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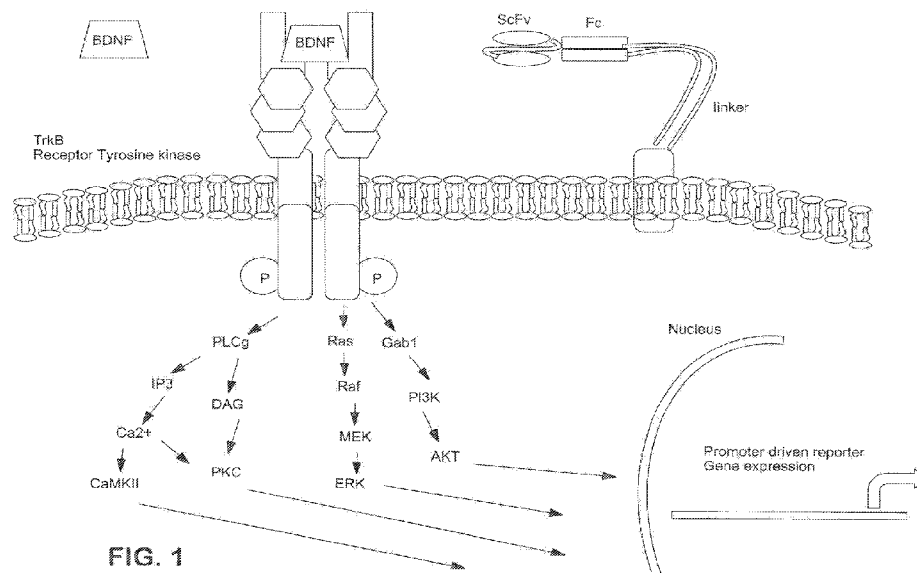
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(54) Title: TRKB AGONIST ANTIBODIES FOR TREATING NEURODEGENERATIVE DISORDERS



(57) Abstract: The invention provides TrkB agonist antibodies or antigen-binding fragments that specifically enhance TrkB signaling activities. The invention also provides therapeutic methods of using such antibodies for promoting survival and regeneration of retinal ganglion cells, and for treating or preventing subjects suffering from or at risk of developing various neurodegenerative conditions such as glaucoma.



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TrkB Agonist Antibodies for Treating Neurodegenerative Disorders

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The subject patent application claims the benefit of priority to U.S. Provisional Patent Application Numbers 62/331,046 (filed May 3, 2016). The full disclosure of the priority application is incorporated herein by reference in its entirety and for all purposes.

BACKGROUND OF THE INVENTION

[0002] The Trk family of receptors are important in physiological and disease processes in both neuronal and non-neuronal tissues. Amongst these receptor kinases, TrkB plays a key role in signaling for brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4 (NT4), which are indispensable for the differentiation, survival and function of neurons in both the peripheral and central nervous systems. Binding of a neurotrophin to a Trk receptor extracellular domain initiates a signal transduction pathway, which leads to dimerization and autophosphorylation of the receptor. Autophosphorylation of TrkB leads to activation of signaling pathways including mitogen-activated protein kinase (MAPK), phospholipase C- γ and phosphatidylinositol-3 kinase (PI3-K).

[0003] Both BDNF and TrkB have been shown to play critical roles in the survival of retinal ganglion cells in the retina. It has been suggested that aberrant TrkB signaling and its compromise contribute to the development of various neuropathies and degenerative conditions such as glaucoma. Glaucoma is the second most common eye disease leading to blindness, affects >60M people worldwide with a US prevalence of approximately 2.3M diagnosed cases. Primarily a “plumbing” problem that leads to elevated intra-ocular pressure (IOP), glaucoma results in damage to retinal ganglion cells (RGCs) and their axons that convey all visual stimuli to the brain. While numerous drugs and devices are approved to lower IOP, as yet no drugs have been developed that prevents, slows or reverses RGC or other retinal neuronal cell atrophy and loss that would stave off blindness that eventually occurs in >10% of glaucoma patients. The ability to specifically modulate TrkB signaling can be critical in various pathological scenarios associated with this pathway.

[0004] There is a strong need in the art for means for promoting TrkB and BDNF signaling activities in the treatment of various retinal diseases and disorders, especially neuropathies including glaucoma. For example, current glaucoma treatment options reduce IOP but do not reverse RGC degeneration. The instant invention is directed to addressing these and other needs.

SUMMARY OF THE INVENTION

[0005] In one aspect, the invention provides TrkB agonist antibodies or antigen-binding fragments that specifically bind to tropomyosin receptor kinase B (TrkB) with the same binding specificity as that of a second antibody. The second antibody contains heavy chain CDRs 1-3 and light chain CDRs 1-3 sequences that are respectively identical to (1) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and SSRDSSRSHLL (SEQ ID NO:73); (2) GYSFTSYW (SEQ ID NO:46), IYPGDSDT (SEQ ID NO:47), ARQGASSTSYDY (SEQ ID NO:48), QSISSY (SEQ ID NO:49), AAS, and QQANSFPVA (SEQ ID NO:50); (3) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56); (4) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKTDSDK (SEQ ID NO:58), and AIWHSSAWV (SEQ ID NO:56); (5) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:55), AIWHSSAWV (SEQ ID NO:56); (6) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSACV (SEQ ID NO:59); (7) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), ARQLLY (SEQ ID NO:60), SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:61), AIWHSSAWV (SEQ ID NO:56); (8) GYSFTSYW (SEQ ID NO:46), IYPGDSDT (SEQ ID NO:47), ATRVLPAGHF (SEQ ID NO:62), NIGDKF (SEQ ID NO:63), YDS, and QVWDNSSNQGV (SEQ ID NO:64); (9) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and NSRDGSGNNVV (SEQ ID NO:69); or (10) GYTFTSYA (SEQ ID NO:70), INTNTGNP (SEQ ID NO:71), ASRLAAAGFDY (SEQ ID NO:72), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56).

[0006] Some antibodies or antigen-binding fragments of the invention harbor a heavy chain variable region sequence and a light chain variable region sequence, one or both of which are identical to a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (1) SEQ ID NO:38 and SEQ ID NO:39; (2) SEQ ID NO:14 and SEQ ID NO:15; (3) SEQ ID NO:16 and SEQ ID NO:17; (4) SEQ ID NO:18 and SEQ ID NO:19; (5) SEQ ID NO:20 and SEQ ID NO:21; (6) SEQ ID NO:22 and SEQ ID NO:23; (7) SEQ ID NO:24 and SEQ ID NO:25; (8) SEQ ID NO:26 and SEQ ID NO:27; (9) SEQ ID NO:28 and SEQ ID NO:29; (10) SEQ ID NO:30 and SEQ ID NO:31; (11) SEQ ID NO:32 and SEQ ID NO:33; (12) SEQ ID NO:34 and SEQ ID NO:35; or (13) SEQ ID NO:36 and SEQ ID NO:37. Some antibodies or antigen-binding fragments contain a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (1) SEQ ID NO:38 and SEQ ID NO:39; (2) SEQ ID NO:14 and SEQ ID NO:15; (3) SEQ ID NO:16 and SEQ ID NO:17; (4) SEQ ID NO:18 and SEQ ID NO:19; (5) SEQ ID NO:20 and SEQ ID NO:21; (6) SEQ ID NO:22 and SEQ ID NO:23; (7) SEQ ID NO:24 and SEQ ID NO:25; (8) SEQ ID NO:26 and SEQ ID NO:27; (9) SEQ ID NO:28 and SEQ ID NO:29; (10) SEQ ID NO:30 and SEQ ID NO:31; (11) SEQ ID NO:32 and SEQ ID NO:33; (12) SEQ ID NO:34 and SEQ ID NO:35; or (13) SEQ ID NO:36 and SEQ ID NO:37.

[0007] Some antibodies or antigen-binding fragments of the invention contain heavy chain CDRs 1-3 and light chain CDRs 1-3 sequences that are substantially identical, respectively, to (1) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and SSRDSSRSHLL (SEQ ID NO:73); (2) GYSFTSYW (SEQ ID NO:46), IYPGDSDT (SEQ ID NO:47), ARQGASSTSYDY (SEQ ID NO:48), QSISSY (SEQ ID NO:49), AAS, and QQANSFPVA (SEQ ID NO:50); (3) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56); (4) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKTDSDK (SEQ ID NO:58), and AIWHSSAWV (SEQ ID NO:56); (5) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:55), AIWHSSAWV (SEQ ID NO:56); (6) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSACV (SEQ ID NO:59); (7)

GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), ARQLLY (SEQ ID NO:60), SGINVGA YR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:61), AIWHSSAWV (SEQ ID NO:56); (8) GYSFTSYW (SEQ ID NO:46), IYPGDSDT (SEQ ID NO:47), ATRVLPAGHF (SEQ ID NO:62), NIGDKF (SEQ ID NO:63), YDS, and QVWDNSSNQGV (SEQ ID NO:64); (9) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and NSRDGSGNNVV (SEQ ID NO:69); or (10) GYTFTSYA (SEQ ID NO:70), INTNTGNP (SEQ ID NO:71), ASRLAAAGFDY (SEQ ID NO:72), SGINVGT YR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56). Some antibodies or antigen-binding fragments harbor a heavy chain CDR sequence selected from the group consisting of SEQ ID NOs:46-48, 51-53, 60, 62, 65-67, and 70-72. Some of these molecules further contain a light chain CDR sequence selected from the group consisting of SEQ ID NOs:49, 50, 54-59, 61, 63, 64, 68, 69 and 73. Some of these molecules contain heavy chain CDRs 1-3 sequences that are respectively identical to (1) SEQ ID Nos:70-72, (2) SEQ ID NOs:46-48, (3) SEQ ID NOs:51-53, (4) SEQ ID Nos:51, 52, 60, (5) SEQ ID Nos:46, 47, and 62, or (6) SEQ ID Nos:65-67. Some of these antibodies or antigen-binding fragments harbor heavy chain CDRs 1-3 and light chain CDRs 1-3 sequences respectively shown in (1) SEQ ID Nos:65-68, GKN, and SEQ ID NO:73; (2) SEQ ID NOs:46-49, AAS, and SEQ ID NO:50; (3) SEQ ID NOs:51-56; (4) SEQ ID Nos:51-53, 57, 58, and 56; (5) SEQ ID Nos:51-53, 57, 55, and 56; (6) SEQ ID Nos:51-55 and 59; (7) SEQ ID Nos:51, 52, 60, 57, 61 and 56; (8) SEQ ID Nos:46, 47, 62, 63, YDS and SEQ ID NO:64; (9) SEQ ID Nos:65-68, GKN, and SEQ ID NO:69; or (10) SEQ ID Nos:70-72 and 54-56.

[0008] Some antibodies or antigen-binding fragments of the invention contain a light chain CDR sequence selected from the group consisting of SEQ ID NOs:49, 50, 54-59, 61, 63, 64, 68, 69 and 73, and sequences AAS, YDS and GKN. Some of these molecules additionally contain a heavy chain CDR sequence selected from the group consisting of SEQ ID NOs:46-48, 51-53, 60, 62, 65-67, and 70-72. Some of these molecules contain light chain CDRs 1-3 sequences that are respectively identical to (1) SEQ ID No:68, GKN and SEQ ID NO:73; (2) SEQ ID NO:49, AAS, and SEQ ID NO:50; (3) SEQ ID NOs:54-56; (4) SEQ ID Nos:57, 58 and 56; (5) SEQ ID Nos:57, 55 and 56; (6) SEQ ID Nos:54, 55 and 59; (7) SEQ ID Nos:57, 61 and 56; (8) SEQ ID No:63, YDS and SEQ ID NO:64; or (8) SEQ ID No:68, GKN and SEQ ID NO:69.

[0009] Some antibodies or antigen-binding fragments of the invention contain a heavy chain variable region that is substantially identical to SEQ ID NO:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 or 38. Some antibodies or antigen-binding fragments are scFv fragments, which contains a linker sequence that connects the heavy chain and light chain variable region sequences. Some exemplary linker sequences are shown in SEQ ID Nos:40-45. Some scFv fragments of the invention contain a sequence as shown in any one of SEQ ID Nos:1-13.

[0010] The antibodies or antigen-binding fragments of the invention can be in any antibody structure format, e.g., IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, IgM, F(ab)2, Fv, scFv, IgGACH2, F(ab')2, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)2, a non-depleting IgG, a diabody, and a bivalent antibody. The molecules can also be conjugated to a synthetic molecule. In some embodiments, the invention provides bispecific compounds (e.g., bispecific antibodies) that contain a TrkB agonist antibody or antigen-binding fragment described herein.

[0011] In another aspect, the invention provides polynucleotides that encode the variable region of the heavy chain and/or light chain of an antibody or antigen-binding fragment of the invention, as well as vectors that harbor such polynucleotides. In another aspect, the invention provides pharmaceutical compositions or kits that contain a therapeutically effective amount of an antibody or antigen-binding fragment described herein, or a polynucleotide or vector expressing the antibody.

[0012] In still another aspect, the invention provides methods for promoting survival, synaptic function or regeneration of retinal ganglion cells, motor neurons or central nervous system (CNS) neurons in a subject. Also provided are methods for treating or preventing an ocular degenerative condition, a motor neuron disease or a central nervous system (CNS) degenerative disease in a subject. These therapeutic methods of the invention entail administering to the subject a pharmaceutical composition that contains a therapeutically effective amount of a TrkB agonist antibody or antigen-binding fragment, or a polynucleotide or expression vector encoding the antibody as described herein. In various embodiments, the pharmaceutical composition can be administered to one or both eyes of the subject. Typically, the administered pharmaceutical composition further contains a pharmaceutically acceptable carrier. The subjects suitable for the methods can be one who suffers from or is at risk of developing an ocular degenerative disorder, a motor neuron

disease or a central nervous system (CNS) degenerative disease. The ocular degenerative disorder suitable for the methods of the invention can be, e.g., glaucoma, optic nerve injury, optic neuritis, optic neuropathy, central retinal artery occlusion, central retinal vein occlusion, diabetic neuropathy, age-related macular degeneration (AMD), anterior ischemic optic neuropathy (AION), or diabetic retinopathy. Examples of motor neuron diseases suitable for treatment with methods of the invention include, e.g., ALS, idiopathic motor neuropathy, hereditary spastic paraplegia (HSP), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy (PBP) and pseudobulbar palsy, Bell's palsy, or spinal muscular atrophies (SMA). Examples of CNS degenerative diseases that can be treated with methods of the invention include, e.g., Alzheimer's Disease, Parkinson's Disease, or Huntington's Disease.

[0013] A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 illustrates the scheme for the construction of a human TrkB reporter for selecting TrkB agonist Abs.

[0015] Figure 2 shows activities of several unpurified scFv-Fc TrkB agonist antibodies in culture supernatant examined via a TrkB reporter cell line.

[0016] Figure 3 shows activities of a number of TrkB agonist IgG antibodies in TrkB reporter cell assay.

[0017] Figure 4 shows selectivity of the identified TrkB agonist antibodies - the lack of effect on TrkA.

[0018] Figure 5 shows that signaling of TrkB agonist Abs resulted in activation of P-PLC γ , P-Akt and P-MAPK in TrkB reporter cells.

[0019] Figure 6 shows agonist Ab-induced TrkB phosphorylation in cultured mouse cortical neurons.

[0020] Figure 7 shows expression of Arc induced by BDNF and TrkB agonist Ab NFAT-85.

[0021] Figure 8 shows TrkB phosphorylation induced by agonist antibody NFAT-85 in mouse embryonic stem cells.

[0022] Figure 9 shows that TrkB agonist Ab NFAT-85 prevents RGC dendrite retraction.

[0023] Figure 10 is an experimental paradigm of efficacy studies of effect of TrkB agonist antibodies in rat model of glaucoma.

[0024] Figure 11 shows IOP characteristics for the OHT model in rats.

[0025] Figure 12 shows Sholl plot analysis of the effect of TrkB agonist Ab85 and BDNF in OHT rats.

[0026] Figure 13 illustrates dendritic metrics for Sholl analysis shown in Figure 12.

DETAILED DESCRIPTION

I. Overview

[0027] The invention is predicated in part on the development by the present inventors of TrkB activating Antibodies which demonstrated utilities in neuroprotective and neuroregenerative treatment of retinal disorders such as glaucoma. Neuroprotection and neuroregeneration activities of BDNF were evidenced herein by that BDNF greatly slows RGC dendritic retraction, and that BDNF can reverse RGC dendritic degeneration.

Importantly, the inventors demonstrated that the TrkB agonist antibodies of the invention showed equal or better properties than BDNF as a neuroprotectant for RGCs.

[0028] As detailed herein, the inventors have generated a family of fully human agonist antibodies by selecting rare activating agonist antibodies from combinatorial libraries. In exemplified studies, the TrkB agonist antibodies were shown to be able to mimic the efficacy of BDNF in maintaining or regenerating the dendritic arbor of RGCs that rapidly retracts in axotomized adult mammalian retina. Specifically, relative to BDNF, the antibodies were shown to be potent and selective in both TrkB cell lines and BDNF responsive primary neuronal cultures. The antibodies were also found to be equivalent to BDNF in activating TrkB signal transduction, and also have cross-species activity (e.g., human and mouse). In situ, the antibodies were found to be active in slowing and restoring RGC degeneration in an adult mouse retinal explant assay. Furthermore, assessed via a rat model of hypertensive glaucoma, the TrkB agonist Abs showed a regenerative effect on dendritic arbors of retinal ganglion neurons.

[0029] Compared to BDNF, the TrkB agonist antibodies of the invention also have greatly improved biophysical properties (e.g., unlike BDNF these antibodies are not “sticky”

proteins) and have much longer half-lives (e.g., better pharmacokinetics) in human subjects than BDNF itself. In addition, all currently approved treatments for glaucoma are focused on reducing intraocular pressure (IOP) in the anterior chamber of the eye. However, these treatments alone are insufficient to halt the progression of the disease. In contrast, the disease-modifying neuroprotective activities of the TrkB agonist antibodies disclosed herein can reduce RGC dendritic and cell body atrophy or loss and thus slow disease progression. The neuroregenerative activities of the antibodies can also restore the functional integrity of impaired RGCs.

[0030] In accordance with these studies, the present invention provides monoclonal antibodies and related antigen-binding fragments that specifically activate the TrkB tyrosine kinase receptor and promote survival or regeneration of RGCs. Also provided in the invention are methods of using these antibody agents in therapeutic applications for treating various neurodegenerative diseases including retinal degenerative disorders that are associated with RGC death or degeneration, or ocular diseases that are mediated by abnormal or impaired TrkB signaling activities, e.g., glaucoma.

