain and may be purified using protein A or protein G affinity, size exclusion chromatography, and lack of binding to lectin (to remove glycosylated forms). Optionally the A protease inhibitor, such as phenyl methyl sulfonyl fluoride (PMSF) may be added to inhibit proteolytic degradation during purification.

[00203] Nucleic acids are "operably linked" when placed into a functional relationship with another nucleic acid sequence. For example, DNA for a signal sequence is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking may be accomplished by ligation at convenient restriction sites or alternatively via a PCR/recombination method familiar to those skilled in the art (Gateway® Technology; Invitrogen, Carlsbad Calif.). If such sites do not exist, the synthetic oligonucleotide adapters or linkers may be used in accordance with conventional practice. Desired nucleic acids (including nucleic acids comprising operably linked sequences) may also be produced by chemical synthesis.

[00204] Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequences to which they are operably linked. Such promoters fall into several classes: inducible, constitutive, and repressible promoters (that increase levels of transcription in response to absence of a repressor). Inducible promoters may initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature.

[00205] The yeast promoter fragment may also serve as the site for homologous recombination and integration of the expression vector into the same site in the yeast genome; alternatively a selectable marker is used as the site for homologous recombination. *Pichia* transformation is described in Cregg et al. (1985) Mol. Cell. Biol. 5:3376-3385, which is incorporated by reference herein in its entirety.

[00206] Examples of suitable promoters from *Pichia* include the CUP1 (induced by the level of copper in the medium), tetracycline inducible promoters, thiamine inducible promoters, AOX1 promoter (Cregg et al. (1989) Mol. Cell. Biol. 9:1316-1323); ICL1 promoter (Menendez et al. (2003) Yeast 20(13):1097-108); glyceraldehyde-3-phosphate dehydrogenase promoter

(GAP) (Waterham et al. (1997) Gene 186(1):37-44); and FLD1 promoter (Shen et al. (1998) Gene 216(1):93-102). The GAP promoter is a strong constitutive promoter and the CUP1, AOX and FLD1 promoters are inducible. Each foregoing reference is incorporated by reference herein in its entirety.

[00207] Other yeast promoters include ADH1, alcohol dehydrogenase II, GAL4, PHO3, PHO5, Pyk, and chimeric promoters derived therefrom. Additionally, non-yeast promoters may be used in the invention such as mammalian, insect, plant, reptile, amphibian, viral, and avian promoters. Most typically the promoter will comprise a mammalian promoter (potentially endogenous to the expressed genes) or will comprise a yeast or viral promoter that provides for efficient transcription in yeast systems.

[00208] The polypeptides of interest may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, e.g. a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the polypeptide coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed through one of the standard pathways available within the host cell. The *S. cerevisiae* alpha factor pre-pro signal has proven effective in the secretion of a variety of recombinant proteins from *P. pastoris*. Other yeast signal sequences include the alpha mating factor signal sequence, the invertase signal sequence, and signal sequences derived from other secreted yeast polypeptides. Additionally, these signal peptide sequences may be engineered to provide for enhanced secretion in diploid yeast expression systems. Other secretion signals of interest also include mammalian signal sequences, which may be heterologous to the protein being secreted, or may be a native sequence for the protein being secreted. Signal sequences include pre-peptide sequences, and in some instances may include propeptide sequences. Many such signal sequences are known in the art, including the signal sequences found on immunoglobulin chains, e.g., K28 preprotoxin sequence, PHA-E, FACE, human MCP-1, human serum albumin signal sequences, human Ig heavy chain, human Ig light chain, and the like. For example, see Hashimoto et. al. Protein Eng 11(2) 75 (1998); and Kobayashi et. al. Therapeutic Apheresis 2(4) 257 (1998), each of which is incorporated by reference herein in its entirety.

[00209] Transcription may be increased by inserting a transcriptional activator sequence into the vector. These activators are cis-acting elements of DNA, usually about from 10 to 300 bp, which act on a promoter to increase its transcription. Transcriptional enhancers are relatively orientation and position independent, having been found 5' and 3' to the transcription unit, within an intron, as well as within the coding sequence itself. The enhancer may be spliced into the expression vector at a position 5' or 3' to the coding sequence, but is preferably located at a site 5' from the promoter.

[00210] Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from 3' to the translation termination codon, in untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA.

[00211] Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques or PCR/recombination methods. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required or via recombination methods. For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform host cells, and successful transformants selected by antibiotic resistance (e.g. ampicillin or Zeocin) where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion and/or sequenced.

[00212] As an alternative to restriction and ligation of fragments, recombination methods based on att sites and recombination enzymes may be used to insert DNA sequences into a vector. Such methods are described, for example, by Landy (1989) *Ann. Rev. Biochem.* 58:913-949; and are known to those of skill in the art. Such methods utilize intermolecular DNA recombination that is mediated by a mixture of lambda and E. coli-encoded recombination proteins. Recombination occurs between specific attachment (att) sites on the interacting DNA molecules. For a description of att sites see Weisberg and Landy (1983) *Site-Specific Recombination in Phage Lambda*, in *Lambda II*, Weisberg, ed. (Cold Spring Harbor, N.Y.: Cold Spring Harbor Press), pp. 211-250. The DNA segments flanking the recombination sites are

switched, such that after recombination, the att sites are hybrid sequences comprised of sequences donated by each parental vector. The recombination can occur between DNAs of any topology. Each foregoing reference is incorporated by reference herein in its entirety.

[00213] Att sites may be introduced into a sequence of interest by ligating the sequence of interest into an appropriate vector; generating a PCR product containing att B sites through the use of specific primers; generating a cDNA library cloned into an appropriate vector containing att sites; and the like.

[00214] *Monocistronic and polycistronic genes.* A monocistronic gene encodes an RNA that contains the genetic information to translate only a single protein. A polycistronic gene encodes an mRNA that contains the genetic information to translate more than one protein. The proteins encoded in a polycistronic gene may have the same or different sequences or a combination thereof. Dicistronic or bicistronic refers to a polycistronic gene that encodes two proteins. Polycistronic genes optionally include one or more internal ribosome entry site (IRES) elements to facilitate cap-independent initiation of translation, which may be situated at a location that can drive translation of the downstream protein coding region independently of the 5'-cap structure bound to the 5' end of the mRNA molecule. Any known IRES sequence (e.g., viral, eukaryotic, or artificial in origin) may be used. For example, the cricket paralysis virus IRES sequence in the intergenic region (IGR) may be used, as described in Thompson *et al.* (2001) PNAS 98:12972-12977. Optionally, IRES function may be potentiated by genetic alteration, e.g., by causing constitutive expression of eIF2 kinase GCN2 or disrupting two initiator tRNA(met) genes disrupted (*id.*).

[00215] *Folding*, as used herein, refers to the three-dimensional structure of polypeptides and proteins, where interactions between amino acid residues act to stabilize the structure. While non-covalent interactions are important in determining structure, usually the proteins of interest will have intra- and/or intermolecular covalent disulfide bonds formed by two cysteine residues. For naturally occurring proteins and polypeptides or derivatives and variants thereof, the proper folding is typically the arrangement that results in optimal biological activity, and can conveniently be monitored by assays for activity, e.g. ligand binding, enzymatic activity, etc.

[00216] In some instances, for example where the desired product is of synthetic origin, assays based on biological activity will be less meaningful. The proper folding of such molecules may be determined on the basis of physical properties, energetic considerations, modeling studies, and the like.

[00217] The expression host may be further modified by the introduction of sequences encoding one or more enzymes that enhance folding and disulfide bond formation, i.e. foldases, chaperoning, etc. Such sequences may be constitutively or inducibly expressed in the yeast host cell, using vectors, markers, etc. as known in the art. Preferably the sequences, including transcriptional regulatory elements sufficient for the desired pattern of expression, are stably integrated in the yeast genome through a targeted methodology.

[00218] For example, the eukaryotic PDI is not only an efficient catalyst of protein cysteine oxidation and disulfide bond isomerization, but also exhibits chaperone activity. Co-expression of PDI can facilitate the production of active proteins having multiple disulfide bonds. Also of interest is the expression of BIP (immunoglobulin heavy chain binding protein); cyclophilin; and the like. In one embodiment of the invention, the multi-subunit complex may be expressed from a yeast strain produced by mating, wherein each of the haploid parental strains expresses a distinct folding enzyme, e.g. one strain may express BIP, and the other strain may express PDI or combinations thereof.

[00219] The terms "desired protein" or "target protein" are used interchangeably and refer generally to a heterologous multi-subunit protein such as an antibody (e.g., a humanized antibody) or a binding portion thereof described herein.

[00220] The term "antibody" includes any polypeptide chain-containing molecular structure with a specific shape that fits to and recognizes an epitope, where one or more non-covalent binding interactions stabilize the complex between the molecular structure and the epitope. The archetypal antibody molecule is the immunoglobulin, and all types of immunoglobulins, IgG, IgM, IgA, IgE, IgD, etc., from all sources, e.g. human, rodent, rabbit, cow, sheep, pig, dog, other mammals, chicken, other avians, etc., are considered to be "antibodies." A preferred source for producing antibodies useful as starting material according to the invention is rabbits. Numerous antibody coding sequences have been described; and others

may be raised by methods well-known in the art. Examples thereof include chimeric antibodies, human antibodies and other non-human mammalian antibodies, humanized antibodies, single chain antibodies such as scFvs, camelbodies, nanobodies, IgNAR (single-chain antibodies derived from sharks), small-modular immunopharmaceuticals (SMIPs), and antibody fragments such as Fabs, Fab', F(ab')₂ and the like. See Streltsov V A, et al., Structure of a shark IgNAR antibody variable domain and modeling of an early-developmental isotype, *Protein Sci.* 2005 November; 14(11):2901-9. Epub 2005 Sep. 30; Greenberg A S, et al., A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks, *Nature.* 1995 Mar. 9; 374(6518):168-73; Nuttall S D, et al., Isolation of the new antigen receptor from wobbegong sharks, and use as a scaffold for the display of protein loop libraries, *Mol Immunol.* 2001 August; 38(4):313-26; Hamers-Casterman C, et al., Naturally occurring antibodies devoid of light chains, *Nature.* 1993 Jun. 3; 363(6428):446-8; Gill D S, et al., Biopharmaceutical drug discovery using novel protein scaffolds, *Curr Opin Biotechnol.* 2006 December; 17(6):653-8. Epub 2006 Oct. 19. Each foregoing reference is incorporated by reference herein in its entirety.

[00221] For example, antibodies or antigen binding fragments may be produced by genetic engineering. In this technique, as with other methods, antibody-producing cells are sensitized to the desired antigen or immunogen. The messenger RNA isolated from antibody producing cells is used as a template to make cDNA using PCR amplification. A library of vectors, each containing one heavy chain gene and one light chain gene retaining the initial antigen specificity, is produced by insertion of appropriate sections of the amplified immunoglobulin cDNA into the expression vectors. A combinatorial library is constructed by combining the heavy chain gene library with the light chain gene library. This results in a library of clones which co-express a heavy and light chain (resembling the Fab fragment or antigen binding fragment of an antibody molecule). The vectors that carry these genes are co-transfected into a host cell. When antibody gene synthesis is induced in the transfected host, the heavy and light chain proteins self-assemble to produce active antibodies that can be detected by screening with the antigen or immunogen.

[00222] Antibody coding sequences of interest include those encoded by native sequences, as well as nucleic acids that, by virtue of the degeneracy of the genetic code, are not identical in sequence to the disclosed nucleic acids, and variants thereof. Variant polypeptides can include

amino acid (aa) substitutions, additions or deletions. The amino acid substitutions can be conservative amino acid substitutions or substitutions to eliminate non-essential amino acids, such as to alter a glycosylation site, or to minimize misfolding by substitution or deletion of one or more cysteine residues that are not necessary for function. Variants can be designed so as to retain or have enhanced biological activity of a particular region of the protein (e.g., a functional domain, catalytic amino acid residues, etc). Variants also include fragments of the polypeptides disclosed herein, particularly biologically active fragments and/or fragments corresponding to functional domains. Techniques for in vitro mutagenesis of cloned genes are known. Also included in the subject invention are polypeptides that have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent.

[00223] Chimeric antibodies may be made by recombinant means by combining the variable light and heavy chain regions (V_L and V_H), obtained from antibody producing cells of one species with the constant light and heavy chain regions from another. Typically chimeric antibodies utilize rodent or rabbit variable regions and human constant regions, in order to produce an antibody with predominantly human domains. The production of such chimeric antibodies is well known in the art, and may be achieved by standard means (as described, e.g., in U.S. Pat. No. 5,624,659, incorporated herein by reference in its entirety). It is further contemplated that the human constant regions of chimeric antibodies of the invention may be selected from IgG1, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgG10, IgG11, IgG12, IgG13, IgG14, IgG15, IgG16, IgG17, IgG18 or IgG19 constant regions.

[00224] Humanized antibodies are engineered to contain even more human-like immunoglobulin domains, and incorporate only the complementarity-determining regions of the animal-derived antibody. This is accomplished by carefully examining the sequence of the hyper-variable loops of the variable regions of the monoclonal antibody, and fitting them to the structure of the human antibody chains. Although facially complex, the process is straightforward in practice. See, e.g., U.S. Pat. No. 6,187,287, incorporated fully herein by reference. Methods of humanizing antibodies have been described previously in issued U.S. Patent No. 7935340, the disclosure of which is incorporated herein by reference in its entirety. In some instances, a determination of whether additional rabbit framework residues are required

to maintain activity is necessary. In some instances the humanized antibodies still requires some critical rabbit framework residues to be retained to minimize loss of affinity or activity. In these cases, it is necessary to change single or multiple framework amino acids from human germline sequences back to the original rabbit amino acids in order to have desired activity. These changes are determined experimentally to identify which rabbit residues are necessary to preserve affinity and activity.

[00225] In addition to entire immunoglobulins (or their recombinant counterparts), immunoglobulin fragments comprising the epitope binding site (e.g., Fab', F(ab')₂, or other fragments) may be synthesized. "Fragment," or minimal immunoglobulins may be designed utilizing recombinant immunoglobulin techniques. For instance "Fv" immunoglobulins for use in the present invention may be produced by synthesizing a fused variable light chain region and a variable heavy chain region. Combinations of antibodies are also of interest, e.g. diabodies, which comprise two distinct Fv specificities. In another embodiment of the invention, SMIPs (small molecule immunopharmaceuticals), camelbodies, nanobodies, and IgNAR are encompassed by immunoglobulin fragments.

[00226] Immunoglobulins and fragments thereof may be modified post-translationally, e.g. to add effector moieties such as chemical linkers, detectable moieties, such as fluorescent dyes, enzymes, toxins, substrates, bioluminescent materials, radioactive materials, chemiluminescent moieties and the like, or specific binding moieties, such as streptavidin, avidin, or biotin, and the like may be utilized in the methods and compositions of the present invention. Examples of additional effector molecules are provided infra.

[00227] *Product-associated variant*: a product other than the desired product (e.g., the desired multi-subunit complex) which is present in a preparation of the desired product and related to the desired product. Exemplary product-associated variants include truncated or elongated peptides, products having different glycosylation than the desired glycosylation (e.g., if an aglycosylated product is desired then any glycosylated product would be considered to be a product-associated variant), complexes having abnormal stoichiometry, improper assembly, abnormal disulfide linkages, abnormal or incomplete folding, aggregation, protease cleavage, or other abnormalities. Exemplary product-associated variants may exhibit alterations in one or

more of molecular mass (e.g., detected by size exclusion chromatography), isoelectric point (e.g., detected by isoelectric focusing), electrophoretic mobility (e.g., detected by gel electrophoresis), phosphorylation state (e.g., detected by mass spectrometry), charge to mass ratio (e.g., detected by mass spectrometry), mass or identity of proteolytic fragments (e.g., detected by mass spectrometry or gel electrophoresis), hydrophobicity (e.g., detected by HPLC), charge (e.g., detected by ion exchange chromatography), affinity (e.g., in the case of an antibody, detected by binding to protein A, protein G, and/or an epitope to which the desired antibody binds), and glycosylation state (e.g., detected by lectin binding affinity). Where the desired protein is an antibody, the term product-associate variant may include a glyco-heavy variant and/or half antibody species (described below).

[00228] Exemplary product-associated variants include variant forms that contain aberrant disulfide bonds. For example, most IgG1 antibody molecules are stabilized by a total of 16 intra-chain and inter-chain disulfide bridges, which stabilize the folding of the IgG domains in both heavy and light chains, while the inter-chain disulfide bridges stabilize the association between heavy and light chains. Other antibody types likewise contain characteristic stabilizing intra-chain and inter-chain disulfide bonds. Further, some antibodies (including Ab-A and Ab-B disclosed herein) contain additional disulfide bonds referred to as non-canonical disulfide bonds. Thus, aberrant inter-chain disulfide bonds may result in abnormal complex stoichiometry, due to the absence of a stabilizing covalent linkage, and/or disulfide linkages to additional subunits. Additionally, aberrant disulfide bonds (whether inter-chain or intra-chain) may decrease structural stability of the antibody, which may result in decreased activity, decreased stability, increased propensity to form aggregates, and/or increased immunogenicity. Product-associated variants containing aberrant disulfide bonds may be detected in a variety of ways, including non-reduced denaturing SDS-PAGE, capillary electrophoresis, cIEX, mass spectrometry (optionally with chemical modification to produce a mass shift in free cysteines), size exclusion chromatography, HPLC, changes in light scattering, and any other suitable methods known in the art. *See, e.g.,* The Protein Protocols Handbook 2002, Part V, 581-583, DOI: 10.1385/1-59259-169-8:581;

[00229] *Half antibody, half-antibody species, or HILL1* refer to a protein complex that includes a single heavy and single light antibody chain, but lacks a covalent linkage to a second

heavy and light antibody chain. Two half antibodies may remain non-covalently associated under some conditions (which may give behavior similar to a full antibody, e.g., apparent molecular weight determined by size exclusion chromatography). Similarly, *H2L1* refers to a protein complex that includes two heavy antibody chains and single light antibody chain, but lacks a covalent linkage to a second light antibody chain; these complexes may also non-covalently associate with another light antibody chain (and likewise give similar behavior to a full antibody). Like full antibodies, half antibody species and H2L1 species can dissociate under reducing conditions into individual heavy and light chains. Half antibody species and H2L1 species can be detected on a non-reduced SDS-PAGE gel as a species migrating at a lower apparent molecular weight than the full antibody, e.g., H1L1 migrates at approximately half the apparent molecular weight of the full antibody (e.g., about 75 kDa).

[00230] *Glyco-heavy variant* refers to a glycosylated product-associated variant sometimes present in antibody preparations and which contains at least a partial Fc sequence. The glyco-heavy variant is characterized by decreased electrophoretic mobility observable by SDS-PAGE (relative to a normal heavy chain), lectin binding affinity, binding to an anti-Fc antibody, and apparent higher molecular weight of antibody complexes containing the glyco-heavy variant as determined by size exclusion chromatography. See U.S. Provisional Application Ser. No. 61/525,307 (Atty. Docket No. 67858.730200), filed August 31, 2011 which is incorporated by reference herein in its entirety.

[00231] The term "polyploid yeast that stably expresses or expresses a desired secreted heterologous polypeptide for prolonged time" refers to a yeast culture that secretes said polypeptide for at least several days to a week, more preferably at least a month, still more preferably at least 1-6 months, and even more preferably for more than a year at threshold expression levels, typically at least 50-500 mg/liter (after about 90 hours in culture) and preferably substantially greater.

[00232] The term "polyploidal yeast culture that secretes desired amounts of recombinant polypeptide" refers to cultures that stably or for prolonged periods secrete at least at least 50-500 mg/liter, and most preferably 500-1000 mg/liter or more.

[00233] A polynucleotide sequence "corresponds" to a polypeptide sequence if translation of the polynucleotide sequence in accordance with the genetic code yields the polypeptide sequence (i.e., the polynucleotide sequence "encodes" the polypeptide sequence), one polynucleotide sequence "corresponds" to another polynucleotide sequence if the two sequences encode the same polypeptide sequence.

[00234] A "heterologous" region or domain of a DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous region is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

[00235] A "coding sequence" is an in-frame sequence of codons that (in view of the genetic code) correspond to or encode a protein or peptide sequence. Two coding sequences correspond to each other if the sequences or their complementary sequences encode the same amino acid sequences. A coding sequence in association with appropriate regulatory sequences may be transcribed and translated into a polypeptide. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence. A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. Promoter sequences typically contain additional sites for binding of regulatory molecules (e.g., transcription factors) which affect the transcription of the coding sequence. A coding sequence is "under the control" of the promoter sequence or "operatively linked" to the promoter when RNA polymerase binds the promoter sequence in a cell and transcribes the coding sequence into mRNA, which is then in turn translated into the protein encoded by the coding sequence.

[00236] Vectors are used to introduce a foreign substance, such as DNA, RNA or protein, into an organism or host cell. Typical vectors include recombinant viruses (for polynucleotides)

and liposomes (for polypeptides). A "DNA vector" is a replicon, such as plasmid, phage or cosmid, to which another polynucleotide segment may be attached so as to bring about the replication of the attached segment. An "expression vector" is a DNA vector which contains regulatory sequences which will direct polypeptide synthesis by an appropriate host cell. This usually means a promoter to bind RNA polymerase and initiate transcription of mRNA, as well as ribosome binding sites and initiation signals to direct translation of the mRNA into a polypeptide(s). Incorporation of a polynucleotide sequence into an expression vector at the proper site and in correct reading frame, followed by transformation of an appropriate host cell by the vector, enables the production of a polypeptide encoded by said polynucleotide sequence.

[00237] "Amplification" of polynucleotide sequences is the in vitro production of multiple copies of a particular nucleic acid sequence. The amplified sequence is usually in the form of DNA. A variety of techniques for carrying out such amplification are described in the following review articles, each of which is incorporated by reference herein in its entirety: Van Brunt 1990, *Bio/Technol.*, 8(4):291-294; and Gill and Ghaemi, *Nucleosides Nucleotides Nucleic Acids*. 2008 Mar;27(3):224-43. Polymerase chain reaction or PCR is a prototype of nucleic acid amplification, and use of PCR herein should be considered exemplary of other suitable amplification techniques.

[00238] The general structure of antibodies in most vertebrates (including mammals) is now well understood (Edelman, G. M., *Ann. N.Y. Acad. Sci.*, 190: 5 (1971)). Conventional antibodies consist of two identical light polypeptide chains of molecular weight approximately 23,000 daltons (the "light chain"), and two identical heavy chains of molecular weight 53,000-70,000 (the "heavy chain"). The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" configuration. The "branch" portion of the "Y" configuration is designated the F_{ab} region; the stem portion of the "Y" configuration is designated the F_C region. The amino acid sequence orientation runs from the N-terminal end at the top of the "Y" configuration to the C-terminal end at the bottom of each chain. The N-terminal end possesses the variable region having specificity for the antigen that elicited it, and is approximately 100 amino acids in length, there being slight variations between light and heavy chain and from antibody to antibody.

[00239] The variable region is linked in each chain to a constant region that extends the remaining length of the chain and that within a particular class of antibody does not vary with the specificity of the antibody (i.e., the antigen eliciting it). There are five known major classes of constant regions that determine the class of the immunoglobulin molecule (IgG, IgM, IgA, IgD, and IgE corresponding to gamma, mu, alpha, delta, and epsilon heavy chain constant regions). The constant region or class determines subsequent effector function of the antibody, including activation of complement (Kabat, E. A., *Structural Concepts in Immunology and Immunochemistry*, 2nd Ed., p. 413-436, Holt, Rinehart, Winston (1976)), and other cellular responses (Andrews, D. W., et al., *Clinical Immunobiology*, pp 1-18, W. B. Sanders (1980); Kohl, S., et al., *Immunology*, 48: 187 (1983)); while the variable region determines the antigen with which it will react. Light chains are classified as either kappa or lambda. Each heavy chain class can be paired with either kappa or lambda light chain. The light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages when the immunoglobulins are generated either by hybridomas or by B cells.

[00240] The expression "variable region" or "VR" refers to the domains within each pair of light and heavy chains in an antibody that are involved directly in binding the antibody to the antigen. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain (V_L) at one end and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain.

[00241] The expressions "complementarity determining region," "hypervariable region," or "CDR" refer to one or more of the hyper-variable or complementarity determining regions (CDRs) found in the variable regions of light or heavy chains of an antibody (See Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, Md., (1987)). These expressions include the hypervariable regions as defined by Kabat et al. ("*Sequences of Proteins of Immunological Interest*," Kabat E., et al., US Dept. of Health and Human Services, 1983) or the hypervariable loops in 3-dimensional structures of antibodies (Chothia and Lesk, *J Mol. Biol.* 196 901-917 (1987)). The CDRs in each chain are held in close

proximity by framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site. Within the CDRs there are select amino acids that have been described as the selectivity determining regions (SDRs) which represent the critical contact residues used by the CDR in the antibody-antigen interaction (Kashmiri, S., Methods, 36:25-34 (2005)).

[00242] The expressions "framework region" or "FR" refer to one or more of the framework regions within the variable regions of the light and heavy chains of an antibody (See Kabat, E. A. et al., Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, Md., (1987)). These expressions include those amino acid sequence regions interposed between the CDRs within the variable regions of the light and heavy chains of an antibody.

[00243] *Anti-NGF Antibodies and Binding Fragments Thereof Having Binding Activity for NGF*

[00244] Antibody Ab1

[00245] The invention contemplates methods of treating pain and the specific pain associated disorders using antibody Ab1 or fragments thereof, or an antibody or antibody fragment that binds to the same or overlapping epitope as Ab1, for example as set forth below, alone or in association with another active agent, e.g., an NSAID or opioid analgesic, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75 and/or inhibits or prevents pain. In one embodiment, the invention includes chimeric antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

ALVMTQTPSSVSAAVGGTVTINCQASQNIYSNLAWYQQRPGQRPKLLIYGASNLDAGV
PSRFRGSGSGTEYTLTISDLECDVGTYYCQSAFSDSTENTFGGGTEVVVKR (SEQ ID
NO: 1).

[00246] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or

opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

ALVMTQTPSSVSAAVGGTVTINCQASQNIYSNLAWYQQRPGQRPKLLIYGASNLDAGV
PSRFRGSGSGTEYTLTISDLECDDVGTYYCQSAFSDSTENTFGGGTEVVVKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 2).

[00247] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or is association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

[00248] QSLEESGGRLVTPGTPLTLTCTVSGFSLSSYAMSWVRQAPGKGLEWIGVI
TSIGSTVYASWAKGRFTISKSTTTVDLKITSPTTEDTATYFCARGYDDYDEMTYFNIWGQ
GTLVTVSS (SEQ ID NO: 3).

[00249] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or is association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

[00250] QSLEESGGRLVTPGTPLTLTCTVSGFSLSSYAMSWVRQAPGKGLEWIGVI
TSIGSTVYASWAKGRFTISKSTTTVDLKITSPTTEDTATYFCARGYDDYDEMTYFNIWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL
YSKLTVDKSRWQQGNVDFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 4).

[00251] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or is association with another active agent, e.g., an NSAID or

opioid analgesic, wherein the antibodies comprise one or more of the polypeptide sequences of SEQ ID NO: 5; SEQ ID NO: 6; and SEQ ID NO: 7 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 1 or the light chain sequence of SEQ ID NO: 2, and/or one or more of the polypeptide sequences of SEQ ID NO: 8; SEQ ID NO: 9; and SEQ ID NO: 10 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 3 or the heavy chain sequence of SEQ ID NO: 4, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00252] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibody is a fragment having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 3 or SEQ ID NO: 4.

[00253] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 5; SEQ ID NO: 6; and SEQ ID NO: 7 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 1 or the light chain sequence of SEQ ID NO: 2.

[00254] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include fragments having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 8; SEQ ID NO: 9; and SEQ ID NO: 10 which correspond to the complementarity-determining

regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 3 or the heavy chain sequence of SEQ ID NO: 4.

[00255] The invention also optionally contemplates antibody fragments which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 1; the variable heavy chain region of SEQ ID NO: 3; the complementarity-determining regions (SEQ ID NO: 5; SEQ ID NO: 6; and SEQ ID NO: 7) of the variable light chain region of SEQ ID NO: 1; and the complementarity-determining regions (SEQ ID NO: 8; SEQ ID NO: 9; and SEQ ID NO: 10) of the variable heavy chain region of SEQ ID NO: 3.

[00256] In a particularly preferred optional embodiment of the invention, the chimeric anti-NGF antibody is Ab1, comprising, or alternatively consisting of, SEQ ID NO: 2 and SEQ ID NO: 4, and having at least one of the biological activities set forth herein.

[00257] In a further particularly preferred optional embodiment of the invention, antibody fragments for use herein comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab1. With respect to antibody Ab1, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 1 and the variable heavy chain sequence of SEQ ID NO: 3. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 1 and/or SEQ ID NO: 3 in said Fab while retaining binding specificity for NGF.

[00258] In one optional embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab1. In another embodiment of the invention, anti-NGF antibodies such as Ab1 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00259] Antibody Ab2

[00260] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or is association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric or humanized antibodies having binding specificity to NGF wherein the antibody is antibody Ab2 or fragments thereof, or an antibody or antibody fragment that binds to the same or overlapping epitope as Ab2, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVTITCQASQNIYSNLAWYQQKPGKAPKLLIYGASNLDAGVP
SRFSGSGSGTEYTLTISSLQPDDFATYYCQSAFSDSTENTFGGGTKVEIKR (SEQ ID NO:
11).

[00261] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVTITCQASQNIYSNLAWYQQKPGKAPKLLIYGASNLDAGVP
SRFSGSGSGTEYTLTISSLQPDDFATYYCQSAFSDSTENTFGGGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 12).

[00262] The invention further optionally includes chimeric or humanized antibodies having binding specificity to NGF for treatment or prevention of pain and pain associated conditions and possessing a variable heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSSYAMSWVRQAPGKGLEWVGIVTSIGSTV
YASSAKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGYDDYDEMTYFNIWGQGT
LVTVSS (SEQ ID NO: 13).

[00263] The invention also optionally includes chimeric or humanized antibodies having binding specificity to NGF for treatment or prevention of pain and pain associated conditions and

possessing a heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSSYAMSWVRQAPGKGLEWVGVITSIGSTV
YASSAKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGYDDYDEMTYFNIWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
KLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID NO: 14).

[00264] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 15; SEQ ID NO: 16; and SEQ ID NO: 17 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 11 or the light chain sequence of SEQ ID NO: 12, and/or one or more of the polypeptide sequences of SEQ ID NO: 18; SEQ ID NO: 19; and SEQ ID NO: 20 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 13 or the heavy chain sequence of SEQ ID NO: 14, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00265] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 11 or SEQ ID NO: 12. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 13 or SEQ ID NO: 14.

[00266] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions

optionally comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 15; SEQ ID NO: 16; and SEQ ID NO: 17 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 11 or the light chain sequence of SEQ ID NO: 12.

[00267] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 18; SEQ ID NO: 19; and SEQ ID NO: 20 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 13 or the heavy chain sequence of SEQ ID NO: 14.

[00268] The invention also optionally contemplates antibody fragments which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 11; the variable heavy chain region of SEQ ID NO: 13; the complementarity-determining regions (SEQ ID NO: 15; SEQ ID NO: 16; and SEQ ID NO: 17) of the variable light chain region of SEQ ID NO: 11; and the complementarity-determining regions (SEQ ID NO: 18; SEQ ID NO: 19; and SEQ ID NO: 20) of the variable heavy chain region of SEQ ID NO: 13.

[00269] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions optionally is Ab2, comprising, or alternatively consisting of, SEQ ID NO: 12 and SEQ ID NO: 14, and having at least one of the biological activities set forth herein.

[00270] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF or a Fab or other monovalent antibody fragment that binds to the same or overlapping epitope as Ab2,. With respect to antibody Ab2, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 11 and the variable heavy chain sequence of SEQ ID NO: 13.

This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 11 and/or SEQ ID NO: 13 in said Fab while retaining binding specificity for NGF.

[00271] In one optional embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab2. In another embodiment of the invention, anti-NGF antibodies such as Ab2 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00272] Antibody Ab3

[00273] The invention contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric or humanized antibodies having binding specificity to NGF wherein the antibody is Ab3 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab3, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75. In one embodiment, the invention includes chimeric antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AVLTQTPSPVSAAMGDTVTIKCQSSQSVYKNNYLSWYQQKPGQPPRLLIYDASNLP SGV
PSRFSGSGSGTQFTLTISGVQCDDAATYYCLGDYDDDADNAFGGGTEVVVKR (SEQ ID
NO: 21).

[00274] The invention also includes chimeric antibodies or treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AVLTQTPSPVSAAMGDTVTIKCQSSQSVYKNNYLSWYQQKPGQPPRLLIYDASNLP SGV
PSRFSGSGSGTQFTLTISGVQCDDAATYYCLGDYDDDADNAFGGGTEVVVKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 22).

[00275] The invention further includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QSVEESGGRLVTPGTPLTLTCTVSGFSLSSYVMIWVRQAPGKGLEYIGITWSAGTYYYAS
WAKGRFTISKTSSTTVDLKITSPTTEDTATYFCAGGGGSIYDIWGPGTLVTVSS (SEQ ID
NO: 23).

[00276] The invention also includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QSVEESGGRLVTPGTPLTLTCTVSGFSLSSYVMIWVRQAPGKGLEYIGITWSAGTYYYAS
WAKGRFTISKTSSTTVDLKITSPTTEDTATYFCAGGGGSIYDIWGPGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 24).

[00277] The invention further contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 25; SEQ ID NO: 26; and SEQ ID NO: 27 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 21 or the light chain sequence of SEQ ID NO: 22, and/or one or more of the polypeptide sequences of SEQ ID NO: 28; SEQ ID NO: 29; and SEQ ID NO: 30 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 23 or the heavy chain sequence of SEQ ID NO: 24, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00278] The invention also contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 21 or SEQ ID NO: 22. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 23 or SEQ ID NO: 24.

[00279] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 25; SEQ ID NO: 26; and SEQ ID NO: 27 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 21 or the light chain sequence of SEQ ID NO: 22.

[00280] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 28; SEQ ID NO: 29; and SEQ ID NO: 30 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 23 or the heavy chain sequence of SEQ ID NO: 24.

[00281] The invention also optionally contemplates antibody fragments which include one or more of the antibody fragments described herein for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 21; the variable heavy chain region of SEQ ID NO: 23; the complementarity-determining regions (SEQ ID NO: 25; SEQ ID NO: 26; and SEQ ID NO: 27) of the variable light chain region of SEQ ID NO: 21; and the complementarity-determining regions (SEQ ID NO: 28; SEQ ID NO: 29; and SEQ ID NO: 30) of the variable heavy chain region of SEQ ID NO: 23.

[00282] In a particularly preferred optional embodiment of the invention, the chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab3,

comprising, or alternatively consisting of, SEQ ID NO: 22 and SEQ ID NO: 24, and having at least one of the biological activities set forth herein.

[00283] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab3. With respect to antibody Ab3, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 21 and the variable heavy chain sequence of SEQ ID NO: 23. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 21 and/or SEQ ID NO: 23 in said Fab while retaining binding specificity for NGF.

[00284] In one optional embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab3. In another embodiment of the invention, anti-NGF antibodies such as Ab3 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00285] Antibody Ab4

[00286] The invention contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric or humanized antibodies having binding specificity to NGF wherein the antibody is antibody Ab4 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab4, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75 and/or for preventing or effectively treating pain. In one embodiment, the invention includes humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

[00287] DIQMTQSPSTLSASVGDRVITTCQSSQSVYKNNYLSWYQQKPGKAPKLLIYDASNLPSGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCLGDYDDDADNAFGGGTKVEIKR (SEQ ID NO: 31).

[00288] The invention also includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:
DIQMTQSPSTLSASVGDRVITTCQSSQSVYKNNYLSWYQQKPGKAPKLLIYDASNLPSGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCLGDYDDDADNAFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRFAKVKWDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 32).

[00289] The invention further includes humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:
EVQLVESGGGLVQPGGSLRLSCAASGFTVSSYVMWVRQAPGKGLYIGITWSAGTYYASSAKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAGGGGSIYDIWGQGTLLTVSS (SEQ ID NO: 33).

[00290] The invention also includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:
EVQLVESGGGLVQPGGSLRLSCAASGFTVSSYVMWVRQAPGKGLYIGITWSAGTYYASSAKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAGGGGSIYDIWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 34).

[00291] The invention further contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID

NO: 35; SEQ ID NO: 36; and SEQ ID NO: 37 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 31 or the light chain sequence of SEQ ID NO: 32, and/or one or more of the polypeptide sequences of SEQ ID NO: 38; SEQ ID NO: 39; and SEQ ID NO: 40 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 33 or the heavy chain sequence of SEQ ID NO: 34, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00292] The invention also contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 31 or SEQ ID NO: 32. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

[00293] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 35; SEQ ID NO: 36; and SEQ ID NO: 37 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 31 or the light chain sequence of SEQ ID NO: 32.

[00294] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 38; SEQ ID NO: 39; and SEQ ID NO: 40 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 33 or the heavy chain sequence of SEQ ID NO: 34.

[00295] The invention also optional contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 31; the variable heavy chain region of SEQ ID NO: 33; the complementarity-determining regions (SEQ ID NO: 35; SEQ ID NO: 36; and SEQ ID NO: 37) of the variable light chain region of SEQ ID NO: 31; and the complementarity-determining regions (SEQ ID NO: 38; SEQ ID NO: 39; and SEQ ID NO: 40) of the variable heavy chain region of SEQ ID NO: 33.

[00296] In a particularly preferred embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab4, comprising, or alternatively consisting of, SEQ ID NO: 32 and SEQ ID NO: 34, and having at least one of the biological activities set forth herein.

[00297] In a further particularly preferred embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab4. With respect to antibody Ab4, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 31 and the variable heavy chain sequence of SEQ ID NO: 33. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 31 and/or SEQ ID NO: 33 in said Fab while retaining binding specificity for NGF.

[00298] In one embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab4. In another embodiment of the invention, anti-NGF antibodies such as Ab4 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00299] Antibody Ab5

[00300] The invention contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies optionally include Ab5 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab5, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AYDMTQTPASVEVAVGGTVTIKCQASQSIYSNLAWYQQRPGQPPKLLIYDASTLESGVP
SRFKGSGSGTEYTLTISGVECADAAASYCQQGFTVSDIDNAFGGGTEVVVKR (SEQ ID
NO: 41).

[00301] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AYDMTQTPASVEVAVGGTVTIKCQASQSIYSNLAWYQQRPGQPPKLLIYDASTLESGVP
SRFKGSGSGTEYTLTISGVECADAAASYCQQGFTVSDIDNAFGGGTEVVVKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 42).

[00302] The invention further optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QSVEESGGRLVTPGTPLTLTCTVSGFSLSNYAVGWVRQAPGKGLEWIGIIGRNGNTWYA
SWARGRFTISKSTTTVDLKITSPTSEDATYFCARGYGRSVAYYVFNIWGPGLTVTVSS
(SEQ ID NO: 43).

[00303] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QSVEESGGRLVTPGTPLTLTCTVSGFSLSNYAVGWVRQAPGKGLEWIGIIGRNGNTWYA
SWARGRFTISKSTTTVDLKITSPTSEDATYFCARGYGRSVAYYVFNIWGPGLTVTVSSA

STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
 EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 44).

[00304] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 45; SEQ ID NO: 46; and SEQ ID NO: 47 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 41 or the light chain sequence of SEQ ID NO: 42, and/or one or more of the polypeptide sequences of SEQ ID NO: 48; SEQ ID NO: 49; and SEQ ID NO: 50 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 43 or the heavy chain sequence of SEQ ID NO: 44, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof optionally comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00305] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 41 or SEQ ID NO: 42. In another optional embodiment of the invention, antibody fragments of the invention for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 43 or SEQ ID NO: 44.

[00306] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 45; SEQ ID NO: 46; and SEQ ID NO: 47 which correspond to the

complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 41 or the light chain sequence of SEQ ID NO: 42.

[00307] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions optionally comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 48; SEQ ID NO: 49; and SEQ ID NO: 50 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 43 or the heavy chain sequence of SEQ ID NO: 44.

[00308] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 41; the variable heavy chain region of SEQ ID NO: 43; the complementarity-determining regions (SEQ ID NO: 45; SEQ ID NO: 46; and SEQ ID NO: 47) of the variable light chain region of SEQ ID NO: 41; and the complementarity-determining regions (SEQ ID NO: 48; SEQ ID NO: 49; and SEQ ID NO: 50) of the variable heavy chain region of SEQ ID NO: 43.

[00309] In a particularly preferred optional embodiment of the invention, the optionally included chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab5, comprising, or alternatively consisting of, SEQ ID NO: 42 and SEQ ID NO: 44, and having at least one of the biological activities set forth herein.

[00310] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF or another Fab or antibody fragment that binds to the same or overlapping epitope as Ab5. With respect to antibody Ab5, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 41 and the variable heavy chain sequence of SEQ ID NO: 43. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 41 and/or SEQ ID NO: 43 in said Fab while retaining binding specificity for NGF.

[00311] In one embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab5. In another embodiment of the invention, anti-NGF antibodies such as Ab5 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00312] Antibody Ab6

[00313] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include Ab6 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab6, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVTITCQASQSIYSNLAWYQQKPGKAPKLLIYDASTLESGVPS
RFGSGSGTEYTLTISSLQPDDFATYYCQQGFTVSDIDNAFGGGTKVEIKR (SEQ ID NO:
51).

[00314] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVTITCQASQSIYSNLAWYQQKPGKAPKLLIYDASTLESGVPS
RFGSGSGTEYTLTISSLQPDDFATYYCQQGFTVSDIDNAFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
SLTTLTKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 52).

[00315] The invention further optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSNYAVGWVRQAPGKGLEWVGIIGRNGNT
WYASSARGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGYGRSVAYYVFNIWGP GT
LVTVSS (SEQ ID NO: 53).

[00316] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSNYAVGWVRQAPGKGLEWVGIIGRNGNT
WYASSARGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGYGRSVAYYVFNIWGP GT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 54).

[00317] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 52, and/or one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 54, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00318] The invention also optionally contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention comprise, or

alternatively consist of, the polypeptide sequence of SEQ ID NO: 51 or SEQ ID NO: 52. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 53 or SEQ ID NO: 54.

[00319] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 52.

[00320] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 54.

[00321] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 51; the variable heavy chain region of SEQ ID NO: 53; the complementarity-determining regions (SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57) of the variable light chain region of SEQ ID NO: 51; and the complementarity-determining regions (SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60) of the variable heavy chain region of SEQ ID NO: 53.

[00322] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab6, comprising, or alternatively consisting of, SEQ ID NO: 52 and SEQ ID NO: 54, and having at least one of the biological activities set forth herein.

[00323] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab6. With respect to antibody Ab6, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 51 and the variable heavy chain sequence of SEQ ID NO: 53. This optional embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 51 and/or SEQ ID NO: 53 in said Fab while retaining binding specificity for NGF.

[00324] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab6. In another embodiment of the invention, anti-NGF antibodies such as Ab6 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00325] Antibody Ab7

[00326] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include Ab7 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab7, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

ADVVMQTQTPASVSQPVGGTVTIKCQASEDIYNLLAWYQQKPGQPPKLLIYSASTLASGV
PSRFKSGSGSTEYTLTISGLECADAATYYCQNNYLVTITYGVAFGGGTEVVVKR (SEQ
ID NO: 61).

[00327] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

ADVVMTQTPASVSQPVGTVTIKCQASEDIYNLLAWYQQKPGQPPLLIYSASTLASGV
PSRFKSGSGTEYTLTISGLECADAATYYCQNNYLVTITYGVAFGGGTEVVKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 62).

[00328] The invention further optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QEQLKESGGRLVTPGTPLTLCTVSGFSLSSYAMIWVRQAPGKGLEYIGYIDTSDTSAYYA
SWVKGRFTISRTSTTVDLKITSPTTEDTATYFCARSYAAAYGGYPATFDPWGPGTLVTVSS
(SEQ ID NO: 63).

[00329] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QEQLKESGGRLVTPGTPLTLCTVSGFSLSSYAMIWVRQAPGKGLEYIGYIDTSDTSAYYA
SWVKGRFTISRTSTTVDLKITSPTTEDTATYFCARSYAAAYGGYPATFDPWGPGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVKDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 64).

[00330] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 65; SEQ ID NO: 66; and SEQ ID NO: 67 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 61 or the light chain sequence of SEQ ID NO: 62, and/or one or

more of the polypeptide sequences of SEQ ID NO: 68; SEQ ID NO: 69; and SEQ ID NO: 70 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 63 or the heavy chain sequence of SEQ ID NO: 64, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00331] The invention also optionally contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 61 or SEQ ID NO: 62. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 63 or SEQ ID NO: 64.

[00332] In a further optional embodiment of the invention, fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 65; SEQ ID NO: 66; and SEQ ID NO: 67 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 61 or the light chain sequence of SEQ ID NO: 62.

[00333] In a further optional embodiment of the invention, fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 68; SEQ ID NO: 69; and SEQ ID NO: 70 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 63 or the heavy chain sequence of SEQ ID NO: 64.

[00334] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more,

including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 61; the variable heavy chain region of SEQ ID NO: 63; the complementarity-determining regions (SEQ ID NO: 65; SEQ ID NO: 66; and SEQ ID NO: 67) of the variable light chain region of SEQ ID NO: 61; and the complementarity-determining regions (SEQ ID NO: 68; SEQ ID NO: 69; and SEQ ID NO: 70) of the variable heavy chain region of SEQ ID NO: 63.

[00335] In a particularly preferred optional embodiment of the invention, the chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab7, comprising, or alternatively consisting of, SEQ ID NO: 62 and SEQ ID NO: 64, and having at least one of the biological activities set forth herein.

[00336] In a further particularly optionally preferred embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab7. With respect to antibody Ab7, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 61 and the variable heavy chain sequence of SEQ ID NO: 63. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 61 and/or SEQ ID NO: 63 in said Fab while retaining binding specificity for NGF.

[00337] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab7. In another embodiment of the invention, anti-NGF antibodies such as Ab7 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00338] Antibody Ab8

[00339] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include Ab8 or fragments thereof, or another antibody

or fragment that binds to the same or overlapping epitope as Ab8, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

DIQMTQSPSSLSASVGDRVTITCQASEDIYNLLAWYQQKPGKVPKLLIYSASTLASGVPS
RFSGSGSGTDYTLTISSLQPEDVATYYCQNNYLVTITYGVAFGGGTKVEIKR (SEQ ID
NO: 71).

[00340] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

DIQMTQSPSSLSASVGDRVTITCQASEDIYNLLAWYQQKPGKVPKLLIYSASTLASGVPS
RFSGSGSGTDYTLTISSLQPEDVATYYCQNNYLVTITYGVAFGGGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYSL
STLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 72).

[00341] The invention further optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMIWVRQAPGKGLEYIGYIDTDTSAYY
ASSVKGRFTISRDN SKNTLYLQMSSLRAEDTAVYYCARSYAAYGGYPATFDPWGQGTL
VTVSS (SEQ ID NO: 73).

[00342] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMIWVRQAPGKGLEYIGYIDTDTSAYY
ASSVKGRFTISRDN SKNTLYLQMSSLRAEDTAVYYCARSYAAYGGYPATFDPWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSK
LTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 74).

[00343] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 75; SEQ ID NO: 76; and SEQ ID NO: 77 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 71 or the light chain sequence of SEQ ID NO: 72, and/or one or more of the polypeptide sequences of SEQ ID NO: 78; SEQ ID NO: 79; and SEQ ID NO: 80 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 73 or the heavy chain sequence of SEQ ID NO: 74, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00344] The invention also optionally contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 71 or SEQ ID NO: 72. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 73 or SEQ ID NO: 74.

[00345] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 75; SEQ ID NO: 76; and SEQ ID NO: 77 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 71 or the light chain sequence of SEQ ID NO: 72.

[00346] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated

conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 78; SEQ ID NO: 79; and SEQ ID NO: 80 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 73 or the heavy chain sequence of SEQ ID NO: 74.

[00347] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 71; the variable heavy chain region of SEQ ID NO: 73; the complementarity-determining regions (SEQ ID NO: 75; SEQ ID NO: 76; and SEQ ID NO: 77) of the variable light chain region of SEQ ID NO: 71; and the complementarity-determining regions (SEQ ID NO: 78; SEQ ID NO: 79; and SEQ ID NO: 80) of the variable heavy chain region of SEQ ID NO: 73.

[00348] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab8, comprising, or alternatively consisting of, SEQ ID NO: 72 and SEQ ID NO: 74, and having at least one of the biological activities set forth herein.

[00349] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab8, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 71 and the variable heavy chain sequence of SEQ ID NO: 73 or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab8. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 71 and/or SEQ ID NO: 73 in said Fab while retaining binding specificity for NGF.

[00350] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab8. In another embodiment of the invention, anti-NGF antibodies such as Ab8 or Fab fragments thereof may be produced via expression in mammalian

cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00351] *Antibody Ab9*

[00352] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include Ab9 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab9, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one optional embodiment, the invention includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AYDMTQTPASVSAAVGGTVTIKCQASENIGSYLAWYQQKPGQPPELLIYRASTLASGVP
SRFKGSGSGTQFTLTISGVECADEAATYYCQQGYNSENLDNAFGGGTEVVVKR (SEQ ID
NO: 81).

[00353] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AYDMTQTPASVSAAVGGTVTIKCQASENIGSYLAWYQQKPGQPPELLIYRASTLASGVP
SRFKGSGSGTQFTLTISGVECADEAATYYCQQGYNSENLDNAFGGGTEVVVKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 82).

[00354] The invention further optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QSVEESGGRLVTPGTPLTLTCTVSGIDLSMYSMGWVRQAPGKGLEYIGWISYGGTAYYA
SWAKGRFTISKSTSTVELKITSPTIEDTATYFCARETPVNYLDIWGQGTLLTVSS (SEQ
ID NO: 83).

[00355] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QSVEESGGRLVTPGTPLTLTCTVSGIDLSMYSMGWVRQAPGKGLEYIGWISYGGTAYYA
 SWAKGRFTISKSTTTVELKITSPTIEDTATYFCARETPVNYLDIWGQGTLTVSSASTKG
 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFL
 FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT
 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQ
 QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 84).

[00356] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 85; SEQ ID NO: 86; and SEQ ID NO: 87 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 81 or the light chain sequence of SEQ ID NO: 82, and/or one or more of the polypeptide sequences of SEQ ID NO: 88; SEQ ID NO: 89; and SEQ ID NO: 90 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 83 or the heavy chain sequence of SEQ ID NO: 84, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00357] The invention also optionally contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 81 or SEQ ID NO: 82. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 83 or SEQ ID NO: 84.

[00358] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 85; SEQ ID NO: 86; and SEQ ID NO: 87 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 81 or the light chain sequence of SEQ ID NO: 82.

[00359] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 88; SEQ ID NO: 89; and SEQ ID NO: 90 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 83 or the heavy chain sequence of SEQ ID NO: 84.

[00360] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 81; the variable heavy chain region of SEQ ID NO: 83; the complementarity-determining regions (SEQ ID NO: 85; SEQ ID NO: 86; and SEQ ID NO: 87) of the variable light chain region of SEQ ID NO: 81; and the complementarity-determining regions (SEQ ID NO: 88; SEQ ID NO: 89; and SEQ ID NO: 90) of the variable heavy chain region of SEQ ID NO: 83.

[00361] In a particularly preferred optional embodiment of the invention, the chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab9, comprising, or alternatively consisting of, SEQ ID NO: 82 and SEQ ID NO: 84, and having at least one of the biological activities set forth herein.

[00362] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab9, the Fab fragment includes the variable light chain sequence

of SEQ ID NO: 81 and the variable heavy chain sequence of SEQ ID NO: 83 or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab9. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 81 and/or SEQ ID NO: 83 in said Fab while retaining binding specificity for NGF.

[00363] In one embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab9. In another optional embodiment of the invention, anti-NGF antibodies such as Ab9 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00364] *Antibody Ab10*

[00365] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include Ab10 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab10, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AYDMTQSPSSLSASVGDRVTITCQASENIGSYLAWYQQKPGKVPKLLIYRASTLASGVPS
RFSGSGSGTDFTLTISLQPEDVATYYCQQGYNSENLDNAFGGGTKVEIKR (SEQ ID
NO: 91).

[00366] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AYDMTQSPSSLSASVGDRVTITCQASENIGSYLAWYQQKPGKVPKLLIYRASTLASGVPS
RFSGSGSGTDFTLTISLQPEDVATYYCQQGYNSENLDNAFGGGTKVEIKRTVAAPSVFI

FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 92).

[00367] The invention further optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:
QVQLVESGGGVVQPGRSLRLSCAASGFTFSMYSMGWVRQAPGKGLEYIGWISYGGTAY
YASSAKGRFTISRDN SKNTLYLQMSSLRAEDTAVYYCARETPVNYLDIWGQGTLVTV
SS (SEQ ID NO: 93).

[00368] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:
QVQLVESGGGVVQPGRSLRLSCAASGFTFSMYSMGWVRQAPGKGLEYIGWISYGGTAY
YASSAKGRFTISRDN SKNTLYLQMSSLRAEDTAVYYCARETPVNYLDIWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 94).

[00369] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 95; SEQ ID NO: 96; and SEQ ID NO: 97 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 91 or the light chain sequence of SEQ ID NO: 92, and/or one or more of the polypeptide sequences of SEQ ID NO: 98; SEQ ID NO: 99; and SEQ ID NO: 100 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 93 or the heavy chain sequence of SEQ ID NO: 94, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist

of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00370] The invention also optionally contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 91 or SEQ ID NO: 92. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 93 or SEQ ID NO: 94.

[00371] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions optionally comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 95; SEQ ID NO: 96; and SEQ ID NO: 97 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 91 or the light chain sequence of SEQ ID NO: 92.

[00372] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 98; SEQ ID NO: 99; and SEQ ID NO: 100 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 93 or the heavy chain sequence of SEQ ID NO: 94.

[00373] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 91; the variable heavy chain region of SEQ ID NO: 93; the complementarity-determining regions (SEQ ID NO: 95; SEQ ID NO: 96; and SEQ ID NO: 97) of the variable light chain region of SEQ ID NO: 91; and the

complementarity-determining regions (SEQ ID NO: 98; SEQ ID NO: 99; and SEQ ID NO: 100) of the variable heavy chain region of SEQ ID NO: 93.

[00374] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab10, comprising, or alternatively consisting of, SEQ ID NO: 92 and SEQ ID NO: 94, and having at least one of the biological activities set forth herein.

[00375] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab10, the Fab fragment for treatment or prevention of pain and pain associated conditions includes the variable light chain sequence of SEQ ID NO: 91 and the variable heavy chain sequence of SEQ ID NO: 93 or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab10. This embodiment of the invention further optionally contemplates additions, deletions, and variants of SEQ ID NO: 91 and/or SEQ ID NO: 93 in said Fab while retaining binding specificity for NGF.

[00376] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab10. In another embodiment of the invention, anti-NGF antibodies such as Ab10 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00377] Antibody Ab11

[00378] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include Ab11 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab11, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with

TrkA and the association of NGF with p75. In one optional embodiment, the invention includes chimeric antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AFELTQTPSSVEAAVGGTVTIKCQASQNIVTNLAWYQQKPGQPPKLLIYGASTLASGVSS
RFKGS GSGTQFTLTISDLECADAAATYFCQSYDGFNSAGFGGGTEVVVKR (SEQ ID NO:
101).

[00379] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AFELTQTPSSVEAAVGGTVTIKCQASQNIVTNLAWYQQKPGQPPKLLIYGASTLASGVSS
RFKGS GSGTQFTLTISDLECADAAATYFCQSYDGFNSAGFGGGTEVVVKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 102).

[00380] The invention further optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QSLEESGGRLVTPGTPLTLTCTASGFSLSGYDMSWVRQAPGKGLEYIGLISYDGNTYYA
TWAKGRFTISKSTSTTVDLKITSPTTEDTATYFCARSLYAGPNAGIGPFNIWGQGLTVTVSS
(SEQ ID NO: 103).

[00381] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QSLEESGGRLVTPGTPLTLTCTASGFSLSGYDMSWVRQAPGKGLEYIGLISYDGNTYYA
TWAKGRFTISKSTSTTVDLKITSPTTEDTATYFCARSLYAGPNAGIGPFNIWGQGLTVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR

EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
SRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 104).

[00382] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 105; SEQ ID NO: 106; and SEQ ID NO: 107 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 101 or the light chain sequence of SEQ ID NO: 102, and/or one or more of the polypeptide sequences of SEQ ID NO: 108; SEQ ID NO: 109; and SEQ ID NO: 110 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 103 or the heavy chain sequence of SEQ ID NO: 104, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof optionally comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00383] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 101 or SEQ ID NO: 102. In another optional embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 103 or SEQ ID NO: 104.

[00384] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 105; SEQ ID NO: 106; and SEQ ID NO: 107 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 101 or the light chain sequence of SEQ ID NO: 102.

[00385] In a further optional embodiment of the invention, fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF

comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 108; SEQ ID NO: 109; and SEQ ID NO: 110 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 103 or the heavy chain sequence of SEQ ID NO: 104.

[00386] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 101; the variable heavy chain region of SEQ ID NO: 103; the complementarity-determining regions (SEQ ID NO: 105; SEQ ID NO: 106; and SEQ ID NO: 107) of the variable light chain region of SEQ ID NO: 101; and the complementarity-determining regions (SEQ ID NO: 108; SEQ ID NO: 109; and SEQ ID NO: 110) of the variable heavy chain region of SEQ ID NO: 103.

[00387] In a particularly preferred optional embodiment of the invention, the chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab11, comprising, or alternatively consisting of, SEQ ID NO: 102 and SEQ ID NO: 104, and having at least one of the biological activities set forth herein.

[00388] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab11, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 101 and the variable heavy chain sequence of SEQ ID NO: 103 or comprises another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab11. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 101 and/or SEQ ID NO: 103 in said Fab while retaining binding specificity for NGF.

[00389] In one optional embodiment of the invention described herein (infra), Fab fragments may for treatment or prevention of pain and pain associated conditions be produced by enzymatic digestion (e.g., papain) of Ab11. In another optional embodiment of the invention,

anti-NGF antibodies such as Ab11 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00390] *Antibody Ab12*

[00391] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include Ab12 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab12, or example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AFQMTQSPSSLSASVGDRVTITCQASQNIVTNLAWYQQKPGKVPKLLIYGASTLASGVP
SRFSGSGSGTDFTLTISSLQPEDVATYYCQSYDGFNSAGFGGGTKVEIKR (SEQ ID NO:
111).