II. Definitions

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention pertains. The following references provide one of skill with a general definition of many of the terms used in this invention: *Academic Press Dictionary of Science and Technology*, Morris (Ed.), Academic Press (1st ed., 1992); *Oxford Dictionary of Biochemistry and Molecular Biology*, Smith et al. (Eds.), Oxford University Press (revised ed., 2000); *Encyclopaedic Dictionary of Chemistry*, Kumar (Ed.), Anmol Publications Pvt. Ltd. (2002); *Dictionary of Microbiology and Molecular Biology*, Singleton et al. (Eds.), John Wiley & Sons (3rd ed., 2002); *Dictionary of Chemistry*, Hunt (Ed.), Routledge (1st ed., 1999); *Dictionary of Pharmaceutical Medicine*, Nahler (Ed.), Springer-Verlag Telos (1994); *Dictionary of Organic Chemistry*, Kumar and Anandand (Eds.), Anmol Publications Pvt. Ltd. (2002); and *A Dictionary of Biology (Oxford Paperback Reference)*, Martin and Hine (Eds.), Oxford University Press (4th ed., 2000). In addition, the following definitions are provided to assist the reader in the practice of the invention.

[0032] The term "antibody" or "antigen-binding fragment" refers to polypeptide chain(s) which exhibit a strong monovalent, bivalent or polyvalent binding to a given antigen, epitope or epitopes. Unless otherwise noted, antibodies or antigen-binding fragments used in the invention can have sequences derived from any vertebrate species, including camelid, avian or pisces species. They can be generated using any suitable technology, e.g., hybridoma technology, ribosome display, phage display, gene shuffling libraries, semi-synthetic or fully synthetic libraries or combinations thereof. Unless otherwise noted, the term "antibody" as used in the present invention includes intact antibodies, antigen-binding polypeptide fragments and other designer antibodies that are described below or well known in the art (see, e.g., Serafini, J Nucl. Med. 34:533-6, 1993).

[0033] An intact "antibody" typically comprises at least two heavy (H) chains (about 50-70 kD) and two light (L) chains (about 25 kD) inter-connected by disulfide bonds. The recognized immunoglobulin genes encoding antibody chains include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0034] Each heavy chain of an antibody is comprised of a heavy chain variable region (V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1} , C_{H2} and C_{H3} . Each light chain is comprised of a light chain variable region (V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L . The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system and the first component (C1q) of the classical complement system.

[0035] The V_H and V_L regions of an antibody can be further subdivided into regions of hypervariability, also termed complementarity determining regions (CDRs), which are interspersed with the more conserved framework regions (FRs). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The locations of CDR and FR regions and a numbering system have been defined by, e.g., Kabat *et al.*, *Sequences*

of Proteins of Immunological Interest, U.S. Department of Health and Human Services, U.S. Government Printing Office (1987 and 1991).

[0036] Binding affinity is generally expressed in terms of equilibrium association or dissociation constants (K_a or K_d , respectively), which are in turn reciprocal ratios of dissociation and association rate constants (k_{off} and k_{on} , respectively). Thus, equivalent affinities may correspond to different rate constants, so long as the ratio of the rate constants remains the same.

[0037] The term "conservatively modified variant" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0038] For polypeptide sequences, "conservatively modified variants" refer to a variant which has conservative amino acid substitutions, amino acid residues replaced with other amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-

branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0039] The term “contacting” has its normal meaning and refers to combining two or more agents (e.g., polypeptides or phage), combining agents and cells, or combining two populations of different cells. Contacting can occur in vitro, e.g., mixing an antibody and a cell or mixing a population of antibodies with a population of cells in a test tube or growth medium. Contacting can also occur in a cell or in situ, e.g., contacting two polypeptides in a cell by coexpression in the cell of recombinant polynucleotides encoding the two polypeptides, or in a cell lysate. Contacting can also occur in vivo inside a subject, e.g., by administering an agent to a subject for delivery the agent to a target cell.

[0040] The term “epitope” refers to any antigenic determinant on an antigen to which the paratope of an antibody binds, and is made up of one or more segments of amino acids. An epitope can be a linear or conformational epitope, and can be continuous or discontinuous. Typically, linear epitopes are continuous, i.e., made up of one continuous stretch of amino acids. Conformational epitopes can be discontinuous i.e., made up of two or more discontinuous segments of amino acids that come together to form an epitope when the antigen is folded. Methods for determining whether antibodies binds to the same epitope are known in the art. Epitopes can be defined or mapped by standard methods well known in art. For example, epitopes can be mapped using assays, such as ELISA assays, utilizing peptide libraries or site-directed mutagenesis of the antigen (such as alanine-scanning of the antigen).

[0041] As used herein, “binds to the same epitope” with reference to two or more antibodies means that the antibodies compete for binding to an antigen and bind to the same, overlapping or encompassing continuous or discontinuous segments of amino acids. Those of skill in the art understand that the phrase “binds to the same epitope” does not necessarily mean that the antibodies bind to exactly the same amino acids. The precise amino acids to which the antibodies bind can differ. For example, a first antibody can bind to a segment of amino acids that is completely encompassed by the segment of amino acids bound by a second antibody. In another example, a first antibody binds one or more segments of amino acids that significantly overlap the one or more segments bound by the second antibody. For the purposes herein, such antibodies are considered to “bind to the same epitope.”

[0042] Antibody competition assays can be used to determine whether an antibody “binds to the same epitope” as another antibody. Such assays are well known on the art. Typically, competition of 70 % or more, such as 70%, 71%, 72%, 73%, 74%, 75%, 80%, 85%, 90%, 95% or more, of an antibody known to interact with the epitope by a second antibody under conditions in which the second antibody is in excess and the first saturates all sites, is indicative that the antibodies “bind to the same epitope.” To assess the level of competition between two antibodies, for example, radioimmunoassays or assays using other labels for the antibodies, such as biotin can be used. For example, an antigen can be incubated with a saturating amount of a first antibody or antigen-binding fragment thereof conjugated to a labeled compound (e.g., ^3H , ^{125}I or biotin) in the presence the same amount of a second unlabeled antibody. The amount of labeled antibody that is bound to the antigen in the presence of the unlabeled blocking antibody is then assessed and compared to binding in the absence of the unlabeled blocking antibody. Competition is determined by the percentage change in binding signals in the presence of the unlabeled blocking antibody compared to the absence of the blocking antibody. Thus, if there is a 70% inhibition of binding of the labeled antibody in the presence of the blocking antibody compared to binding in the absence of the blocking antibody, then there is competition between the two antibodies of 70%. Thus, reference to competition between a first and second antibody of 70 % or more, such as 70%, 71%, 72%, 73%, 74%, 75%, 80%, 85%, 90%, 95% or more, means that the first antibody inhibits binding of the second antibody (or *vice versa*) to the antigen by 70%, 71%, 72%, 73%, 74%, 75%, 80%, 85%, 90%, 95% or more (compared to binding of the antigen by the second antibody in the absence of the first antibody). Thus, inhibition of binding of a first antibody to an antigen by a second antibody of 70%, 71%, 72%, 73%, 74%, 75%, 80%, 85%, 90%, 95% or more indicates that the two antibodies bind to the same epitope. The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. Two sequences are “substantially identical” if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual

inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

[0043] Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.* 2:482c, 1970; by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; by the search for similarity method of Pearson and Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444, 1988; by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI); or by manual alignment and visual inspection (see, *e.g.*, Brent et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., *Nuc. Acids Res.* 25:3389-3402, 1977; and Altschul et al., *J. Mol. Biol.* 215:403-410, 1990, respectively.

[0044] As used herein, ocular degenerative conditions or disorders refer to eye diseases that are characterized by or associated with degeneration or death of retinal ganglion cells (RGCs), or ocular disorders that are mediated by or associated with impaired TrkB signaling activities. They encompass various clinical presentations and etiologies. Examples of these disorders include glaucoma, optic nerve injury, optic neuritis, optic neuropathies, central retinal artery occlusion, and central retinal vein occlusion.

[0045] Glaucoma is an exemplary ocular degenerative condition which is characterized by pathological changes in the optic nerve, visible on the optic disk, and corresponding visual field loss, resulting in blindness if untreated. Glaucoma is associated with increased intraocular pressure, but other factors are involved. Glaucoma includes the more common type of open-angle glaucoma and less common types such as closed-angle glaucoma and normal-tension glaucoma. Current therapies for glaucoma are directed at decreasing intraocular pressure. Medical therapy includes topical ophthalmic drops or oral medications that reduce the production or increase the outflow of intraocular fluid. However, these drug therapies for glaucoma are sometimes associated with significant side effects, such as headache, blurred vision, allergic reactions, death from cardiopulmonary complications and

potential interactions with other drugs. Surgical therapies also are used, but they also have numerous disadvantages and modest success rates.

[0046] A motor neuron disease (MND) is any of several neurological disorders that selectively affect motor neurons, the cells that control voluntary muscles of the body. They include, e.g., amyotrophic lateral sclerosis (ALS), hereditary spastic paraplegia (HSP), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy (PBP) and pseudobulbar palsy. Spinal muscular atrophies are also sometimes included in the group. Motor neuron diseases are neurodegenerative in nature and cause increasing disability and eventually death. The TrkB agonist antibodies disclosed herein can be employed to treat or ameliorate symptoms of any of these disorders, e.g., ALS.

[0047] The term "subject" refers to human and non-human animals (especially non-human mammals). The term "subject" is used herein, for example, in connection with therapeutic and diagnostic methods, to refer to human or animal subjects. Animal subjects include, but are not limited to, animal models, such as, mammalian models of conditions or disorders associated with degeneration or death of retinal ganglion cells. Other specific examples of non-human subjects include, e.g., cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys.

[0048] The term "target," "target molecule," or "target cell" refers to a molecule or biological cell of interest that is to be analyzed or detected, e.g., a eukaryotic cell the death of which is to be modulated.

[0049] The terms "treat," "treating," "treatment," and "therapeutically effective" used herein do not necessarily imply 100% or complete treatment. Rather, there are varying degrees of treatment recognized by one of ordinary skill in the art as having a potential benefit or therapeutic effect. In this respect, the inventive method can provide any amount of any level of treatment. Furthermore, the treatment provided by the inventive method can include the treatment of one or more conditions or symptoms of the disease being treated.

[0050] Tropomyosin receptor kinase B (TrkB), also known as Tyrosine receptor kinase B, BDNF/NT-3 growth factors receptor or type 2 neurotrophic receptor tyrosine kinase, is a protein that in humans is encoded by the NTRK2 gene. TrkB is a receptor for brain-derived neurotrophic factor (BDNF). Klein et al., Cell 61: 647-56, 1990; and Squinto et al., Cell 65: 885-93, 1991. TrkB is the high affinity catalytic receptor for several "neurotrophins", which are small protein growth factors that induce the survival and differentiation of distinct cell

populations. The neurotrophins that activate TrkB are: BDNF (Brain Derived Neurotrophic Factor), neurotrophin-4 (NT-4), and neurotrophin-3 (NT-3). As such, TrkB mediates the multiple effects of these neurotrophic factors, which includes neuronal differentiation and survival. Research has shown that activation of the TrkB receptor can lead to down regulation of the KCC2 chloride transporter in cells of the CNS.

[0051] A "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. Vectors capable of directing the expression of genes encoding for one or more polypeptides are referred to as "expression vectors".

III. TrkB agonist antibodies and related antigen-binding molecules

[0052] The invention provides antibodies or antigen-binding fragments that specifically bind to TrkB (e.g., human TrkB) and activate the TrkB signaling pathway. Preferably, TrkB agonist antibodies of the invention have the same binding specificity as that of an TrkB agonist antibody exemplified herein (e.g., antibody NFAT-85 or antibody CRE-30 show in Table 1). Thus, the invention encompasses antibodies or antigen-binding fragments with the same binding specificity as that of an antibody having heavy chain CDRs 1-3 and light chain CDRs 1-3 sequences that are respectively identical to (1) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and SSRDSSRSHHLL (SEQ ID NO:73); (2) GYSFTSYW (SEQ ID NO:46), IYPGSDT (SEQ ID NO:47), ARQGASSTSYDY (SEQ ID NO:48), QSISSY (SEQ ID NO:49), AAS, and QQANSFPVA (SEQ ID NO:50); (3) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56); (4) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKTDSDK (SEQ ID NO:58), and AIWHSSAWV (SEQ ID NO:56); (5) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:55), AIWHSSAWV (SEQ ID NO:56); (6) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSACV (SEQ ID NO:59); (7) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), ARQLLY (SEQ ID NO:60), SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID

NO:61), AIWHSSAWV (SEQ ID NO:56); (8) GYSFTSYW (SEQ ID NO:46), IYPGDSDT (SEQ ID NO:47), ATRVLPAGHF (SEQ ID NO:62), NIGDKF (SEQ ID NO:63), YDS, and QVWDNSSNQGV (SEQ ID NO:64); (9) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and NSRDGSGNNVV (SEQ ID NO:69); or (10) GYTFTSYA (SEQ ID NO:70), INTNTGNP (SEQ ID NO:71), ASRLAAAGFDY (SEQ ID NO:72), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56).

[0053] Some antibodies of the invention can compete with the exemplified antibodies for binding to TrkB. Some antibodies of the invention bind to the same epitope as that recognized by one of the TrkB antibodies exemplified herein. Some antibodies of the invention bind to the same epitope on TrkB with the same or similar affinity as that of one of the TrkB antibodies exemplified herein. Upon binding to TrkB, some antibodies of the invention are capable of modulating one or more of TrkB biological activities or generating cellular responses (e.g., activation of MAPK, phospholipase C- γ and/or PI3-K signaling pathways) in the same or similar manner as that by one of the TrkB antibodies exemplified herein.

[0054] Antibodies of the invention include intact antibodies (e.g., IgG1 antibodies), antibody fragments or antigen-binding fragments (e.g., scFv antibodies exemplified herein) which contain the antigen-binding portions of an intact antibody that retain capacity to bind the cognate antigen. A typical intact antibody interacts with target antigen predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDR's). The functional TrkB agonist antibodies of the invention encompass antibodies or antigen-binding fragments having at least one of their heavy chain CDR sequences and light chain CDR sequences that is the same as or substantially identical to the corresponding CDR sequence of exemplified TrkB agonist antibodies. In some embodiments, the TrkB agonists of the invention are antibody fragments or antigen-binding molecules that are derived from the exemplified antibodies. Examples of such antibody fragments include (i) a Fab fragment, a monovalent fragment consisting of the V_L , V_H , C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an intact antibody; (v) disulfide stabilized Fvs (dsFvs) which

have an interchain disulfide bond engineered between structurally conserved framework regions; (vi) a single domain antibody (dAb) which consists of a V_H or V_L domain (see, e.g., Ward et al., Nature 341:544-546, 1989); and (vii) an isolated complementarity determining region (CDR) as a linear or cyclic peptide.

[0055] Antibodies of the invention also encompass single chain antibodies. The term "single chain antibody" refers to a polypeptide comprising a V_H domain and a V_L domain in polypeptide linkage, generally linked via a spacer peptide or linker sequence, and which may comprise additional domains or amino acid sequences at the amino- and/or carboxyl-termini. For example, a single-chain antibody may comprise a tether segment for linking to the encoding polynucleotide. As an example, a single chain variable region fragment (scFv) is a single-chain antibody. Compared to the V_L and V_H domains of the Fv fragment which are coded for by separate genes, a scFv has the two domains joined (e.g., via recombinant methods) by a synthetic linker. This enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules. Some scFv TrkB agonist antibodies of the invention are shown in listed in Table 1. The V_L and V_H sequences, as well as the connecting linker sequence, of these scFv antibodies are indicated in Table 2.

[0056] As exemplified herein, the scFv TrkB agonist antibodies can be fused to the Fc domain of an IgG to provide scFv-Fc fusion molecules. Fusion with an Fc-domain provides a number of beneficial biological and pharmacological properties. For example, the presence of the Fc domain can markedly increase the plasma half-life of the hybrid molecule, which prolongs therapeutic activity, owing to its interaction with the salvage neonatal Fc-receptor, as well as to the slower renal clearance for larger sized molecules. Further, addition of the Fc region also can improve the solubility and stability of the fused partner, and allows for easy cost-effective purification by protein-G/A affinity chromatography during manufacture. Hybrid or fusion molecules containing an scFv antibody fragment and a fusion partner such as Fc domain can be readily generated in accordance with standard recombination techniques, routinely practiced protein synthesis methods or the protocols described herein.

[0057] Antibodies of the present invention also encompass single domain antigen-binding units which have a camelid scaffold. Animals in the camelid family include camels, llamas, and alpacas. Camelids produce functional antibodies devoid of light chains. The heavy chain variable (V_H) domain folds autonomously and functions independently as an

antigen-binding unit. Its binding surface involves only three CDRs as compared to the six CDRs in classical antigen-binding molecules (Fabs) or single chain variable fragments (scFvs). Camelid antibodies are capable of attaining binding affinities comparable to those of conventional antibodies.

[0058] In addition to the TrkB agonist antibodies exemplified herein which were initially identified via functional screening, various other antibodies or antigen-binding fragments of the invention can also be produced by enzymatic or chemical modification of the intact antibodies, or synthesized de novo using recombinant DNA methodologies, or identified using phage display libraries. As exemplification, the specific scFv antibodies shown in Table I can be readily converted into scFv-Fc fusions via standard recombinant techniques, e.g., by cloning into a pFUSE IgG backbone vector described herein. Additional methods that may be employed for generating the other TrkB agonist antibodies or antigen-binding molecules of the invention are all well known in the art. For example, single chain antibodies can be identified using phage display libraries or ribosome display libraries, gene shuffled libraries (see, e.g., McCafferty et al., *Nature* 348:552-554, 1990; and U.S. Pat. No. 4,946,778). In particular, scFv antibodies can be obtained using methods described in, e.g., Bird et al., *Science* 242:423-426, 1988; and Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988. Fv antibody fragments can be generated as described in Skerra and Plückthun, *Science* 240:1038-41, 1988. Disulfide-stabilized Fv fragments (dsFvs) can be made using methods described in, e.g., Reiter et al., *Int. J. Cancer* 67:113-23, 1996. Similarly, single domain antibodies (dAbs) can be produced by a variety of methods described in, e.g., Ward et al., *Nature* 341:544-546, 1989; and Cai and Garen, *Proc. Natl. Acad. Sci. USA* 93:6280-85, 1996. Camelid single domain antibodies can be produced using methods well known in the art, e.g., Dumoulin et al., *Nat. Struct. Biol.* 11:500-515, 2002; Ghahroudi et al., *FEBS Letters* 414:521-526, 1997; and Bond et al., *J. Mol. Biol.* 332:643-55, 2003. Other types of antigen-binding fragments (e.g., Fab, F(ab')₂ or Fd fragments) can also be readily produced with routinely practiced immunology methods. See, e.g., Harlow & Lane, *Using Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1998.