[00392] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AFQMTQSPSSLSASVGDRVTITCQASQNIVTNLAWYQQKPGKVPKLLIYGASTLASGVP
SRFSGSGSGTDFTLTISSLQPEDVATYYCQSYDGFNSAGFGGGTKVEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSST
LTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 112).

[00393] The invention further optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QVQLVESGGGVVQPGRSLRLSCAASGFSLSGYDMSWVRQAPGKGLEWVGLISYDGNT

YYATSAKGRFTISRDN SKNTLYLQMSSLRAEDTAVYYCARSLYAGPNAGIGPFNIWGQG
TLVTVSS (SEQ ID NO: 113).

[00394] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QVQLVESGGGVVQPGRSLRLSCAASGFSLSGYDMSWVRQAPGKGLEWVGLISYDGNT
YYATSAKGRFTISRDN SKNTLYLQMSSLRAEDTAVYYCARSLYAGPNAGIGPFNIWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCP
APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 114).

[00395] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 115; SEQ ID NO: 116; and SEQ ID NO: 117 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 111 or the light chain sequence of SEQ ID NO: 112, and/or one or more of the polypeptide sequences of SEQ ID NO: 118; SEQ ID NO: 119; and SEQ ID NO: 120 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 113 or the heavy chain sequence of SEQ ID NO: 114, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00396] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one optional embodiment of the invention, antibody fragments of the invention comprise, or

alternatively consist of, the polypeptide sequence of SEQ ID NO: 111 or SEQ ID NO: 112. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 113 or SEQ ID NO: 114.

[00397] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 115; SEQ ID NO: 116; and SEQ ID NO: 117 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 111 or the light chain sequence of SEQ ID NO: 112.

[00398] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 118; SEQ ID NO: 119; and SEQ ID NO: 120 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 113 or the heavy chain sequence of SEQ ID NO: 114.

[00399] The invention also optionally contemplates antibody fragments which include one or more of the antibody fragments described herein. In one optional embodiment of the invention, fragments of the antibodies having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 111; the variable heavy chain region of SEQ ID NO: 113; the complementarity-determining regions (SEQ ID NO: 115; SEQ ID NO: 116; and SEQ ID NO: 117) of the variable light chain region of SEQ ID NO: 111; and the complementarity-determining regions (SEQ ID NO: 118; SEQ ID NO: 119; and SEQ ID NO: 120) of the variable heavy chain region of SEQ ID NO: 113.

[00400] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab12, comprising, or alternatively consisting of, SEQ ID NO: 112 and SEQ ID NO: 114, and having at least one of the biological activities set forth herein.

[00401] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab12, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 111 and the variable heavy chain sequence of SEQ ID NO: 113 or comprises another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab12. This optional embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 111 and/or SEQ ID NO: 113 in said Fab while retaining binding specificity for NGF.

[00402] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab12. In another embodiment of the invention, anti-NGF antibodies such as Ab12 or Fab fragments thereof for treatment or prevention of pain and pain associated conditions may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00403] Antibody Ab13

[00404] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include Ab13 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab13, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AAVLTQTPSPVSAAVGGTVSISCQSSQNVYKNNYLSWYQQKPGQPPKLLIYKASTLASG

VPSRFKGGGSGTDFTLTISDVQCDAATYYCAGGYTSSSDNAFGGGTEVVVKR (SEQ ID NO: 121).

[00405] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AAVLTQTPSPVSAAVGGTVSISCQSSQNVYKNNYLSWYQQKPGQPPKLLIYKASTLASG
VPSRFKGGGSGTDFTLTISDVQCDAATYYCAGGYTSSSDNAFGGGTEVVVKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 122).

[00406] The invention further optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:
QSVEASGGRLVTPGTPLTLTCTASGFSLSITYWMSWVRQAPGKGLEWIGDIYFSNEETNY
ASWAKGRFTISKSTTTVDLNVISPTTEDTATYFCARGSPDVDIGIDMWGPGLTVTVSS
(SEQ ID NO: 123).

[00407] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:
QSVEASGGRLVTPGTPLTLTCTASGFSLSITYWMSWVRQAPGKGLEWIGDIYFSNEETNY
ASWAKGRFTISKSTTTVDLNVISPTTEDTATYFCARGSPDVDIGIDMWGPGLTVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 124).

[00408] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 125; SEQ ID NO: 126; and SEQ ID NO: 127 which correspond to the

complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 121 or the light chain sequence of SEQ ID NO: 122, and/or one or more of the polypeptide sequences of SEQ ID NO: 128; SEQ ID NO: 129; and SEQ ID NO: 130 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 123 or the heavy chain sequence of SEQ ID NO: 124, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00409] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 121 or SEQ ID NO: 122. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 123 or SEQ ID NO: 124.

[00410] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 125; SEQ ID NO: 126; and SEQ ID NO: 127 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 121 or the light chain sequence of SEQ ID NO: 122.

[00411] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 128; SEQ ID NO: 129; and SEQ ID NO: 130 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 123 or the heavy chain sequence of SEQ ID NO: 124.

[00412] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 121; the variable heavy chain region of SEQ ID NO: 123; the complementarity-determining regions (SEQ ID NO: 125; SEQ ID NO: 126; and SEQ ID NO: 127) of the variable light chain region of SEQ ID NO: 121; and the complementarity-determining regions (SEQ ID NO: 128; SEQ ID NO: 129; and SEQ ID NO: 130) of the variable heavy chain region of SEQ ID NO: 123.

[00413] In a particularly preferred optional embodiment of the invention, the chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab13, comprising, or alternatively consisting of, SEQ ID NO: 122 and SEQ ID NO: 124, and having at least one of the biological activities set forth herein.

[00414] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab13, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 121 and the variable heavy chain sequence of SEQ ID NO: 123 or comprises another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as Ab13. This optional embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 121 and/or SEQ ID NO: 123 in said Fab while retaining binding specificity for NGF.

[00415] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab13. In another embodiment of the invention, anti-NGF antibodies such as Ab13 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00416] Antibody Ab14

[00417] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or is association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF wherein the antibody is Ab14 or fragments thereof, or another antibody or fragment that binds to the same or overlapping epitope as Ab14, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one optional embodiment, the invention includes chimeric or humanized antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

DIQMTQSPSSLSASVGDRVTITCQSSQNVYKNNYLSWYQQKPGKVPKLLIYKASTLASG
VPSRFSGSGSGTDFTLTISLQPEDVATYYCAGGYTSSSDNAFGGGTKVEIKR (SEQ ID
NO: 131).

[00418] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

DIQMTQSPSSLSASVGDRVTITCQSSQNVYKNNYLSWYQQKPGKVPKLLIYKASTLASG
VPSRFSGSGSGTDFTLTISLQPEDVATYYCAGGYTSSSDNAFGGGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 132).

[00419] The invention further optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSTYWMSWVRQAPGKGLEWVGDIYFSNEET
NYASSAKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGSPDVIDIGIDMWGPGLV
TVSS (SEQ ID NO: 133).

[00420] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSTYWMSWVRQAPGKGLEWVGDIYFSNEET
 NYASSAKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGSPDVDIGIDMWGPGLTV
 TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE
 LLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
 EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 134).

[00421] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 135; SEQ ID NO: 136; and SEQ ID NO: 137 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 131 or the light chain sequence of SEQ ID NO: 132, and/or one or more of the polypeptide sequences of SEQ ID NO: 138; SEQ ID NO: 139; and SEQ ID NO: 140 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 133 or the heavy chain sequence of SEQ ID NO: 134, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00422] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 131 or SEQ ID NO: 132. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 133 or SEQ ID NO: 134.

[00423] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated

conditions optionally comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 135; SEQ ID NO: 136; and SEQ ID NO: 137 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 131 or the light chain sequence of SEQ ID NO: 132.

[00424] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 138; SEQ ID NO: 139; and SEQ ID NO: 140 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 133 or the heavy chain sequence of SEQ ID NO: 134.

[00425] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 131; the variable heavy chain region of SEQ ID NO: 133; the complementarity-determining regions (SEQ ID NO: 135; SEQ ID NO: 136; and SEQ ID NO: 137) of the variable light chain region of SEQ ID NO: 131; and the complementarity-determining regions (SEQ ID NO: 138; SEQ ID NO: 139; and SEQ ID NO: 140) of the variable heavy chain region of SEQ ID NO: 133.

[00426] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab14, comprising, or alternatively consisting of, SEQ ID NO: 132 and SEQ ID NO: 134, and having at least one of the biological activities set forth herein.

[00427] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab14, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 131 and the variable heavy chain sequence of SEQ ID NO: 133 or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as

Ab14. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 131 and/or SEQ ID NO: 133 in said Fab while retaining binding specificity for NGF.

[00428] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab14. In another embodiment of the invention, anti-NGF antibodies such as Ab14 or Fab fragments thereof for treatment or prevention of pain and pain associated conditions may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00429] *Antibody Ab15*

[00430] The invention contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF wherein the antibody is Ab15 or fragments thereof, for example as set forth below, or comprises another antibody or antibody fragment that binds to the same or overlapping epitope as Ab15, in a therapeutically effective amount which inhibits the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75. In one embodiment, the invention includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AAVLTQTPSPVSAAVGDTVTIKCQSSQSVYKNNYLSWYQQKPGQPPKLLIYDASNLP
SGVPSRFSGSGSGTQFTLTISGVQCDDAATYYCLGDYDDDTDNGFGGGTEVVVKR (SEQ ID NO: 141).

[00431] The invention also includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AAVLTQTPSPVSAAVGDTVTIKCQSSQSVYKNNYLSWYQQKPGQPPKLLIYDASNLP

VPSRFSGSGSGTQFTLTISGVQCDDAATYYCLGDYDDDDTDNGFGGGTEVVVKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 142).

[00432] The invention further includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QSVEESGGRLVTPGTPLTLTCTVSGIDLSSYAMIWVRQAPGKGLEYIGIIWSGGTTYATW
AKGRFTISKSTTTVDLQITSPTTEDAATYFCAAGGGSYDVWGPGLTVTVSS (SEQ ID
NO: 143).

[00433] The invention also includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QSVEESGGRLVTPGTPLTLTCTVSGIDLSSYAMIWVRQAPGKGLEYIGIIWSGGTTYATW
AKGRFTISKSTTTVDLQITSPTTEDAATYFCAAGGGSYDVWGPGLTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTPVSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 144).

[00434] The invention further contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 145; SEQ ID NO: 146; and SEQ ID NO: 147 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 141 or the light chain sequence of SEQ ID NO: 142, and/or one or more of the polypeptide sequences of SEQ ID NO: 148; SEQ ID NO: 149; and SEQ ID NO: 150 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 143 or the heavy chain sequence of SEQ ID NO: 144, or combinations of these polypeptide sequences. In another embodiment of the invention,

the antibodies of the invention or fragments thereof for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00435] The invention also contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 141 or SEQ ID NO: 142. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 143 or SEQ ID NO: 144.

[00436] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 145; SEQ ID NO: 146; and SEQ ID NO: 147 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 141 or the light chain sequence of SEQ ID NO: 142.

[00437] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 148; SEQ ID NO: 149; and SEQ ID NO: 150 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 143 or the heavy chain sequence of SEQ ID NO: 144.

[00438] The invention also contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 141; the variable heavy chain region of SEQ ID NO: 143; the complementarity-determining regions (SEQ ID NO: 145; SEQ

ID NO: 146; and SEQ ID NO: 147) of the variable light chain region of SEQ ID NO: 141; and the complementarity-determining regions (SEQ ID NO: 148; SEQ ID NO: 149; and SEQ ID NO: 150) of the variable heavy chain region of SEQ ID NO: 143.

[00439] In a particularly preferred embodiment of the invention, the chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab15, comprising, or alternatively consisting of, SEQ ID NO: 142 and SEQ ID NO: 144, and having at least one of the biological activities set forth herein.

[00440] In a further particularly preferred embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab15, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 141 and the variable heavy chain sequence of SEQ ID NO: 143 or comprises another Fab that binds to the same or overlapping epitope as Ab15,. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 141 and/or SEQ ID NO: 143 in said Fab while retaining binding specificity for NGF.

[00441] In one embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab15. In another embodiment of the invention, anti-NGF antibodies for treatment or prevention of pain and pain associated conditions such as Ab15 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00442] *Antibody Ab16*

[00443] The invention contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF wherein the antibody is Ab16 or fragments thereof, for example as set forth below, or comprises

another antibody or antibody fragment that binds to the same or overlapping epitope as Ab16, in a therapeutically effective amount which inhibits the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

ALVMTQTPSSTSEPVGGTVTINCQASQNIGNDLSWYQQKPGQPPELLIYSTSKLATGVPK
RFSGSRSGTQFTLTISDLECDAAATYYCLGVYSYISDDGNAFGGGTEVVVKR (SEQ ID
NO: 151).

[00444] The invention also includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

ALVMTQTPSSTSEPVGGTVTINCQASQNIGNDLSWYQQKPGQPPELLIYSTSKLATGVPK
RFSGSRSGTQFTLTISDLECDAAATYYCLGVYSYISDDGNAFGGGTEVVVKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 152).

[00445] The invention further includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

QSVEEFGGRLVTPGTPLTLTCTVSGFSLNNYAMTWVRQAPGKGLEWIGIISIGTTTYAS
WAKGRFFISKSTSTTVDLKIISPTTEDTATYFCARDAGVTVDGYGYFNIWGPGLTVTVSS
(SEQ ID NO: 153).

[00446] The invention also includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

QSVEEFGGRLVTPGTPLTLTCTVSGFSLNNYAMTWVRQAPGKGLEWIGIISIGTTTYAS
WAKGRFFISKSTSTTVDLKIISPTTEDTATYFCARDAGVTVDGYGYFNIWGPGLTVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG

PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 154).

[00447] The invention further contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 155; SEQ ID NO: 156; and SEQ ID NO: 157 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 151 or the light chain sequence of SEQ ID NO: 152, and/or one or more of the polypeptide sequences of SEQ ID NO: 158; SEQ ID NO: 159; and SEQ ID NO: 160 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 153 or the heavy chain sequence of SEQ ID NO: 154, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00448] The invention also contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 151 or SEQ ID NO: 152. In another embodiment of the invention, antibody fragments of the invention for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 153 or SEQ ID NO: 154.

[00449] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 155; SEQ ID NO: 156; and SEQ ID NO: 157 which correspond to the complementarity-

determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 151 or the light chain sequence of SEQ ID NO: 152.

[00450] In a further embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 158; SEQ ID NO: 159; and SEQ ID NO: 160 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 153 or the heavy chain sequence of SEQ ID NO: 154.

[00451] The invention also contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 151; the variable heavy chain region of SEQ ID NO: 153; the complementarity-determining regions (SEQ ID NO: 155; SEQ ID NO: 156; and SEQ ID NO: 157) of the variable light chain region of SEQ ID NO: 151; and the complementarity-determining regions (SEQ ID NO: 158; SEQ ID NO: 159; and SEQ ID NO: 160) of the variable heavy chain region of SEQ ID NO: 153.

[00452] In a particularly preferred embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab16, comprising, or alternatively consisting of, SEQ ID NO: 152 and SEQ ID NO: 154, and having at least one of the biological activities set forth herein.

[00453] In a further particularly preferred embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab16, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 151 and the variable heavy chain sequence of SEQ ID NO: 153 or comprises another Fab or another bivalent or monovalent antibody fragment that binds to the same or overlapping epitope as Ab16. This embodiment of the invention further contemplates

additions, deletions, and variants of SEQ ID NO: 151 and/or SEQ ID NO: 153 in said Fab while retaining binding specificity for NGF.

[00454] In one embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab16. In another embodiment of the invention, anti-NGF antibodies for treatment or prevention of pain and pain associated conditions such as Ab16 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00455] *Antibody Ab17*

[00456] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF wherein the antibody is Ab17 or fragments thereof, for example as set forth below, or comprises another antibody or antibody fragment that binds to the same or overlapping epitope as Ab17, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AIEMTQTPFSVSAAVGGTVTIKCQASQTISNYLAWYQQKPGQPPKLLIYGASNLESGVPS
RFKGS GSGTQFTLTISDLECDAAATYYCQQGYTISNVDNNVFGGGTEVVVKR (SEQ ID
NO: 161).

[00457] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AIEMTQTPFSVSAAVGGTVTIKCQASQTISNYLAWYQQKPGQPPKLLIYGASNLESGVPS
RFKGS GSGTQFTLTISDLECDAAATYYCQQGYTISNVDNNVFGGGTEVVVKRTVAAPSV

FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 162).

[00458] The invention further optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:
QSLEESGGRLVTPGGSLTLTCAASGFSLTGYNLVWVRQAPGKGLEWIGFISYGDTTTYA
SWAKGRFTISKSTSTTVTLTITDLQPSDTGTIFYCARETANTYDYGIWGPGLTVTVSS (SEQ
ID NO: 163).

[00459] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:
QSLEESGGRLVTPGGSLTLTCAASGFSLTGYNLVWVRQAPGKGLEWIGFISYGDTTTYA
SWAKGRFTISKSTSTTVTLTITDLQPSDTGTIFYCARETANTYDYGIWGPGLTVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 164).

[00460] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 165; SEQ ID NO: 166; and SEQ ID NO: 167 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 161 or the light chain sequence of SEQ ID NO: 162, and/or one or more of the polypeptide sequences of SEQ ID NO: 168; SEQ ID NO: 169; and SEQ ID NO: 170 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 163 or the heavy chain sequence of SEQ ID NO: 164, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof for treatment

or prevention of pain and pain associated conditions comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00461] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 161 or SEQ ID NO: 162. In another optional embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 163 or SEQ ID NO: 164.

[00462] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 165; SEQ ID NO: 166; and SEQ ID NO: 167 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 161 or the light chain sequence of SEQ ID NO: 162.

[00463] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 168; SEQ ID NO: 169; and SEQ ID NO: 170 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 163 or the heavy chain sequence of SEQ ID NO: 164.

[00464] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 161; the variable heavy chain region of SEQ ID NO: 163; the complementarity-determining regions (SEQ ID NO: 165; SEQ ID NO: 166; and SEQ ID NO: 167) of the variable light chain region of SEQ ID NO: 161; and

the complementarity-determining regions (SEQ ID NO: 168; SEQ ID NO: 169; and SEQ ID NO: 170) of the variable heavy chain region of SEQ ID NO: 163.

[00465] In a particularly preferred optional embodiment of the invention, the chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab17, comprising, or alternatively consisting of, SEQ ID NO: 162 and SEQ ID NO: 164, and having at least one of the biological activities set forth herein.

[00466] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab17, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 161 and the variable heavy chain sequence of SEQ ID NO: 163 or comprises another Fab or monovalent or bivalent antibody fragment that binds to the same or overlapping epitope as Ab15,. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 161 and/or SEQ ID NO: 163 in said Fab while retaining binding specificity for NGF.

[00467] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab17. In another embodiment of the invention, anti-NGF antibodies such as Ab17 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

[00468] Antibody Ab18

[00469] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF wherein the antibody is Ab18 or fragments thereof, for example as set forth below, or comprises another antibody or antibody fragment that binds to the same or overlapping epitope

as Ab18, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVTITCQASQTISNYLAWYQQKPGKAPKLLIYGASNLESGVPS
RFSGSGSGTEFTLTISLQPDDEFATYYCQQGYTISNVDNNVFGGGTKVEIKR (SEQ ID
NO: 171).

[00470] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVTITCQASQTISNYLAWYQQKPGKAPKLLIYGASNLESGVPS
RFSGSGSGTEFTLTISLQPDDEFATYYCQQGYTISNVDNNVFGGGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPPREKVKWQVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 172).

[00471] The invention further optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSGYNLVWVRQAPGKGLEWVGFISYGDFTY
YASSAKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARETANTYDYGIWGQGTLVTV
SS (SEQ ID NO: 173).

[00472] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSGYNLVWVRQAPGKGLEWVGFISYGDFTY
YASSAKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARETANTYDYGIWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE

QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 174).

[00473] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 175; SEQ ID NO: 176; and SEQ ID NO: 177 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 171 or the light chain sequence of SEQ ID NO: 172, and/or one or more of the polypeptide sequences of SEQ ID NO: 178; SEQ ID NO: 179; and SEQ ID NO: 180 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 173 or the heavy chain sequence of SEQ ID NO: 174, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00474] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 171 or SEQ ID NO: 172. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 173 or SEQ ID NO: 174.

[00475] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 175; SEQ ID NO: 176; and SEQ ID NO: 177 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 171 or the light chain sequence of SEQ ID NO: 172.

[00476] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 178; SEQ ID NO: 179; and SEQ ID NO: 180 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 173 or the heavy chain sequence of SEQ ID NO: 174.

[00477] The invention also optional contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 171; the variable heavy chain region of SEQ ID NO: 173; the complementarity-determining regions (SEQ ID NO: 175; SEQ ID NO: 176; and SEQ ID NO: 177) of the variable light chain region of SEQ ID NO: 171; and the complementarity-determining regions (SEQ ID NO: 178; SEQ ID NO: 179; and SEQ ID NO: 180) of the variable heavy chain region of SEQ ID NO: 173.

[00478] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab18, comprising, or alternatively consisting of, SEQ ID NO: 172 and SEQ ID NO: 174, and having at least one of the biological activities set forth herein.

[00479] In a further particularly preferred optional embodiment of the invention, antibody fragments comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab18, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 171 and the variable heavy chain sequence of SEQ ID NO: 173 or comprise another Fab or antibody fragment that binds to the same or overlapping epitope as Ab18. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 171 and/or SEQ ID NO: 173 in said Fab while retaining binding specificity for NGF.

[00480] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab18. In another embodiment of the invention, anti-NGF antibodies such as Ab18 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00481] *Antibody Ab19*

[00482] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF wherein the antibody is Ab19 or fragments thereof, for example as set forth below, or comprises another antibody or antibody fragment that binds to the same or overlapping epitope as Ab19, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

AAVLTQTPSPVSAAVGGTVSISCQSSQNVYKNNYLSWYQQKPGQPPKLLIYKASTLASG
VPSRFKSGSGTDFLTISDVQCDAATYYCAGGYSSSSDNAFGGGTEVVKR (SEQ ID
NO: 181).

[00483] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

AAVLTQTPSPVSAAVGGTVSISCQSSQNVYKNNYLSWYQQKPGQPPKLLIYKASTLASG
VPSRFKSGSGTDFLTISDVQCDAATYYCAGGYSSSSDNAFGGGTEVVKRTVAAPS
VFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 182).

[00484] The invention further optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:
 QSVEASGGRLVMPGGSLTLTCTASGFSLSSTYWMSWVRQAPGKGLEWIGDIYFSNEETN
 YATWAKGRFTISKSTTTVDLNVISPTTEDTATYFCARGSPDVEIAIDMWGQGTLTVTVSS
 (SEQ ID NO: 183).

[00485] The invention also optionally includes chimeric antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:
 QSVEASGGRLVMPGGSLTLTCTASGFSLSSTYWMSWVRQAPGKGLEWIGDIYFSNEETN
 YATWAKGRFTISKSTTTVDLNVISPTTEDTATYFCARGSPDVEIAIDMWGQGTLTVTVSSA
 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGP
 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
 EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 184).

[00486] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 185; SEQ ID NO: 186; and SEQ ID NO: 187 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 181 or the light chain sequence of SEQ ID NO: 182, and/or one or more of the polypeptide sequences of SEQ ID NO: 188; SEQ ID NO: 189; and SEQ ID NO: 190 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 183 or the heavy chain sequence of SEQ ID NO: 184, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00487] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 181 or SEQ ID NO: 182. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 183 or SEQ ID NO: 184.

[00488] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 185; SEQ ID NO: 186; and SEQ ID NO: 187 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 181 or the light chain sequence of SEQ ID NO: 182.

[00489] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 188; SEQ ID NO: 189; and SEQ ID NO: 190 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 183 or the heavy chain sequence of SEQ ID NO: 184.

[00490] The invention also optionally contemplates antibody fragments which include one or more of the antibody fragments described herein for treatment or prevention of pain and pain associated conditions. In one optional embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 181; the variable heavy chain region of SEQ ID NO: 183; the complementarity-determining regions (SEQ ID NO: 185; SEQ ID NO: 186; and SEQ ID NO: 187) of the variable light chain region of SEQ ID NO: 181; and the complementarity-determining regions (SEQ ID NO: 188; SEQ ID NO: 189; and SEQ ID NO: 190) of the variable heavy chain region of SEQ ID NO: 183.

[00491] In a particularly preferred optional embodiment of the invention, the chimeric anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab19,

comprising, or alternatively consisting of, SEQ ID NO: 182 and SEQ ID NO: 184, and having at least one of the biological activities set forth herein.

[00492] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab19, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 181 and the variable heavy chain sequence of SEQ ID NO: 183 or comprises another Fab or antibody fragment that binds to the same or overlapping epitope as Ab19. This optional embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 181 and/or SEQ ID NO: 183 in said Fab while retaining binding specificity for NGF.

[00493] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab19. In another embodiment of the invention, anti-NGF antibodies such as Ab19 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00494] Antibody Ab20

[00495] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF wherein the antibody is Ab20 or fragments thereof, for example as set forth below, or comprises another antibody or antibody fragment that binds to the same or overlapping epitope as Ab20, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

DIQMTQSPSSLSASVGDRVTITCQSSQNVYKNNYLSWYQQKPGKVPKLLIYKASTLASG
VPSRFSGSGSGTDFTLTISLQPEDVATYYCAGGYTSSSDNAFGGGTKVEIKR (SEQ ID
NO: 191).

[00496] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

DIQMTQSPSSLSASVGDRVTITCQSSQNVYKNNYLSWYQQKPGKVPKLLIYKASTLASG
VPSRFSGSGSGTDFTLTISLQPEDVATYYCAGGYTSSSDNAFGGGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCCLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 192).

[00497] The invention further optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSTYWMWVRQAPGKGLEWVGDIYFSNEET
NYATSAKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGSPDVEIAIDMWGQGLTV
TVSS (SEQ ID NO: 193).

[00498] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSTYWMWVRQAPGKGLEWVGDIYFSNEET
NYATSAKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGSPDVEIAIDMWGQGLTV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO: 194).

[00499] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide

sequences of SEQ ID NO: 195; SEQ ID NO: 196; and SEQ ID NO: 197 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 191 or the light chain sequence of SEQ ID NO: 192, and/or one or more of the polypeptide sequences of SEQ ID NO: 198; SEQ ID NO: 199; and SEQ ID NO: 200 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 193 or the heavy chain sequence of SEQ ID NO: 194, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention or fragments thereof for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00500] The invention also optionally contemplates fragments of the antibody having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 191 or SEQ ID NO: 192. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 193 or SEQ ID NO: 194.

[00501] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 195; SEQ ID NO: 196; and SEQ ID NO: 197 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 191 or the light chain sequence of SEQ ID NO: 192.

[00502] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 198; SEQ ID NO: 199; and SEQ ID NO: 200 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 193 or the heavy chain sequence of SEQ ID NO: 194.

[00503] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 191; the variable heavy chain region of SEQ ID NO: 193; the complementarity-determining regions (SEQ ID NO: 195; SEQ ID NO: 196; and SEQ ID NO: 197) of the variable light chain region of SEQ ID NO: 191; and the complementarity-determining regions (SEQ ID NO: 198; SEQ ID NO: 199; and SEQ ID NO: 200) of the variable heavy chain region of SEQ ID NO: 193.

[00504] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab20, comprising, or alternatively consisting of, SEQ ID NO: 192 and SEQ ID NO: 194, and having at least one of the biological activities set forth herein.

[00505] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab20, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 191 and the variable heavy chain sequence of SEQ ID NO: 193. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 191 and/or SEQ ID NO: 193 in said Fab while retaining binding specificity for NGF.

[00506] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab20. In another embodiment of the invention, anti-NGF antibodies for treatment or prevention of pain and pain associated conditions such as Ab20 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00507] *Antibody Ab21*

[00508] The invention optionally contemplates methods of treating pain and the specific pain associated disorders alone or in association with another active agent, e.g., an NSAID or opioid analgesic, wherein the antibodies include chimeric antibodies having binding specificity to NGF wherein the antibody is Ab21 or fragments thereof, or another antibody or antibody fragment that binds to the same or overlapping epitope as Ab5, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes chimeric or humanized antibodies having binding specificity to NGF and possessing a variable light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVTITCQASQSIYSNLAWYQQKPGKAPKLLIYDASTLESGVPS
RFGSGSGTEYTLTISSLQPDDFATYYCQQGFTVSDIDNAFGGGTKVEIKR (SEQ ID NO:
51).

[00509] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVTITCQASQSIYSNLAWYQQKPGKAPKLLIYDASTLESGVPS
RFGSGSGTEYTLTISSLQPDDFATYYCQQGFTVSDIDNAFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYSLSS
TLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 401).

[00510] The invention further optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a variable heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSNYAVGWVRQAPGKGLEWVGIIGRNGNT
WYASSARGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGYGRSVAYYVFNIWGP
LVTVSS (SEQ ID NO: 53).

[00511] The invention also optionally includes chimeric or humanized antibodies for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSNYAVGWVRQAPGKGLEWVGIIGRNGNT

WYASSARGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGYGRSVAYYVFNIWGPGL
 LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
 AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTCPPCPA
 PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
 PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
 YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
 KLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID NO: 402).

[00512] The invention further optionally contemplates antibodies for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 401, and/or one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 402, or combinations of these polypeptide sequences. In another optional embodiment of the invention, the antibodies of the invention or fragments thereof comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00513] The invention also optionally contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 51 or SEQ ID NO: 401. In another embodiment of the invention, antibody fragments of the invention for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 53 or SEQ ID NO: 402.

[00514] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated

conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 401.

[00515] In a further optional embodiment of the invention, fragments of the antibody having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 402.

[00516] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 51; the variable heavy chain region of SEQ ID NO: 53; the complementarity-determining regions (SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57) of the variable light chain region of SEQ ID NO: 51; and the complementarity-determining regions (SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60) of the variable heavy chain region of SEQ ID NO: 53.

[00517] In a particularly preferred optional embodiment of the invention, the chimeric or humanized anti-NGF antibody for treatment or prevention of pain and pain associated conditions is Ab21, comprising, or alternatively consisting of, SEQ ID NO: 401 and SEQ ID NO: 402, and having at least one of the biological activities set forth herein.

[00518] In a further particularly preferred optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab21, the Fab fragment includes the variable light chain sequence of SEQ ID NO: 51 and the variable heavy chain sequence of SEQ ID NO: 53 or another Fab or monovalent antibody fragment that binds to the same or overlapping epitope as

Ab5,. This embodiment of the invention further contemplates additions, deletions, and variants of SEQ ID NO: 51 and/or SEQ ID NO: 53 in said Fab while retaining binding specificity for NGF.

[00519] In one optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab21. In another embodiment of the invention, anti-NGF antibodies for treatment or prevention of pain and pain associated conditions such as Ab21 or Fab fragments thereof may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

[00520] Antibody fragment Fab1

[00521] The invention optionally contemplates methods of treating pain using antibody fragment Fab1 or fragments thereof, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention optionally includes Fab antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

[00522]

DIQMTQSPSTLSASVGDRVITTCQASQSIYSNLAWYQQKPGKAPKLLIYDASTLESGVPS
RFGSGSGTEYTLTISSLQPDDFATYYCQQGFTVSDIDNAFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDDSTYSLS
TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 405).

[00523] The invention further optionally includes Fab antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSNYAVGWVRQAPGKGLEWVGIIGRNGNT
WYASSARGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGYGRSVAYYVFNIWGP

LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTH (SEQ ID
NO: 406).

[00524] The invention further optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 405, and/or one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 406, or combinations of these polypeptide sequences. In another optional embodiment of the invention, antibody fragments of the invention for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00525] The invention also optionally contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 51 or SEQ ID NO: 405. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 53 or SEQ ID NO: 406.

[00526] In a further optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 405.

[00527] In a further optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 406.

[00528] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 51; the variable heavy chain region of SEQ ID NO: 53; the complementarity-determining regions (SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57) of the variable light chain region of SEQ ID NO: 51; and the complementarity-determining regions (SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60) of the variable heavy chain region of SEQ ID NO: 53.

[00529] In a particularly preferred optional embodiment of the invention, the anti-NGF antibody fragment for treatment or prevention of pain and pain associated conditions is Fab1, comprising SEQ ID NO: 405 and SEQ ID NO: 406, or another Fab or antibody fragment that binds to the same or overlapping epitope as Fab1, and having at least one of the biological activities set forth herein. In one embodiment of the invention, antibody fragment Fab1 may be produced by enzymatic digestion (e.g., papain) of Ab21.

[00530] Antibody fragment Fab2

[00531] The invention optionally contemplates methods of treating pain using antibody fragment Fab2 or fragments thereof, for example as set forth below, in a therapeutically effective amount which inhibits the association of NGF with TrkA and the association of NGF with p75. In one embodiment, the invention includes Fab antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a light chain sequence comprising the sequence set forth below:

DIQMTQSPSTLSASVGDRVITTCQASQSIYSNLAWYQQKPGKAPKLLIYDASTLESGVPS

RFSGSGSGTEYTLTISSLQPDDFATYYCQQGFTVSDIDNAFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS
TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 407).

[00532] The invention further optionally includes Fab antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF and possessing a heavy chain sequence comprising the sequence set forth below:

EVQLVESGGGLVQPGGSLRLSCAASGFTVSNYAVGWVRQAPGKGLEWVGIIGRNGNT
WYASSARGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGYGRSVAYYVFNIWGP
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
P
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTH (SEQ ID
NO: 408).

[00533] The invention further optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions comprising one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 407, and/or one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 408, or combinations of these polypeptide sequences. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, combinations of one or more of the CDRs, the variable heavy and variable light chain sequences, and the heavy and light chain sequences set forth above, including all of them.

[00534] The invention also optionally contemplates fragments of the antibody for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, antibody fragments of the invention for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 51 or SEQ ID NO: 407. In another embodiment of the invention, antibody fragments of the invention for treatment or prevention of pain and pain

associated conditions comprise, or alternatively consist of, the polypeptide sequence of SEQ ID NO: 53 or SEQ ID NO: 408.

[00535] In a further optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 407.

[00536] In a further optional embodiment of the invention, antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polypeptide sequences of SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 408.

[00537] The invention also optionally contemplates antibody fragments for treatment or prevention of pain and pain associated conditions which include one or more of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to NGF for treatment or prevention of pain and pain associated conditions comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 51; the variable heavy chain region of SEQ ID NO: 53; the complementarity-determining regions (SEQ ID NO: 55; SEQ ID NO: 56; and SEQ ID NO: 57) of the variable light chain region of SEQ ID NO: 51; and the complementarity-determining regions (SEQ ID NO: 58; SEQ ID NO: 59; and SEQ ID NO: 60) of the variable heavy chain region of SEQ ID NO: 53.

[00538] In a particularly preferred optional embodiment of the invention, the anti-NGF antibody fragment for treatment or prevention of pain and pain associated conditions is Fab2, comprising SEQ ID NO: 407 and SEQ ID NO: 408, or another Fab or antibody fragment that binds to the same or overlapping epitope as Fab2, and having at least one of the biological activities set forth herein.

[00539] In another optional embodiment of the invention described herein (infra), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced via expression in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention, antibody fragment Fab2 may be produced by expression in *Pichia pastoris* using protocols set forth herein in the examples.

[00540] In another embodiment, antibody fragments may be present in one or more of the following non-limiting forms: Fab, Fab', F(ab')₂, Fv and single chain Fv antibody forms. In a preferred embodiment, the anti-NGF antibodies described herein further comprises the kappa constant light chain sequence comprising the sequence set forth below:

[00541] VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 412).

[00542] In another preferred optional embodiment, the anti-NGF antibodies described herein for treatment or prevention of pain and pain associated conditions further comprises the gamma-1 constant heavy chain polypeptide sequence comprising the sequence set forth below:

[00543] ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 413).

[00544] In another optional embodiment, the invention contemplates an isolated anti-NGF antibody for treatment or prevention of pain and pain associated conditions comprising a V_H polypeptide sequence selected from: SEQ ID NO: 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, 133, 143, 153, 163, 173, 183, 193, or 402, or a variant thereof; and further comprising a V_L polypeptide sequence selected from: SEQ ID NO: 1, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111,

121, 131, 141, 151, 161, 171, 181, 191, or 401, or a variant thereof, wherein one or more of the framework residues (FR residues) in said V_H or V_L polypeptide has been substituted with another amino acid residue resulting in an anti-NGF antibody that specifically binds NGF. The invention contemplates humanized and chimeric forms of these antibodies for treatment or prevention of pain and pain associated conditions. The chimeric antibodies may include an Fc derived from IgG1, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgG10, IgG11, IgG12, IgG13, IgG14, IgG15, IgG16, IgG17, IgG18 or IgG19 constant regions.

[00545] In one embodiment of the invention, the antibodies or V_H or V_L polypeptides originate or are selected from one or more rabbit B cell populations prior to initiation of the humanization process referenced herein.

[00546] In another embodiment of the invention, the anti-NGF antibodies and fragments thereof for treatment or prevention of pain and pain associated conditions do not have binding specificity for p75 or TrkA. In a further embodiment of the invention, there is contemplated methods for treating pain comprising using the anti-NGF antibodies and fragments thereof to inhibit the association of NGF with p75 and/or TrkA. In another embodiment of the invention, there is contemplated methods for treating pain comprising using anti-NGF antibodies and fragments thereof to inhibit the association of NGF with TrkA and/or multimers thereof and/or antagonizes the biological effects thereof. In another embodiment of the invention, there is contemplated methods for treating pain comprising using anti-NGF antibodies and fragments thereof to inhibit the association of NGF with p75 and/or multimers thereof and the association of NGF with TrkA and/or multimers thereof, and antagonizes the biological effects of p75 and TrkA.

[00547] As stated supra, antibodies and fragments thereof may be modified post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent moieties, or functional moieties such as for example streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, and radioactive materials.

[00548] Regarding detectable moieties, further exemplary enzymes include, but are not limited to, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, beta-galactosidase

and luciferase. Further exemplary fluorescent materials include, but are not limited to, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin and dansyl chloride. Further exemplary chemiluminescent moieties include, but are not limited to, luminol. Further exemplary bioluminescent materials include, but are not limited to, luciferin and aequorin. Further exemplary radioactive materials include, but are not limited to, Iodine 125 (^{125}I), Carbon 14 (^{14}C), Sulfur 35 (^{35}S), Tritium (^3H) and Phosphorus 32 (^{32}P).

[00549] Regarding functional moieties, exemplary cytotoxic agents include, but are not limited to, methotrexate, aminopterin, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine; alkylating agents such as mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU), mitomycin C, lomustine (CCNU), 1-methylnitrosourea, cyclophosphamide, mechlorethamine, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin and carboplatin (paraplatin); anthracyclines include daunorubicin (formerly daunomycin), doxorubicin (adriamycin), detorubicin, carminomycin, idarubicin, epirubicin, mitoxantrone and bisantrene; antibiotics include dactinomycin (actinomycin D), bleomycin, calicheamicin, mithramycin, and anthramycin (AMC); and antimetabolic agents such as the vinca alkaloids, vincristine and vinblastine. Other cytotoxic agents include paclitaxel (taxol), ricin, pseudomonas exotoxin, gemcitabine, cytochalasin B, gramicidin D, ethidium bromide, emetine, etoposide, teniposide, colchicin, dihydroxy anthracin dione, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons, and mixtures of these cytotoxic agents.

[00550] Further cytotoxic agents include, but are not limited to, chemotherapeutic agents such as carboplatin, cisplatin, paclitaxel, gemcitabine, calicheamicin, doxorubicin, 5-fluorouracil, mitomycin C, actinomycin D, cyclophosphamide, vincristine and bleomycin. Toxic enzymes from plants and bacteria such as ricin, diphtheria toxin and Pseudomonas toxin may be conjugated to the humanized or chimeric antibodies, or binding fragments thereof, to generate cell-type-specific-killing reagents (Youle, et al., Proc. Nat'l Acad. Sci. USA 77:5483 (1980); Gilliland, et al., Proc. Nat'l Acad. Sci. USA 77:4539 (1980); Krolick, et al., Proc. Nat'l Acad. Sci. USA 77:5419 (1980)).

[00551] Other cytotoxic agents include cytotoxic ribonucleases as described by Goldenberg in U.S. Pat. No. 6,653,104. Embodiments of the invention also relate to radioimmunoconjugates where a radionuclide that emits alpha or beta particles is stably coupled to the antibody, or binding fragments thereof, with or without the use of a complex-forming agent. Such radionuclides include beta-emitters such as Phosphorus-32 (^{32}P), Scandium-47 (^{47}Sc), Copper-67 (^{67}Cu), Gallium-67 (^{67}Ga), Yttrium-88 (^{88}Y), Yttrium-90 (^{90}Y), Iodine-125 (^{125}I), Iodine-131 (^{131}I), Samarium-153 (^{153}Sm), Lutetium-177 (^{177}Lu), Rhenium-186 (^{186}Re) or Rhenium-188 (^{188}Re), and alpha-emitters such as Astatine-211 (^{211}At), Lead-212 (^{212}Pb), Bismuth-212 (^{212}Bi) or -213 (^{213}Bi) or Actinium-225 (^{225}Ac).

[00552] Further exemplary radioactive materials include, but are not limited to, Iodine 125 (^{125}I), Carbon 14 (^{14}C), Sulfur 35 (^{35}S), Tritium (^3H) and Phosphorus 32 (^{32}P).

[00553] Regarding functional moieties, exemplary cytotoxic agents include, but are not limited to, methotrexate, aminopterin, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine; alkylating agents such as mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU), mitomycin C, lomustine (CCNU), 1-methylnitrosourea, cyclophosphamide, mechlorethamine, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin and carboplatin (paraplatin); anthracyclines include daunorubicin (formerly daunomycin), doxorubicin (adriamycin), detorubicin, carminomycin, idarubicin, epirubicin, mitoxantrone and bisantrene; antibiotics include dactinomycin (actinomycin D), bleomycin, calicheamicin, mithramycin, and anthramycin (AMC); and antimetabolic agents such as the vinca alkaloids, vincristine and vinblastine. Other cytotoxic agents include paclitaxel (taxol), ricin, pseudomonas exotoxin, gemcitabine, cytochalasin B, gramicidin D, ethidium bromide, emetine, etoposide, teniposide, colchicin, dihydroxy anthracin dione, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotate (O,P'-(DDD)), interferons, and mixtures of these cytotoxic agents.

[00554] Further cytotoxic agents include, but are not limited to, chemotherapeutic agents such as carboplatin, cisplatin, paclitaxel, gemcitabine, calicheamicin, doxorubicin, 5-fluorouracil, mitomycin C, actinomycin D, cyclophosphamide, vincristine and bleomycin. Toxic

enzymes from plants and bacteria such as ricin, diphtheria toxin and Pseudomonas toxin may be conjugated to the humanized or chimeric antibodies, or binding fragments thereof, to generate cell-type-specific-killing reagents (Youle, et al., Proc. Nat'l Acad. Sci. USA 77:5483 (1980); Gilliland, et al., Proc. Nat'l Acad. Sci. USA 77:4539 (1980); Krolick, et al., Proc. Nat'l Acad. Sci. USA 77:5419 (1980)).

[00555] Other cytotoxic agents include cytotoxic ribonucleases as described by Goldenberg in U.S. Pat. No. 6,653,104. Embodiments of the invention also relate to radioimmunoconjugates where a radionuclide that emits alpha or beta particles is stably coupled to the antibody, or binding fragments thereof, with or without the use of a complex-forming agent. Such radionuclides include beta-emitters such as Phosphorus-32 (^{32}P), Scandium-47 (^{47}Sc), Copper-67 (^{67}Cu), Gallium-67 (^{67}Ga), Yttrium-88 (^{88}Y), Yttrium-90 (^{90}Y), Iodine-125 (^{125}I), Iodine-131 (^{131}I), Samarium-153 (^{153}Sm), Lutetium-177 (^{177}Lu), Rhenium-186 (^{186}Re) or Rhenium-188 (^{188}Re), and alpha-emitters such as Astatine-211 (^{211}At), Lead-212 (^{212}Pb), Bismuth-212 (^{212}Bi) or -213 (^{213}Bi) or Actinium-225 (^{225}Ac). .

[00556] Methods are known in the art for conjugating an antibody or binding fragment thereof to a detectable moiety and the like, such as for example those methods described by Hunter et al, Nature 144:945 (1962); David et al, Biochemistry 13:1014 (1974); Pain et al, J. Immunol. Meth. 40:219 (1981); and Nygren, J., Histochem. and Cytochem. 30:407 (1982).

[00557] Embodiments described herein further include variants and equivalents that are substantially homologous to the antibodies, antibody fragments, diabodies, SMIPs, camelbodies, nanobodies, IgNAR, polypeptides, variable regions and CDRs set forth herein. These may contain, e.g., conservative substitution mutations, (i.e., the substitution of one or more amino acids by similar amino acids). For example, conservative substitution refers to the substitution of an amino acid with another within the same general class, e.g., one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid, or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art.

[00558] In another embodiment, the invention contemplates polypeptide sequences having at least 90% or greater sequence homology to any one or more of the polypeptide sequences of

antibody fragments, variable regions and CDRs set forth herein. More preferably, the invention contemplates polypeptide sequences having at least 95% or greater sequence homology, even more preferably at least 98% or greater sequence homology, and still more preferably at least 99% or greater sequence homology to any one or more of the polypeptide sequences of antibody fragments, variable regions and CDRs set forth herein. Methods for determining homology between nucleic acid and amino acid sequences are well known to those of ordinary skill in the art.

[00559] In another embodiment, the invention further contemplates the above-recited polypeptide homologs of the antibody fragments, variable regions and CDRs set forth herein further having anti-NGF activity. Non-limiting examples of anti-NGF activity are set forth herein.

[00560] In another embodiment, the invention further contemplates the generation and use of anti-idiotypic antibodies that bind any of the foregoing sequences. In an exemplary embodiment, such an anti-idiotypic antibody could be administered to a subject who has received an anti-NGF antibody to modulate, reduce, or neutralize, the effect of the anti-NGF antibody. Such anti-idiotypic antibodies could also be useful for treatment of an autoimmune disease characterized by the presence of anti-NGF antibodies. A further exemplary use of such anti-idiotypic antibodies is for detection of the anti-NGF antibodies of the present invention, for example to monitor the levels of the anti-NGF antibodies present in a subject's blood or other bodily fluids.

[00561] The present invention also contemplates anti-NGF antibodies comprising any of the polypeptide or polynucleotide sequences described herein substituted for any of the other polynucleotide sequences described herein. For example, without limitation thereto, the present invention contemplates antibodies comprising the combination of any of the variable light chain and variable heavy chain sequences described herein, and further contemplates antibodies resulting from substitution of any of the CDR sequences described herein for any of the other CDR sequences described herein.

[00562]

[00563] *Polynucleotides Encoding Anti-NGF Antibody Polypeptides***[00564] *Antibody Ab1***

[00565] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab1 polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab1 polypeptides. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 1:

[00566] GCCCTTGTGATGACCCAGACTCCATCCTCCGTGTCTGCAGCTGTGGGA
GGCACAGTCACCATCAATTGCCAGGCCAGTCAGAACATTTACAGCAATTTAGCCTGG
TATCAACAGAGACCAGGGCAGCGTCCCAAGCTCCTGATCTATGGTGCATCCAATCTG
GATGCTGGGGTCCCATCGCGGTTTCAGAGGCAGTGGATCTGGGACAGAGTACACTCT
CACCATCAGCGACCTGGAGTGTGACGATGTTGGCACTTACTACTGTCAAAGTGCTTT
TGATAGTGATAGTACTGAAAATACTTTTCGGCGGAGGGACCGAGGTGGTGGTCAAAC
GT (SEQ ID NO: 201).

[00567] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 2:

[00568] GCCCTTGTGATGACCCAGACTCCATCCTCCGTGTCTGCAGCTGTGGGA
GGCACAGTCACCATCAATTGCCAGGCCAGTCAGAACATTTACAGCAATTTAGCCTGG
TATCAACAGAGACCAGGGCAGCGTCCCAAGCTCCTGATCTATGGTGCATCCAATCTG
GATGCTGGGGTCCCATCGCGGTTTCAGAGGCAGTGGATCTGGGACAGAGTACACTCT
CACCATCAGCGACCTGGAGTGTGACGATGTTGGCACTTACTACTGTCAAAGTGCTTT
TGATAGTGATAGTACTGAAAATACTTTTCGGCGGAGGGACCGAGGTGGTGGTCAAAC
GTACGGTAGCGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAG

TACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 202).

[00569] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 3:

[00570] CAGTCGCTGGAGGAGTCCGGGGGTGCGCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGCTTCTCCCTCAGTAGCTATGCAATGAGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTACTAGTATTG
GTAGCACAGTCTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCG
ACCACGGTGGATCTGAAAATCACCAGTCCGACAACCGAGGACACGGCCACCTATTT
CTGTGCCAGAGGCTACGATGACTATGATGAGATGACCTACTTTAACATCTGGGGCCA
GGGGACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 203).

[00571] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 4:

[00572] CAGTCGCTGGAGGAGTCCGGGGGTGCGCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGCTTCTCCCTCAGTAGCTATGCAATGAGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGTCATTACTAGTATTG
GTAGCACAGTCTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCG
ACCACGGTGGATCTGAAAATCACCAGTCCGACAACCGAGGACACGGCCACCTATTT
CTGTGCCAGAGGCTACGATGACTATGATGAGATGACCTACTTTAACATCTGGGGCCA
GGGGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCT
GGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCA
AGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGC
GGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC
GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAA

AACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTT
CCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC
ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG
TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGC
CAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGG
CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCA
TCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC
ACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGC
TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
TGA (SEQ ID NO: 204).

[00573] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 205; SEQ ID NO: 206; and SEQ ID NO: 207 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 1 or the light chain sequence of SEQ ID NO: 2.

[00574] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 208; SEQ ID NO: 209; and SEQ ID NO: 210 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 3 or the heavy chain sequence of SEQ ID NO: 4.

[00575] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or

prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 201 encoding the light chain variable sequence of SEQ ID NO: 1; the polynucleotide SEQ ID NO: 202 encoding the light chain sequence of SEQ ID NO: 2; the polynucleotide SEQ ID NO: 203 encoding the heavy chain variable sequence of SEQ ID NO: 3; the polynucleotide SEQ ID NO: 204 encoding the heavy chain sequence of SEQ ID NO: 4; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 205; SEQ ID NO: 206; and SEQ ID NO: 207) of the light chain variable sequence of SEQ ID NO: 1 or the light chain sequence of SEQ ID NO: 2; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 208; SEQ ID NO: 209; and SEQ ID NO: 210) of the heavy chain variable sequence of SEQ ID NO: 3 or the heavy chain sequence of SEQ ID NO: 4.

[00576] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments for treatment or prevention of pain and pain associated conditions having binding specificity for NGF. With respect to antibody Ab1, the polynucleotides encoding the full length Ab1 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 202 encoding the light chain sequence of SEQ ID NO: 2 and the polynucleotide SEQ ID NO: 204 encoding the heavy chain sequence of SEQ ID NO: 4.

[00577] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (*infra*), Fab fragments for treatment or prevention of pain and pain associated conditions may be produced by enzymatic digestion (e.g., papain) of Ab1 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab1 or Fab fragments thereof may be produced via expression of Ab1 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example

diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00578] Antibody Ab2

[00579] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab2 polypeptides which inhibit the association of NGF with TrkA and the association of NGF with p75, for treatment or prevention of pain and pain associated conditions having binding specificity to NGF in methods of treating pain in an individual comprising administering to said individual antibody Ab2 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to NGF for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 11:

[00580] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAACATTTACAGCAACTTAGCCTG
GTATCAGCAGAAACCAGGAAAAGCCCCCTAAGCTCCTGATCTATGGTGCATCCAATCT
GGATGCTGGAGTCCCATCAAGGTTCTCTGGCAGTGGATCTGGGACAGAGTACACTCT
CACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGCCAAAGTGCTTT
TGATAGTGATAGTACTGAAAACACTTTCGGCGGAGGAACCAAGGTGGAAATCAAAC
GT (SEQ ID NO: 211).

[00581] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 12:

[00582] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAACATTTACAGCAACTTAGCCTG
GTATCAGCAGAAACCAGGAAAAGCCCCCTAAGCTCCTGATCTATGGTGCATCCAATCT
GGATGCTGGAGTCCCATCAAGGTTCTCTGGCAGTGGATCTGGGACAGAGTACACTCT
CACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGCCAAAGTGCTTT

TGATAGTGATAGTACTGAAAACACTTTCGGCGGAGGAACCAAGGTGGAAATCAAAC
GTACGGTAGCGGCCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAG
TACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTGACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 212).

[00583] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 13:

[00584] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCGTCAGTAGCTATGCAATGAG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAGTCATTACTAGTA
TTGGTAGCACAGTCTACGCGAGCAGCGCGAAAGGCCGATTCACCATCTCCAGAGAC
AATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCT
GTGTATTACTGTGCTAGAGGCTACGATGACTATGATGAGATGACCTACTTTAACATC
TGGGGCCAAGGGACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 213).

[00585] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 14:

[00586] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCGTCAGTAGCTATGCAATGAG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAGTCATTACTAGTA
TTGGTAGCACAGTCTACGCGAGCAGCGCGAAAGGCCGATTCACCATCTCCAGAGAC
AATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCT
GTGTATTACTGTGCTAGAGGCTACGATGACTATGATGAGATGACCTACTTTAACATC
TGGGGCCAAGGGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGT
CTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTG
CCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCT

GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCT
CAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCA
ACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCT
TGTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACC
GTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCC
TGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCA
ACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGA
GCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTG
GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCA
TCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACC
CTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGT
CAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
AGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCT
ACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT
CCGGGTAAATGA (SEQ ID NO: 214).

[00587] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 215; SEQ ID NO: 216; and SEQ ID NO: 217 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 11 or the light chain sequence of SEQ ID NO: 12.

[00588] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 218; SEQ ID NO: 219; and SEQ ID NO: 220 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 13 or the heavy chain sequence of SEQ ID NO: 14.

[00589] The invention also optional contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 211 encoding the light chain variable sequence of SEQ ID NO: 11; the polynucleotide SEQ ID NO: 212 encoding the light chain sequence of SEQ ID NO: 12; the polynucleotide SEQ ID NO: 213 encoding the heavy chain variable sequence of SEQ ID NO: 13; the polynucleotide SEQ ID NO: 214 encoding the heavy chain sequence of SEQ ID NO: 14; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 215; SEQ ID NO: 216; and SEQ ID NO: 217) of the light chain variable sequence of SEQ ID NO: 11 or the light chain sequence of SEQ ID NO: 12; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 218; SEQ ID NO: 219; and SEQ ID NO: 220) of the heavy chain variable sequence of SEQ ID NO: 13 or the heavy chain sequence of SEQ ID NO: 14.

[00590] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments for treatment or prevention of pain and pain associated conditions having binding specificity for NGF. With respect to antibody Ab2, the polynucleotides encoding the full length Ab2 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 212 encoding the light chain sequence of SEQ ID NO: 12 and the polynucleotide SEQ ID NO: 214 encoding the heavy chain sequence of SEQ ID NO: 14.

[00591] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab2 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab2 or Fab fragments thereof may be produced via expression of Ab2 polynucleotides in mammalian

cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00592] *Antibody Ab3*

[00593] The invention is further directed to the use of polynucleotides set forth below to produce antibody Ab3 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab3 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 21:

[00594] GCAGCCGTGCTGACCCAGACACCATCGCCCGTGTCTGCAGCTATGGGA
GACACAGTCACCATCAAGTGCCAGTCCAGTCAGAGTGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAGGCTCCTGATCTATGATGCATCC
AATCTGCCATCTGGGGTCCCATCACGGTTCAGCGGCAGTGGATCTGGGACACAGTTC
ACTCTCACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGC
GATTATGATGATGATGCTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGT (SEQ ID NO: 221).

[00595] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 22:

[00596] GCAGCCGTGCTGACCCAGACACCATCGCCCGTGTCTGCAGCTATGGGA
GACACAGTCACCATCAAGTGCCAGTCCAGTCAGAGTGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAGGCTCCTGATCTATGATGCATCC
AATCTGCCATCTGGGGTCCCATCACGGTTCAGCGGCAGTGGATCTGGGACACAGTTC
ACTCTCACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGC

GATTATGATGATGATGCTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGTACGGTAGCGGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 222).

[00597] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 23:

[00598] CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTAGCTATGTAATGATCTGG
GTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGAATCACTTGGAGTGCTGG
TACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGA
CCACGGTGGATCTGAAAATCACCAGTCCGACAACCGAGGACACGGCCACCTATTTC
TGTGCCCGAGGTGGTGGTAGTATTTATGATATTTGGGGCCCGGGCACCCCTGGTCACC
GTCTCGAGC (SEQ ID NO: 223).

[00599] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 24:

[00600] CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTAGCTATGTAATGATCTGG
GTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGAATCACTTGGAGTGCTGG
TACATACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGA
CCACGGTGGATCTGAAAATCACCAGTCCGACAACCGAGGACACGGCCACCTATTTC
TGTGCCCGAGGTGGTGGTAGTATTTATGATATTTGGGGCCCGGGCACCCCTGGTCACC
GTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAG
AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGA
ACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCC

CGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCT
 CCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC
 ACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCC
 ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCC AAA
 ACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGG
 ACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
 GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGT
 GGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGT
 GCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCC
 AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
 GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACA
 TCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCT
 CCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAG
 AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC
 AACC ACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:
 224).

[00601] In a further embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 225; SEQ ID NO: 226; and SEQ ID NO: 227 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 21 or the light chain sequence of SEQ ID NO: 22.

[00602] In a further embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 228; SEQ ID NO: 229; and SEQ ID NO: 230 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 23 or the heavy chain sequence of SEQ ID NO: 24.

[00603] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein for treatment or prevention of pain and pain associated conditions. In one embodiment of the invention, polynucleotides encoding antibody fragments having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 221 encoding the light chain variable sequence of SEQ ID NO: 21; the polynucleotide SEQ ID NO: 222 encoding the light chain sequence of SEQ ID NO: 22; the polynucleotide SEQ ID NO: 223 encoding the heavy chain variable sequence of SEQ ID NO: 23; the polynucleotide SEQ ID NO: 224 encoding the heavy chain sequence of SEQ ID NO: 24; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 225; SEQ ID NO: 226; and SEQ ID NO: 227) of the light chain variable sequence of SEQ ID NO: 21 or the light chain sequence of SEQ ID NO: 22; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 228; SEQ ID NO: 229; and SEQ ID NO: 230) of the heavy chain variable sequence of SEQ ID NO: 23 or the heavy chain sequence of SEQ ID NO: 24.