[0059] In some embodiments, the antibodies or antigen binding fragments of the invention have its heavy chain CDR1, CDR2 and CDR3 sequences and/or its light chain CDR1, CDR2 and CDR3 sequences that are substantially identical to that of the antibodies

shown in Table 1. The light chain and heavy chain CDR sequences of the exemplified antibodies are all indicated in Table 2. In some of these embodiments, the antibodies or antigen-binding fragments have heavy chain CDR1-3 sequences and light chain CDR1-3 sequences that are substantially identical to (1) SEQ ID NOs:46-49, AAS, and SEQ ID NO:50; (2) SEQ ID NOs:51-56; (3) SEQ ID Nos:51-53, 57, 58, and 56; (4) SEQ ID Nos:51-53, 57, 55, and 56; (5) SEQ ID Nos:51-55 and 59; (6) SEQ ID Nos:51, 52, 60, 57, 61 and 56; (7) SEQ ID Nos:46, 47, 62, 63, YDS and SEQ ID NO:64; (8) SEQ ID Nos:65-68, GKN, and SEQ ID NO:69; (9) SEQ ID Nos:70-72 and 54-56; or (10) SEQ ID Nos:65-68, GKN, and SEQ ID NO:73. In some of these embodiments, the percentage of sequence identity can be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or even 100%. Thus, some antibodies or antigen binding fragments of the invention have heavy chain CDRs 1-3 and/or light chain CDRs 1-3 sequences that are at least 90% identical, respectively, to the corresponding CDRs of one of the exemplified antibodies. Some of these antibodies contain heavy chain CDR sequences and/or light chain CDR sequences that are at least 95% identical, respectively, to the corresponding CDRs of the exemplified antibody. In some other antibodies or antigen binding fragments of the invention, the heavy chain CDR sequences and/or light chain CDR sequences are at least 95% identical, respectively, to the corresponding CDRs of one of the exemplified antibodies. In some embodiments, the heavy chain CDR1-CDR3 and light chain CDR1-CDR3 sequences of the antibody are respectively identical to the sequences shown in (1) SEQ ID NOs:46-49, AAS, and SEQ ID NO:50; (2) SEQ ID NOs:51-56; (3) SEQ ID Nos:51-53, 57, 58, and 56; (4) SEQ ID Nos:51-53, 57, 55, and 56; (5) SEQ ID Nos:51-55 and 59; (6) SEQ ID Nos:51, 52, 60, 57, 61 and 56; (7) SEQ ID Nos:46, 47, 62, 63, YDS and SEQ ID NO:64; (8) SEQ ID Nos:65-68, GKN, and SEQ ID NO:69; (9) SEQ ID Nos:70-72 and 54-56; or (10) SEQ ID Nos:65-68, GKN, and SEQ ID NO:73.

[0060] In other embodiments, the antibodies or antigen-binding fragments that specifically bind to human TrkB contain (a) a heavy chain variable region that is substantially identical to SEQ ID NO:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 or 38, (b) a light chain variable region that is substantially identical to SEQ ID NO:15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39, or (c) both a heavy chain variable region of (a) and a light chain variable region of (b). In some embodiments, the antibody comprises both a heavy chain of (a) and a light chain of (b). In some embodiments, the antibody or antigen-binding

fragment contains (a) a heavy chain variable domain having at least 90% identity to any one of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 and 38, (b) a light chain variable domain having at least 90% sequence identity to any one of SEQ ID NOs:15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, or (c) both a heavy chain of (a) and a light chain of (b). In some embodiments, the antibody or antigen-binding fragment contains a light chain variable domain having at least 90% identity to any one of SEQ ID NOs:15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39. In some of these embodiments, the percentage of sequence identity can be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or even 100%. In some embodiments, the light chain variable domain has at least 95% identity to any one of SEQ ID NOs:15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the light chain variable domain has 100% identity to any one of SEQ ID NOs:15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39. In some other embodiments, the antibody or antigen-binding fragment contains a heavy chain variable domain having at least 90% identity to any one of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 and 38. In other embodiments, the percentage identity can be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or even 100%. In some embodiments, the heavy chain variable domain has at least 95% identity to any one of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 and 38. In some embodiments, the heavy chain variable domain has 100% identity to any one of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 and 38.

[0061] In addition to a heavy chain as described above, the antibody or antigen-binding fragment of the invention can further comprise a light chain selected from a Fab library using sequential naive chain shuffling. Likewise, in addition to a light chain as described above, the antibody of the invention can further comprise a heavy chain selected from a Fab library using sequential naive chain shuffling. In some embodiments, the antibody or antigen-binding fragment of the invention can contain any heavy chain as described herein (e.g., heavy chains shown in SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 and 38) in combination with any suitable light chain, such as those exemplified herein. Likewise, the antibody can comprise any of the light chains as described above (e.g., light chains shown in 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39) in combination with any suitable heavy chain, such as those exemplified herein. For example, in some preferred

embodiments, the antibody contains a heavy chain variable region sequence and a light chain variable region sequence that are at least 90% identical, respectively, to SEQ ID NOs:14 and 15, SEQ ID NOs:16 and 17, SEQ ID NOs:18 and 19, SEQ ID NOs:20 and 21, SEQ ID NOs:22 and 23, SEQ ID NOs:24 and 25, SEQ ID NOs:26 and 27, SEQ ID NOs:28 and 29, SEQ ID NOs:30 and 31, SEQ ID NOs:32 and 33, SEQ ID NOs:34 and 35, SEQ ID NOs:36 and 37, or SEQ ID NOs:38 and 39. In some embodiments, the antibody can contain the heavy chain and light chain sequences respectively shown in SEQ ID NOs:14 and 15, SEQ ID NOs:16 and 17, SEQ ID NOs:18 and 19, SEQ ID NOs:20 and 21, SEQ ID NOs:22 and 23, SEQ ID NOs:24 and 25, SEQ ID NOs:26 and 27, SEQ ID NOs:28 and 29, SEQ ID NOs:30 and 31, SEQ ID NOs:32 and 33, SEQ ID NOs:34 and 35, SEQ ID NOs:36 and 37, or SEQ ID NOs:38 and 39. In the various embodiments, percent (%) identity of peptide sequences can be calculated, for example, as $100 \times [(\text{identical positions})/\min(\text{TGA}, \text{TGB})]$, where TGA and TGB are the sum of the number of residues and internal gap positions in peptide sequences A and B in the alignment that minimizes TGA and TGB. See, e.g., Russell et al, *J. Mol. Biol.*, 244: 332-350 (1994).

[0062] The antibody or antigen-binding fragment of the invention can be any antibody including a full length antibody or an antibody fragment that specifically recognizes or binds to the extracellular domain of TrkB (e.g., human TrkB) with the same specificity as one of the TrkB agonist antibodies exemplified herein (Table 1). The TrkB antibodies of the invention are preferably monoclonal. They can be recombinant, chimeric, humanized or fully human antibodies. Furthermore, the antibody can be of any isotype including without limitation IgA, IgD, IgE, IgG, or IgM. Thus, for example, the antibody can be any IgA such as IgA1 or IgA2, or any IgG such as IgG1, IgG2, IgG3, IgG4, or synthetic IgG. The antibody can also be any antibody fragment having specificity for the extracellular domain of human TrkB, such as F(ab)₂, Fv, scFv, IgGACH2, F(ab')₂, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)₂, a diabody, and a bivalent antibody. The antibody can be any modified or synthetic antibody, including, but not limited to, non-depleting IgG antibodies, CARs, or other Fc or Fab variants of antibodies.

[0063] In some embodiments, the invention provides antibodies or antigen-binding fragments that are conservatively modified variants of the anti-TrkB antibodies exemplified herein. Typically, the variable regions of these variants have an amino acid sequence that is identical to one of these exemplified sequences except for conservative substitutions at one

or more amino acid residues. In some embodiments, the antibody or antigen-binding fragment of the invention specifically binds to TrkB and contains (a) at least one HCDR having a sequence selected from the group consisting of SEQ ID NOs:46-48, 51-53, 60, 62, 65-67, and 70-72; and/or (b) at least one LCDR having a sequence selected from the group consisting of SEQ ID NOs:49, 50, 54-59, 61, 63, 64, 68, 69 and 73, and sequences AAS, YDS and GKN. The invention also provides TrkB agonist antibodies that specifically bind to TrkB and contain one or more variants of the foregoing CDR sequences or substantially identically CDR sequences. The variant CDR sequences in these antibodies can include 1, 2, or 3 substitutions, insertions, deletions, or combinations thereof in a sequence selected from the group consisting of SEQ ID NOs:46-73 and sequences AAS, YDS and GKN. For example, a recombinant chimeric or humanized antibody (or fragment thereof) can include one, two, three, four, five, or six of the foregoing CDR sequences.

[0064] In some embodiments, the TrkB agonist antibodies or antigen-binding fragments of the invention include three CDR sequences of the same light or heavy chain of the antibodies in Table 1. These include, e.g., light chain CDRs shown in (1) SEQ ID NO:49, AAS, and SEQ ID NO:50, (2) SEQ ID NOs:54-56, (3) SEQ ID Nos:57, 58 and 56, (4) SEQ ID Nos:57, 55 and 56, (5) SEQ ID Nos:54, 55 and 59, (6) SEQ ID Nos:57, 61 and 56, (7) SEQ ID No:63, YDS and SEQ ID NO:64, (8) SEQ ID No:68, GKN and SEQ ID NO:69, and (9) SEQ ID No:68, GKN and SEQ ID NO:73; or heavy chain CDRs shown in (1) SEQ ID NOs:46-48, (2) SEQ ID NOs:51-53, (3) SEQ ID Nos:51, 52, 60, (4) SEQ ID Nos:46, 47, and 62, (5) SEQ ID Nos:65-67, and (6) SEQ ID Nos:70-72. In some embodiments, the TrkB agonist antibodies or antigen-binding fragments of the invention can contain six CDR sequences of the same antibody, e.g., (1) SEQ ID NOs:46-49, AAS, and SEQ ID NO:50 (antibody CRE-6); (2) SEQ ID NOs:51-56 (antibody CRE-30 or CRE-83); (3) SEQ ID Nos:51-53, 57, 58, and 56 (antibody CRE-31 or CRE-53); (4) SEQ ID Nos:51-53, 57, 55, and 56 (antibody CRE-39 or NFAT-79); (5) SEQ ID Nos:51-55 and 59 (antibody CRE-87); (6) SEQ ID Nos:51, 52, 60, 57, 61 and 56 (antibody CRE-93); (7) SEQ ID Nos:46, 47, 62, 63, YDS and SEQ ID NO:64 (antibody NFAT-27); (8) SEQ ID Nos:65-68, GKN, and SEQ ID NO:69 (antibody NFAT-40); (9) SEQ ID Nos:70-72 and 54-56 (antibody NFAT-44); or (10) SEQ ID Nos:65-68, GKN, and SEQ ID NO:73 (antibody NFAT-85).

[0065] In some embodiments, the invention provides antibodies or antigen-binding fragments with avidity for TrkB of about 10 μ M or less, 5 μ M or less, 2 μ M or less, 1 μ M or

less, 500 nM or less, 400 nM or less, 300 nM or less, or 200 nM or less. In some embodiments, the antibodies or antigen-binding fragments bind to TrkB with an avidity of about 100 nM or less, about 75 nM or less, about 50 nM or less, about 25 nM or less, about 10 nM or less, or about 5 nM or less. In some embodiments, the antibodies or antigen-binding fragments bind to TrkB with an avidity of about 1 nM or less, about 800 pM or less, about 700 pM or less, about 600 pM or less, about 500 pM or less, about 400 pM or less, about 300 pM or less, about 200 pM or less, or about 100 pM or less. Avidity can be measured using art-known techniques, such as ELISA or surface plasmon resonance.

[0066] The antibody of the invention can be produced by any suitable technique, for example, using any suitable eukaryotic or non-eukaryotic expression system. In certain embodiments, the antibody is produced using a mammalian expression system. Some specific techniques for generating the antibodies or antigen-binding fragments of the invention are exemplified herein. In some embodiments, the antibodies or antigen-binding fragments of the invention can be produced using a suitable non-eukaryotic expression system such as a bacterial expression system. Bacterial expression systems can be used to produce fragments such as a F(ab)₂, Fv, scFv, IgGACH₂, F(ab')₂, scFv2CH₃, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)₂, and diabodies. Techniques for altering DNA coding sequences to produce such fragments are known in the art.

[0067] The antibodies or antigen-binding fragments of the invention can be conjugated to a synthetic molecule using any type of suitable conjugation. Recombinant engineering and incorporated selenocysteine (e.g., as described in U.S. Patent 8,916,159 issued on December 23, 2014) can be used to conjugate a synthetic molecule. Other methods of conjugation can include covalent coupling to native or engineered lysine side-chain amines or cysteine side-chain thiols. See, e.g., Wu et al., Nat. Biotechnol, 23: 1 137-1 146 (2005). The synthetic molecule can be any molecule such as one targeting a molecule of interest (e.g., a cell surface receptor). In some embodiments, the synthetic molecule for conjugation to the antibody is a protein (e.g., an antibody) or an RNA or DNA aptamer. In some embodiments, a TrkB agonist antibody (e.g., a scFv molecule) of the invention is present as a component in a bispecific molecule or multispecific molecule. Typically, the bispecific or multispecific compounds of the invention contain the TrkB agonist antibody or antigen-binding fragment that is covalently or non-covalently conjugated to at least one other binding moiety that specifically recognizes a target of interest (e.g., TrkC or TNF α). The binding moiety can be

a molecule of any chemical nature, e.g., another antibody or antigen binding fragment, a polypeptide or peptide agent, or a small molecule agent. In some embodiments, the multispecific molecules of the invention are bispecific antibodies that contain the TrkB agonist antibody and another antibody or antigen-binding fragment that is well known in the art, e.g., TNF α antibody adalimumab as exemplified herein. In various embodiments, the other binding moiety in these bispecific or multiple specific molecules can be edrecolomab, capromab, ibritumomab, blinatumomab, abciximab, rituximab, basiliximab, infliximab, cetuximab, brentuximab, siltuximab, palivizumab, trastuzumab, alemtuzumab, omalizumab, bevacizumab, natalizumab, ranibizumab, eculizumab, certolizumab, pertuzumab, obinutuzumab, pembrolizumab, vedolizumab, elotuzumab, idarucizumab, mepolizumab, adalimumab, panitumumab, canakinumab, golimumab, ofatumumab, ustekinumab, denosumab, belimumab, ipilimumab, raxibacumab, nivolumab, ramucirumab, alirocumab, daratumumab, evolocumab, necitumumab, and secukinumab. In some other embodiments, the multispecific molecules of the invention are bispecific small molecule-antibody conjugates. Relative to a monospecific antibody, such bispecific compounds can lead to enhanced drug efficacy and/or desired targeting manipulation.

IV. Polynucleotides, vectors and host cells for producing TrkB antibodies

[0068] The invention provides substantially purified polynucleotides (DNA or RNA) that are identical or complementary to sequences encoding polypeptides comprising segments or domains of the antibody chains or antigen-binding molecules described herein. In some embodiments, the polynucleotides of the invention encode the heavy chain and/or light chain domains sequences shown in Table 1. When expressed from appropriate expression vectors, polypeptides encoded by these polynucleotides are capable of exhibiting antigen binding capacity. Also provided in the invention are polynucleotides which encode at least one CDR region and usually all three CDR regions from the heavy or light chain of the antibodies described herein. Some other polynucleotides encode all or substantially all of the variable region sequence of the heavy chain and/or the light chain of the exemplified antibodies. For example, some of these polynucleotides encode the amino acid sequence of the heavy chain variable region shown in any one SEQ ID NO:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 or 38, and/or the amino acid sequence of the light chain variable region shown in any one SEQ ID NO:15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39. Because of the

degeneracy of the code, a variety of nucleic acid sequences will encode each of the immunoglobulin amino acid sequences.

[0069] The polynucleotides of the invention can encode only the variable region sequence of an exemplified antibody. They can also encode both a variable region and a constant region of the antibody. Some of polynucleotide sequences of the invention nucleic acids encode a mature heavy chain variable region sequence that is substantially identical (e.g., at least 80%, 90%, or 99%) to the mature heavy chain variable region sequence shown in any one SEQ ID NOs: 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 or 38. Some other polynucleotide sequences encode a mature light chain variable region sequence that is substantially identical to the mature light chain variable region sequence shown in any one SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39. Some of the polynucleotide sequences encode a polypeptide that comprises variable regions of both the heavy chain and the light chain of one of the exemplified antibody. Some other polynucleotides encode two polypeptide segments that respectively are substantially identical to the variable regions of the heavy chain and the light chain of one of the exemplified antibodies.

[0070] The polynucleotide sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence (e.g., sequences as described in the Examples below) encoding an exemplified functional antibody. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang et al., Meth. Enzymol. 68:90, 1979; the phosphodiester method of Brown et al., Meth. Enzymol. 68:109, 1979; the diethylphosphoramidite method of Beaucage et al., Tetra. Lett., 22:1859, 1981; and the solid support method of U.S. Patent No. 4,458,066. Introducing mutations to a polynucleotide sequence by PCR can be performed as described in, e.g., *PCR Technology: Principles and Applications for DNA Amplification*, H.A. Erlich (Ed.), Freeman Press, NY, NY, 1992; *PCR Protocols: A Guide to Methods and Applications*, Innis et al. (Ed.), Academic Press, San Diego, CA, 1990; Mattila et al., Nucleic Acids Res. 19:967, 1991; and Eckert et al., PCR Methods and Applications 1:17, 1991.

[0071] Also provided in the invention are expression vectors and host cells for producing the functional antibodies described herein. Specific examples of lentiviral based vectors for expressing the antibodies are described in the Examples below. Various other expression vectors can also be employed to express the polynucleotides encoding the functional

antibody chains or binding fragments. Both viral-based and nonviral expression vectors can be used to produce the antibodies in a mammalian host cell. Nonviral vectors and systems include plasmids, episomal vectors, typically with an expression cassette for expressing a protein or RNA, and human artificial chromosomes (see, e.g., Harrington et al., *Nat. Genet.* 15:345, 1997). For example, nonviral vectors useful for expression of the antibody polynucleotides and polypeptides in mammalian (e.g., human) cells include pCEP4, pREP4, pThioHis A, B & C, pcDNA3.1/His, pEBVHis A, B & C (Invitrogen, San Diego, CA), MPSV vectors, and numerous other vectors known in the art for expressing other proteins. Other useful nonviral vectors include Sleeping Beauty, PiggyBack and other transposon systems. Useful viral vectors include vectors based on lentiviruses or other retroviruses, adenoviruses, adenoassociated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent et al., *supra*; Smith, *Annu. Rev. Microbiol.* 49:807, 1995; and Rosenfeld et al., *Cell* 68:143, 1992.

[0072] The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences (e.g., enhancers) that are operably linked to the polynucleotides encoding a functional antibody chain or fragment. In some embodiments, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, e.g., arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under noninducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of a functional antibody chain or fragment. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, e.g., Scharf et al., *Results Probl. Cell Differ.* 20:125, 1994; and Bittner et al., *Meth. Enzymol.*, 153:516, 1987). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

[0073] The expression vectors may also provide a secretion signal sequence position to form a fusion protein with polypeptides encoded by inserted functional antibody sequences.

More often, the inserted functional antibody sequences are linked to a signal sequences before inclusion in the vector. Vectors to be used to receive sequences encoding the functional antibody light and heavy chain variable domains sometimes also encode constant regions or parts thereof. Such vectors allow expression of the variable regions as fusion proteins with the constant regions thereby leading to production of intact antibodies or fragments thereof. Typically, such constant regions are human.