[00604] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments for treatment or prevention of pain and pain associated conditions having binding specificity for NGF. With respect to antibody Ab3, the polynucleotides encoding the full length Ab3 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 222 encoding the light chain sequence of SEQ ID NO: 22 and the polynucleotide SEQ ID NO: 224 encoding the heavy chain sequence of SEQ ID NO: 24.

[00605] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab3 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab3 or Fab fragments thereof may be produced via expression of Ab3 polynucleotides in mammalian

cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00606] *Antibody Ab4*

[00607] The invention is further directed to the use of polynucleotides set forth below to produce antibody Ab4 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab4 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 31:

[00608] GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATAAGGCATCC
ACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTC
ACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTGCAGGC
GGTTATACCAGTAGTAGTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAA
ACGT (SEQ ID NO: 231).

[00609] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 32:

[00610] GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATAAGGCATCC
ACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTC
ACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTGCAGGC

GGTTATACCAGTAGTAGTGATAATGCTTTTCGGCGGAGGAACCAAGGTGGAAATCAA
ACGTACGGTAGCGGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 232).

[00611] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 33:

[00612] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCGTCAGTAGCTATGTAATGAT
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTACATCGGAATCACTTGGAGTG
CTGGTACATACTACGCGAGCAGTGCGAAAGGCCGATTACCATCTCCAGAGACAAT
TCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTG
TATTACTGTGCTGGAGGTGGTGGTAGTATCTATGATATTTGGGGCCAAGGGACCCTC
GTCACCGTCTCGAGC (SEQ ID NO: 233).

[00613] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 34:

[00614] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCGTCAGTAGCTATGTAATGAT
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTACATCGGAATCACTTGGAGTG
CTGGTACATACTACGCGAGCAGTGCGAAAGGCCGATTACCATCTCCAGAGACAAT
TCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTGTG
TATTACTGTGCTGGAGGTGGTGGTAGTATCTATGATATTTGGGGCCAAGGGACCCTC
GTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCC
TCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTT
CCCCGAACCGGTGACGGTGTCGTGGAATCAGGCGCCCTGACCAGCGGCGTGCACA

CCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCG
TGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACA
TGCCACACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAGCACGTA
CCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGT
ACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGA
GGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGAC
CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGT
GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG
CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ
ID NO: 234).

[00615] In a further embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 235; SEQ ID NO: 236; and SEQ ID NO: 237 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 31 or the light chain sequence of SEQ ID NO: 32.

[00616] In a further embodiment of the invention, polynucleotides encoding antibody fragments h for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 238; SEQ ID NO: 239; and SEQ ID NO: 240 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 33 or the heavy chain sequence of SEQ ID NO: 34.

[00617] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 231 encoding the light chain variable sequence of SEQ ID NO: 31; the polynucleotide SEQ ID NO: 232 encoding the light chain sequence of SEQ ID NO: 32; the polynucleotide SEQ ID NO: 233 encoding the heavy chain variable sequence of SEQ ID NO: 33; the polynucleotide SEQ ID NO: 234 encoding the heavy chain sequence of SEQ ID NO: 34; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 235; SEQ ID NO: 236; and SEQ ID NO: 237) of the light chain variable sequence of SEQ ID NO: 31 or the light chain sequence of SEQ ID NO: 32; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 238; SEQ ID NO: 239; and SEQ ID NO: 240) of the heavy chain variable sequence of SEQ ID NO: 33 or the heavy chain sequence of SEQ ID NO: 34.

[00618] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments for treatment or prevention of pain and pain associated conditions having binding specificity for NGF. With respect to antibody Ab4, the polynucleotides encoding the full length Ab4 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 232 encoding the light chain sequence of SEQ ID NO: 32 and the polynucleotide SEQ ID NO: 234 encoding the heavy chain sequence of SEQ ID NO: 34.

[00619] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab4 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies for treatment or prevention of pain and pain associated conditions such as Ab4 or Fab fragments thereof may be

produced via expression of Ab4 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00620] *Antibody Ab5*

[00621] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab5 polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab5 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 41:

[00622] GCCTATGATATGACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGA
GGCACAGTCACCATCAAGTGCCAGGCCAGTCAGAGCATTTACAGCAATTTAGCCTG
GTATCAGCAGAGACCAGGGCAGCCTCCCAAGCTCCTGATCTATGATGCATCCACTCT
GGAATCTGGGGTCCCATCGCGTTCAAAGGCAGTGGATCTGGGACAGAGTACACTC
TCACCATCAGCGGCGTGGAGTGTGCCGATGCTGCCTCTTACTACTGTCAACAGGGTT
TACTGTTAGTGATATTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAAC
GT (SEQ ID NO: 241).

[00623] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 42:

[00624] GCCTATGATATGACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGA
GGCACAGTCACCATCAAGTGCCAGGCCAGTCAGAGCATTTACAGCAATTTAGCCTG
GTATCAGCAGAGACCAGGGCAGCCTCCCAAGCTCCTGATCTATGATGCATCCACTCT

GGAATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGAGTACACTC
TCACCATCAGCGGCGTGGAGTGTGCCGATGCTGCCTCTTACTACTGTCAACAGGGTT
TACTGTTAGTGATATTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAAC
GTACGGTAGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAG
TACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 242).

[00625] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 43:

[00626] CAGTCGGTGGAGGAGTCCGGGGGTGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTAACTATGCAGTGGGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATCATTGGTCGTAATG
GTAACACATGGTACGCGAGCTGGGCAAGAGGCCGATTACCATCTCCAAAACCTCG
ACCACGGTGGATCTGAAAATCACCAGTCCGACAAGCGAGGACACGGCCACATATTT
CTGTGCCAGAGGATATGGCCGTAGTGTTGCTTATTACGTCTTTAACATCTGGGGCCC
AGGCACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 243).

[00627] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 44:

[00628] CAGTCGGTGGAGGAGTCCGGGGGTGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTAACTATGCAGTGGGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATCATTGGTCGTAATG
GTAACACATGGTACGCGAGCTGGGCAAGAGGCCGATTACCATCTCCAAAACCTCG
ACCACGGTGGATCTGAAAATCACCAGTCCGACAAGCGAGGACACGGCCACATATTT
CTGTGCCAGAGGATATGGCCGTAGTGTTGCTTATTACGTCTTTAACATCTGGGGCCC
AGGCACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCT

GGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCA
 AGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGC
 GGC GTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC
 GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
 CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAA
 AACTCACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTT
 CCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC
 ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG
 TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGC
 CAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGG
 CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
 CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCA
 TCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTT
 CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACT
 ACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGC
 TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
 CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
 TGA (SEQ ID NO: 244).

[00629] In a further optional embodiment of the invention, polynucleotides encoding for treatment or prevention of pain and pain associated conditions fragments having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 245; SEQ ID NO: 246; and SEQ ID NO: 247 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 41 or the light chain sequence of SEQ ID NO: 42.

[00630] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 248; SEQ ID NO: 249; and SEQ ID NO: 250 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or

hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 43 or the heavy chain sequence of SEQ ID NO: 44.

[00631] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 241 encoding the light chain variable sequence of SEQ ID NO: 41; the polynucleotide SEQ ID NO: 242 encoding the light chain sequence of SEQ ID NO: 42; the polynucleotide SEQ ID NO: 243 encoding the heavy chain variable sequence of SEQ ID NO: 43; the polynucleotide SEQ ID NO: 244 encoding the heavy chain sequence of SEQ ID NO: 44; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 245; SEQ ID NO: 246; and SEQ ID NO: 247) of the light chain variable sequence of SEQ ID NO: 41 or the light chain sequence of SEQ ID NO: 42; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 248; SEQ ID NO: 249; and SEQ ID NO: 250) of the heavy chain variable sequence of SEQ ID NO: 43 or the heavy chain sequence of SEQ ID NO: 44.

[00632] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab5, the polynucleotides encoding the full length Ab5 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 242 encoding the light chain sequence of SEQ ID NO: 42 and the polynucleotide SEQ ID NO: 244 encoding the heavy chain sequence of SEQ ID NO: 44.

[00633] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by

enzymatic digestion (e.g., papain) of Ab5 following expression of the full-length polynucleotides in a suitable host. In another optional embodiment of the invention, anti-NGF antibodies such as Ab5 or Fab fragments thereof may be produced via expression of Ab5 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00634] *Antibody Ab6*

[00635] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab6 polypeptides having binding specificity to NGF, which inhibits the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab6 polypeptides. The invention is further directed to polynucleotides encoding for treatment or prevention of pain and pain associated conditions polypeptides having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 51:

[00636] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAGCATTTACAGCAATCTTGCCTGG
TATCAGCAGAAACCAGGAAAAGCCCCTAAGCTCCTGATCTATGATGCATCCACTCTG
GAATCTGGAGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAGTACACTCT
CACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGCCAACAGGGTTT
TACTGTTAGTGATATTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAAC
GT (SEQ ID NO: 251).

[00637] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 52:

[00638] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAGCATTTACAGCAATCTTGCCTGG

TATCAGCAGAAACCAGGAAAAGCCCCTAAGCTCCTGATCTATGATGCATCCACTCTG
GAATCTGGAGTCCCATCAAGGTTACGCGGCAGTGGATCTGGGACAGAGTACACTCT
CACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGCCAACAGGGTTT
TACTGTTAGTGATATTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAAC
GTACGGTAGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAG
TACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 252).

[00639] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 53:

[00640] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCGTCAGTAACTATGCAGTGGG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAATCATTGGTCGTA
ATGGTAACACATGGTACGCGAGCTCTGCAAGAGGCCGATTCACCATCTCCAGAGAC
AATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCT
GTGTATTACTGTGCTAGAGGATATGGCCGTAGTGTTGCTTATTACGTCTTTAACATCT
GGGGCCCAGGGACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 253).

[00641] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 54:

[00642] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCGTCAGTAACTATGCAGTGGG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAATCATTGGTCGTA
ATGGTAACACATGGTACGCGAGCTCTGCAAGAGGCCGATTCACCATCTCCAGAGAC
AATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCT
GTGTATTACTGTGCTAGAGGATATGGCCGTAGTGTTGCTTATTACGTCTTTAACATCT

GGGGCCCAGGGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCT
TCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG
ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTT
GTGACAAAACCTCACACATGCCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCG
TCAGTCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT
GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAA
CTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC
AGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGC
TGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC
GAGAAAACCATCTCCAAGGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTAC
AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC
GGGTAAATGA (SEQ ID NO: 254).

[00643] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 255; SEQ ID NO: 256; and SEQ ID NO: 257 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 52.

[00644] In a further optional embodiment of the invention, polynucleotides encoding for treatment or prevention of pain and pain associated conditions fragments having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 258; SEQ ID NO: 259; and SEQ ID NO: 260 which correspond to

polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 54.

[00645] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 251 encoding the light chain variable sequence of SEQ ID NO: 51; the polynucleotide SEQ ID NO: 252 encoding the light chain sequence of SEQ ID NO: 52; the polynucleotide SEQ ID NO: 253 encoding the heavy chain variable sequence of SEQ ID NO: 53; the polynucleotide SEQ ID NO: 254 encoding the heavy chain sequence of SEQ ID NO: 54; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 255; SEQ ID NO: 256; and SEQ ID NO: 257) of the light chain variable sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 52; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 258; SEQ ID NO: 259; and SEQ ID NO: 260) of the heavy chain variable sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 54.

[00646] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab6, the polynucleotides encoding the full length Ab6 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 252 encoding the light chain sequence of SEQ ID NO: 52 and the polynucleotide SEQ ID NO: 254 encoding the heavy chain sequence of SEQ ID NO: 54.

[00647] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab6 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab6 or Fab fragments thereof may be produced via expression of Ab6 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00648] Antibody Ab7

[00649] The invention optionally is further directed to the use of polynucleotides set forth below to produce antibody Ab7 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75. in methods of treating pain in an individual comprising administering to said individual antibody Ab7 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 61:

[00650] GCCGATGTTGTGATGACCCAGACTCCAGCCTCCGTGTCTCAACCTGTG
GGAGGCACAGTCACCATCAAGTGCCAGGCCAGTGAGGACATTTATAACTTATTGGC
CTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATTCTGCATCCAC
TCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGAGTACA
CTCTCACCATCAGCGGCCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAAAACA
ATTATCTTGTTACTACTTATGGTGTGCTTTCGGCGGAGGGACCGAGGTGGTGGTCA
AACGT (SEQ ID NO: 261).

[00651] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 62:

[00652] GCCGATGTTGTGATGACCCAGACTCCAGCCTCCGTGTCTCAACCTGTG
GGAGGCACAGTCACCATCAAGTGCCAGGCCAGTGAGGACATTTATAACTTATTGGC
CTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATTCTGCATCCAC
TCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGAGTACA
CTCTCACCATCAGCGGCCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAAAACA
ATTATCTTGTTACTACTTATGGTGTGCTTTCGGCGGAGGGACCGAGGTGGTGGTCA
AACGTACGGTAGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGA
AATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA
AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTC
ACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAG
CAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 262).

[00653] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 63:

[00654] CAGGAGCAGCTGAAGGAGTCCGGGGGTGCGCTGGTCACGCCTGGGAC
ACCCCTGACACTCACCTGTACAGTCTCTGGATTCTCCCTCAGTAGCTATGCAATGAT
CTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGATACATTGATACTG
ATACTAGCGCATACTACGCGAGCTGGGTGAAAGGCCGATTACCATCTCCAGAACC
TCGACCACGGTGGATCTCAAAATCACTAGTCCGACAACCGAGGACACGGCCACCTA
TTTCTGTGCCAGATCTTATGCTGCTTATGGTGGTTATCCTGCTACTTTTGATCCCTGG
GGCCCAGGCACCCTGGTCACCGTCTCGAGC (SEQ ID NO: 263).

[00655] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 64:

[00656] CAGGAGCAGCTGAAGGAGTCCGGGGGTGCGCTGGTCACGCCTGGGAC
ACCCCTGACACTCACCTGTACAGTCTCTGGATTCTCCCTCAGTAGCTATGCAATGAT
CTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGATACATTGATACTG
ATACTAGCGCATACTACGCGAGCTGGGTGAAAGGCCGATTACCATCTCCAGAACC

TCGACCACGGTGGATCTCAAAATCACTAGTCCGACAACCGAGGACACGGCCACCTA
TTTCTGTGCCAGATCTTATGCTGCTTATGGTGGTTATCCTGCTACTTTTGATCCCTGG
GGCCCAGGCACCCTGGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTC
CCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGAC
CAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAG
CAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGT
GAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG
ACAAAACCTCACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGGACCGTCA
GTCTTCCTCTTCCCCCCTCAAGGACACCCTCATGATCTCCCGGACCCCTGAG
GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG
AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGA
GAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGC
CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCG
TGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAATGA (SEQ ID NO: 264).

[00657] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 265; SEQ ID NO: 266; and SEQ ID NO: 267 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 61 or the light chain sequence of SEQ ID NO: 62.

[00658] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having

binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 268; SEQ ID NO: 269; and SEQ ID NO: 270 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 63 or the heavy chain sequence of SEQ ID NO: 64.

[00659] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 261 encoding the light chain variable sequence of SEQ ID NO: 61; the polynucleotide SEQ ID NO: 262 encoding the light chain sequence of SEQ ID NO: 62; the polynucleotide SEQ ID NO: 263 encoding the heavy chain variable sequence of SEQ ID NO: 63; the polynucleotide SEQ ID NO: 264 encoding the heavy chain sequence of SEQ ID NO: 64; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 265; SEQ ID NO: 266; and SEQ ID NO: 267) of the light chain variable sequence of SEQ ID NO: 61 or the light chain sequence of SEQ ID NO: 62; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 268; SEQ ID NO: 269; and SEQ ID NO: 270) of the heavy chain variable sequence of SEQ ID NO: 63 or the heavy chain sequence of SEQ ID NO: 64.

[00660] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab7, the polynucleotides encoding the full length Ab7 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 262 encoding the light chain sequence of SEQ ID NO: 62 and the polynucleotide SEQ ID NO: 264 encoding the heavy chain sequence of SEQ ID NO: 64.

[00661] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such

as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab7 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab7 or Fab fragments thereof may be produced via expression of Ab7 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00662] Antibody Ab8

[00663] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab8 polypeptides having binding specificity to NGF, which inhibits the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab8 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 71:

[00664] GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTGAGGACATTTACAACCTTATTGGCCTGG
TATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATTCTGCATCCACTCTG
GCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTACACTCTC
ACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTCAAACAACATAT
CTTGTTACTACTTATGGTGTGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAACGT
(SEQ ID NO: 271).

[00665] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 72:

[00666] GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTGAGGACATTTACAACTTATTGGCCTGG
TATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATTCTGCATCCACTCTG
GCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTACACTCTC
ACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTCAAAACAACATAT
CTTGTTACTACTTATGGTGTGTGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAACGT
ACGGTAGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA
CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAG
CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGC
TCGCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 272).

[00667] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 73:

[00668] CAGGTACAGCTGGTGGAGTCTGGTGGAGGCGTGGTCCAGCCTGGGAG
GTCCCTGAGACTCTCCTGTGCAGCTTCTGGATTACCTTCAGTAGCTATGCAATGATC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGATACATTGATACTGA
TACTAGCGCATACTACGCAAGCAGTGTGAAAGGCCGATTCACCATCTCCAGAGACA
ATTCCAAGAACACGCTGTACCTGCAAATGTCTAGCCTGAGAGCCGAGGACACGGCT
GTGTATTACTGTGCTAGATCTTATGCTGCTTATGGTGGTTATCCTGCTACTTTTGATC
CCTGGGGCCAAGGTACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 273).

[00669] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 74:

[00670] CAGGTACAGCTGGTGGAGTCTGGTGGAGGCGTGGTCCAGCCTGGGAG
GTCCCTGAGACTCTCCTGTGCAGCTTCTGGATTACCTTCAGTAGCTATGCAATGATC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGATACATTGATACTGA
TACTAGCGCATACTACGCAAGCAGTGTGAAAGGCCGATTCACCATCTCCAGAGACA

ATTCCAAGAACACGCTGTACCTGCAAATGTCTAGCCTGAGAGCCGAGGACACGGCT
GTGTATTACTGTGCTAGATCTTATGCTGCTTATGGTGGTTATCCTGCTACTTTTGATC
CCTGGGGCCAAGGTACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGG
TCTTCCCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCT
GCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCC
CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATC
TTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGAC
CGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC
AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG
AGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCC
ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACAC
CCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG
GAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTC
TACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTC
TCCGGGTAAATGA (SEQ ID NO: 274).

[00671] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 275; SEQ ID NO: 276; and SEQ ID NO: 277 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 71 or the light chain sequence of SEQ ID NO: 72.

[00672] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having

binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 278; SEQ ID NO: 279; and SEQ ID NO: 280 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 73 or the heavy chain sequence of SEQ ID NO: 74.

[00673] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 271 encoding the light chain variable sequence of SEQ ID NO: 71; the polynucleotide SEQ ID NO: 272 encoding the light chain sequence of SEQ ID NO: 72; the polynucleotide SEQ ID NO: 273 encoding the heavy chain variable sequence of SEQ ID NO: 73; the polynucleotide SEQ ID NO: 274 encoding the heavy chain sequence of SEQ ID NO: 74; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 275; SEQ ID NO: 276; and SEQ ID NO: 277) of the light chain variable sequence of SEQ ID NO: 71 or the light chain sequence of SEQ ID NO: 72; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 278; SEQ ID NO: 279; and SEQ ID NO: 280) of the heavy chain variable sequence of SEQ ID NO: 73 or the heavy chain sequence of SEQ ID NO: 74.

[00674] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab8, the polynucleotides encoding the full length Ab8 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 272 encoding the light chain sequence of SEQ ID NO: 72 and the polynucleotide SEQ ID NO: 274 encoding the heavy chain sequence of SEQ ID NO: 74.

[00675] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such

as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab8 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab8 or Fab fragments thereof may be produced via expression of Ab8 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00676] *Antibody Ab9*

[00677] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab9 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab9 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 81:

[00678] GCCTATGATATGACCCAGACTCCAGCCTCCGTGTCTGCAGCTGTGGGA
GGCACAGTCACCATCAAGTGCCAGGCCAGTGAGAACATTGGTAGCTACTTAGCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCGAACTCCTGATCTACAGGGCGTCCACTCT
GGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCT
CACCATCAGCGGCGTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAACAGGGTTA
TAATAGTGAGAATCTTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAAC
GT (SEQ ID NO: 281).

[00679] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 82:

[00680] GCCTATGATATGACCCAGACTCCAGCCTCCGTGTCTGCAGCTGTGGGA
GGCACAGTCACCATCAAGTGCCAGGCCAGTGAGAACATTGGTAGCTACTTAGCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCGAACTCCTGATCTACAGGGCGTCCACTCT
GGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCT
CACCATCAGCGGCGTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAACAGGGTTA
TAATAGTGAGAATCTTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAAC
GTACGGTAGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAG
TACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 282).

[00681] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 83:

[00682] CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGAATCGACCTCAGTATGTATTCAATGGGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGATGGATTAGTTATGGTG
GTACTGCATATTACGCGAGCTGGGCGAAGGGCCGATTCACCATCTCCAAAACCTCG
ACCACGGTGGAGCTGAAGATCACAGTCCGACAATCGAGGACACGGCCACCTATTT
CTGTGCCAGAGAGACTCCTGTTAATTATTATTTGGACATTTGGGGCCAGGGGACCCT
CGTCACCGTCTCGAGC (SEQ ID NO: 283).

[00683] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 84:

[00684] CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGAATCGACCTCAGTATGTATTCAATGGGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGATGGATTAGTTATGGTG
GTACTGCATATTACGCGAGCTGGGCGAAGGGCCGATTCACCATCTCCAAAACCTCG

ACCACGGTGGAGCTGAAGATCACCAAGTCCGACAATCGAGGACACGGCCACCTATTT
CTGTGCCAGAGAGACTCCTGTTAATTATTATTTGGACATTTGGGGCCAGGGGACCCT
CGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTC
CTCCAAGAGCACCTCTGGGGGACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
TCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCAC
ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACC
GTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCC
AGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACAC
ATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCC
CCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAGCACGTA
CCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGT
ACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGA
GGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGAC
CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGT
GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG
CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ
ID NO: 284).

[00685] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 285; SEQ ID NO: 286; and SEQ ID NO: 287 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 81 or the light chain sequence of SEQ ID NO: 82.

[00686] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having

binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 288; SEQ ID NO: 289; and SEQ ID NO: 290 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 83 or the heavy chain sequence of SEQ ID NO: 84.

[00687] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 281 encoding the light chain variable sequence of SEQ ID NO: 81; the polynucleotide SEQ ID NO: 282 encoding the light chain sequence of SEQ ID NO: 82; the polynucleotide SEQ ID NO: 283 encoding the heavy chain variable sequence of SEQ ID NO: 83; the polynucleotide SEQ ID NO: 284 encoding the heavy chain sequence of SEQ ID NO: 84; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 285; SEQ ID NO: 286; and SEQ ID NO: 287) of the light chain variable sequence of SEQ ID NO: 81 or the light chain sequence of SEQ ID NO: 82; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 288; SEQ ID NO: 289; and SEQ ID NO: 290) of the heavy chain variable sequence of SEQ ID NO: 83 or the heavy chain sequence of SEQ ID NO: 84.

[00688] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab9, the polynucleotides encoding the full length Ab9 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 282 encoding the light chain sequence of SEQ ID NO: 82 and the polynucleotide SEQ ID NO: 284 encoding the heavy chain sequence of SEQ ID NO: 84.

[00689] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such

as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab9 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab9 or Fab fragments thereof may be produced via expression of Ab9 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00690] *Antibody Ab10*

[00691] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab10 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab10 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 91:

[00692] GCCTATGATATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAG
ACAGAGTCACCATCACTTGCCAGGCCAGTGAGAACATTGGTAGCTACTTAGCCTGGT
ATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATAGGGCTTCCACTCTGG
CATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCA
CCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTCAACAGGGTTACA
ATAGTGAGAATCTTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAACGT
(SEQ ID NO: 291).

[00693] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 92:

[00694] GCCTATGATATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAG
ACAGAGTCACCATCACTTGCCAGGCCAGTGAGAACATTGGTAGCTACTTAGCCTGGT
ATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATAGGGCTTCCACTCTGG
CATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCA
CCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTCAACAGGGTTACA
ATAGTGAGAATCTTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAACGT
ACGGTAGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT
GGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA
CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAG
CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGC
TCGCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 292).

[00695] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 93:

[00696] CAGGTACAGCTGGTGGAGTCTGGTGGAGGCGTGGTCCAGCCTGGGAG
GTCCCTGAGACTCTCCTGTGCAGCTTCTGGATTACCTTCAGTATGTATTCAATGGGC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGATGGATTAGTTATGG
TGGTACTGCATACTACGCTAGCAGCGCTAAGGGCCGATTCACCATCTCCAGAGACA
ATTCCAAGAACACGCTGTACCTGCAAATGTCTAGCCTGAGAGCCGAGGACACGGCT
GTGTATTACTGTGCTAGAGAGACTCCTGTTAATTACTACTTGGACATTTGGGGCCAA
GGTACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 293).

[00697] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 94:

[00698] CAGGTACAGCTGGTGGAGTCTGGTGGAGGCGTGGTCCAGCCTGGGAG
GTCCCTGAGACTCTCCTGTGCAGCTTCTGGATTACCTTCAGTATGTATTCAATGGGC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGATGGATTAGTTATGG
TGGTACTGCATACTACGCTAGCAGCGCTAAGGGCCGATTCACCATCTCCAGAGACA

ATTCCAAGAACACGCTGTACCTGCAAATGTCTAGCCTGAGAGCCGAGGACACGGCT
GTGTATTACTGTGCTAGAGAGACTCCTGTTAATTACTACTTGGACATTTGGGGCCAA
GGTACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG
GCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAA
GGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCG
GCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCG
TGGTGACCGTGCCCTCCAGCAGCTTGGGACCCAGACCTACATCTGCAACGTGAATC
ACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAA
ACTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTC
CTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACA
TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGT
GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCC
AGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGC
AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAAC
CATCTCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAATA
CAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCT
CACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC
ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT
GA (SEQ ID NO: 294).

[00699] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 295; SEQ ID NO: 296; and SEQ ID NO: 297 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 91 or the light chain sequence of SEQ ID NO: 92.

[00700] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having

binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 298; SEQ ID NO: 299; and SEQ ID NO: 300 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 93 or the heavy chain sequence of SEQ ID NO: 94.

[00701] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 291 encoding the light chain variable sequence of SEQ ID NO: 91; the polynucleotide SEQ ID NO: 292 encoding the light chain sequence of SEQ ID NO: 92; the polynucleotide SEQ ID NO: 293 encoding the heavy chain variable sequence of SEQ ID NO: 93; the polynucleotide SEQ ID NO: 294 encoding the heavy chain sequence of SEQ ID NO: 94; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 295; SEQ ID NO: 296; and SEQ ID NO: 297) of the light chain variable sequence of SEQ ID NO: 91 or the light chain sequence of SEQ ID NO: 92; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 298; SEQ ID NO: 299; and SEQ ID NO: 300) of the heavy chain variable sequence of SEQ ID NO: 93 or the heavy chain sequence of SEQ ID NO: 94.

[00702] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab10, the polynucleotides encoding the full length Ab10 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 292 encoding the light chain sequence of SEQ ID NO: 92 and the polynucleotide SEQ ID NO: 294 encoding the heavy chain sequence of SEQ ID NO: 94.

[00703] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such

as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab10 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab10 or Fab fragments thereof may be produced via expression of Ab10 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00704] *Antibody Ab11*

[00705] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab11 polypeptides having binding specificity to NGF, which inhibits the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab11 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 101:

[00706] GCATTCGAATTGACCCAGACTCCATCCTCCGTGGAGGCAGCTGTGGGA
GGCACAGTCACCATCAAGTGCCAGGCCAGTCAGAACATTGTTACCAATTTAGCCTGG
TATCAACAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACTCTG
GCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTC
ACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTATTTCTGTGAGAGCTATGAT
GGTTTTAATAGTGCTGGGTTCGGCGGAGGGACCGAGGTGGTGGTCAAACGT (SEQ
ID NO: 301).

[00707] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 102:

[00708] GCATTCGAATTGACCCAGACTCCATCCTCCGTGGAGGCAGCTGTGGGA
GGCACAGTCACCATCAAGTGCCAGGCCAGTCAGAACATTGTTACCAATTTAGCCTGG
TATCAACAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACTCTG
GCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTC
ACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTATTTCTGTCAGAGCTATGAT
GGTTTTAATAGTGCTGGGTTCTGGCGGAGGGACCGAGGTGGTGGTCAAACGTACGGT
AGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC
TGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG
GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGG
ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGAC
TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 302).

[00709] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 103:

[00710] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGCCTCTGGATTCTCCCTCAGTGGCTACGACATGAGCTG
GGTCCGCCAGGCTCCAGGAAAGGGGCTGGAATACATCGGACTCATTAGTTATGATG
GTAACACATACTACGCGACCTGGGCGAAAGGCCGATTACCATCTCCAAAACCTCG
ACCACGGTGGATCTGAAAATCACCAGTCCGACAACCGAGGACACGGCCACCTATTT
CTGTGCCAGAAGTCTTTATGCTGGTCCTAATGCTGGTATCGGACCGTTTAACATCTG
GGGCCAGGGGACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 303).