[0074] The host cells for harboring and expressing the functional antibody chains can be either prokaryotic or eukaryotic. In some preferred embodiments, mammalian host cells are used to express and produce the antibody polypeptides of the present invention. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes or a mammalian cell line harboring an exogenous expression vector. These include any normal mortal or normal or abnormal immortal animal or human cell. In addition to the cell lines exemplified herein, a number of other suitable host cell lines capable of secreting intact immunoglobulins are also known in the art. These include, e.g., the CHO cell lines, various HEK 293 cell lines, various Cos cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, e.g., Winnacker, *From Genes to Clones*, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. These expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, EF1 α and human UbC promoters exemplified herein, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPSV promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

[0075] Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transformation is commonly utilized for prokaryotic cells, whereas calcium

phosphate treatment or electroporation may be used for other cellular hosts (see generally Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (3rd ed., 2001)). Other methods include, e.g., electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation:nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, *Cell* 88:223, 1997), agent-enhanced uptake of DNA, and *ex vivo* transduction. For long-term, high-yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express the antibody chains or binding fragments can be prepared using expression vectors of the invention which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate for the cell type.

V. Therapeutic applications

[0076] The TrkB antibodies disclosed herein can be used in various therapeutic applications. Some methods of the invention are directed to induce activation or enhance signaling activities of the TrkB signaling pathway in a TrkB expressing cell, e.g., retinal ganglion cell. These methods can be employed for *in vitro* or *in vivo* modification of the cell. In these methods, a therapeutically effective amount of a TrkB agonist of the invention is typically contacted with the cell. Some other methods of the invention are directed to promoting regeneration or survival of retinal ganglion cells in a mammalian subject. For example, these methods can be used for restoring, improving or preserving visual function of the eyes of a subject afflicted with or at risk of developing an ocular degenerative condition. Some other methods of the invention relate to treating or preventing a subject having or at risk of developing an ocular degenerative condition. In these methods, a pharmaceutical composition containing a therapeutically effective amount of a TrkB agonist of the invention is administered to the subject. In addition to pharmaceutical compositions containing a TrkB antibody of the invention, therapeutic applications of the invention can also utilize the

polynucleotides or vectors discussed herein that encode and express the antibodies. For example, compositions that harbor the antibody-encoding polynucleotides or vectors of the invention can be employed in gene therapy for various neurodegenerative diseases described herein.

[0077] Many ocular degenerative conditions are suitable for therapeutic or prophylactic treatment with the therapeutic methods of the invention. These include any ocular disease or disorder that is characterized by or associated with degeneration or death of retinal ganglion cells (RGCs). Specific examples of such diseases or conditions include, e.g., glaucoma, optic nerve injury, optic neuritis, optic neuropathies, central retinal artery occlusion, and central retinal vein occlusion. Other ocular disorders that may be amenable for treatment with the therapeutic antibody compositions of the invention include, e.g., diabetic neuropathy, macular degeneration including both the wet and dry forms of age-related macular degeneration (AMD), anterior ischemic ocular neuropathy (AION), ischemic retinopathy, diabetic retinopathy, retinopathy of prematurity, retinitis pigmentosa, and retinal degeneration.

[0078] In some embodiments, the invention provides methods for enhancing TrkB signaling activities in cells that express TrkB (e.g., retinal ganglion cells) by contacting the cells with an antibody or antigen-binding fragment of the invention. In some related embodiments, the TrkB agonists of the invention can be used for promoting the survival or regeneration of retinal ganglion cells in a subject. In these embodiments, functional integrity of retinal ganglion cells can be preserved or restored as a result of updated TrkB signaling activities that are elicited by the administered TrkB agonist agents. In various embodiments, the administered TrkB agonist antibody or antigen-binding fragment can be a naked (unconjugated) molecule or an antibody molecule conjugated to a synthetic molecule, e.g., a targeting moiety or another therapeutic agent. The methods can be used to upregulate TrkB signaling in the cells in vitro or in a subject (i.e., in vivo). The contacted TrkB-expressing cells can be present in, for example, a cell culture or animal model of a disorder associated with aberrant or impaired TrkB signaling activation or signaling activities or a disease characterized by degeneration or death of retinal ganglion cells.

[0079] In some other embodiments, the invention provides methods for treating a subject that is in need of enhanced TrkB signaling. This includes subjects who have, are suspected to have, or are at risk of developing a disease associated with impaired TrkB signaling

activities or a disease that can be treated via enhanced TrkB signaling, e.g., motor neuron diseases such as amyotrophic lateral sclerosis (ALS). In some embodiments, the invention provides methods for promoting survival, regeneration and synaptic function of motor neurons. These methods can be employed for treating subjects afflicted with various motor neuron diseases including ALS, idiopathic motor neuropathies, hereditary spastic paraplegia (HSP), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy (PBP) and pseudobulbar palsy, Bell's palsy, or spinal muscular atrophies (SMA). In some other embodiments, the invention provides methods for promoting survival, regeneration and synaptic function of central nervous system (CNS) neurons. These methods can be readily employed for treating subjects who are suffering from CNS degenerative diseases. Many CNS degenerative diseases or disorders are suitable for treatment with the therapeutic compositions of the invention. These include, e.g., Alzheimer's Disease, Parkinson's Disease, Huntington's Disease and Tourette's syndrome.

[0080] Some therapeutic methods of the invention are specifically directed to treating subjects having or are at risk of developing an ocular disorder characterized by degeneration or death of retinal ganglion cells. These include a number of ocular degenerative conditions, e.g., glaucoma, optic nerve injury, optic neuritis, optic neuropathies, central retinal artery occlusion, central retinal vein occlusion, diabetic neuropathy, macular degeneration including both the wet and dry forms of age-related macular degeneration (AMD), anterior ischemic ocular neuropathy (AION), and diabetic retinopathy.

[0081] In generally, the various therapeutic methods described herein involve administering to the subject a pharmaceutical composition that contains a therapeutically effective amount of an isolated or purified TrkB agonist antibody or antigen-binding fragment (or a polynucleotide or vector expressing the antibody) of the invention. The antibody can be any anti-TrkB agonist antibody of the invention as described herein in Table 1 or a derivative therefrom, e.g., an antibody derived from antibody NFAT-85 and/or having the same binding specificity as that of NFAT-85. Thus, the administered antibody can be a synthetic, a chimeric, a humanized, or a fully human antibody or antigen-binding fragment. It can be F(ab)₂, Fv, scFv, IgGACH2, F(ab')₂, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, or (scFv)₂. In some embodiments, the method includes administering an IgG, an scFv, a dsFv, a F(ab')₂, a diabody, or a bivalent antibody. In some embodiments, the administered antibody or antigen-binding fragment can be conjugated to another therapeutic

agent (e.g., a known drug or compound for treating ocular degenerative conditions) or other agents (e.g., a targeting agent).

[0082] The TrkB agonist antibodies of the invention can be used in combination with other known agents for promoting TrkB signaling and for treating ocular degenerative conditions. Such known TrkB agonist compounds include, e.g., neurotrophic factor BDNF and neurotrophic factor mimetics. Specific examples of known TrkB agonist compounds include, e.g., L-783,281 adenosine, CGS 21680, and etc. See, e.g., Pollack et al. *Curr. Drug Targ-CNS and Neurol. Disorders* 1:59-80 2002. The TrkB agonist compounds can also be small molecules that mimic critical regions of neurotrophins. For example, the small molecule can be a mimetic of a BDNF β -turn loop. Particular examples of such small molecule mimetics that may be used according to the invention are described in, e.g., U.S. Published Application No. 2007/0060526. Preferably, the TrkB agonist antibodies and the other known TrkB agonist compounds used in the methods of the invention are selective for TrkB and activate TrkB to a greater extent than TrkA or TrkC. In some embodiments, the employed agents are specific for TrkB and do not activate TrkA or TrkC.

[0083] In some therapeutic applications, the TrkB agonist antibodies of the invention can be used in combination with other known medications or regimens for treating optic neuropathies or retinal degenerative disorders such as glaucoma. For example, the antibodies can be used together with any of the existing IOP lowering drugs. These known drugs include, e.g., prostaglandin analogues such as Xalatan (latanoprost) and Lumigan (bimatoprost), Beta blockers such as timolol, betaxolol and metipranolol. In addition to therapeutic IOP lowering drugs, there are many approved drainage devices which can be used in combination with antibodies of the invention.

[0084] In some embodiments, the TrkB agonist antibody or antigen-binding molecules of the invention can be conjugated to a targeting moiety when employed in the therapeutic methods described herein. The targeting moieties can be a protein or a peptide which directs localization to a certain part of the body, e.g., to the brain or compartments therein. In some embodiments, the TrkB antibody agonists can be attached or fused to a brain targeting moiety. The brain targeting moieties can be attached covalently (e.g., direct, translational fusion, or by chemical linkage either directly or through a spacer molecule, which can be optionally cleavable) or non-covalently attached (e.g., through reversible interactions such as avidin, biotin, protein A, IgG, etc.). A brain targeting moiety conjugated to a TrkB antibody

agonist of the invention can enhance brain delivery of the TrkB agonist. A number of agents can be employed as the brain targeting moiety in the practice of the invention. These include polypeptides or antibody fragments which can deliver a fused protein or therapeutic agent through the blood brain barrier (BBB). Examples of such agents include single domain antibody FC5 (Abulrob et al. J. Neurochem. 95, 1201-1214, 2005); mAB 83-14, a monoclonal antibody to the human insulin receptor (Pardridge et al. Pharmacol. Res. 12, 807-816, 1995); the B2, B6 and B8 peptides which bind to the human transferrin receptor (hTfR) (Xia et al. J. Virol. 74, 11359-11366, 2000); the OX26 monoclonal antibody to the transferrin receptor (Pardridge et al. J. Pharmacol. Exp. Ther. 259, 66-70, 1991); and several polypeptides described in U.S. Pat. No. 6,306,365.

VI. Pharmaceutical compositions and combinations

[0085] The invention provides methods of using a TrkB agonist of the invention in the manufacture of a medicament for treating ocular degenerative disorders such as glaucoma. Subjects in need of treatment or alleviation of such a condition can be administered with a TrkB agonist of the invention alone. However, the administration of a pharmaceutical composition that contains the TrkB agonist and a pharmaceutically acceptable carrier is more preferred. Examples of TrkB agonist of the inventions that can be employed in the pharmaceutical compositions include any of the TrkB agonist antibodies or antigen-binding molecules described herein. Exemplary compositions include one or more of antibodies that have mature heavy chain and light chain variable domain sequences respectively shown in SEQ ID NOs:14 and 15, SEQ ID NOs:16 and 17, SEQ ID NOs:18 and 19, SEQ ID NOs:20 and 21, SEQ ID NOs:22 and 23, SEQ ID NOs:24 and 25, SEQ ID NOs:26 and 27, SEQ ID NOs:28 and 29, SEQ ID NOs:30 and 31, SEQ ID NOs:32 and 33, SEQ ID NOs:34 and 35, SEQ ID NOs:36 and 37, or SEQ ID NOs:38 and 39. In some embodiments, the antibody can contain the heavy chain and light chain sequences respectively shown in SEQ ID NOs:14 and 15, SEQ ID NOs:16 and 17, SEQ ID NOs:18 and 19, SEQ ID NOs:20 and 21, SEQ ID NOs:22 and 23, SEQ ID NOs:24 and 25, SEQ ID NOs:26 and 27, SEQ ID NOs:28 and 29, SEQ ID NOs:30 and 31, SEQ ID NOs:32 and 33, SEQ ID NOs:34 and 35, SEQ ID NOs:36 and 37, or SEQ ID NOs:38 and 39. Other antibodies suitable for the pharmaceutical compositions of the invention include those having a mature chain sequence as shown in

SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 or 38 and/or a mature light chain sequence as shown in SEQ ID NOs:15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39.

[0086] Additional exemplary therapeutic compositions of the invention can contain an antibody having one, two, three, four, five, or six CDRs selected from the group consisting of SEQ ID NOs:46-73 and sequences AAS, YDS and GKN. In some embodiments, however, the antibody includes three CDR sequences of the same exemplified light or heavy chains shown in Table 1. In some embodiments, the pharmaceutical composition includes an antibody having six CDR sequences of the same antibody exemplified in Table 1, e.g., (1) SEQ ID NOs:46-49, AAS, and SEQ ID NO:50 (antibody CRE-6); (2) SEQ ID NOs:51-56 (antibody CRE-30 or CRE-83); (3) SEQ ID Nos:51-53, 57, 58, and 56 (antibody CRE-31 or CRE-53); (4) SEQ ID Nos:51-53, 57, 55, and 56 (antibody CRE-39 or NFAT-79); (5) SEQ ID Nos:51-55 and 59 (antibody CRE-87); (6) SEQ ID Nos:51, 52, 60, 57, 61 and 56 (antibody CRE-93); (7) SEQ ID Nos:46, 47, 62, 63, YDS and SEQ ID NO:64 (antibody NFAT-27); (8) SEQ ID Nos:65-68, GKN, and SEQ ID NO:69 (antibody NFAT-40); (9) SEQ ID Nos:70-72 and 54-56 (antibody NFAT-44); or (10) SEQ ID Nos:65-68, GKN, and SEQ ID NO:73 (antibody NFAT-85). Still another exemplary pharmaceutical composition includes a dsFv fragment, which can include one or more modifications to the amino acid sequence as appropriate and understood by one of ordinary skill in the art.

[0087] The invention also provides for a pharmaceutical combination, e.g. a kit. Such pharmaceutical combination can contain an active agent which is a TrkB agonist disclosed herein, in free form or in a composition, one or more inactive agents or other components, as well as instructions for administration of the agents. In some embodiments, therapeutic kits of the invention can contain one or more doses of a TrkB agonist (e.g., antibody NFAT-85) present in a pharmaceutical composition described herein, a suitable device for intravitreal injection of the pharmaceutical composition, and an instruction detailing suitable subjects and protocols for carrying out the injection.

[0088] The pharmaceutical compositions that comprise a TrkB agonist antibody can be prepared in various forms. Suitable solid or liquid pharmaceutical preparation forms are, e.g., granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solution in ampule form and also preparations with protracted release of active compounds. The antibody compositions of the invention can also be prepared and administered to subjects in the form of surfactants.

The pharmaceutical compositions of the invention can be prepared in accordance with the standard protocols well known in the art, e.g., *Remington: The Science and Practice of Pharmacy*, Gennaro (ed.), Lippincott Williams & Wilkins (20th ed., 2003). The pharmaceutical compositions typically contain an effective amount of the TrkB agonist antibody that is sufficient to lessen or ameliorate symptoms of an ocular degenerative disorder. In addition to the TrkB agonist antibody, the pharmaceutical compositions can also contain certain pharmaceutically acceptable carriers which enhance or stabilize the composition, or facilitate preparation of the composition. For example, the TrkB agonist antibody can be complexed with carrier proteins such as ovalbumin or serum albumin prior to their administration in order to enhance stability or pharmacological properties. The various forms of pharmaceutical compositions can also contain excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners and elixirs containing inert diluents commonly used in the art, such as purified water.

[0089] Pharmaceutically acceptable carriers are determined in part by the particular composition being administered as well as by the particular method used to administer the composition. They should also be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the subject. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral, sublingual, rectal, nasal, intravenous, or parenteral. For example, examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers for occlusive dressings can be used to increase skin permeability and enhance absorption. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form.

[0090] For therapeutic or prophylactic applications, a pharmaceutical composition containing a TrkB agonist antibody can be administered locally or systemically in a therapeutically effective amount or dose. For example, they may be administered parenterally, enterically, by injection, rapid infusion, nasopharyngeal absorption, dermal absorption, and orally. However, in preferred embodiments, local administration of the composition is desired in order to achieve the intended therapeutic effect. In some of these embodiments, the compositions can be administered to the subject in need of treatment via

intravitreal injection. This can be performed in accordance with standard procedures known in the art. See, e.g., Russelakis-Carneiro et al., *Neuropathol. Appl. Neurobiol.* 25:196-206, 1999; and Wray et al., *Arch. Neurol.* 33:183-5, 1976. Other local administration routes that may be used for delivering a pharmaceutical composition of the invention to the eyes of a subject include, e.g., intraocular, intraorbital, subconjunctival, subretinal or transscleral routes. It is contemplated that local modes of administration may reduce or eliminate the incidence of potential side effects (e.g., systemic toxicity) that may occur during systemic administration. In some other embodiments, a pharmaceutical composition of the invention may also be administered to patients systemically, e.g., by oral or parenteral routes. Parenteral routes include, for example, intravenous, intrarterial, intramuscular, intradermal, subcutaneous, intranasal and intraperitoneal routes.

[0091] A therapeutically effective amount means an amount that is sufficient to reduce or inhibit the symptoms of the disorder or condition to be treated in a subject. In the practice of the present invention, the amount of the administered TrkB agonist antibody should be effective for slowing, stopping or reversing ocular degeneration, e.g., degeneration or death of RGCs. Such effective amount will vary from subject to subject depending on the ocular disorder afflicted by the subject, stage and severity of the disorder, the subject's general conditions (such as height, weight, age, and health), the particular compound administered, and other factors. For a given TrkB agonist antibody, one skilled in the art can easily identify the effective amount of the compound by using routinely practiced pharmaceutical methods. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders in human subjects. More often, a suitable therapeutic dose can be determined by clinical studies on mammalian species to determine maximum tolerable dose and on normal human subjects to determine safe dosage.

[0092] In general, except under certain circumstances when higher dosages may be required, the preferred dosage of a TrkB agonist antibody usually lies within the range of from about 0.001 to about 1000 mg, more usually from about 0.01 to about 500 mg per day. As a general rule, the quantity of a TrkB agonist antibody administered is the smallest dosage which effectively and reliably prevents or minimizes the conditions of the subjects. Also, the dosages to be administered and frequency of administration can vary depending on

whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively lower dosage may be administered at relatively infrequent intervals over a long period of time. Some subjects may continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively higher dosage at relatively short intervals may be required until progression of the disease is reduced or terminated, and preferably until the subject shows partial or complete amelioration of symptoms of the ocular vascular disease. Thereafter, the subject can be administered a prophylactic regime. As is readily apparent, the above dosage ranges are intended to provide general guidance and support for the teachings herein, but are not intended to limit the scope of the invention. Additional guidance for preparation and administration of the pharmaceutical compositions of the invention has also been described in the art. See, e.g., *Goodman & Gilman's The Pharmacological Bases of Therapeutics*, Hardman et al., eds., McGraw-Hill Professional (10th ed., 2001); *Remington: The Science and Practice of Pharmacy*, Gennaro, ed., Lippincott Williams & Wilkins (20th ed., 2003); and *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Ansel et al. (eds.), Lippincott Williams & Wilkins (7th ed., 1999).

EXAMPLES

[0093] The following examples are provided to further illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims.

Example 1 Development of TrkB agonist Abs

[0094] *Cell lines:* The specific TrkB agonist antibodies exemplified herein were selected and identified via a function-based screen of a combinatorial library of scFv antibodies. Detailed procedures for carrying out the screen are described in Zhang et al., *Proc. Natl. Acad. Sci. USA* 109:15728-33, 2012; and Zhang et al., *Chem. Biol.* 20:734-41, 2013. Briefly, a two-stage process was employed. First, a reporter cell line was generated by transfecting human TrkB into CHO cells using a lentiviral vector. Cells were also transfected with a reporter cassette using an NFAT promoter linked to a GFP reporter, such that, upon activation with the cognate ligand, BDNF, a fluorescent signal is generated. Second, a 10¹¹ scFv phage library was used to pan the ecto domain of human TrkB. Positives (hits) from the panning process were cloned into a lentiviral vector and clones were infected into the

reporter cell at a multiplicity of infection (MOI) of ~1 (Figure 1). In a second screen, instead of using NFAT activation as readout, the reporter cassette utilized the CRE promoter linked to the GFP reporter.

[0095] Hits from this functional selection were harvested and three rounds of infection/selection yielded multiple scFv clones upon deep sequencing. The scFv sequences were cloned into a pFUSE IgG backbone (human IgG₁) to generate scFv-Fc fusion antibodies. In a preliminary study of the activities of the selected TrkB antibodies, the ScFv-Fc constructs were transfected into HEK293 cells. Following 2 days of culturing, the supernatant of each cultured clone was used to examine the effect on activating a TrkB reporter cell line. Results from the study are shown in Figure 2. As shown in the figure, the identified TrkB scFv agonist antibodies (e.g., scFv CRE-30) showed varying degree of activities in activating the TrkB reporter cell line.

[0096] To further characterize activities of the identified antibodies, the selected scFv-Fc antibodies were purified using a Protein G affinity column and tested in the reporter cell assay to measure potency relative to BDNF (Figure 3). As indicated in the figure, several Abs were full agonists as compared to BDNF while others showed partial agonism. Of the Abs purified and tested, potencies varied from 0.3-30 nM. Ab NFAT-85 showed the highest potency (EC_{50} = 1 nM) and displayed full efficacy. All TrkB agonist Abs were tested for selectivity in a TrkA reporter cell. Figure 4 shows that neither BDNF nor any of the Abs activated TrkA while NGF gave the expected robust response, confirming selectivity.

[0097] Amino acid sequences of some of the selected TrkB agonist scFv antibodies are shown in Table 1. Identified antibody clones from the "CRE" reporter screen are denoted "CRE-" in the table, and antibodies identified from the "NFAT" reporter screen are designated "NFAT-". The heavy chain and light chain variable region sequences and other sequence elements (e.g., CDRs) of the scFv antibodies are designated by the sequence identifiers listed in Table 2.

Table 1. TrkB agonist scFV antibodies identified from functional screen

CRE-6 (SEQ ID NO:1)
 MAQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIYP
 GDSDIRYSPSFQGQVTISADKSISTAYLQWRSLKASDTAMYYCARQGASSTSYDYW

GQGPRSPSPQSGGGSGGGSGGGGSEIVLTQSPSSLSASVGDRVTTTCRASOSISSYLN
WYQQKPGKAPKLLIYAASSLQNGVPSRFSGSGSGTDFTLTISSLPEDFATYYCQQA
NSEPVAFGQGTKVEIKR

CRE-30 (SEQ ID NO:2)

MAQVQLVESGAALVQPGGSLRLSCAASGFIFSRYNMNVWRQAPGKRPEWISFINTD
GSVIHYADSVEGRFTVSRDNVNNSLYLQMNDLRDDDTAVYYCYRQMLFWGQGT
VTVSSGGGGGGSQAVLTQPSSLASPGASASLTCTLRSGINVGTYRIYWYQQKPGSP
PQYLLRYKSDSDKHQSGVPSRFSGSKAASANAGILLISGLQSEDEADYYCAIWHSS
AWYFGGGTQLTVL

CRE-31 (SEQ ID NO:3)

MAQVQLVQSGAALVQPGGSLRLSCAASGFIFSRYNMNVWRQAPGKRPEWISFINTD
GSVIHYADSVEGRFTVSRDNVNNSLYLQMNDLRDDDTAVYYCYRQMLFWGQGT
VTVSSGGGGGGSQAVLTQPSSLASPGASVSLTCTLRSGINVGAYRVYWYQQKPGSP
PQYLLRYKSDSDKQQSGVSSRFSGSRDASANAGILLISGLRSEDEADYYCAIWHSSA
WYFGGGTQLTVL

CRE-39 (SEQ ID NO:4)

MAQVQLQESGAALVQPGGSLRLSCAASGFIFSRYNMNVWRQAPGKRPEWISFINTD
GSVIHYADSVEGRFVSRDNVNNSLYLQMNDLRDDDTAVYYCYRQMLFWGQGT
VTVSSGGGGGGSQAVLTQPSSLASPGASVSLTCTLRSGINVGAYRIYWYQQKPGSP
PQYLLRYKSDSDKQQSGVPSRFSGSRDASANAGILLISGLRSEDEADYYCAIWHSSA
WYFGGGTQLTVL

CRE-53 (SEQ ID NO:5)

MAQVQLVESGAALVQPGGSLRLSCAASGFIFSRYNMNVWRQAPGKRPEWISFINTD
GSVIHYADSVEGRFTVSRDNVNNSLYLQMNDLRDDDTAVYYCYRQMLFWGQGT
VTVSSGGGGGGSQAVLTQPSSLASPGASVSLTCTLRSGINVGAYRVYWYQQKPGSP
PQYLLRYKSDSDKQQSGVPSRFSGSRDASANAGILLISGLRSEDEADYYCAIWHSS
AWYFGGGTKLTVL

CRE-83 (SEQ ID NO:6)

MAQVQLVESGAALVQPGGSLRLSCAASGFIFSRYNMNVWRQAPGKRPEWISFINTD
GSVIHYADSVEGRFTVSRDNVNNSLYLQMNDLRDDDTAVYYCYRQMLFWGQGT
VTVSSGGGGGGSQAVLTQPSSLASPGASASLTCTLRSGINVGTYRIYWYQQKPGSP
PQYLLRYKSDSDKQQSGVPSRFSGSRDASANAGILLISGLRSEDEADYYCAIWHSS
AWYFGGGTKLTVL

CRE-87 (SEQ ID NO:7)

MAQVQLVESGAALVQPGGSLRLSCAASGFIFSRYNMNVWRQAPGKRPEWISFINTD
GSVIHYADSVEGRFTVSRDNVNNSLYLQMNDLRDDDTAVYYCYRQMLFWGQGT
VTVSSGGGGGGSQAVLTQPSSLASPGASASLTCTLRSGINVGTYRIYWYQQKPGSP
PQYLLRYKSDSDKQQSGVPSRFSGSRDASANAGILLISGLRSEDEADYYCAIWHSS
ACVFGGGTKLTVL

CRE-93 (SEQ ID NO:8)

MAQVQLQESGAALVQPGGSLRLSCAASGFI~~FSRY~~NMNVWRQAPGKRPEWISFINTD
~~GS~~VIHYADSV~~EG~~RFSVSRD~~V~~NNSLYLQMNDLRDDDTAVYYCARQLLYWGQGTV
 VTVSSGGGGGSQAVLTQPSSLSASPGASVSLTCTLR~~SGIN~~VGAYRIY~~WY~~QKPGSP
 QFLRLRYKSD~~SDK~~QQGS~~GV~~PSRFS~~GS~~KDASANAGILLISGLRSEDEADYYCAIWHSSA
 WVF~~GGGT~~QLTVL

NFAT-27 (SEQ ID NO:9)

QVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGHIYPGDS
 DTRYSPSFQGGVTISVDNSVSTTYLQLNNLQASDTAMYYCATRVLPAGHFYTL~~DVW~~
 GQGT~~LV~~TVSSGGGGSGGGGSSSYVLTQPPSVSVAPGETAILTCVGN~~NIGD~~KFV
 HWYQKPGQAPVLMYYDS~~DR~~PSGIPERFS~~GS~~NSGNTATLTISRVEAGDEGEYYCQ
 VWDN~~SSNOGY~~VFGGGTQLTVL

NFAT-40 (SEQ ID NO:10)

MAQVQLQQSGPGLVKPSQTLSTCVISGDSVSDNSGAWNWI~~RQ~~SPSRGLEWLGR~~TY~~
 YRSKWYTDYADSVKSRIT~~IP~~DIPKNQFSLHLNSVTPEDTAVYYCVRGYY~~YAF~~HIWG
 QGTMVTVSSGSGGGGSSSEL~~TQ~~DPAVSVALGQTVRITCQGD~~SLRT~~YYASWYQKPG
 QAPLLVIY~~GK~~NNRPSGIPDRFS~~GS~~SSGNTASLTITGAQAEDEADYYCNS~~RD~~GS~~GN~~NV
 VF~~GGGT~~KLTVL

NFAT-44 (SEQ ID NO:11)

MAQVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNVWRQAPGQGLEWMGW~~I~~
 NTNTGNPTYAQGFTGRFVFS~~LD~~TSVSTAYLQISSLKAEDTAVYYCASRLAAAGFDY
 WGQGT~~LV~~TVSSGGGGSGGGGSGGGGSQAVLTQPSSLSASPGASASLTCTLR~~SGIN~~VG
 TYRIY~~WY~~QKPGSP~~PQY~~LLRYKSD~~SDK~~QQGS~~GV~~PSRFS~~GS~~KDASANAGILLISGLRS
 EDEADYYCAIWHSSA~~WVF~~GGGTQLTVL

NFAT-79 (SEQ ID NO:12)

MAQVQLVESGAALVQPGGSLRLSCAASGFI~~FSRY~~NMNVWRQAPGKRPEWISFINTD
~~GS~~VIHYADSV~~EG~~RFTVSRD~~V~~NNSLYLQMNDLRDDDTAVYYCV~~ROML~~FWGQGT~~L~~
 VTVSSGSGGGGSQAVLTQPSSLSASPGASVSLTCTLR~~SGIN~~VGAYRIY~~WY~~QKPGSP
 PQYLLRYKSD~~SDK~~QQGS~~GV~~PSRFS~~GS~~KDASANAGILLISGLRSEDEADYYCAIWHSS
 A~~WVF~~GGGTKLTVL

NFAT-85 (SEQ ID NO:13)

MAQVQLQQSGPGLVKPSQTLSTCVISGDSVSDNSGAWNWI~~RQ~~SPSRGLEWLGR~~TY~~
 YRSKWYTDYADSVKSRIT~~IP~~DIPKNQFSLHLNSVTPEDTAVYYCVRGYY~~YAF~~HIWG
 QGTMVTVSSGSGGGGSSSEL~~TQ~~DPAVSVALGQTVRITCQGD~~SLRT~~YYASWYQKPG
 QAPVLVAAG~~GK~~NNRPSGIPDRFS~~AS~~SSGNTASLTITGAQAEDEADYYC~~SSRD~~SS~~SR~~SHH
 LLF~~GGGT~~KVTVL

Each scFV sequence includes the heavy chain variable region sequence connected to the light chain variable region sequence via a GS rich linker (italicized). The heavy chain CDR sequences are underlined, and the light chain CDR sequences double underlined.

Table 2. Sequence components of TrkB agonist scFV antibodies

scFv (SEQ ID NO:)	H chain variable region (SEQ ID NO:)	L chain variable region (SEQ ID NO:)	Linker (SEQ ID NO:)	HCDRs 1-3 (SEQ ID NOs:)	LCDRs 1-3 (SEQ ID NOs:)
CRE-6 (1)	14	15	40	46, 47, 48	49, AAS, 50
CRE-30 (2)	16	17	41	51, 52, 53	54, 55, 56
CRE-31 (3)	18	19	42	51, 52, 53	57, 58, 56
CRE-39 (4)	20	21	43	51, 52, 53	57, 55, 56
CRE-53 (5)	22	23	44	51, 52, 53	57, 58, 56
CRE-83 (6)	24	25	41	51, 52, 53	54, 55, 56
CRE-87 (7)	26	27	41	51, 52, 53	54, 55, 59
CRE-93 (8)	28	29	43	51, 52, 60	57, 61, 56
NFAT-27 (9)	30	31	45	46, 47, 62	63, YDS, 64
NFAT-40 (10)	32	33	44	65, 66, 67	68, GKN, 69
NFAT-44 (11)	34	35	45	70, 71, 72	54, 55, 56
NFAT-79 (12)	36	37	44	51, 52, 53	57, 55, 56
NFAT-85 (13)	38	39	44	65, 66, 67	68, GKN, 73

Example 2. Function of TrkB agonist antibodies in signal transduction

[0098] Additional characterization of TrkB signaling by agonist Abs was carried out in the CHO reporter cell and compared to that of BDNF. Agonist Abs stimulated phosphorylation of P-PLC γ , P-Akt and P-MAPK signaling at 5 and 30 min, albeit to varying degrees (Figure B5); the levels and time course varied compared to BDNF but signal strength tended to correlate with effects on fluorescent signal in the reporter cell (see Figure 5).

[0099] Canonical signaling of BDNF/TrkB involves phosphorylation cascades through Akt, MAPK and PLC γ pathways. BDNF functions, such as survival, differentiation, neurite outgrowth, and synaptic plasticity have been ascribed to various aspects of these pathways. The identified TrkB agonist Abs were also tested for trophic activity and signaling in

primary neurons. It was found that Abs NFAT-85, NFAT-44 and NFAT-27 maintained survival of mouse sensory neurons over 3 days in culture, similar to BDNF (not shown). Incubation of mouse cortical neurons with Ab NFAT-85 resulted in time-dependent tyrosine phosphorylation of TrkB. However, the pattern of TrkB phospho-proteins was distinct from that of BDNF, possibly revealing cleaved TrkB products (Figure 6). Potential differential signal transduction with TrkB agonist Abs is currently under investigation.

[00100] To examine downstream targets of BDNF activation, the synaptic protein Arc was measured in mouse primary cortical neurons after Ab or BDNF treatment. Arc was originally identified as a cytoskeleton-associated protein. Recent studies link transient increases in Arc expression to stable expansion of the F-actin network in dendritic spines, which is believed to underlie morphological enlargement of the synapse and stable LTP. BDNF, long implicated in synaptic plasticity and memory storage, activates Arc-dependent consolidation and is necessary for actin-dependent spine enlargement. Current evidence further suggests a possible reciprocal interaction between F-actin formation, Arc, and translation during LTP consolidation. The results from this study are shown in Figure 7. As with BDNF, antibody NFAT-85 stimulated Arc after 2-4 hr of incubation.

[00101] Since anti-TrkB Agonist Abs were discovered by panning and selection with the human TrkB receptor, we determined the relative potency of Abs to rodent cells for future *in vitro* and *in vivo* studies. To examine this, we utilized a mouse embryonic stem cell line cell shown previously to respond to BDNF and compared TrkB phosphorylation stimulated by Ab NFAT-85 and BDNF. It was found that Ab NFAT-85 increased phosphorylation in a concentration-dependent fashion. However, the potency was at least two orders of magnitude less than its effect on the human reporter cell (~1 nM). In both mouse and human cells BDNF gave robust signaling at ~2 nM (Figure 8).

[00102] Collectively, the signal transduction and downstream marker studies are consistent with TrkB engagement by the agonist Abs in a selective manner. Concentration-response in mouse cells defines a relative potency of Ab NFAT-85 for further rodent studies.

Example 3. Effect of TrkB agonist Abs in retinal culture systems

[00103] To initiate studies on the effects of TrkB agonists on retinal physiology *in vitro*, a mouse retinal explant culture system was established. Briefly, retinae from adult mice were dissected and placed in culture medium (RGC layer facing up) for periods of 0-14 days. By

definition the RGC are axotomized; during this period there is progressive loss of various retinal layers and of RGC dendritic fields. RGC dendritic retraction is evident as early 6 hr in culture, as assessed by Sholl analysis, which measures dendritic field parameters as a function of distance from the cell soma. By 3 days in culture there is significant loss of dendrite intersections and dendrite branches.

[00104] The 3-day culture period provides a good dynamic range to test for neuroprotectant or neuroregenerative effects of BDNF and of TrkB agonist Abs on dendritic arbors of RGCs. It was observed that incubation of explants with 100 ng/ml BDNF significantly blocks dendrite retraction, such that the area under the Sholl curve and dendritic branchings after 3 days in culture resemble those of time-zero explants, suggesting complete protection of the dendritic retraction response to axotomy. To determine if BDNF was capable of reversing the axotomy-induced dendritic retraction, explants were maintained in culture for 3 days, at which time BDNF was added, and Sholl analysis performed. Strikingly, delayed application of BDNF reversed the retraction response to parameters, again resembling those of time-zero explants, suggesting that BDNF induces a neuroregenerative sprouting response in the damaged neurons.

[00105] This paradigm establishes the groundwork to test whether TrkB agonist Abs can prevent dendritic retraction in retinal explants similar to that observed for BDNF. Figure 9 shows that Ab NFAT-85 maintained dendritic parameters of axotomized RGC dendrites identical in magnitude to those of BDNF, corroborating biochemical and signal transduction parameters observed for Ab NFAT-85 in other cell types. Future experiments will determine the effects of delayed Ab NFAT-85 administration, as well as the durability of the effects of agonist Abs in this culture system.

[00106] In corroboration of the above, unpublished results, two recent reports have shown trophic effects of BDNF on RGC. First, Johnson et al. (Invest. Ophthalmol. Vis. Sci. 57:253–264, 2016) showed that administration of BDNF + CNTF (ciliary neurotrophic factor) to mouse retinal explants was capable of improving the number of branch segments, junctions and terminal branches of RGCs at 7 days. Also, Domenici et al. (PloS One 9(12):e115579, 2014) showed that intravitreal or topical application of BDNF improved visual function as assessed electrophysiologically in the DBA/2J mouse model of spontaneous glaucoma (not shown). Interestingly these effects occurred independently of elevated IOP.

Example 4. Efficacy of TrkB agonist Abs in a rat model of glaucoma.

[00107] The ocular hypertensive (OHT) model of glaucoma was performed in Brown Norway rats by injection of sterile 5 μ m magnetic microbeads into the anterior eye chamber. The experimental procedures were essentially carried out as described in Samsel et al., Invest. Ophthalmol. 52:1671, 2011; and Morgan and Tribble, Exp. Eye Res. 141:9-14, 2015. A magnet was used to draw the beads to the iridocorneal canal, disrupting the trabecular meshwork, preventing outflow of vitreous and subsequently elevating intra-ocular pressure (IOP). Elevations in IOP were measured periodically with a rebound tonometer. Bead injections were made in the left eye with the right eye serving as a control.

[00108] At 5 weeks post bead injection, the rats were administered via intravitreal injection into the left eye a phosphate buffered saline (PBS), 500ng of TrkB agonist antibody NFAT-85 (Ab85), or 500 ng of BDNF in a volume of 1 μ l. In some animals the effect of Ab85 or BDNF were determined in normotensive (NT) eyes (not receiving magnetic microbead injections). Two weeks later (7 weeks post microbead injection) rats were sacrificed, retinæ were removed and prepared for diolistic labeling of retinal ganglion cells and their dendritic arbors. Arbor morphology (dendritic branching index, dendritic length and dendrite intersections) was quantified by Sholl analysis. An average of 6-10 cells were analyzed per retina. The experiment paradigm is outlined in Figure 10.