[00711] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 104:

[00712] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGCCTCTGGATTCTCCCTCAGTGGCTACGACATGAGCTG
GGTCCGCCAGGCTCCAGGAAAGGGGCTGGAATACATCGGACTCATTAGTTATGATG
GTAACACATACTACGCGACCTGGGCGAAAGGCCGATTACCATCTCCAAAACCTCG

ACCACGGTGGATCTGAAAATCACCAGTCCGACAACCGAGGACACGGCCACCTATTT
 CTGTGCCAGAAGTCTTTATGCTGGTCCTAATGCTGGTATCGGACCGTTTAACATCTG
 GGGCCAGGGGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTT
 CCCCCTGGCAcCCTCCTCCaAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCT
 GGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGA
 CCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCA
 GCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
 GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCCAAATCTTG
 TGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGT
 CAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTG
 AGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
 TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC
 AGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGC
 TGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC
 GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
 GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCA
 AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
 AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTAC
 AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
 CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC
 GGGTAAATGA (SEQ ID NO: 304).

[00713] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 305; SEQ ID NO: 306; and SEQ ID NO: 307 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 101 or the light chain sequence of SEQ ID NO: 102.

[00714] In a further optional embodiment of the invention, polynucleotides encoding for treatment or prevention of pain and pain associated conditions fragments having binding

specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 308; SEQ ID NO: 309; and SEQ ID NO: 310 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 103 or the heavy chain sequence of SEQ ID NO: 104.

[00715] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 301 encoding the light chain variable sequence of SEQ ID NO: 101; the polynucleotide SEQ ID NO: 302 encoding the light chain sequence of SEQ ID NO: 102; the polynucleotide SEQ ID NO: 303 encoding the heavy chain variable sequence of SEQ ID NO: 103; the polynucleotide SEQ ID NO: 304 encoding the heavy chain sequence of SEQ ID NO: 104; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 305; SEQ ID NO: 306; and SEQ ID NO: 307) of the light chain variable sequence of SEQ ID NO: 101 or the light chain sequence of SEQ ID NO: 102; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 308; SEQ ID NO: 309; and SEQ ID NO: 310) of the heavy chain variable sequence of SEQ ID NO: 103 or the heavy chain sequence of SEQ ID NO: 104.

[00716] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab11, the polynucleotides encoding the full length Ab11 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 302 encoding the light chain sequence of SEQ ID NO: 102 and the polynucleotide SEQ ID NO: 304 encoding the heavy chain sequence of SEQ ID NO: 104.

[00717] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such

as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab11 following expression of the full-length polynucleotides in a suitable host. In another optional embodiment of the invention, anti-NGF antibodies such as Ab11 or Fab fragments thereof may be produced via expression of Ab11 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00718] *Antibody Ab12*

[00719] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab12 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab12 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 111:

[00720] GCATTCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAACATTGTTACCAACTTAGCCTGG
TATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATGGTGCATCCACTCTG
GCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTC
ACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTCAGAGCTATGAT
GGTTTCAATAGTGCTGGTTTCGGCGGAGGAACCAAGGTGGAAATCAAACGT (SEQ
ID NO: 311).

[00721] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 112:

[00722] GCATTCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAACATTGTTACCAACTTAGCCTGG
TATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATGGTGCATCCACTCTG
GCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTC
ACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTCAGAGCTATGAT
GGTTTCAATAGTGCTGGTTTCGGCGGAGGAACCAAGGTGGAAATCAAACGTACGGT
AGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC
TGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG
GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGG
ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGAC
TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 312).

[00723] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 113:

[00724] CAGGTACAGCTGGTGGAGTCTGGTGGAGGCGTGGTCCAGCCTGGGAG
GTCCTGAGACTCTCCTGTGCAGCTTCTGGATTCTCCCTCAGTGGCTACGACATGAG
CTGGGTCCGTCAGGCTCCAGGCAAGGGACTGGAGTGGGTGGGACTCATTAGTTATG
ATGGTAACACATACTACGCGACCTCCGCGAAAGGCCGATTACCATCTCCAGAGAC
AATTCCAAGAACACGCTGTACCTGCAAATGTCTAGCCTGAGAGCCGAGGACACGGC
TGTGTATTACTGTGCTAGAAAGTCTTTATGCTGGTCCTAATGCTGGTATCGGACCGTTT
AACATCTGGGGCCAAGGTACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 313).

[00725] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 114:

[00726] CAGGTACAGCTGGTGGAGTCTGGTGGAGGCGTGGTCCAGCCTGGGAG
GTCCTGAGACTCTCCTGTGCAGCTTCTGGATTCTCCCTCAGTGGCTACGACATGAG
CTGGGTCCGTCAGGCTCCAGGCAAGGGACTGGAGTGGGTGGGACTCATTAGTTATG
ATGGTAACACATACTACGCGACCTCCGCGAAAGGCCGATTACCATCTCCAGAGAC

AATTCCAAGAACACGCTGTACCTGCAAATGTCTAGCCTGAGAGCCGAGGACACGGC
TGTGTATTACTGTGCTAGAAAGTCTTTATGCTGGTCCTAATGCTGGTATCGGACCGTTT
AACATCTGGGGCCAAGGTACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCC
ATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCT
GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAG
GCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT
ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA
TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
AAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGG
ACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAA
GTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG
AGGAGCAGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGC
CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT
ACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC
CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA
GCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT
CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCT
CATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCC
TGTCTCCGGGTAAATGA (SEQ ID NO: 314).

[00727] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 315; SEQ ID NO: 316; and SEQ ID NO: 317 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 111 or the light chain sequence of SEQ ID NO: 112.

[00728] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having

binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 318; SEQ ID NO: 319; and SEQ ID NO: 320 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 113 or the heavy chain sequence of SEQ ID NO: 114.

[00729] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 311 encoding the light chain variable sequence of SEQ ID NO: 111; the polynucleotide SEQ ID NO: 312 encoding the light chain sequence of SEQ ID NO: 112; the polynucleotide SEQ ID NO: 313 encoding the heavy chain variable sequence of SEQ ID NO: 113; the polynucleotide SEQ ID NO: 314 encoding the heavy chain sequence of SEQ ID NO: 114; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 315; SEQ ID NO: 316; and SEQ ID NO: 317) of the light chain variable sequence of SEQ ID NO: 111 or the light chain sequence of SEQ ID NO: 112; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 318; SEQ ID NO: 319; and SEQ ID NO: 320) of the heavy chain variable sequence of SEQ ID NO: 113 or the heavy chain sequence of SEQ ID NO: 114.

[00730] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab12, the polynucleotides encoding the full length Ab12 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 312 encoding the light chain sequence of SEQ ID NO: 112 and the polynucleotide SEQ ID NO: 314 encoding the heavy chain sequence of SEQ ID NO: 114.

[00731] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such

as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab12 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab12 or Fab fragments thereof may be produced via expression of Ab12 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00732] *Antibody Ab13*

[00733] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab13 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab13 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 121:

[00734] GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAGCTGTGGGA
GGCACAGTCAGCATCAGTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAAGGCATCC
ACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCGGTGGATCTGGGACAGATTTC
ACTCTACCATCAGCGACGTGCAGTGTGACGCTGCTGCCACTTACTACTGTGCAGGC
GGTTATACCAGTAGTAGTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGT (SEQ ID NO: 321).

[00735] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 122:

[00736] GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAGCTGTGGGA
GGCACAGTCAGCATCAGTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAAGGCATCC
ACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCGGTGGATCTGGGACAGATTTC
ACTCTACCATCAGCGACGTGCAGTGTGACGCTGCTGCCACTTACTACTGTGCAGGC
GGTTATACCAGTAGTAGTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGTACGGTAGCGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTA (SEQ ID NO: 322).

[00737] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 123:

[00738] CAGTCGGTGGAGGCGTCCGGGGGTGCGCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGCCTCTGGATTCTCCCTCAGTACCTACTGGATGAGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGACATTTATTTTAGTA
ATGAAGAAACAACTACGCGAGCTGGGCGAAAGGCCGATTTACCATCTCCAAAACC
TCGACCACGGTGGATCTGAATGTCATCAGTCCGACAACCGAGGACACGGCCACCTA
TTTCTGTGCCAGAGGTTCTCCTGATGTTGATATTGGTATAGATATGTGGGGCCCGGG
CACCTCGTCACCGTCTCGAGC (SEQ ID NO: 323).

[00739] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 124:

[00740] CAGTCGGTGGAGGCGTCCGGGGGTGCGCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGCCTCTGGATTCTCCCTCAGTACCTACTGGATGAGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGACATTTATTTTAGTA
ATGAAGAAACAACTACGCGAGCTGGGCGAAAGGCCGATTTACCATCTCCAAAACC

TCGACCACGGTGGATCTGAATGTCATCAGTCCGACAACCGAGGACACGGCCACCTA
 TTTCTGTGCCAGAGGTTCTCCTGATGTTGATATTGGTATAGATATGTGGGGCCCCGGG
 CACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGC
 ACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
 ACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGC
 GTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG
 GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC
 AAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAC
 TCACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCT
 CTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATG
 CGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG
 ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAG
 CACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAA
 GGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCA
 TCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCC
 CGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTA
 TCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACA
 AGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA
 CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
 GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG
 A (SEQ ID NO: 324).

[00741] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 325; SEQ ID NO: 326; and SEQ ID NO: 327 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 121 or the light chain sequence of SEQ ID NO: 122.

[00742] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having

binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 328; SEQ ID NO: 329; and SEQ ID NO: 330 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 123 or the heavy chain sequence of SEQ ID NO: 124.

[00743] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 321 encoding the light chain variable sequence of SEQ ID NO: 121; the polynucleotide SEQ ID NO: 322 encoding the light chain sequence of SEQ ID NO: 122; the polynucleotide SEQ ID NO: 323 encoding the heavy chain variable sequence of SEQ ID NO: 123; the polynucleotide SEQ ID NO: 324 encoding the heavy chain sequence of SEQ ID NO: 124; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 325; SEQ ID NO: 326; and SEQ ID NO: 327) of the light chain variable sequence of SEQ ID NO: 121 or the light chain sequence of SEQ ID NO: 122; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 328; SEQ ID NO: 329; and SEQ ID NO: 330) of the heavy chain variable sequence of SEQ ID NO: 123 or the heavy chain sequence of SEQ ID NO: 124.

[00744] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab13, the polynucleotides encoding the full length Ab13 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 322 encoding the light chain sequence of SEQ ID NO: 122 and the polynucleotide SEQ ID NO: 324 encoding the heavy chain sequence of SEQ ID NO: 124.

[00745] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such

as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab13 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab13 or Fab fragments thereof may be produced via expression of Ab13 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00746] *Antibody Ab14*

[00747] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab14 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab14 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 131:

[00748] GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATAAGGCATCC
ACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTC
ACTCTACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTGCAGGC
GGTTATACCAGTAGTAGTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAA
ACGT (SEQ ID NO: 331).

[00749] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 132:

[00750] GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATAAGGCATCC
ACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTC
ACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTGCAGGC
GGTTATACCAGTAGTAGTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAA
ACGTACGGTAGCGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 332).

[00751] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 133:

[00752] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCGTCAGTACCTACTGGATGAG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAGACATTTACTTTA
GTAATGAAGAAACAACTACGCGAGCAGCGCGAAAGGCCGATTACCATCTCCAGA
GACAATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACAC
TGCTGTGTATTACTGTGCTAGAGGTTCTCCTGATGTTGATATTGGTATAGATATGTGG
GGCCCAGGGACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 333).

[00753] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 134:

[00754] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCGTCAGTACCTACTGGATGAG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAGACATTTACTTTA
GTAATGAAGAAACAACTACGCGAGCAGCGCGAAAGGCCGATTACCATCTCCAGA

GACAATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACAC
TGCTGTGTATTACTGTGCTAGAGGTTCTCCTGATGTTGATATTGGTATAGATATGTGG
GGCCCAGGGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTC
CCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGAC
CAGCGGCGTGACACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAG
CAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGT
GAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG
ACAAAACCTCACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCA
GTCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAG
GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACGCCAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG
AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGA
GAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGC
CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCG
TGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAATGA (SEQ ID NO: 334).

[00755] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 335; SEQ ID NO: 336; and SEQ ID NO: 337 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 131 or the light chain sequence of SEQ ID NO: 132.

[00756] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having

binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 338; SEQ ID NO: 339; and SEQ ID NO: 340 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 133 or the heavy chain sequence of SEQ ID NO: 134.

[00757] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 331 encoding the light chain variable sequence of SEQ ID NO: 131; the polynucleotide SEQ ID NO: 332 encoding the light chain sequence of SEQ ID NO: 132; the polynucleotide SEQ ID NO: 333 encoding the heavy chain variable sequence of SEQ ID NO: 133; the polynucleotide SEQ ID NO: 334 encoding the heavy chain sequence of SEQ ID NO: 134; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 335; SEQ ID NO: 336; and SEQ ID NO: 337) of the light chain variable sequence of SEQ ID NO: 131 or the light chain sequence of SEQ ID NO: 132; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 338; SEQ ID NO: 339; and SEQ ID NO: 340) of the heavy chain variable sequence of SEQ ID NO: 133 or the heavy chain sequence of SEQ ID NO: 134.

[00758] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab14, the polynucleotides encoding the full length Ab14 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 332 encoding the light chain sequence of SEQ ID NO: 132 and the polynucleotide SEQ ID NO: 334 encoding the heavy chain sequence of SEQ ID NO: 134.

[00759] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such

as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab14 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab14 or Fab fragments thereof may be produced via expression of Ab14 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00760] *Antibody Ab15*

[00761] The invention is further directed to the use of polynucleotides set forth below to produce antibody Ab15 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab15 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 141:

[00762] GCAGCCGTGCTGACCCAGACACCATCGCCCGTGTCTGCAGCTGTGGGA
GACACAGTCACCATCAAGTGCCAGTCCAGTCAGAGTGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGATGCATCC
AATCTGCCATCTGGGGTCCCATCACGGTTCAGCGGCAGTGGATCTGGGACACAGTTC
ACTCTCACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGC
GATTATGATGATGATACTGATAATGGTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGT (SEQ ID NO: 341).

[00763] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 142:

[00764] GCAGCCGTGCTGACCCAGACACCATCGCCCGTGTCTGCAGCTGTGGGA
GACACAGTCACCATCAAGTGCCAGTCCAGTCAGAGTGTTTATAAGAACAACACTTA
TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGATGCATCC
AATCTGCCATCTGGGGTCCCATCACGGTTCAGCGGCAGTGGATCTGGGACACAGTTC
ACTCTACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGC
GATTATGATGATGATACTGATAATGGTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGTACGGTAGCGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 342).

[00765] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 143:

[00766] CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGAATCGACCTCAGTAGCTATGCAATGATCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGAATCATTTGGAGTGGTG
GCACCTACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGACC
ACGGTGGATCTGCAAATCACCAGTCCGACAACCGAGGACGCGGCCACCTATTTCTGT
GCCGCAGGTGGTGGTAGTATTTATGATGTTTGGGGCCCGGGCACCCCTGGTCACCGTC
TCGAGC (SEQ ID NO: 343).

[00767] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 144:

[00768] CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACAGTCTCTGGAATCGACCTCAGTAGCTATGCAATGATCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGAATCATTTGGAGTGGTG
GCACCTACTACGCGACCTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGACC

ACGGTGGATCTGCAAATCACCAGTCCGACAACCGAGGACGCGGCCACCTATTTCTGT
GCCGCAGGTGGTGGTAGTATTTATGATGTTTGGGGCCCGGGCACCCTGGTCACCGTC
TCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGC
ACCTCTGGGGGACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACC
GGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGG
CTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCA
GCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACC
GTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACC
CAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGT
GAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC
ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAA
GGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAG
GGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC
AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGT
GCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAG
GTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCA
CTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 344).

[00769] In a further embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 345; SEQ ID NO: 346; and SEQ ID NO: 347 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 141 or the light chain sequence of SEQ ID NO: 142.

[00770] In a further embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide

sequences of SEQ ID NO: 348; SEQ ID NO: 349; and SEQ ID NO: 350 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 143 or the heavy chain sequence of SEQ ID NO: 144.

[00771] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 341 encoding the light chain variable sequence of SEQ ID NO: 141; the polynucleotide SEQ ID NO: 342 encoding the light chain sequence of SEQ ID NO: 142; the polynucleotide SEQ ID NO: 343 encoding the heavy chain variable sequence of SEQ ID NO: 143; the polynucleotide SEQ ID NO: 344 encoding the heavy chain sequence of SEQ ID NO: 144; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 345; SEQ ID NO: 346; and SEQ ID NO: 347) of the light chain variable sequence of SEQ ID NO: 141 or the light chain sequence of SEQ ID NO: 142; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 348; SEQ ID NO: 349; and SEQ ID NO: 350) of the heavy chain variable sequence of SEQ ID NO: 143 or the heavy chain sequence of SEQ ID NO: 144.

[00772] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab15, the polynucleotides encoding the full length Ab15 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 342 encoding the light chain sequence of SEQ ID NO: 142 and the polynucleotide SEQ ID NO: 344 encoding the heavy chain sequence of SEQ ID NO: 144.

[00773] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast

Pichia. Suitable Pichia species include, but are not limited to, Pichia pastoris. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab15 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab15 or Fab fragments thereof may be produced via expression of Ab15 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid Pichia) and other yeast strains. Suitable Pichia species include, but are not limited to, Pichia pastoris.

[00774] Antibody Ab16

[00775] The invention is further directed to the use of polynucleotides set forth below to produce antibody Ab16 polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF, which inhibit the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab16 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 151:

[00776] GCCCTGGTGATGACCCAGACTCCATCCTCCACGTCTGAACCAGTGGGA
GGCACAGTCACCATCAATTGCCAGGCTAGTCAGAATATTGGTAACGACCTATCCTGG
TATCAGCAGAAACCAGGGCAGCCTCCCGAGCTCCTAATCTATTCTACATCCAACTG
GCAACTGGGGTCCCAAAGCGGTTTCAGTGGCAGCAGATCTGGGACACAGTTCACTCT
CACCATCAGCGACCTGGAGTGTGACGATGCTGCCACTTACTACTGTCTAGGTGTTTA
TAGTTATATTAGTGATGATGGTAATGCTTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGT (SEQ ID NO: 351).

[00777] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 152:

[00778] GCCCTGGTGATGACCCAGACTCCATCCTCCACGTCTGAACCAGTGGGA
GGCACAGTCACCATCAATTGCCAGGCTAGTCAGAATATTGGTAACGACCTATCCTGG
TATCAGCAGAAACCAGGGCAGCCTCCCGAGCTCCTAATCTATTCTACATCCAAACTG
GCAACTGGGGTCCCAAAGCGGTTCAGTGGCAGCAGATCTGGGACACAGTTCACTCT
CACCATCAGCGACCTGGAGTGTGACGATGCTGCCACTTACTACTGTCTAGGTGTTTA
TAGTTATATTAGTGATGATGGTAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGTACGGTAGCGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 352).

[00779] In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 153:

[00780] CAGTCGGTGGAGGAGTTCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTCAATAACTATGCAATGACCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGGATCATTGGTAGTATTG
GTACCACATACTACGCGAGCTGGGCGAAAGGCCGATTCTTCATCTCCAAAACCTCGA
CCACTGTGGATCTGAAAATCATTAGTCCGACAACCGAGGACACGGCCACCTATTTCT
GTGCCAGAGATGCTGGCGTTACTGTTGATGGTTATGGCTACTACTTTAACATCTGGG
GCCCAGGCACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 353).

[00781] In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 154:

[00782] CAGTCGGTGGAGGAGTTCGGGGGTCGCCTGGTCACGCCTGGGACACC
CCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTCAATAACTATGCAATGACCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGGATCATTGGTAGTATTG
GTACCACATACTACGCGAGCTGGGCGAAAGGCCGATTCTTCATCTCCAAAACCTCGA

CCACTGTGGATCTGAAAATCATTAGTCCGACAACCGAGGACACGGCCACCTATTTCT
GTGCCAGAGATGCTGGCGTTACTGTTGATGGTTATGGCTACTACTTTAACATCTGGG
GCCCAGGCACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCC
CCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGACAGCGGCCCTGGGCTGCCTGG
TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACC
AGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC
AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTG
AATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGA
CAAAACTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAG
TCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG
TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG
TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGT
ACGCCAGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGA
ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG
AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC
CCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGT
GATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG
TAAATGA (SEQ ID NO: 354).

[00783] In a further embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 355; SEQ ID NO: 356; and SEQ ID NO: 357 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 151 or the light chain sequence of SEQ ID NO: 152.

[00784] In a further embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding

specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 358; SEQ ID NO: 359; and SEQ ID NO: 360 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 153 or the heavy chain sequence of SEQ ID NO: 154.

[00785] The invention also contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 351 encoding the light chain variable sequence of SEQ ID NO: 151; the polynucleotide SEQ ID NO: 352 encoding the light chain sequence of SEQ ID NO: 152; the polynucleotide SEQ ID NO: 353 encoding the heavy chain variable sequence of SEQ ID NO: 153; the polynucleotide SEQ ID NO: 354 encoding the heavy chain sequence of SEQ ID NO: 154; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 355; SEQ ID NO: 356; and SEQ ID NO: 357) of the light chain variable sequence of SEQ ID NO: 151 or the light chain sequence of SEQ ID NO: 152; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 358; SEQ ID NO: 359; and SEQ ID NO: 360) of the heavy chain variable sequence of SEQ ID NO: 153 or the heavy chain sequence of SEQ ID NO: 154.

[00786] In a preferred embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab16, the polynucleotides encoding the full length Ab16 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 352 encoding the light chain sequence of SEQ ID NO: 152 and the polynucleotide SEQ ID NO: 354 encoding the heavy chain sequence of SEQ ID NO: 154.

[00787] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO,

HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab16 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab16 or Fab fragments thereof may be produced via expression of Ab16 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00788] *Antibody Ab17*

[00789] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab17 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA without appreciably inhibiting the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab17 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 161:

[00790] GCCATCGAAATGACCCAGACTCCATTCTCCGTGTCTGCAGCTGTGGGA
GGCACAGTCACCATCAAGTGCCAGGCCAGTCAGACCATTAGCAACTACTTAGCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCAATCT
GGAATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCT
CACCATCAGCGACCTGGAGTGTGACGATGCTGCCACTTACTACTGTCAACAGGGTTA
TACTATCAGTAATGTTGATAACAATGTTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGT (SEQ ID NO: 361).

[00791] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 162:

[00792] GCCATCGAAATGACCCAGACTCCATTCTCCGTGTCTGCAGCTGTGGGA
GGCACAGTCACCATCAAGTGCCAGGCCAGTCAGACCATTAGCAACTACTTAGCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCAATCT
GGAATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCT
CACCATCAGCGACCTGGAGTGTGACGATGCTGCCACTTACTACTGTCAACAGGGTTA
TACTATCAGTAATGTTGATAACAATGTTTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGTACGGTAGCGGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 362).

[00793] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 163:

[00794] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGGGATC
CCTGACACTCACCTGCGCAGCCTCTGGATTCTCCCTCACTGGCTACAACCTTGGTCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATTCATTAGTTATGGTG
ATACCACATACTACGCGAGCTGGGCGAAAGGCCGATTACCATCTCCAAAACCTCG
ACCACGGTGACTCTGACGATACCGATCTGCAACCTTCAGACACGGGCACCTATTTC
TGTGCCAGAGAGACTGCTAATACTTATGATTATGGCATCTGGGGCCCAGGCACCCTC
GTCACCGTCTCGAGC (SEQ ID NO: 363).

[00795] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 164:

[00796] CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGGGATC
CCTGACACTCACCTGCGCAGCCTCTGGATTCTCCCTCACTGGCTACAACTTGGTCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATTCATTAGTTATGGTG
ATACCACATACTACGCGAGCTGGGCGAAAGGCCGATTACCATCTCCAAAACCTCG
ACCACGGTGACTCTGACGATACCGATCTGCAACCTTCAGACACGGGCACCTATTTC
TGTGCCAGAGAGACTGCTAATACTTATGATTATGGCATCTGGGGCCCAGGCACCCTC
GTCACCGTCTCGAGCGCCTCCACCAAGGGGCCATCGGTCTTCCCCCTGGCACCCCTCC
TCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTT
CCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACA
CCTTCCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCG
TGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACA
TGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG
TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAGCACGTA
CCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGT
ACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGA
GGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCA
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGAC
CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGT
GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG
CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ
ID NO: 364).

[00797] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 365; SEQ ID NO: 366; and SEQ ID NO: 367 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or

hypervariable regions) of the light chain variable sequence of SEQ ID NO: 161 or the light chain sequence of SEQ ID NO: 162.

[00798] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 368; SEQ ID NO: 369; and SEQ ID NO: 370 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 163 or the heavy chain sequence of SEQ ID NO: 164.

[00799] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 361 encoding the light chain variable sequence of SEQ ID NO: 161; the polynucleotide SEQ ID NO: 362 encoding the light chain sequence of SEQ ID NO: 162; the polynucleotide SEQ ID NO: 363 encoding the heavy chain variable sequence of SEQ ID NO: 163; the polynucleotide SEQ ID NO: 364 encoding the heavy chain sequence of SEQ ID NO: 164; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 365; SEQ ID NO: 366; and SEQ ID NO: 367) of the light chain variable sequence of SEQ ID NO: 161 or the light chain sequence of SEQ ID NO: 162; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 368; SEQ ID NO: 369; and SEQ ID NO: 370) of the heavy chain variable sequence of SEQ ID NO: 163 or the heavy chain sequence of SEQ ID NO: 164.

[00800] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab17, the polynucleotides encoding the full length Ab17 antibody comprise, or alternatively consist of, the

polynucleotide SEQ ID NO: 362 encoding the light chain sequence of SEQ ID NO: 162 and the polynucleotide SEQ ID NO: 364 encoding the heavy chain sequence of SEQ ID NO: 164.

[00801] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one optional embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab17 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab17 or Fab fragments thereof may be produced via expression of Ab17 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00802] Antibody Ab18

[00803] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab18 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab18 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 171:

[00804] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGTCAGGCTAGTCAGACCATTAGCAACTACTTAGCCTGG
TATCAGCAGAAACCAGGAAAAGCCCCTAAGCTCCTGATCTATGGTGCATCCAATCTG
GAATCTGGAGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGAACAGAATTCACCTCTC
ACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGTCAACAGGGTTAT

ACTATCAGTAATGTTGATAACAATGTTTTTCGGCGGAGGAACCAAGGTGGAAATCAA
ACGT (SEQ ID NO: 371).

[00805] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 172:

[00806] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGTCAGGCTAGTCAGACCATTAGCAACTACTTAGCCTGG
TATCAGCAGAAACCAGGAAAAGCCCCTAAGCTCCTGATCTATGGTGCATCCAATCTG
GAATCTGGAGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGAACAGAATTCACTCTC
ACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGTCAACAGGGTTAT
ACTATCAGTAATGTTGATAACAATGTTTTTCGGCGGAGGAACCAAGGTGGAAATCAA
ACGTACGGTAGCGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 372).

[00807] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 173:

[00808] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCGTCAGTGGCTACAACCTTGGT
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGATTCATTAGTTATG
GTGATACCACATACTACGCTAGCTCTGCTAAAGGCCGATTCACCATCTCCAGAGACA
ATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTG
TGTATTACTGTGCTAGAGAGACTGCTAATACTTATGATTATGGCATCTGGGGCCAAG
GGACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 373).

[00809] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 174:

[00810] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCGTCAGTGGCTACAACCTTGGT
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGATTCATTAGTTATG
GTGATACCACATACTACGCTAGCTCTGCTAAAGGCCGATTCACCATCTCCAGAGACA
ATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCTG
TGTATTACTGTGCTAGAGAGACTGCTAATACTTATGATTATGGCATCTGGGGCCAAG
GGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGG
CACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG
GACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGG
CGTGCACACCTTCCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGT
GGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA
CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAA
CTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCC
TCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACAT
GCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTG
GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCA
GCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATC
CCGGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCT
ATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTAC
AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTC
ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG
A (SEQ ID NO: 374).

[00811] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having

binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 375; SEQ ID NO: 376; and SEQ ID NO: 377 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 171 or the light chain sequence of SEQ ID NO: 172.

[00812] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 378; SEQ ID NO: 379; and SEQ ID NO: 380 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 173 or the heavy chain sequence of SEQ ID NO: 174.

[00813] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 371 encoding the light chain variable sequence of SEQ ID NO: 171; the polynucleotide SEQ ID NO: 372 encoding the light chain sequence of SEQ ID NO: 172; the polynucleotide SEQ ID NO: 373 encoding the heavy chain variable sequence of SEQ ID NO: 173; the polynucleotide SEQ ID NO: 374 encoding the heavy chain sequence of SEQ ID NO: 174; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 375; SEQ ID NO: 376; and SEQ ID NO: 377) of the light chain variable sequence of SEQ ID NO: 171 or the light chain sequence of SEQ ID NO: 172; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 378; SEQ ID NO: 379; and SEQ ID NO: 380) of the heavy chain variable sequence of SEQ ID NO: 173 or the heavy chain sequence of SEQ ID NO: 174.

[00814] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments for treatment or prevention of pain and pain associated conditions having binding specificity for NGF. With respect to antibody Ab18, the polynucleotides encoding the full length Ab18 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 372 encoding the light chain sequence of SEQ ID NO: 172 and the polynucleotide SEQ ID NO: 374 encoding the heavy chain sequence of SEQ ID NO: 174.

[00815] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab18 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab18 or Fab fragments thereof may be produced via expression of Ab18 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00816] Antibody Ab19

[00817] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab19 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75. in methods of treating pain in an individual comprising administering to said individual antibody Ab19 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 181:

[00818] GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAGCTGTGGGA
GGCACAGTCAGCATCAGTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTATTTA
TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAAGGCTTCC
ACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGATTTC
ACTCTACCATCAGCGACGTGCAGTGTGACGCTGCTGCCACTTACTACTGTGCAGGC
GGTTATAGTAGTAGTAGTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGT (SEQ ID NO: 381).

[00819] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 182:

[00820] GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAGCTGTGGGA
GGCACAGTCAGCATCAGTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTATTTA
TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAAGGCTTCC
ACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGATTTC
ACTCTACCATCAGCGACGTGCAGTGTGACGCTGCTGCCACTTACTACTGTGCAGGC
GGTTATAGTAGTAGTAGTGATAATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
ACGTACGGTAGCGGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 382).

[00821] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 183:

[00822] CAGTCGGTGGAGGCGTCCGGGGGTCGTCTGGTCATGCCTGGAGGATCC
CTGACACTCACCTGCACAGCCTCTGGATTCTCCCTCAGTACCTACTGGATGTCCTGG
GTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGACATTTATTTTAGTAA
TGAGGAAACAACTACGCGACCTGGGCGAAAGGCCGATTTACCATCTCCAAAACCT

CGACCACGGTGGATCTGAATGTCATCAGTCCGACAACCGAGGACACGGCCACCTAT
TTCTGTGCAAGAGGTTCTCCTGATGTTGAGATTGCTATAGATATGTGGGGCCAGGGC
ACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 383).

[00823] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 184:

[00824] CAGTCGGTGGAGGCGTCCGGGGGTCGTCTGGTCATGCCTGGAGGATCC
CTGACACTCACCTGCACAGCCTCTGGATTCTCCCTCAGTACCTACTGGATGTCCTGG
GTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAGACATTTATTTTAGTAA
TGAGGAAACAACTACGCGACCTGGGCGAAAGGCCGATTTACCATCTCCAAAACCT
CGACCACGGTGGATCTGAATGTCATCAGTCCGACAACCGAGGACACGGCCACCTAT
TTCTGTGCAAGAGGTTCTCCTGATGTTGAGATTGCTATAGATATGTGGGGCCAGGGC
ACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGGCCATCGGTCTTCCCCCTGGCA
CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGA
CTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGT
GCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACA
AGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCT
CACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TCCCCCCCCAAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGA
CGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAGC
ACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCAT
CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCC
GGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACA
AGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA
CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT

GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG
A (SEQ ID NO: 384).

[00825] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 385; SEQ ID NO: 386; and SEQ ID NO: 387 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 181 or the light chain sequence of SEQ ID NO: 182.

[00826] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 388; SEQ ID NO: 389; and SEQ ID NO: 390 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 183 or the heavy chain sequence of SEQ ID NO: 184.

[00827] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 381 encoding the light chain variable sequence of SEQ ID NO: 181; the polynucleotide SEQ ID NO: 382 encoding the light chain sequence of SEQ ID NO: 182; the polynucleotide SEQ ID NO: 383 encoding the heavy chain variable sequence of SEQ ID NO: 183; the polynucleotide SEQ ID NO: 384 encoding the heavy chain sequence of SEQ ID NO: 184; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 385; SEQ ID NO: 386; and SEQ ID NO: 387) of the light chain variable sequence of SEQ ID NO: 181 or the light chain sequence of SEQ ID NO: 182; and

polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 388; SEQ ID NO: 389; and SEQ ID NO: 390) of the heavy chain variable sequence of SEQ ID NO: 183 or the heavy chain sequence of SEQ ID NO: 184.

[00828] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab19, the polynucleotides encoding the full length Ab19 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 382 encoding the light chain sequence of SEQ ID NO: 182 and the polynucleotide SEQ ID NO: 384 encoding the heavy chain sequence of SEQ ID NO: 184.

[00829] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one optional embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab19 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab19 or Fab fragments thereof may be produced via expression of Ab19 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00830] Antibody Ab20

[00831] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab20 polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF, which inhibits the association of NGF with TrkA and the association of NGF with p75, in methods of treating pain in an individual comprising administering to said individual antibody Ab20 polypeptides. The invention is further directed to polynucleotides encoding antibody polypeptides having binding specificity to NGF. In one optional embodiment of the invention, polynucleotides of the invention comprise,

or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 191:

[00832] GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATAAGGCATCC
ACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTC
ACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTGCAGGC
GGTTATACCAGTAGTAGTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAA
ACGT (SEQ ID NO: 391).

[00833] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 192:

[00834] GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGTCCAGTCAGAATGTTTATAAGAACAACACTACTTA
TCCTGGTATCAGCAGAAACCAGGGAAAGTCCCTAAGCTCCTGATCTATAAGGCATCC
ACTCTGGCATCTGGGGTCCCATCTCGTTTCAGTGGCAGTGGATCTGGGACAGATTTC
ACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTGCAGGC
GGTTATACCAGTAGTAGTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAA
ACGTACGGTAGCGGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
ATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC
AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 392).

[00835] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 193:

[00836] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCGTCAGTACCTACTGGATGAG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAGACATTTACTTTA
GTAATGAAGAAACAACTACGCGACCAGCGCGAAAGGCCGATTACCATCTCCAGA
GACAATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACAC
TGCTGTGTATTACTGTGCTAGAGGTTCTCCTGATGTTGAGATTGCTATAGATATGTGG
GGCCAAGGGACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 393).

[00837] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 194:

[00838] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCGTCAGTACCTACTGGATGAG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAGACATTTACTTTA
GTAATGAAGAAACAACTACGCGACCAGCGCGAAAGGCCGATTACCATCTCCAGA
GACAATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACAC
TGCTGTGTATTACTGTGCTAGAGGTTCTCCTGATGTTGAGATTGCTATAGATATGTGG
GGCCAAGGGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTC
CCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGAC
CAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAG
CAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGT
GAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG
ACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGGACCGTCA
GTCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAG
GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACGCCAGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTG
AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGA
GAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGC
CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA

GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCG
TGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAATGA (SEQ ID NO: 394).

[00839] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 395; SEQ ID NO: 396; and SEQ ID NO: 397 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 191 or the light chain sequence of SEQ ID NO: 192.

[00840] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 398; SEQ ID NO: 399; and SEQ ID NO: 400 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 193 or the heavy chain sequence of SEQ ID NO: 194.

[00841] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 391 encoding the light chain variable sequence of SEQ ID NO: 191; the polynucleotide SEQ ID NO: 392 encoding the light chain sequence of SEQ ID NO: 192; the polynucleotide SEQ ID NO: 393 encoding the heavy chain variable sequence of SEQ ID NO: 193; the polynucleotide SEQ ID NO: 394 encoding the heavy chain

sequence of SEQ ID NO: 194; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 395; SEQ ID NO: 396; and SEQ ID NO: 397) of the light chain variable sequence of SEQ ID NO: 191 or the light chain sequence of SEQ ID NO: 192; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 398; SEQ ID NO: 399; and SEQ ID NO: 400) of the heavy chain variable sequence of SEQ ID NO: 193 or the heavy chain sequence of SEQ ID NO: 194.

[00842] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab20, the polynucleotides encoding the full length Ab20 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 392 encoding the light chain sequence of SEQ ID NO: 192 and the polynucleotide SEQ ID NO: 394 encoding the heavy chain sequence of SEQ ID NO: 194.

[00843] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab20 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab20 or Fab fragments thereof may be produced via expression of Ab20 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00844] Antibody Ab21

[00845] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody Ab21 polypeptides having binding specificity to NGF, which inhibit the association of NGF with TrkA and the association of NGF with p75 in methods of treating pain in an individual comprising administering to said individual antibody Ab21 polypeptides. The invention is further optionally directed to polynucleotides encoding antibody polypeptides

for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 51:

[00846] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAGCATTTACAGCAATCTTGCCTGG
TATCAGCAGAAACCAGGAAAAGCCCCTAAGCTCCTGATCTATGATGCATCCACTCTG
GAATCTGGAGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAGTACACTCT
CACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGCCAACAGGGTTT
TACTGTTAGTGATATTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAAC
GT (SEQ ID NO: 251).

[00847] In one embodiment of the invention, polynucleotides of the invention optionally comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 401:

[00848] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAGCATTTACAGCAATCTTGCCTGG
TATCAGCAGAAACCAGGAAAAGCCCCTAAGCTCCTGATCTATGATGCATCCACTCTG
GAATCTGGAGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAGTACACTCT
CACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGCCAACAGGGTTT
TACTGTTAGTGATATTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAAC
GTACGGTAGCGGCCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAG
TACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 403).

[00849] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 53:

[00850] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCGTCAGTAACTATGCAGTGGG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAATCATTGGTCGTA
ATGGTAACACATGGTACGCGAGCTCTGCAAGAGGCCGATTACCATCTCCAGAGAC
AATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCT
GTGTATTACTGTGCTAGAGGATATGGCCGTAGTGTGCTTATTACGTCTTTAACATCT
GGGGCCCAGGGACCCTCGTCACCGTCTCGAGC (SEQ ID NO: 253).

[00851] In one optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 402:

[00852] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCGTCAGTAACTATGCAGTGGG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTCGGAATCATTGGTCGTA
ATGGTAACACATGGTACGCGAGCTCTGCAAGAGGCCGATTACCATCTCCAGAGAC
AATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCT
GTGTATTACTGTGCTAGAGGATATGGCCGTAGTGTGCTTACTACGTCTTTAACATCT
GGGGCCCAGGGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGGCCATCGGTCT
TCCCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTG
ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACGCGAGAGTTGAGCCCAAATCTT
GTGACAAAACCTCACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGGACCG
TCAGTCTTCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT
GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAA
CTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC
AGTACGCCAGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGC
TGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC
GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCA

AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTAC
AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC
GGGTAAATGA (SEQ ID NO: 404).

[00853] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 255; SEQ ID NO: 256; and SEQ ID NO: 257 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 401.

[00854] In a further optional embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one or more of the polynucleotide sequences of SEQ ID NO: 258; SEQ ID NO: 259; and SEQ ID NO: 260 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 402.

[00855] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 251 encoding the light chain variable sequence of SEQ ID NO: 51; the polynucleotide SEQ ID NO: 403 encoding the light chain sequence of SEQ ID NO: 401; the polynucleotide SEQ ID NO: 253 encoding the heavy chain variable sequence of SEQ ID NO: 53; the polynucleotide SEQ ID NO: 404 encoding the heavy chain sequence of

SEQ ID NO: 402; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 255; SEQ ID NO: 256; and SEQ ID NO: 257) of the light chain variable sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 401; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 258; SEQ ID NO: 259; and SEQ ID NO: 260) of the heavy chain variable sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 402.

[00856] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody Ab21, the polynucleotides encoding the full length Ab21 antibody comprise, or alternatively consist of, the polynucleotide SEQ ID NO: 403 encoding the light chain sequence of SEQ ID NO: 401 and the polynucleotide SEQ ID NO: 404 encoding the heavy chain sequence of SEQ ID NO: 402.

[00857] Another optional embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one optional embodiment of the invention described herein (infra), Fab fragments may be produced by enzymatic digestion (e.g., papain) of Ab21 following expression of the full-length polynucleotides in a suitable host. In another embodiment of the invention, anti-NGF antibodies such as Ab21 or Fab fragments thereof may be produced via expression of Ab21 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00858] *Antibody fragment Fab2*

[00859] The invention is further optionally directed to the use of polynucleotides set forth below to produce antibody fragment Fab2 polypeptides that inhibit the association of NGF with TrkA and p75 for treatment or prevention of pain and pain associated conditions having binding specificity to NGF in methods of treating pain in an individual comprising administering to said individual antibody Ab1 polypeptides. The invention is further directed to polynucleotides

encoding antibody fragment polypeptides for treatment or prevention of pain and pain associated conditions having binding specificity to NGF. In one embodiment of the invention, Fab polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the light chain polypeptide sequence of SEQ ID NO: 407:

[00860] GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGA
GACAGAGTCACCATCACTTGCCAGGCCAGTCAGAGCATTTACAGCAATCTTGCCTGG
TATCAGCAGAAACCAGGAAAAGCCCCTAAGCTCCTGATCTATGATGCATCCACTCTG
GAATCTGGAGTCCCATCAAGGTTTACGCGGCAGTGGATCTGGGACAGAGTAACTCT
CACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTACTACTGCCAACAGGGTTT
TACTGTTAGTGATATTGATAATGCTTTCGGCGGAGGAACCAAGGTGGAAATCAAAC
GTACGGTAGCGGCCCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAAT
CTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAG
TACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTGACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 409).

[00861] In another optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the heavy chain polypeptide sequence of SEQ ID NO: 408:

[00862] GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGG
GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCGTCAGTAACTATGCAGTGGG
CTGGGTCCGTCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCCGAATCATTGGTCGTA
ATGGTAACACATGGTACGCGAGCTCTGCAAGAGGCCGATTCACCATCTCCAGAGAC
AATTCCAAGAACACCCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACTGCT
GTGTATTACTGTGCTAGAGGATATGGCCGTAGTGTGCTTACTACGTCTTTAACATCT
GGGGCCCAGGGACCCTCGTCACCGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCT
TCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG
ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC

AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACGCGAGAGTTGAGCCCAAATCTT
GTGACAAAACCTCACTAG (SEQ ID NO: 410).

[00863] In a further optional embodiment of the invention, polynucleotides encoding Fab antibody fragments having binding specificity to NGF comprise one or more of the polynucleotide sequences of SEQ ID NO: 255; SEQ ID NO: 256; and SEQ ID NO: 257 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 409.

[00864] In a further optional embodiment of the invention, polynucleotides encoding Fab antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise one or more of the polynucleotide sequences of SEQ ID NO: 258; SEQ ID NO: 259; and SEQ ID NO: 260 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 410.

[00865] The invention also optionally contemplates polynucleotide sequences including one or more of the polynucleotide sequences encoding antibody fragments for treatment or prevention of pain and pain associated conditions described herein. In one embodiment of the invention, polynucleotides encoding antibody fragments for treatment or prevention of pain and pain associated conditions having binding specificity to NGF comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 251 encoding the light chain variable sequence of SEQ ID NO: 51; the polynucleotide SEQ ID NO: 409 encoding the light chain sequence of SEQ ID NO: 407; the polynucleotide SEQ ID NO: 253 encoding the heavy chain variable sequence of SEQ ID NO: 53; the polynucleotide SEQ ID NO: 410 encoding the heavy chain sequence of SEQ ID NO: 408; polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 255; SEQ ID NO: 256; and SEQ ID NO: 257) of the light chain variable sequence of SEQ ID NO: 51 or the light chain sequence of SEQ ID NO: 407; and polynucleotides encoding the complementarity-determining regions (SEQ ID NO: 258; SEQ ID NO: 259; and SEQ ID NO:

260) of the heavy chain variable sequence of SEQ ID NO: 53 or the heavy chain sequence of SEQ ID NO: 408.

[00866] In a preferred optional embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, polynucleotides encoding Fab (fragment antigen binding) fragments having binding specificity for NGF. With respect to antibody fragment Fab2, the polynucleotides encoding the Fab fragment include the polynucleotide SEQ ID NO: 409 encoding the light chain sequence of SEQ ID NO: 407 and the polynucleotide SEQ ID NO: 410 encoding the heavy chain sequence of SEQ ID NO: 408.

[00867] Another embodiment of the invention contemplates these polynucleotides incorporated into an expression vector for expression in mammalian cells such as CHO, NSO, HEK-293, or in fungal, insect, plant or microbial systems such as yeast cells such as the yeast *Pichia*. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*. In one embodiment of the invention described herein (infra), Fab fragments may be produced via expression of Fab2 polynucleotides in mammalian cells such as CHO, NSO or HEK 293 cells, fungal, insect, plant or microbial systems such as yeast cells (for example diploid yeast such as diploid *Pichia*) and other yeast strains. Suitable *Pichia* species include, but are not limited to, *Pichia pastoris*.

[00868] In one embodiment, the invention is optionally directed to an isolated polynucleotide comprising a polynucleotide encoding an anti-NGF V_H antibody amino acid sequence selected from SEQ ID NO: 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, 133, 143, 153, 163, 173, 183, 193, or 402, or encoding a variant thereof wherein at least one framework residue (FR residue) has been substituted with an amino acid present at the corresponding position in a rabbit anti-NGF antibody V_H polypeptide or a conservative amino acid substitution.

[00869] In another optional embodiment, the invention is directed to an isolated polynucleotide comprising the polynucleotide sequence encoding an anti-NGF V_L antibody amino acid sequence of 1, 11, 21, 31, 41, 51, 61, 71, 81, 91, 101, 111, 121, 131, 141, 151, 161, 171, 181, 191, or 401, or encoding a variant thereof wherein at least one framework residue (FR

residue) has been substituted with an amino acid present at the corresponding position in a rabbit anti-NGF antibody V_L polypeptide or a conservative amino acid substitution.

[00870] In yet another optional embodiment, the invention is directed to one or more heterologous polynucleotides comprising a sequence encoding the polypeptides contained in SEQ ID NO:1 and SEQ ID NO:3; SEQ ID NO:11 and SEQ ID NO:13; SEQ ID NO:21 and SEQ ID NO:23; SEQ ID NO:31 and SEQ ID NO:33; SEQ ID NO:411 and SEQ ID NO:43; SEQ ID NO:51 and SEQ ID NO:53, SEQ ID NO:61 and SEQ ID NO:63; SEQ ID NO:71 and SEQ ID NO:73; SEQ ID NO:81 and SEQ ID NO:83; SEQ ID NO:91 and SEQ ID NO:93; SEQ ID NO:101 and SEQ ID NO:103; SEQ ID NO:111 and SEQ ID NO:113; SEQ ID NO:121 and SEQ ID NO:123; SEQ ID NO:131 and SEQ ID NO:133; SEQ ID NO:141 and SEQ ID NO:143; SEQ ID NO:151 and SEQ ID NO:153; SEQ ID NO:161 and SEQ ID NO:163; SEQ ID NO:171 and SEQ ID NO:173; SEQ ID NO:181 and SEQ ID NO:183; SEQ ID NO:191 and SEQ ID NO:193; or SEQ ID NO:401 and SEQ ID NO:403.

[00871] In another embodiment, the invention is optionally directed to an isolated polynucleotide that expresses a polypeptide containing at least one CDR polypeptide derived from an anti-NGF antibody wherein said expressed polypeptide alone specifically binds NGF or specifically binds NGF when expressed in association with another polynucleotide sequence that expresses a polypeptide containing at least one CDR polypeptide derived from an anti-NGF antibody for treatment or prevention of pain and pain associated conditions wherein said at least one CDR is selected from those contained in the V_L or V_H polypeptides of SEQ ID NO: 1, 3, 11, 13, 21, 23, 31, 33, 41, 43, 51, 53, 61, 63, 71, 73, 81, 83, 91, 93, 101, 103, 111, 113, 121, 123, 131, 133, 141, 143, 151, 153, 161, 163, 171, 173, 181, 183, 191, 193, 401 or SEQ ID NO:403.

[00872] Host cells and vectors comprising said polynucleotides are also contemplated.

[00873] The invention further optionally contemplates vectors comprising the polynucleotide sequences encoding the variable heavy and light chain polypeptide sequences, as well as the individual complementarity-determining regions (CDRs, or hypervariable regions), as set forth herein, as well as host cells comprising said vector sequences. In one embodiment of the invention, the host cell is a yeast cell. In another embodiment of the invention, the yeast host cell belongs to the genus *Pichia*.

EXAMPLES

[00874] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (e.g. amounts, temperature, concentrations, etc.) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

[00875] EXAMPLE 1

[00876] This example describes culture methods that improved the purity of recombinant antibodies produced from cultured *P. pastoris* cells. When a bolus of ethanol was added during culturing, the resulting antibodies exhibited a great decrease in the concentration of an undesired product-associated variant.

[00877] Methods

[00878] To generate the inoculum, diploid *P. pastoris* was grown using a medium composed of the following nutrients (percentages are given as w/v): yeast extract 3%, anhydrous dextrose 2%, YNB 1.34%, Biotin 0.004% and 100 mM potassium phosphate (pH 6.0). The inoculum medium for runs L355, L357, L358, L359 and L360 was composed of the following nutrients (percentages are given as w/v): yeast extract 3%, glycerol 2%, YNB 1.34%, Biotin 0.004%, 200 mM potassium phosphate (pH 6.0) The inoculum was grown for approximately 24 hours to 29 hours in a shaking incubator at 30°C and 300 rpm. A 10% inoculum was then added to Labfors 2.5L working volume vessels containing 1 L sterile growth medium. The growth medium was composed of the following nutrients: potassium sulfate 18.2 g/L, ammonium phosphate monobasic 36.4 g/L, potassium phosphate dibasic 12.8 g/L, magnesium sulfate heptahydrate 3.72 g/L, sodium citrate dihydrate 10 g/L, glycerol 40 g/L, yeast extract 30 g/L, PTM1 trace metals 4.35 mL/L, and antifoam 204 1.67 mL/L. The PTM1 trace metal solution was comprised of the following components: cupric sulfate pentahydrate 6 g/L, sodium iodide

0.08 g/L, manganese sulfate hydrate 3 g/L, sodium molybdate dihydrate 0.2 g/L, boric acid 0.02 g/L, cobalt chloride 0.5 g/L, zinc chloride 20 g/L, ferrous sulfate heptahydrate 65 g/L, biotin 0.2 g/L, and sulfuric acid 5 mL/L. The yeast strain was engineered to express Ab-A antibody from four integrated genomic copies of the heavy chain coding sequence (SEQ ID NO: 441) and 3 copies of the light chain coding sequence (SEQ ID NO: 440). The heavy chain gene copies were integrated into the pGAP locus (3 copies) and HIS4 TT locus (1 copy) while the 3 light chain gene copies were integrated into the pGAP locus. The antibody chain gene copies were each under the control of the GAP promoter. The bioreactor process control parameters were set as follows: Agitation 1000 rpm, airflow 1.35 standard liter per minute, temperature 28°C and pH was controlled (at 6) using ammonium hydroxide. No oxygen supplementation was provided.

[00879] Following addition of the inoculum, fermentation cultures were grown for approximately 12 to 16 hours (the “growth phase”). The growth phase ended when the initial glycerol in the medium was consumed, as which was detected by a dissolved oxygen (“DO”) spike (a sudden increase in the dissolved oxygen concentration). The cultures were then starved for approximately three hours after the dissolved oxygen spike (“starvation phase”) for run L306. For other runs, the ethanol bolus was added immediately after DO spike. A bolus of ethanol was then added to the reactor to give a final concentration of 1% ethanol (w/v). Control cultures were treated identically, except that the bolus addition of ethanol was omitted. The fermentation cultures were allowed to equilibrate for 15 to 30 minutes (“equilibration phase”). After the equilibration phase, feed was added at a constant rate of 30 g/L/hr for 40 minutes (“transition phase”). For the remainder of the culture (“production phase”) the ethanol concentration was detected using an ethanol sensing probe (Raven Biotech) which was used to control the feed rate, with the feed rate being set at 15 g/L/hr when the ethanol concentration was below the set point, or 7.5 g/L/hr when the ethanol concentration was above the set point. In instances in which the high feed rate of 15 g/L/hr was not high enough to maintain ethanol at set point (which occurred in the L315 fermentation run), the high feed rate was set to 22.5 g/L/hr while the low feed rate was set to 15 g/L/hr. The same set point was maintained whether or not an ethanol bolus had been added to the culture (production of ethanol by the yeast caused the set point to be reached without the bolus addition of ethanol). The feed was composed of the following components: yeast extract 50 g/L, anhydrous dextrose 500 g/L, magnesium sulfate heptahydrate 3 g/L, PTM1 trace metals 12 mL/L, and sodium citrate dihydrate 0.5g/L. The total fermentation time was

typically 85 hours to 97 hours in these experiments, though longer and shorter times can also be used.

[00880] After the production phase, fermentation cultures had PEI (polyethyleneimine) and EDTA (ethylenediaminetetraacetic acid) added to 0.05% w/v and 3 mM final concentrations respectively. The cultures were then spun in a centrifuge and antibodies were purified from the culture supernatant by Protein A affinity. Briefly, approximately 20 mL of 0.2 μ clarified supernatants from harvested fermentation broth were diluted with the same volume of equilibration buffer (20 mM Histidine pH6). From this diluted broth, 20 mL were then loaded onto a pre-equilibrated 1 mL HiTrap MabSelect Sure column (GE, Piscataway, NJ). The column was subsequently washed using 40 column volumes of equilibration buffer. The antibody bound onto the column was eluted using a step gradient into 100% elution buffer (100 mM Citric Acid pH 3.0). One mL fractions were collected and immediately neutralized using 100 μ L of 2M Tris buffer pH 8.0. Protein containing fractions were determined by measuring absorbance at 280nm and protein-containing fractions were pooled.

[00881] Protein A purified antibodies were analyzed for purity by SDS-PAGE. For non-reduced samples, SDS-PAGE was carried out using precast polyacrylamide gels (NuPAGE® Bis-Tris Gels) containing a 4%-12% polyacrylamide gradient, using NuPAGE® MES SDS running buffer and NuPAGE® LDS Sample Buffer (all from Invitrogen, Carlsbad, Ca.) in accord with the manufacturer's instructions. Proteins were then visualized by Coomassie blue staining. Reduced samples were processed in the same manner except that samples were reduced prior to loading using the NuPAGE® Sample Reducing Agent (Invitrogen, Carlsbad, Ca.) in accord with the manufacturer's instructions.

[00882] *Results*

[00883] To determine the effect of a bolus addition of ethanol during culture on antibody purity, the antibody Ab-A was produced from yeast cultures with or without the addition of a bolus of ethanol to a final concentration of 1% (10 g/L) at the end of the growth phase and prior to the production phase. The production phase was continued for 97 hours (**FIG. 1**), 87 hours (**FIG. 2**), or 86 hours (**FIG. 3**). The antibody produced by each culture was then harvested from the culture media, purified by Protein A affinity chromatography.

[00884] SDS-PAGE was used to detect the relative abundance of the full antibody, the “half antibody” or H1L1 complex (containing one heavy and one light chain) and the H2L1 complex (containing two heavy chains and one light chain). The abundance of the H1L1 and H2L1 complexes was greatly decreased in the cultures that were produced with the bolus addition of ethanol. This improvement was reproduced in three experiments shown in **FIG. 1A**, compare lanes 2 and 3 (with bolus) to 5 (no bolus); **FIG. 2A**, compare lane 2 (with bolus) to 3 (no bolus); **FIG. 3A**, compare lanes 2 and 4 (with bolus) to lanes 5-7 (no bolus). Under reducing conditions, the H1L1, H2L1, and full antibody species were each separated into individual heavy and light chains, confirming the identity of the 75 kDa band as consisting of one light and one heavy chain joined by a disulfide-linkage (**FIGS. 1B and 2B**; lane order is the same as **FIGS. 1A and 1B**, respectively).

[00885] The decrease in abundance of the H1L1 species was then quantified using ImageJ to plot the gel band density along the length of the non-reduced gels (**FIGS. 1C-E and 2C-D**, respectively corresponding to **FIG. 1A**, lanes 2, 3, and 5, and **FIG. 2A**, lanes 2 and 3, and **FIG. 3B**, corresponding to lanes 2 and 4-6 of **FIG. 3A**). The area under the H1L1 peak was quantified and results are tabulated in **FIGS. 1F, 2E, and 3B**. Based on these measurements, the addition of an ethanol bolus prior to antibody production decreased the relative abundance of the 75 kDa band by about 90% in **FIG. 1A**, by about 85% in **FIG. 2A**, and by about 87% in **FIG. 3A**.

[00886] In summary, these results demonstrate that the concentration of the H1L1 species was greatly decreased by the bolus addition of ethanol to the culture, resulting in decreased production of the H1L1 species between about 85% to 90%.

[00887] **EXAMPLE 2**

[00888] This example extends the results obtained in Example 1 by demonstrating that the same methods produced a similar improvement in antibody purity when used during production of two additional antibodies.

[00889] *Methods*

[00890] Antibodies Ab-B and Ab-C were recombinantly produced using the methods described in Example 1. Antibody Ab-C was expressed from a yeast strain engineered contain

four copies of the heavy chain coding sequence (SEQ ID NO: 439) and three copies of the light chain coding sequence (SEQ ID NO: 438). Samples were taken from the reactors and culture supernatant containing antibodies was collected after a total fermentation time of 67 hours (T67) or 87 hours (T87) for the Ab-B antibody and for 86 hours (T86) for the Ab-C antibody, purified by Protein A affinity, and analyzed by SDS-PAGE as described in Example 1.

[00891] *Results*

[00892] Antibodies Ab-B (FIG. 4) and Ab-C (FIG. 5) were produced with or without a bolus addition of ethanol to a final concentration of 1% at the end of the growth phase and prior to the production phase. For the antibodies produced without the bolus addition of ethanol, the H1L1 or half antibody species, and the H2L1 species were each observed as a prominent band (FIG. 4A, lines 6 and 7; FIG. 4C, lanes 6 and 7; FIG. 5A, lanes 5 and 6). The intensity of these bands were greatly decreased for the culture produced with a bolus addition of ethanol (FIG. 4A, lanes 2-3; FIG. 4C, lanes 2-3; FIG. 5A, lane 3). Under reducing conditions, the H1L1 and H2L1 bands were separated into individual heavy and light chains, confirming the identity of these species as consisting full length heavy and light chains (FIGS. 4B, 4D, and 5B; lane order is the same as in FIGS. 4A, 4D, and 5A, respectively).