[00109] Figure 11 shows the Mean IOP and Peak IOP levels (in mm Hg) for the OHT and NT groups of rats treated with PBS, Ab85 or BDNF. In this method IOP was elevated early after microbead injection, remained elevated for ~3 weeks and returned to normal levels by 4 weeks.

[00110] Figure 12 shows quantification of average dendritic arbor intersections as assessed by Sholl analysis. The top graph compares OHT rats treated with various agents compared to a normotensive eye (NT) control. Both BDNF and to a greater degree Ab85 show increased intersections compared to PBS-treated eyes, and are not different from NT eyes. The bottom plot shows that treatment of NT eyes with BDNF or Ab85 did not significantly alter average intersections (OHT plotted as a point of comparison).

[00111] Figure 13 represents statistical comparisons of the area under the curve (AUC), branching index and dendritic length for the Sholl analyses shown in figure 12. For all measures, OHT + PBS rats were significantly reduced compared to NT eyes. In addition,

OHT + either Ab85 or BDNF ameliorated these measures to values equal to NT eyes.

Neither Ab85 nor BDNF treatment of NT eyes were different from NT controls.

[00112] The results from these studies demonstrated a positive, regenerative effect of TrkB agonist Abs on dendritic arbors of rat retinal ganglion neurons in a model of hypertensive glaucoma. Importantly, these regenerative effects occur after the induction of injury caused by the elevated intraocular pressure, similar to post-lesion treatment paradigm as is practiced clinically for glaucoma.

Example 5. Bispecific antibody targeting TrkB and TNF

[00113] We then attempted to generate a bispecific antibody from the TrkB agonist antibody Ab85 described herein and the well-known anti-TNF antibody Humira (Adalimumab), and a proof of concept bispecific antibody configuration was created. This bispecific molecule configuration contains one arm of Ab85 (TrkB antibody) and the other arm of Adalimumab. Briefly, Ab85 scFv fragment was fused to a human IgG1 Fc fragment with Y407T mutation, the resulting fusion protein is shown in SEQ ID NO:74 (Ab85-Fc(407)). Meanwhile, Adalimumab Fab heavy chain was fused to a human IgG1 Fc fragment with T366Y mutation. The resulting fusion protein is shown in SEQ ID NO:75 (Adalimumab-heavy-Fc (366)).

[00114] For expression and assembly of the bispecific antibody, these two fusion sequences (Ab85-Fc(407), Adalimumab-heavy-Fc (366)) along with an Adalimumab light coding encoding vector are to be transfected together into Expi 293 cells. The assembled antibodies can then purified via Protein G affinity chromatography.

[00115] SEQ ID NO:74 (Ab85-Fc(407))

MAQVQLQQSGPGLVKPSQTLSTCVISGDSVSDNSGAWNWRQSPSRGLEWLGRTY
YRSKWYTDYADSVKSRITIPDIPKNQFSLHLNSVTPEDTAVYYCVRGYYYAFHIWG
QGTMVTVSSSGSGGGSSSELTQDPAVSVALGQTVRITCQGDRLRTYYASWYQQKP
GQAPVLVAAGKNNRPSGIPDRFSASSSGNTASLTITGAQAEDEADYYCSSRDSSRSH
HLLFGGGTKVTVLGGGLGLASEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLTSLKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00116] SEQ ID NO:75 (Adalimumab-heavy-Fc (366))

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNS
GHIDYADSVGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAKVSYSYSTASSLDYW

GQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHITCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLYCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPG
K

[00117] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[00118] All publications, databases, GenBank sequences, patents, and patent applications cited in this specification are herein incorporated by reference as if each was specifically and individually indicated to be incorporated by reference.

WE CLAIM:

1. An antibody or an antigen-binding fragment that specifically binds to tropomyosin receptor kinase B (TrkB) with the same binding specificity as that of a second antibody, wherein the second antibody comprises heavy chain CDRs 1-3 and light chain CDRs 1-3 sequences that are respectively identical to (1) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and SSRDSSRSHLL (SEQ ID NO:73); (2) GYSFTSYW (SEQ ID NO:46), IYPGDSDT (SEQ ID NO:47), ARQGASSTSYDY (SEQ ID NO:48), QSISSY (SEQ ID NO:49), AAS, and QQANSFPVA (SEQ ID NO:50); (3) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56); (4) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKTDSDK (SEQ ID NO:58), and AIWHSSAWV (SEQ ID NO:56); (5) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:55), AIWHSSAWV (SEQ ID NO:56); (6) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSACV (SEQ ID NO:59); (7) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), ARQLLY (SEQ ID NO:60), SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:61), AIWHSSAWV (SEQ ID NO:56); (8) GYSFTSYW (SEQ ID NO:46), IYPGDSDT (SEQ ID NO:47), ATRVLPAGHF (SEQ ID NO:62), NIGDKF (SEQ ID NO:63), YDS, and QVWDNSSNQGV (SEQ ID NO:64); (9) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and NSRDGSGNNVV (SEQ ID NO:69); or (10) GYTFTSYA (SEQ ID NO:70), INTNTGNP (SEQ ID NO:71), ASRLAAAGFDY (SEQ ID NO:72), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56).
2. The antibody or antigen-binding fragment of claim 1, comprising a heavy chain variable region sequence and a light chain variable region sequence, one or both of which are identical to a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (1) SEQ ID NO:38 and SEQ ID NO:39; (2) SEQ ID

NO:14 and SEQ ID NO:15; (3) SEQ ID NO:16 and SEQ ID NO:17; (4) SEQ ID NO:18 and SEQ ID NO:19; (5) SEQ ID NO:20 and SEQ ID NO:21; (6) SEQ ID NO:22 and SEQ ID NO:23; (7) SEQ ID NO:24 and SEQ ID NO:25; (8) SEQ ID NO:26 and SEQ ID NO:27; (9) SEQ ID NO:28 and SEQ ID NO:29; (10) SEQ ID NO:30 and SEQ ID NO:31; (11) SEQ ID NO:32 and SEQ ID NO:33; (12) SEQ ID NO:34 and SEQ ID NO:35; or (13) SEQ ID NO:36 and SEQ ID NO:37.

3. The antibody or antigen-binding fragment of claim 1, comprising a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (1) SEQ ID NO:38 and SEQ ID NO:39; (2) SEQ ID NO:14 and SEQ ID NO:15; (3) SEQ ID NO:16 and SEQ ID NO:17; (4) SEQ ID NO:18 and SEQ ID NO:19; (5) SEQ ID NO:20 and SEQ ID NO:21; (6) SEQ ID NO:22 and SEQ ID NO:23; (7) SEQ ID NO:24 and SEQ ID NO:25; (8) SEQ ID NO:26 and SEQ ID NO:27; (9) SEQ ID NO:28 and SEQ ID NO:29; (10) SEQ ID NO:30 and SEQ ID NO:31; (11) SEQ ID NO:32 and SEQ ID NO:33; (12) SEQ ID NO:34 and SEQ ID NO:35; or (13) SEQ ID NO:36 and SEQ ID NO:37.

4. The antibody or antigen-binding fragment of claim 1, comprising heavy chain CDRs 1-3 and light chain CDRs 1-3 sequences that are substantially identical, respectively, to (1) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and SSRDSSRSHLL (SEQ ID NO:73); (2) GYSFTSYW (SEQ ID NO:46), IYPGSDT (SEQ ID NO:47), ARQGASSTSYDY (SEQ ID NO:48), QSISSY (SEQ ID NO:49), AAS, and QQANSFPVA (SEQ ID NO:50); (3) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56); (4) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKTDSDK (SEQ ID NO:58), and AIWHSSAWV (SEQ ID NO:56); (5) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:55), AIWHSSAWV (SEQ ID NO:56); (6) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), VRQMLF (SEQ ID NO:53), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSACV (SEQ ID NO:59); (7) GFIFSRYN (SEQ ID NO:51), INTDGSVI (SEQ ID NO:52), ARQLLY (SEQ ID NO:60),

SGINVGAYR (SEQ ID NO:57), YKSDSDK (SEQ ID NO:61), AIWHSSAWV (SEQ ID NO:56); (8) GYSFTSYW (SEQ ID NO:46), IYPGSDST (SEQ ID NO:47), ATRVLPAGHF (SEQ ID NO:62), NIGDKF (SEQ ID NO:63), YDS, and QVWDNSSNQGV (SEQ ID NO:64); (9) VSDNSGAWN (SEQ ID NO:65), YRSKWYT (SEQ ID NO:66), VRGYYYAFHI (SEQ ID NO:67), SLRTYY (SEQ ID NO:68), GKN, and NSRDGSGNNVV (SEQ ID NO:69); or (10) GYTFTSYA (SEQ ID NO:70), INTNTGNP (SEQ ID NO:71), ASRLAAAGFDY (SEQ ID NO:72), SGINVGTYR (SEQ ID NO:54), YKSDSDK (SEQ ID NO:55), and AIWHSSAWV (SEQ ID NO:56).

5. The antibody or antigen-binding fragment of claim 1, comprising a heavy chain CDR sequence selected from the group consisting of SEQ ID NOs:46-48, 51-53, 60, 62, 65-67, and 70-72.

6. The antibody or antigen-binding fragment of claim 5, further comprising a light chain CDR sequence selected from the group consisting of SEQ ID NOs:49, 50, 54-59, 61, 63, 64, 68, 69 and 73.

7. The antibody or antigen-binding fragment of claim 5, comprising heavy chain CDRs 1-3 sequences that are respectively identical to (1) SEQ ID Nos:70-72, (2) SEQ ID NOs:46-48, (3) SEQ ID NOs:51-53, (4) SEQ ID Nos:51, 52, 60, (5) SEQ ID Nos:46, 47, and 62, or (6) SEQ ID Nos:65-67.

8. The antibody or antigen-binding fragment of claim 7, comprising heavy chain CDRs 1-3 and light chain CDRs 1-3 sequences respectively shown in (1) SEQ ID Nos:65-68, GKN, and SEQ ID NO:73; (2) SEQ ID NOs:46-49, AAS, and SEQ ID NO:50; (3) SEQ ID NOs:51-56; (4) SEQ ID Nos:51-53, 57, 58, and 56; (5) SEQ ID Nos:51-53, 57, 55, and 56; (6) SEQ ID Nos:51-55 and 59; (7) SEQ ID Nos:51, 52, 60, 57, 61 and 56; (8) SEQ ID Nos:46, 47, 62, 63, YDS and SEQ ID NO:64; (9) SEQ ID Nos:65-68, GKN, and SEQ ID NO:69; or (10) SEQ ID Nos:70-72 and 54-56.

9. The antibody or antigen-binding fragment of claim 1, comprising a light chain CDR sequence selected from the group consisting of SEQ ID NOs:49, 50, 54-59, 61, 63, 64, 68, 69 and 73, and sequences AAS, YDS and GKN.

10. The antibody or antigen-binding fragment of claim 9, further comprising a heavy chain CDR sequence selected from the group consisting of SEQ ID NOs:46-48, 51-53, 60, 62, 65-67, and 70-72.
11. The antibody or antigen-binding fragment of claim 9, comprising light chain CDRs 1-3 sequences that are respectively identical to (1) SEQ ID No:68, GKN and SEQ ID NO:73; (2) SEQ ID NO:49, AAS, and SEQ ID NO:50; (3) SEQ ID NOs:54-56; (4) SEQ ID Nos:57, 58 and 56; (5) SEQ ID Nos:57, 55 and 56; (6) SEQ ID Nos:54, 55 and 59; (7) SEQ ID Nos:57, 61 and 56; (8) SEQ ID No:63, YDS and SEQ ID NO:64; or (8) SEQ ID No:68, GKN and SEQ ID NO:69.
12. The antibody or antigen-binding fragment of claim 1, comprising a heavy chain variable region that is substantially identical to SEQ ID NO:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36 or 38.
13. The antibody or antigen-binding fragment of claim 1, which is a scFv fragment, wherein the heavy chain and light chain variable region sequences are connected via a linker sequence.
14. The antibody or antigen-binding fragment of claim 13, wherein the linker sequence comprises a sequence shown in any one of SEQ ID Nos:40-45.
15. The antibody or antigen-binding fragment of claim 13, comprising a sequence shown in any one of SEQ ID Nos:1-13.
16. The antibody or antigen-binding fragment of claim 1, which is IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, IgM, F(ab)2, Fv, scFv, IgGACH2, F(ab')2, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)2, a non-depleting IgG, a diabody, and a bivalent antibody.
17. The antibody or antigen-binding fragment of claim 16, which is an IgG selected from the group consisting of IgG1, IgG2, IgG3, IgG4, and synthetic IgG.
18. The antibody or antigen-binding fragment of claim 16, which is a Fab, a scFv, or a dsFv.

19. The antibody or antigen-binding fragment of claim 16, which is conjugated to a synthetic molecule.
20. A pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding fragment of claim 1 and a pharmaceutically acceptable carrier.
21. A bispecific compound comprising the antibody or antigen-binding fragment of claim 1.
22. A kit comprising an antibody or antigen-binding fragment of claim 1.
23. A polynucleotide encoding the variable region of the heavy chain and/or light chain of an antibody or antigen-binding fragment of claim 1.
24. A vector harboring the polynucleotide of claim 23.
25. A method for promoting survival, synaptic function or regeneration of retinal ganglion cells, motor neurons or central nervous system (CNS) neurons in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a TrkB agonist antibody or antigen-binding fragment of claim 1 or the vector of claim 24, thereby promoting survival or regeneration of retinal ganglion cells in the subject.
26. The method of claim 25, wherein the pharmaceutical composition is administered to one or both eyes of the subject.
27. The method of claim 25, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.
28. The method of claim 25, wherein the subject suffers from or is at risk of developing an ocular degenerative disorder, a motor neuron disease or a central nervous system (CNS) degenerative disease.

29. The method of claim 28, wherein the ocular degenerative disorder is glaucoma, optic nerve injury, optic neuritis, optic neuropathy, central retinal artery occlusion, central retinal vein occlusion, diabetic neuropathy, age-related macular degeneration (AMD), anterior ischemic ocular neuropathy (AION), or diabetic retinopathy.

30. The method of claim 28, wherein the motor neuron disease is ALS, idiopathic motor neuropathy, hereditary spastic paraplegia (HSP), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy (PBP) and pseudobulbar palsy, Bell's palsy, or spinal muscular atrophies (SMA).

31. The method of claim 28, wherein the central nervous system (CNS) degenerative disease is Alzheimer's Disease, Parkinson's Disease, or Huntington's Disease.

32. A method for treating or preventing an ocular degenerative condition in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding fragment of claim 1 or the vector of claim 24, thereby treating or preventing the ocular neuropathy in the subject.

33. The method of claim 32, wherein the pharmaceutical composition is administered to one or both eyes of the subject.

34. The method of claim 32, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

35. The method of claim 32, wherein the subject is afflicted with or at risk of developing an ocular degenerative condition

36. The method of claim 35, wherein the ocular degenerative condition is glaucoma, optic nerve injury, optic neuritis, optic neuropathy, central retinal artery occlusion, central retinal vein occlusion, diabetic neuropathy, age-related macular degeneration (AMD), anterior ischemic ocular neuropathy (AION), or diabetic retinopathy.

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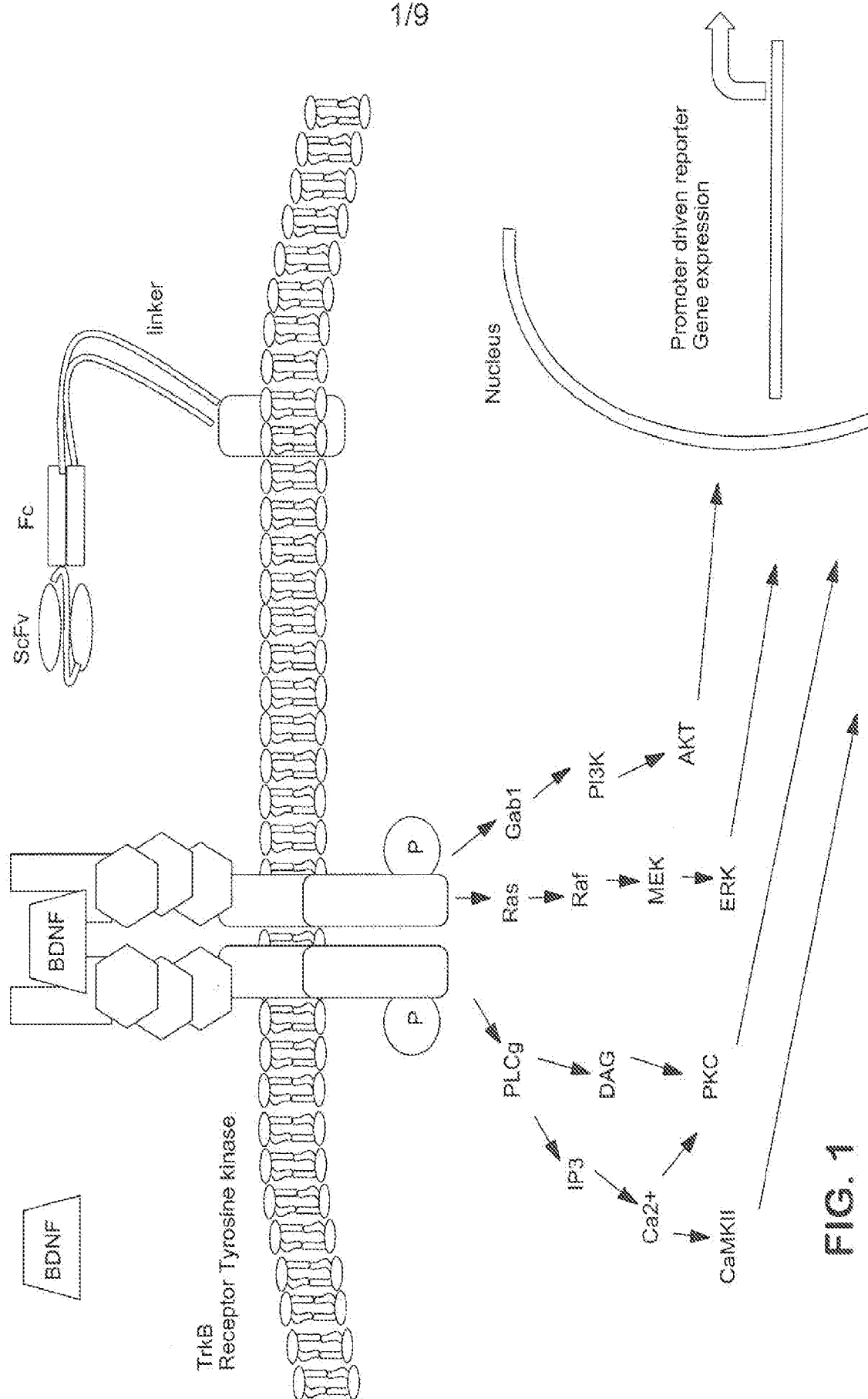