[00893] The decrease in abundance of the H1L1 species was then quantified using ImageJ to plot the gel band density along the length of the non-reduced gels. FIGS. 4E and 4F tabulate the area contained in the H1L1 peaks shown in FIGS. 4A (T67) and 4C (T87), respectively, demonstrating that the bolus addition of ethanol produced about a 73% reduction in the relative abundance of H1L1 complexes at the earlier time point shown FIG. 4A and about a 34% average reduction in the relative abundance of H1L1 complexes at the later time point shown in FIG. 4C. Similarly, FIG. 5C tabulates the area contained in the H1L1 peaks shown in FIGS. 5A, demonstrating about a 61% average reduction in the relative abundance of H1L1 complexes by the bolus addition of ethanol.

[00894] In summary, these results demonstrate that the concentration of the H1L1 and H2L1 species were decreased by between about 61% and 73% by the bolus addition of ethanol to the culture for two additional antibodies having binding specificity for different targets.

[00895] EXAMPLE 3

[00896] This example further describes the improved purity of recombinant antibodies produced from cultured *P. pastoris* cells through addition of a bolus of ethanol during culturing. In addition to greatly decreasing the abundance of the H1L1 and H2L1 species, this example further demonstrates a decrease in the concentration of other product-associated variants.

[00897] Methods

[00898] Recombinant antibodies Ab-A, Ab-B, and Ab-C were prepared and purified from *P. pastoris* cultures as described in Examples 1 and 2. Antibodies were produced either with or without a bolus addition of ethanol to a final concentration of 1% (w/v) at the end of the growth phase and prior to the production phase. To analyze the purity of protein A purified antibody preparations, size exclusion high-performance liquid chromatography (SE-HPLC) was used. Briefly, an Agilent (Santa Clara, CA) 1200 Series HPLC with UV detection instrument was used. For sample separation, a TSKgel 3000SW_{XL} 7.8x300 mm column connected with a TSKgel Guard SW_{XL} 6x40 mm from Tosoh Bioscience (King of Prussia, PA) was used. A solution of 100 mM sodium phosphate, 200 mM sodium chloride pH 6.5 was used as mobile phase with a flow rate of 0.5 mL/min in isocratic mode and absorbance at UV 215nm was monitored. Before injection of samples the column was equilibrated until a stable baseline was achieved. Samples were diluted to a concentration of 1 mg/mL using mobile phase and a 30 µL volume was injected. To monitor column performance, BioRad (Hercules, CA) gel filtration standards were used.

[00899] Results

[00900] The Ab-A antibody preparations described in Example 1 and additional preparations produced using the same methods were expressed in yeast, purified by protein A affinity, and then analyzed for purity using size exclusion chromatography (SEC). Under the condition used, the half antibody (H1L1) species co-elutes with the full antibody, which is thought to be due to non-covalent association between pairs of half antibodies. However, this method allows purity to be assessed with respect to other product-associated variants, such complexes having aberrant stoichiometry, fragments, glycosylated forms, and aggregates.

[00901] SE-HPLC data are shown for Ab-A samples produced without (FIGS. 6A, 6C, and 6E) or with (FIGS. 6B, 6D, and 6F) a bolus addition of ethanol. A product-associated variant consisting of an aggregate of two full antibodies (containing four heavy and four light chains) was detected (arrow), and the abundance thereof was reduced on average in the samples prepared with the bolus addition. FIG. 7 shows quantification of the purity of Ab-A by determining the percentage of the antibody preparation contained in the main peak (containing the full antibody). The bolus addition of ethanol increased the average percentage contained in the main peak from 80.3% up to 90.6%.

[00902] Similar analysis was performed for the Ab-B and Ab-C antibody preparations described in Example 2, quantified in FIGS. 8 and 9, respectively. Overall purity of the Ab-B antibody was improved, with the average fraction in the main peak increasing from 76% to 79% at T67 and from 60% to 73% at T87. For the Ab-C antibody, there was little detectable difference in antibody purity assessed by this method, apparently due to the high initial purity of the Ab-C antibody even without the bolus addition.

[00903] In summary, these results demonstrate that a bolus addition of ethanol to the culture can decrease the concentration of other product-associated variants in addition to the half-antibody species.

[00904] **EXAMPLE 4**

[00905] This example describes the further confirmation of the identity of the 75 kDa product associated variant as a half antibody species containing only one antibody heavy chain and only one antibody light chain. This hypothesis was based on several observations. First, the 75 kDa band was present in protein A purified samples (see Example 1) indicating that it contained at least the protein A binding portion of an antibody heavy chain. Second, the 75 kDa band was prominent in non-reduced samples analyzed by SDS-PAGE (see, e.g., **FIG. 1A**, lanes 2-3), but under reducing conditions the same samples did not contain any bands of comparable intensity (other than the expected light and heavy chains), indicating that the 75 kDa band does not include any components other than antibody heavy and light chains (or other species having the same electrophoretic mobility). Third, disappearance of the 75 kDa band from the reduced samples also indicates that its constituents are linked by at least one disulfide bond. Fourth, SEC

analysis had demonstrated co-elution of the 75 kDa species with the full antibody, strongly suggesting that the 75 kDa species can non-covalently self-associate to form a full antibody (or another complex of the same apparent hydrodynamic radius). Finally, the apparent molecular weight of about 75 kDa (determined by reference to electrophoresis standards), taken together with the observation (from denaturing gels) that this complex was only made up of full-length antibody chains, was consistent with complex containing only one heavy chain and only one light chain, but was inconsistent with other complexes.

[00906] *Methods*

[00907] Mass spectrometry was used to detect the relative abundance of heavy chains lacking the inter-heavy chain disulfide bonds (normally found at amino acids 220 and 223) in different samples. Two-hundred and fifty micrograms of each sample was added into an Eppendorf tube. An appropriate amount (~450 μ L) of denaturing buffer (6 M Guanidine-HCl, 1 mM EDTA, 0.25 M Tris, pH 7.5) was added to the tube to obtain a final volume of 500 μ L and a sample concentration of 0.5 mg/mL. Twelve and a half microliters of 2 M Iodoacetamide was added into each sample to alkylate any free cysteine. The samples were vortexed then incubated at room temperature, in the dark, for 30 ± 5 minutes. The samples were then desalted using NAP-5 columns pre-equilibrated with digestion buffer (0.1 M Tris-HCl, pH 7.5). Each sample solution was added to separate (pre-equilibrated) columns and allowed to enter the column bed. One milliliter of digestion buffer was added to each column and the eluent was collected into Eppendorf tubes. The samples were divided into equal aliquots containing approximately 50 μ g of material (five 200 μ L aliquots). The alkylated and desalted aliquots were stored at -20°C until needed. One aliquot of each sample (alkylated, and desalted) was used for each digestion. Trypsin solution at 0.5 mg/mL was added to each sample aliquot at a 1:25 w:w ratio of trypsin:protein (4 μ L). All trypsin tubes were incubated at $37 \pm 2^\circ\text{C}$ for 4 hours. After incubation, the enzymatic digestion was quenched by adding 1 μ L of Trifluoroacetic acid to each tube. The samples were then divided into two equal portions, reduced and non-reduced. Half the samples were reduced in the presence of 1 M DTT for 1 hour at $37 \pm 2^\circ\text{C}$. The contents of both reduced and nonreduced samples were transferred to HPLC vials and placed in the autosampler for analysis.

[00908] MS and MS/MS data was collected on a Micromass Q-TOF Ultima mass spectrometer using electrospray ionization (ESI) in positive ion mode. Data was acquired from m/z 200-1950 in MS mode. Prior to analysis, the mass spectrometer was calibrated using a 5th order fit on fragment ions of [Glu¹]-Fibrinopeptide covering a range from m/z 175 to 1285. The injections volumes were adjusted to achieve an on-column load of approximately 20 pmoles of protein.

[00909] *Results*

[00910] Based on the observations discussed above, Applicants hypothesized that the 75 kDa band was a “half antibody” species containing one heavy antibody chain and one light antibody chain covalently linked to one another through disulfide bonds, and that pairs of half antibody complexes could non-covalently associate to form a complex having the same stoichiometry as a full antibody (two heavy chains and two light chains) but lacking the disulfide linkages between the two heavy chains (“unlinked heavy chains”). Based thereon, it was predicted the relative abundance of the 75 kDa band would correlate with the relative proportion of unlinked heavy chains, which was determined using mass spectrometry analysis of trypsin digested antibody samples.

[00911] The peptide fragments of interest for this study were the T17 trypsin fragment of the heavy chain (T17H) which is composed of amino acids 217-242, respectively. Amino acids 220 and 223 are responsible for disulfide bonding between antibody heavy chains. Typically, free cysteine analysis can be conducted by determining a ratio of alkylated cysteine residues to non-alkylated residues in reduced samples after a tryptic digestion. However, alkylated species were not present in either lot of material. It was hypothesized the two cysteine residues of the peptide fragment of interest, T17H, were bonding to each other, or the cysteines were protected by the antibody’s quaternary structure. In either case, the cysteine residues would not be accessible for alkylation. Instead, the analytical approach was to utilize the ratio of non-reduced species to reduced species in both lots to calculate a percent difference. It was observed that the non-reduced samples had a 2 Da decrease in molecular weight, indicative of disulfide bonding. This behavior was exploited to calculate the percent free T17H. The theoretical mass of the non-

reduced T17H species is 2727.41 Da (disulfide bonding between Cys220 & Cys223) and 2729.41 Da for reduced peptide.

[00912] The extracted ion chromatograms of reduced and non-reduced samples were analyzed for representative charge states. ratio of counts between the non-reduced to reduced samples calculates the free T17H species to be 2.3% in the antibody produced with a bolus addition of ethanol, and 26.1% in the sample produced without a bolus addition of ethanol. These results are presented in tabular form in **Fig. 9**.

[00913] Thus, the abundance of heavy chains lacking the inter-heavy chain disulfide bond was greatly increased in the sample produced without the bolus addition of ethanol, further confirming the identity of this species as containing one heavy chain and one light chain but lacking an inter-heavy chain disulfide bond. Moreover, the detection of a species 2 Da lighter than the expected mass indicated that heavy chain in the H1L1 species may contain an extra disulfide bond to itself which may interfere with the formation of the normal inter-heavy chain disulfide bond.

[00914] **EXAMPLE 5**

[00915] This example demonstrates a correlation between cell viability and antibody purity. The addition of an ethanol bolus generally improved cell viability and antibody purity for Ab-A and the Ab-B antibody. Further, the Ab-C antibody, which already exhibited high purity even without the bolus addition, also exhibited higher culture viability. Taken together, these results suggest that the improvement in antibody purity resulting from a bolus addition of ethanol is at least partially attributable to increased culture viability.

[00916] *Methods*

[00917] Culture viability was determined using a Cellometer (Nexcelom). Culture samples were diluted with PBS so that the final cell count was within 1×10^7 to 5×10^7 cells/mL. One half of the sample was then treated to heat conditions of 75°C for 10 minutes as positive control for propidium iodide (PI) staining. The untreated sample and treated sample were then mixed with PI (20 uL of sample plus 20 uL of PI). The sample was then placed in a slide cassette and viability was determined by counting the number of non-fluorescing cells then dividing by the

total number of cells. Cells that are dead have taken up the propidium iodide and are fluorescing so the positive control heat killed sample should show less than 1% cells alive.

[00918] *Results*

[00919] Cell viability was determined for antibody-producing cultures grown with or without a bolus addition of ethanol as described in Examples 1 and 2. As described above, purity of the Ab-A and Ab-B antibodies were greatly improved by a bolus addition of ethanol to the yeast culture (see Examples 1-2 and FIGS. 1-4). The addition of an ethanol bolus improved cell viability for these cultures as well. For the Ab-A antibody, viability improved from 91.9% to 97.2% on average (FIG. 11), while for the Ab-B antibody, viability improved from 84.8% to 95.1% on average (FIG. 12). Due to the already high purity of the Ab-C antibody produced even without the bolus addition of ethanol, the improvements in the purity of this antibody resulting from the bolus addition of ethanol were more modest (see Example 3 and FIG. 5). Consistent with the observation that high cell viability correlated with higher antibody purity, the Ab-C antibody cultures exhibited high cell viability (95.8%% on average) in the absence of a bolus addition of ethanol, which was little changed by the bolus addition of ethanol (96.8%).

[00920] Taken together, these results indicate that the improvement in antibody purity resulting from the addition of an ethanol bolus may be in part caused by (or at least correlates with) an improvement in cell viability.

[00921] **EXAMPLE 6**

[00922] This example demonstrates that a similar improvement in antibody purity can be attained with varying ethanol bolus concentrations.

[00923] *Methods*

[00924] The Ab-A antibody was produced as in Example 1, except that the bolus addition of ethanol was 5 g/L (0.5% w/v), 10 g/L (1% w/v), or 15 g/L (1.5% w/v). Antibodies samples were purified from the culture media at 63 and 86 hours and purified by protein A affinity, then the purity was analyzed by non-reduced SDS-PAGE as in Example 1.

[00925] *Results*

[00926] Antibody purity was similarly high irrespective of the bolus concentration added (between 0.5% and 1.5% w/v), at both time points tested, 63 hours (FIG. 14A) and 86 hours (FIG. 14B). Detected levels of the H1L1 and H2L1 species were similarly low in each culture.

[00927] These results indicate that the improvement in antibody purity can be attained while varying the ethanol bolus concentration.

[00928] **EXAMPLE 7**

[00929] This example demonstrates that similar improvements in antibody purity can be attained while varying the duration of the “starvation period” between the dissolved oxygen spike and the addition of the ethanol bolus to the cultures.

[00930] *Methods*

[00931] The Ab-A antibody was produced as in Example 1, except that the duration of the starvation period, the time between the dissolved oxygen spike (indicating exhaustion of the carbon source in the culture) and the bolus addition of ethanol, was either 0 hours or 3 hours. Antibodies samples were purified from the culture media and purified by protein A affinity, then the purity was analyzed by non-reduced SDS-PAGE as in Example 1.

[00932] *Results*

[00933] Antibody purity was similarly high irrespective of the variation of the starvation period between 0 and 3 hours (FIG. 15A, compare lanes 5 (0 hours starvation) and 6 (3 hours starvation)). Detected levels of the H1L1 and H2L1 species were similarly low in each culture.

[00934] These results indicate that the improvement in antibody purity can be attained with a varying the duration of the starvation period.

[00935] EXAMPLE 8

[00936] This example tests the effect on antibody purity from varying the duration of the “equilibration period” between the addition of the ethanol bolus and commencement of adding the feed to the cultures.

[00937] *Methods*

[00938] The Ab-B antibody was produced as in Example 2, except that the duration of the equilibration period, the time between addition of the ethanol bolus and the commencement of feeding the culture, was 0, 30, or 60 minutes. Additionally, the yeast strain from which the Ab-B antibody was produced contained three copies of the light chain gene instead of four. Antibodies samples were purified from the culture media and purified by protein A affinity, then the purity was analyzed by non-reduced SDS-PAGE as in Example 1. Viability was also assessed using the methods described in Example 5.

[00939] *Results*

[00940] Antibody purity was similarly high for an equilibration period of 0 or 30 minutes (FIG. 16A, lanes 7 and 8 (0 minutes equilibration time) and lane 3 (30 minutes equilibration time)). However, detected levels of the H1L1 and H2L1 species were increased in the culture with the 60 minute equilibration period (FIG. 16A, lanes 5 and 6).

[00941] Viability was also assessed for each culture at the 23 hour and 85 hour time points. For the 60 minute equilibration period, viability was between 75% and 80% at 23 hours, while at the same time point viability was approximately 88-90% for the 0 and 30 minute equilibration periods (FIG. 16B). Subsequently, at 85 hours, viability had improved but remained somewhat reduced for the 60 minute equilibration period relative to the 0 and 30 minute equilibration periods (FIG. 16C).

[00942] These results indicate that the improvement in antibody purity can be attained while varying the equilibration period at least between 0 and 30 minutes, while some loss of viability and purity may occur for an equilibration period of 60 minutes or longer (though purity may still be improved relative to a control culture without a bolus addition of ethanol).

[00943] Certain teachings related to methods for obtaining a clonal population of antigen-specific B cells were disclosed in U.S. Provisional patent application no. 60/801,412, filed May 19, 2006.

[00944] The disclosure of U.S. Provisional patent application no. 60/801,412 is herein incorporated by reference in its entirety.

[00945] Certain teachings related to humanization of rabbit-derived monoclonal antibodies and preferred sequence modifications to maintain antigen binding affinity were disclosed in International Application No. PCT/US2008/064421.

[00946] This corresponds to International Publication No. WO/2008/144757, entitled "Novel Rabbit Antibody Humanization Methods and Humanized Rabbit Antibodies".

[00947] This was filed May 21, 2008, the disclosure of which is herein incorporated by reference in its entirety.

[00948] Certain teachings related to producing antibodies or fragments thereof using mating competent yeast and corresponding methods were disclosed in U.S. Patent application no. 11/429,053, filed May 8, 2006, (U.S. Patent Application Publication No. US2006/0270045), the disclosure of which is herein incorporated by reference in its entirety.

[00949] The entire disclosure of each document cited herein (including patents, patent applications, journal articles, abstracts, manuals, books, or other disclosures), including each document cited in the Background, Summary, Detailed Description, and Examples, is hereby incorporated by reference herein in its entirety.

CLAIMS

1. A method of producing secreted full length antibodies, comprising:
 - (a) providing a growth culture comprising *Pichia pastoris* yeast cells comprising genes that provide for the expression and secretion of full length antibodies by said yeast cells , and growing said Pichia yeast for 12-16 hours (“growth phase”);
 - (b) adding ethanol to said culture after said growth phase in a single bolus addition (“bolus”) which bolus results in a concentration of ethanol in said culture of between 0.5% and 1.5% w/v, and
 - (c) culturing said culture to which ethanol bolus has been added to produce said secreted, full length antibodies, wherein said culturing comprises adding a feed comprising a fermentable carbon source to said culture.
2. The method of **claim 1**, wherein the ethanol bolus enhances the formation of stable disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.
3. The method of any one of **claims 1 or 2**, which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.
4. The method of any one of **claims 1 to 3**, which decreases the relative abundance of product-associated variants having a higher or lower apparent molecular weight than said desired full length antibodies as detected by size exclusion chromatography or gel electrophoresis relative to the same method effected in the absence of the bolus of ethanol.
5. The method of any one of **claims 1 to 3** which decreases the relative abundance of complexes having aberrant stoichiometry relative to the same method effected in the absence of the bolus of ethanol.

6. The method of any one of **claims 1 to 3**, which decreases the relative abundance of complexes having aberrant disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.
7. The method of any one of **claims 1 to 3**, which decreases the relative abundance of complexes having reduced cysteines relative to the same method effected in the absence of the bolus of ethanol.
8. The method of any one of **claims 1 to 3**, which decreases the relative abundance of complexes having aberrant glycosylation relative to the same method effected in the absence of the bolus of ethanol.
9. The method of **claim 1**, which modulates the formation or stability of inter-heavy chain disulfide bonds.
10. The method of **claim 9**, which modulates the formation or stability of disulfide bonds linking the light and heavy chains.
11. The method of any one of **claims 1, 9 or 10** which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.
12. The method of **claim 11**, wherein said product-associated variants comprise one or more of the H1L1, H2L1, and H4L4 product-associate variants.
13. The method of any one of **claims 1 to 12**, which increases the purity of said antibody relative to said method effected in the absence of said bolus of ethanol.
14. The method of any one of the **foregoing claims**, wherein step (b) is effected about 15-30 minutes, allowing the ethanol bolus containing culture to equilibrate prior to step (c).

15. The method of any one of the **foregoing claims**, wherein step (c) comprises providing oxygen to said cells.
16. The method of **claim 15**, wherein said providing oxygen comprises agitating said culture.
17. The method of **claim 15 or 16**, wherein said providing oxygen comprises contacting said culture with a gas mixture comprising oxygen.
18. The method of any one of the **foregoing claims**, wherein said fermentable carbon source comprises glucose or galactose.
19. The method of **claim 18**, wherein said fermentable carbon source comprises glucose.
20. The method of **claim 18**, wherein said feed comprises one or more of glucose, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.
21. The method of any one of the **foregoing claims**, wherein the time between step (b) and step (c) is less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 90 minutes, less than 80 minutes, less than 70 minutes, less than 60 minutes, less than 50 minutes, less than 40 minutes, less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, or less than 1 minute.
22. The method of any one of the **foregoing claims**, wherein the culture of step (a) is produced by adding a carbon source to said culture, and culturing said culture until the carbon source is depleted.
23. The method of **claim 22**, wherein said carbon source comprises one or more of: glucose, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

24. The method of **claim 22 or 23**, wherein the depletion of the carbon source is determined by detecting a decrease in the metabolic activity of said cells.

25. The method of **claim 24**, wherein said decrease in the metabolic activity of said cells is identified by detecting a decrease in the consumption of oxygen by said cells, by detecting an increase in pH in the culture, by detecting stabilization of the wet cell mass, or by detecting an increase in the concentration of ammonia in the culture.

26. The method of **claim 25**, wherein said decrease in the consumption of oxygen by said cells is identified by detecting an increase in the concentration of dissolved oxygen in said culture.

27. The method of any one of the **foregoing claims**, wherein said bolus of ethanol results in a concentration of ethanol in said culture of between 1% and 1.5% w/v.

28. The method of **claim 1**, wherein the genes that provide for expression of said full length antibody are integrated into one or more genomic loci.

29. The method of **claim 28**, wherein at least one of said genomic loci are selected from the group consisting of the pGAP locus, 3' AOX TT locus; PpURA5; OCH1; AOX1; HIS4; GAP; pGAP; 3' AOX TT; ARG; and the HIS4 TT locus.

30. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are expressed under control of an inducible or constitutive promoter.

31. The method of **claim 30**, wherein said inducible promoter is selected from the group consisting of the AOX1, CUP1, tetracycline inducible, thiamine inducible, and FLD1 promoters.

32. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are expressed under control of a promoter selected from the group consisting of: the CUP1, AOX1, ICL1, glyceraldehyde-3-phosphate dehydrogenase (GAP), FLD1, ADH1, alcohol dehydrogenase II, GAL4, PHO3, PHO5, and Pyk promoters, tetracycline inducible promoters, thiamine inducible promoters, chimeric promoters derived therefrom, yeast promoters, mammalian promoters, insect promoters, plant promoters, reptile promoters, amphibian promoters, viral promoters, and avian promoters.
33. The method of any one of the **foregoing claims**, wherein said *Pichia pastoris* cells are diploid, tetraploid cell, or polyploid.
34. The method of any one of the **foregoing claims**, further comprising purifying said full length antibody from said cells or from the culture medium.
35. The method of **claim 34**, wherein said cells secrete said full length antibody into the culture medium.
36. The method of **claim 35**, wherein said full length antibody is purified from said culture medium.
37. The method of any one of the **foregoing claims**, wherein said full length antibody comprises a monospecific or bispecific antibody.
38. The method of any one of the **foregoing claims**, wherein said full length antibody comprises a human antibody or a humanized antibody or fragment thereof.
39. The method of **claim 38**, wherein said humanized antibody is of mouse, rat, rabbit, goat, sheep, or cow origin.
40. The method of **claim 38**, wherein said humanized antibody is of rabbit origin.

41. The method of any one of the **foregoing claims**, wherein said full length antibody comprises a monovalent, bivalent, or multivalent antibody.
42. The method of any one of the **foregoing claims**, wherein said antibody is purified from said culture by protein A and/or protein G affinity.
43. The method of any one of the **foregoing claims**, wherein at least one of the genes that provide for expression of a subunit of said antibody is optimized for expression in said cell.
44. The method of any one of the **foregoing claims**, wherein the purity of said antibody is assessed by measuring the fraction of the antibody produced by said cell that is contained in antibody complexes having the expected apparent hydrodynamic radius, is contained in antibody complexes having the expected molecular weight, and / or specifically binds a target of said antibody.
45. The method of any one of the **foregoing claims**, wherein the yield of said antibody is assessed by determining the amount of antibody produced by said cell discounting any product-associated variants that are abnormally glycosylated, contained in antibody complexes other than complexes having the expected apparent hydrodynamic radius, contained in antibody complexes having the expected molecular weight, and / or that fail to specifically bind to the target of said antibody.
46. The method of **claim 44 or 45**, wherein the molecular weight of said antibody complexes is determined by non-reducing SDS-PAGE.
47. The method of any one of the **foregoing claims**, further comprising purifying said antibody.
48. The method of any one of the **foregoing claims**, wherein said culture cell produces a supernatant antibody titer of at least 100 mg / L, at least 150 mg / L, at least 200 mg / L, at

least 250 mg / L, at least 300 mg / L, between 100 and 300 mg / L, between 100 and 500 mg / L, between 100 and 1000 mg / L, at least 1000 mg / L, at least 1250 mg/liter, at least 1500 mg/liter, at least 1750 mg/liter, at least 2000 mg/liter, at least 10000 mg/liter, or more.

49. The method of any one of the **foregoing claims**, wherein one or more subunits of said multi-subunit complex are expressed from more than one gene copy.

50. The method of any one of the **foregoing claims**, wherein said antibody is expressed from between 1-10 copies of a gene encoding the light chain of said antibody and from 1-10 copies of a gene encoding the heavy chain of said antibody.

51. The method of any one of the **foregoing claims**, wherein genes that provide for expression of said antibody are integrated into genome of said cells.

52. The method of any one of the **foregoing claims**, wherein genes that provide for expression of said antibody are contained on an extrachromosomal element, plasmid, or artificial chromosome.

53. The method of **claim 51 or 52**, wherein said cells comprise more copies of the gene that provide for the expression of the light chain of said antibody than copies of the gene that provide for expression of the heavy chain of said antibody.

54. The method of **claim 51 or 52**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 2, 2 and 3, 3 and 3, 3 and 4, 3 and 5, 4 and 3, 4 and 4, 4 and 5, 4 and 6, 5 and 4, 5 and 5, 5 and 6, or 5 and 7.

55. The method of **claim 51 or 52**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 1, 3 and 1, 4 and 1, 5 and 1, 6 and 1, 7 and

1, 8 and 1, 9 and 1, 10 and 1, 1 and 2, 2 and 2, 3 and 2, 4 and 2, 5 and 2, 6 and 2, 7 and 2, 8 and 2, 9 and 2, 10 and 2, 1 and 3, 2 and 3, 3 and 3, 4 and 3, 5 and 3, 6 and 3, 7 and 3, 8 and 3, 9 and 3, 10 and 3, 1 and 4, 2 and 4, 3 and 4, 4 and 4, 5 and 4, 6 and 4, 7 and 4, 8 and 4, 9 and 4, 10 and 4, 1 and 5, 2 and 5, 3 and 5, 4 and 5, 5 and 5, 6 and 5, 7 and 5, 8 and 5, 9 and 5, 10 and 5, 1 and 6, 2 and 6, 3 and 6, 4 and 6, 5 and 6, 6 and 6, 7 and 6, 8 and 6, 9 and 6, 10 and 6, 1 and 7, 2 and 7, 3 and 7, 4 and 7, 5 and 7, 6 and 7, 7 and 7, 8 and 7, 9 and 7, 10 and 7, 1 and 8, 2 and 8, 3 and 8, 4 and 8, 5 and 8, 6 and 8, 7 and 8, 8 and 8, 9 and 8, 10 and 8, 1 and 9, 2 and 9, 3 and 9, 4 and 9, 5 and 9, 6 and 9, 7 and 9, 8 and 9, 9 and 9, 10 and 9, 1 and 10, 2 and 10, 3 and 10, 4 and 10, 5 and 10, 6 and 10, 7 and 10, 8 and 10, 9 and 10, 10 and 10.

56. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown in a production medium.

57. The method of **claim 56**, wherein said production medium is a minimal medium.

58. The method of **claim 56**, wherein said minimal medium lacks selective agents.

59. The method of **claim 58**, wherein said minimal medium lacks pre-formed amino acids or other complex biomolecules.

60. The method of **claim 58**, wherein the production medium is a complex medium.

61. The method of **claim 60**, wherein the complex medium comprises one or more of yeast extract, soy peptones, and other plant peptones.

62. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown to a high cell density.

63. The method of **claim 62**, wherein said high cell density is at least 50 g/L.

64. The method of **claim 62**, wherein said high cell density is at least 100 g/L.

65. The method of **claim 62**, wherein said high cell density is at least 300 g/L.

66. The method of **claim 62**, wherein said high cell density is at least 400 g/L.
67. The method of **claim 62**, wherein said high cell density is at least 500 g/L.
68. The method of **claim 62**, wherein said high cell density is at least 750 g/L.
69. The method of any one of the **foregoing claims**, wherein the yeast cells are cultured for at least 20 doublings and maintain high levels of expression of said full length antibodies after said at least 20 doublings.
70. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 50 doublings and maintain high levels of expression of said full length antibodies after said at least 50 doublings.
71. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 100 doublings and maintain high levels of expression of said full length antibodies after said at least 100 doublings.
72. The method of any one of the **foregoing claims**, wherein at least one subunit of said full length antibodies comprises a secretion signal.
73. The method of **claim 72**, wherein said antibody is humanized.