FIG. 1

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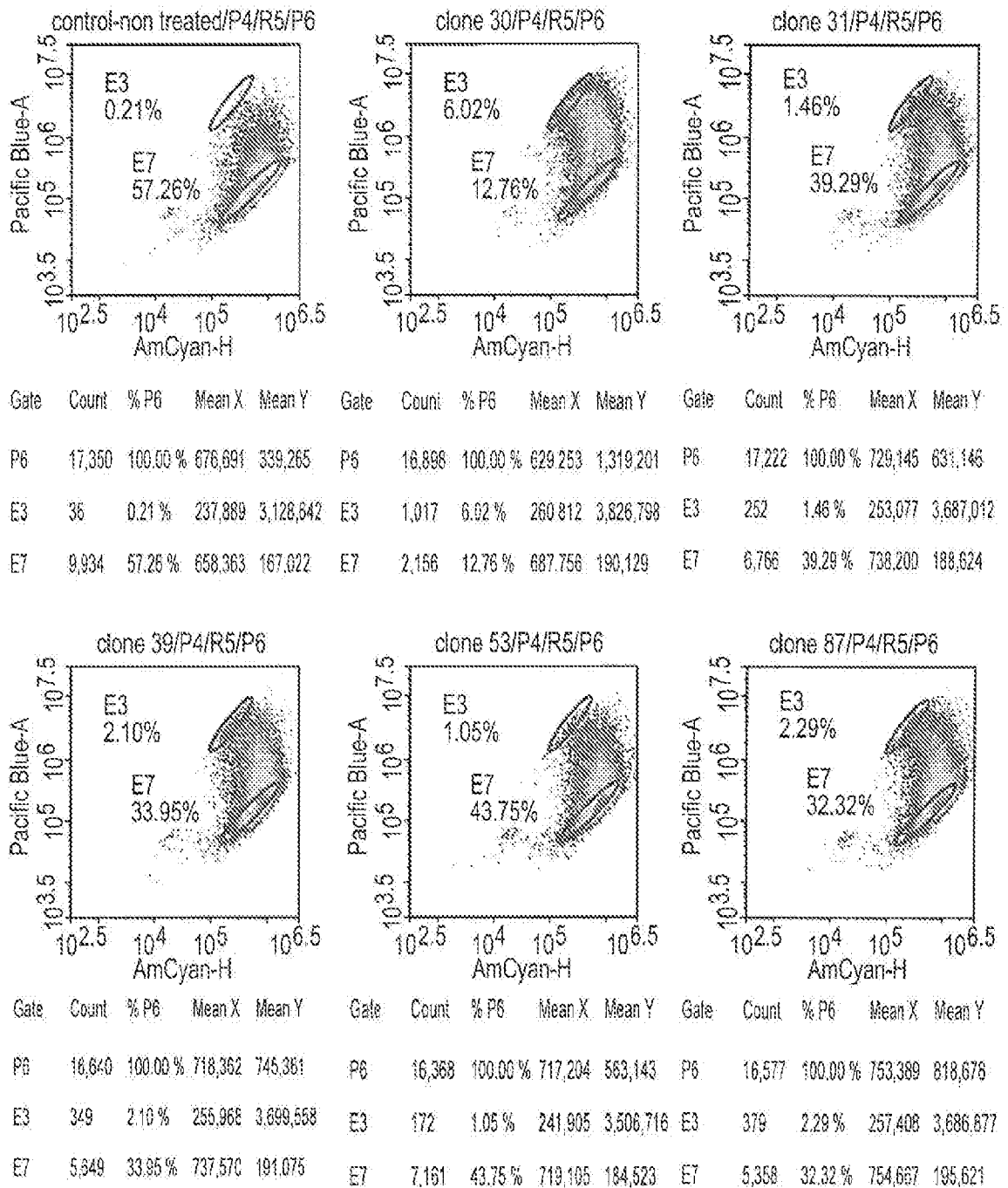


FIG. 2

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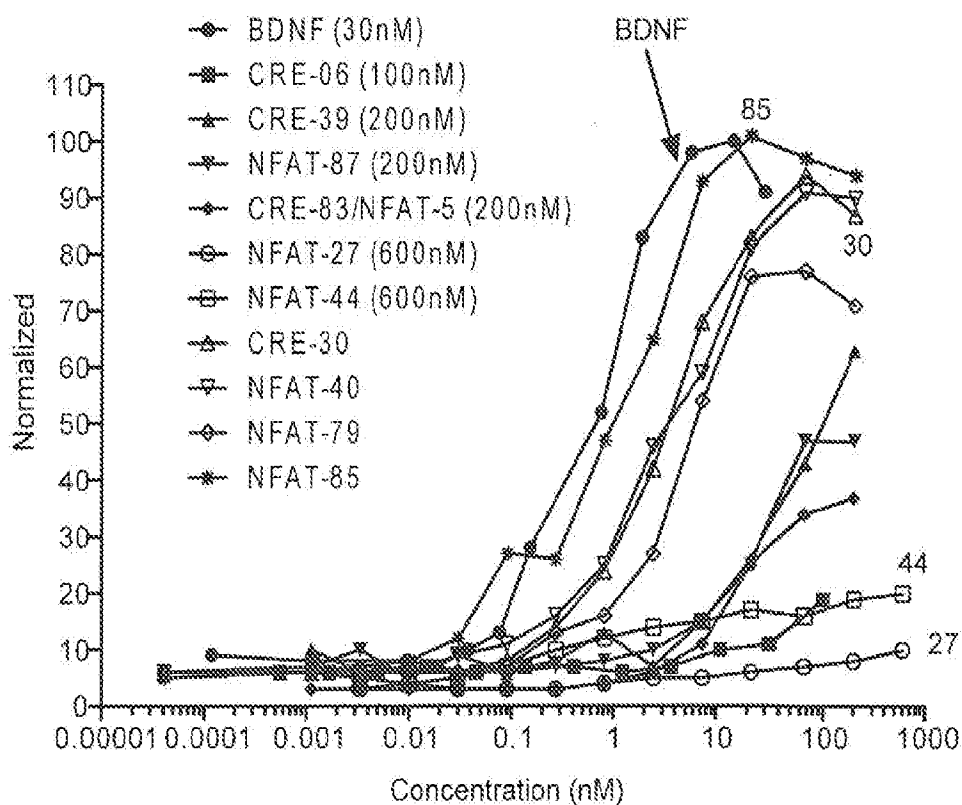


FIG. 3

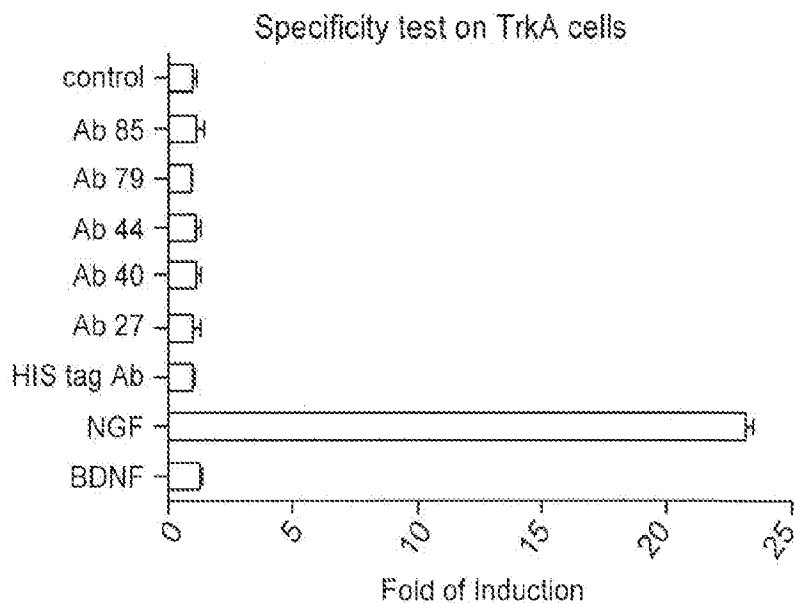


FIG. 4

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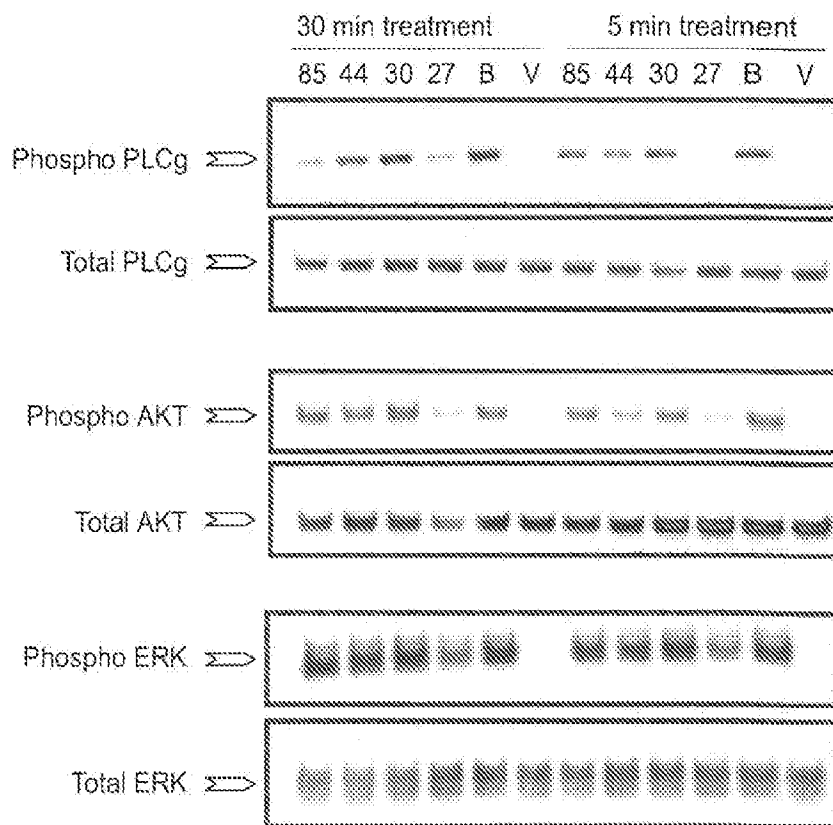


FIG. 5

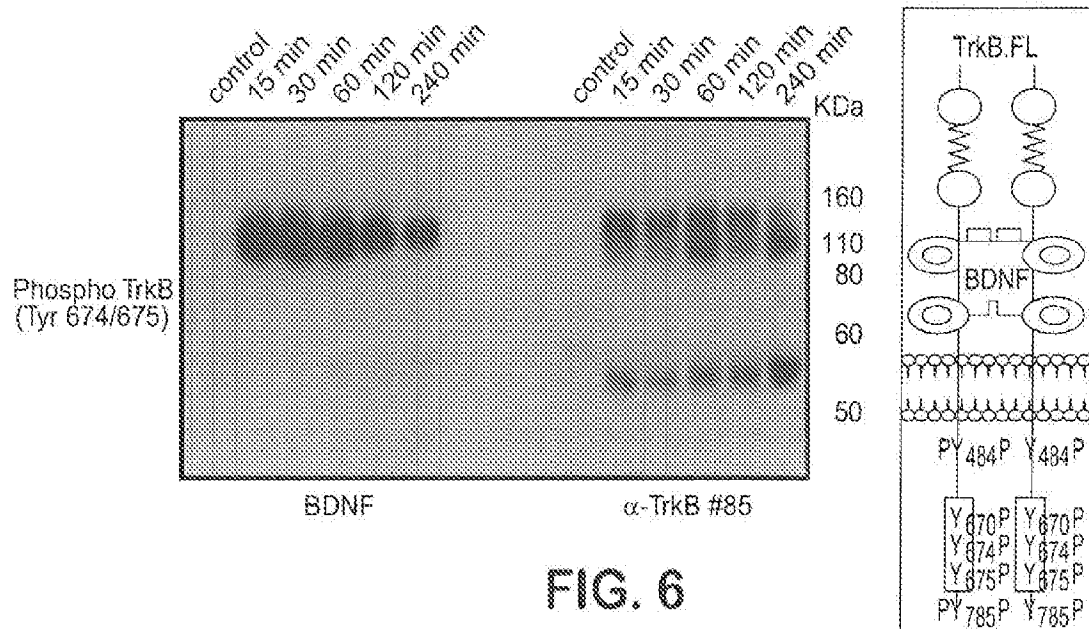


FIG. 6

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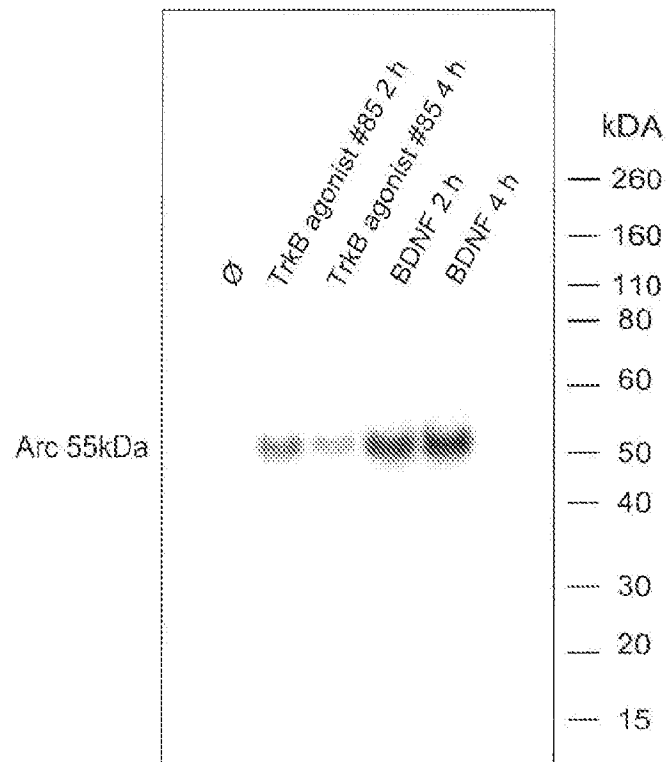


FIG. 7

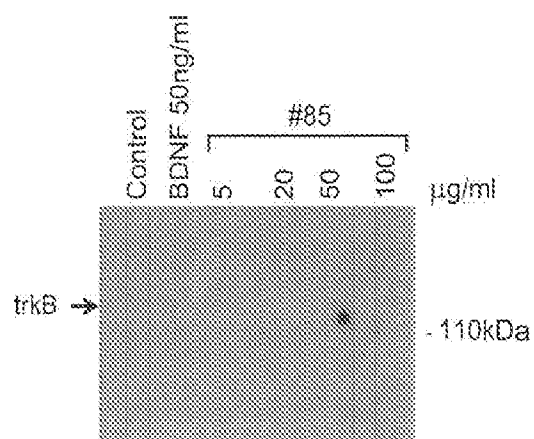
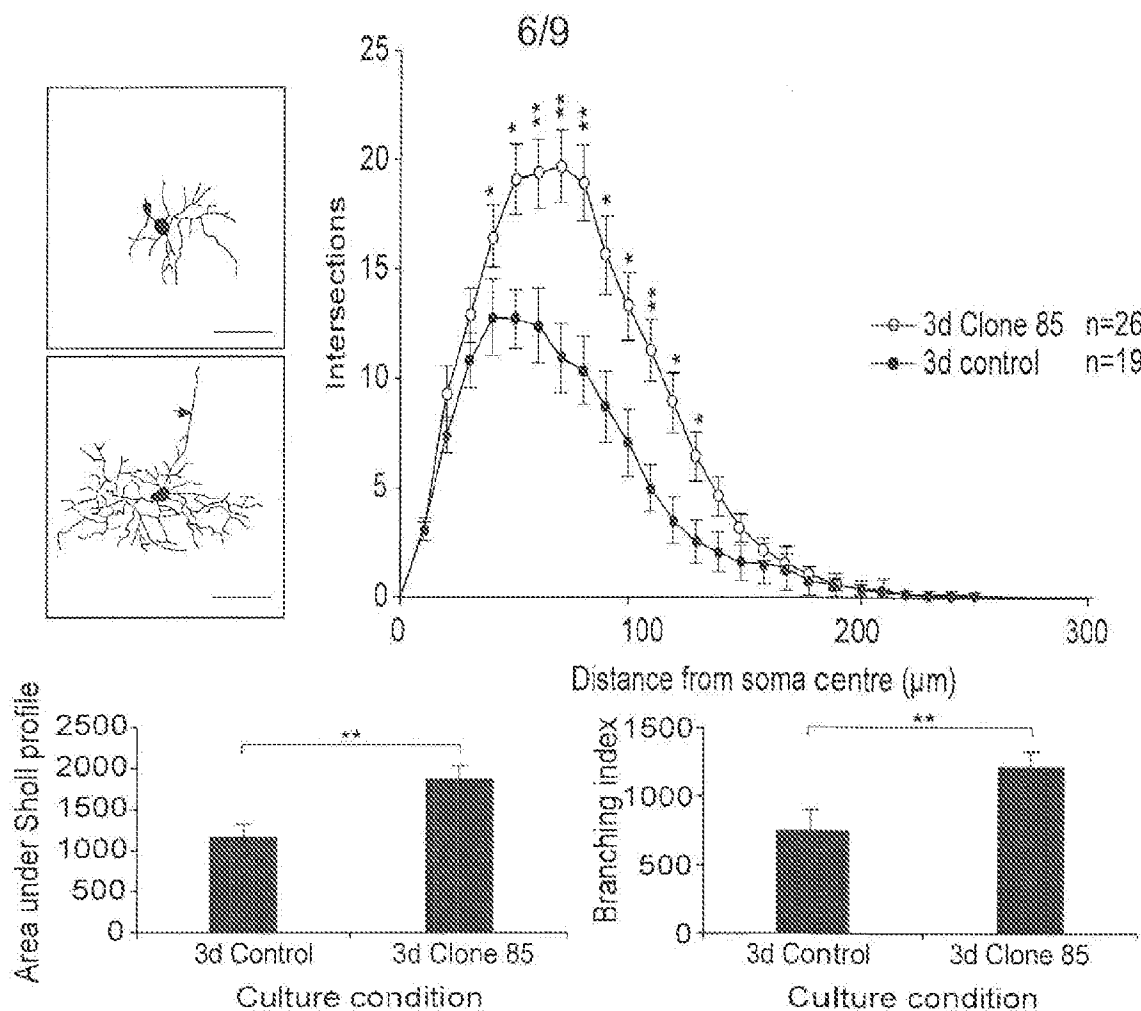
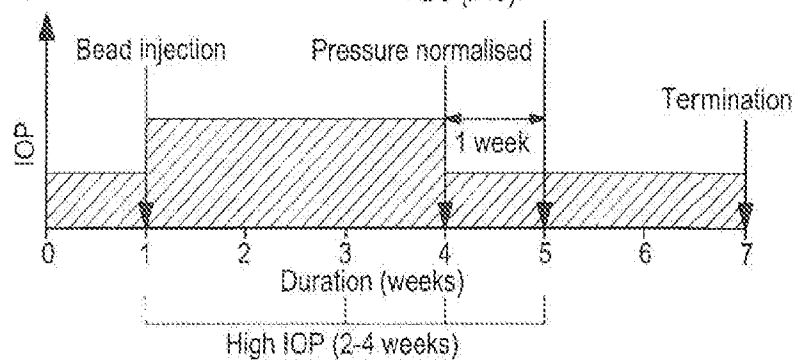
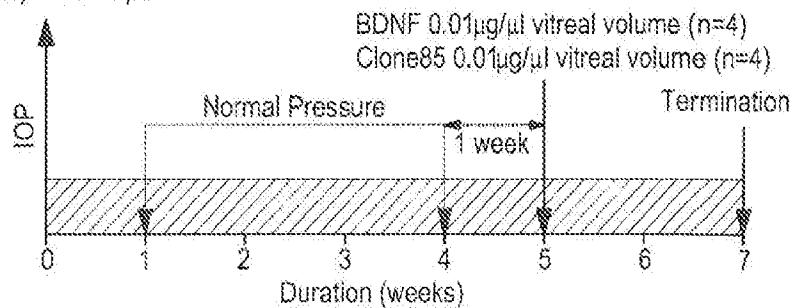


FIG. 8

**FIG. 9**

BDNF $0.01\mu\text{g}/\mu\text{l}$ vitreal volume (n=10)
 Clone85 $0.01\mu\text{g}/\mu\text{l}$ vitreal volume (n=7)
 PBS (n=9)

A) OHT Groups**B) NT Groups****FIG. 10**

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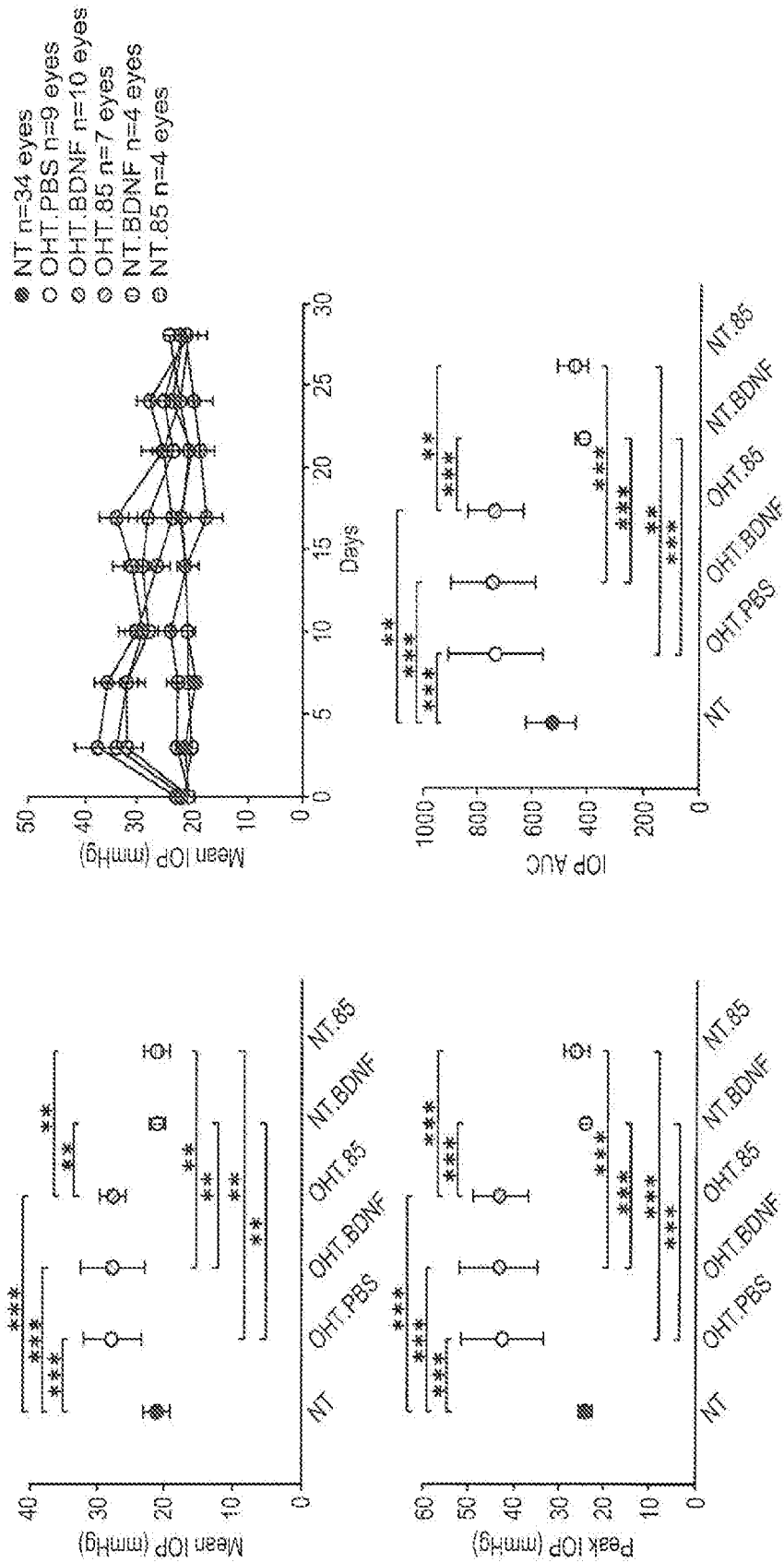


FIG. 11

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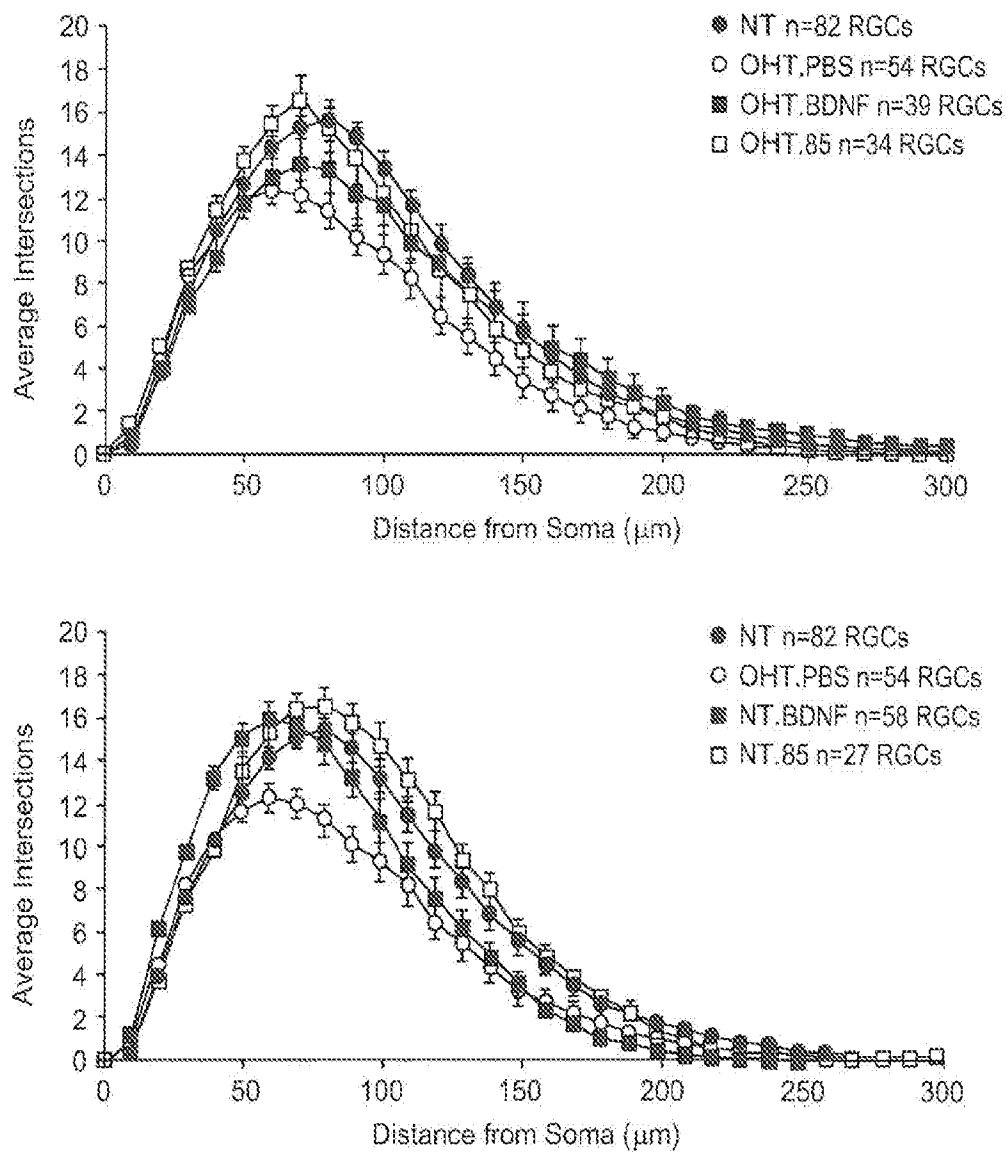


FIG. 12

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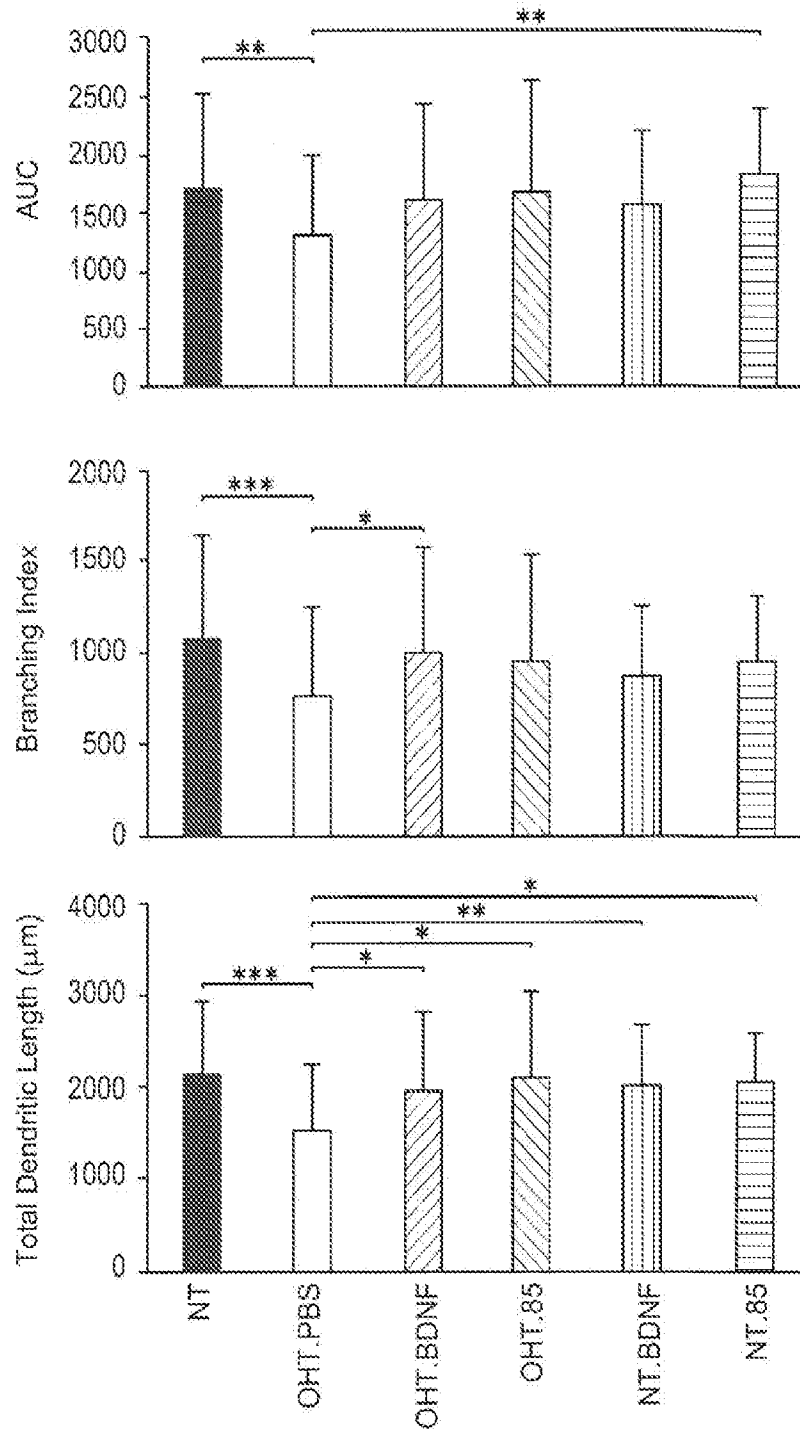


FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/30570

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395, C07K 16/28, A61P 25/00 (2017.01)

CPC - A61K 2039/505, C07K 16/2863, C07K 16/28, A61K 39/395, C07K 16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0196390 A1 (LIN et al.) 5 August 2010 (05.08.2010) para [0012], [0016], [0017], [0018], [0029], [0154], [0238]	1-14, 16-22
A	US 2005/0220795 A1 (WITTRUP et al.) 6 October 2005 (06.10.2005) SEQ ID NO: 292	1-14, 16-22
A	US 2015/0010542 A1 (UNIVERSITY HEALTH NETWORK) 8 January 2015 (08.01.2015) SEQ ID NO: 230	1-14, 16-22
A	US 5,929,041 A (MAGAL) 27 July 1999 (27.07.1999) SEQ ID NO: 1	1-14, 16-22
A	A2N0U1, UniProtKB/TrEMBL Accession No. A2N0U1, 9 December 2015 [online]. [Retrieved on 9 September 2017]. Retrieved from the internet: <URL: http://www.uniprot.org/uniprot/A2N0U1.txt?version=29 >. Entire document	1-14, 16-22
A	US 2014/0243506 A1 (MORPHOSYS AG) 28 August 2014 (28.08.2014) SEQ ID NO: 111	1-14, 16-22
A	US 2013/0323249 A1 (ZHOU et al.) 5 December 2013 (05.12.2013) SEQ ID NO: 19	1-14, 16-22

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 September 2017

Date of mailing of the international search report

04 OCT 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

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Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/30570

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/089344 A1 (GENENTECH INC.) 18 June 2015 (18.06.2015) SEQ ID NO: 110	1-14, 16-22
A	S34014, PIR Accession No. S34014, 16 August 1996 [online]. [Retrieved on 9 September 2017]. Retrieved from the internet: <URL: https://www.ncbi.nlm.nih.gov/protein/S34014 >. Entire document	1-14, 16-22
A	US 2004/0123343 A1 (LA ROSA et al.) 24 June 2004 (24.06.2004) SEQ ID NO: 154332	1-14, 16-22
A	A0A072VBU1, UniProtKB/TrEMBL Accession No. A0A072VBU1, 9 December 2015 [online]. [Retrieved on 9 September 2017]. Retrieved from the internet: <URL: http://www.uniprot.org/uniprot/A0A072VBU1.txt?version=8 >. Entire document	1-14, 16-22
A	G8PQJ0, UniProtKB/TrEMBL Accession No. G8PQJ0, 9 December 2015 [online]. [Retrieved on 9 September 2017]. Retrieved from the internet: <URL: http://www.uniprot.org/uniprot/G8PQJ0.txt?version=18 >. Entire document	1-14, 16-22
A	US 2009/0324797 A1 (BOBZIN et al.) 31 December 2009 (31.12.2009) SEQ ID NO: 333	1-14, 16-22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/30570

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*. 1(a)).

☒ on paper or in the form of an image file (Rule 13*ter*. 1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/30570

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

----- please see extra sheet -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-14, 16-22, limited to SEQ ID NOs. 65-67, 68, GKN, 73, 3A-39, 40

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/30570

Continuation of: Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-22, drawn to an antibody that specifically binds to tropomyosin receptor kinase B (TrkB), and to compositions and kits comprising said antibody. The antibody will be searched to the extent that:

- the heavy chain CDRs 1-3 sequences comprise SEQ ID NOs: 65, 66, 67 (claims 1, 4, 5, 7, 8, 10),
- the light chain CDRs 1 and 3 sequences comprise SEQ ID NOs: 68, 73, and the light chain CDR 2 sequence comprises Gly Lys Asn (GKN) (claims 1, 4, 6, 8, 9, 11),
- the heavy chain variable region sequence comprises SEQ ID NO: 38 (claims 2, 3, 12),
- the light chain variable region sequence comprises SEQ ID NO: 39 (claims 2, 3),
- the linker sequence comprises SEQ ID NO: 40 (claim 14).

It is believed that claims 1-14, 16-22, limited to SEQ ID NOs: 65-67, 68, GKN, 73, 38-39, 40, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a TrkB antibody that comprises SEQ ID NOs: 65-67, 68, GKN, 73, 38-39, 40. [Note that claim 15 is not included in the first named invention because none of the sequences of SEQ ID NOs: 1-13 comprise the heavy and light chain CDRs sequences, heavy and light chain variable region sequences, and linker sequence as described above. The closest antibody, namely the sequence of SEQ ID NO: 13, requires a linker sequence of SEQ ID NO: 44, which is not the linker of the first named invention.]. Additional TrkB antibodies will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected TrkB antibodies. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a TrkB antibody that encompasses an antibody wherein:

- the heavy chain CDRs 1-3 sequences comprise SEQ ID NOs: 46-48 (claims 1, 4, 5, 7, 8, 10),
 - the light chain CDRs 1 and 3 sequences comprise SEQ ID NOs: 49, 50, and the light chain CDR 2 sequence comprises Ala Ala Ser (AAS) (claims 1, 4, 6, 8, 9, 11),
 - the heavy chain variable region sequence comprises SEQ ID NO: 14 (claims 2, 3, 12),
 - the light chain variable region sequence comprises SEQ ID NO: 15 (claims 2, 3),
 - the linker sequence comprises SEQ ID NO: 40 (claim 14),
 - the antibody sequence comprises SEQ ID NO: 1 (claim 15),
- i.e. claims 1-22, limited to SEQ ID NOs: 46-48, 49, AAS, 50, 14, 15, 40, 1.

Group II: Claims 23-24, drawn to a polynucleotide encoding the variable region of the heavy chain and/or light chain of an antibody.

Group III: Claims 25-36, drawn to methods for promoting survival, synaptic function, or regeneration of neurons, and for treating or preventing an ocular degenerative condition in a subject.

The inventions listed as Groups I+, II, and III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I+ requires an antibody, antibody compositions, and kit compositions, not required by Groups II and III. Further, the technical feature of each of the inventions listed as Group I+ is the specific TrkB antibody recited therein. Each invention requires a TrkB antibody, not required by any of the other inventions.

Group II requires a polynucleotide and vector comprising said polynucleotide, not required by Groups I+ and III.

Group III requires method steps for administering a therapeutic composition to promote survival, synaptic function, or regeneration of neurons, and to treat or prevent an ocular degenerative condition in a subject, not required by Groups I+ and II.

Common Technical Features

The feature shared by Groups I+, II, and III is the antibody or an antigen-binding fragment that specifically binds to tropomyosin receptor kinase B (TrkB) as described in claim 1.

Another feature shared by the inventions listed as Group I+ is the pharmaceutical composition of claim 20, the bispecific compound of claim 21, and the kit of claim 22.

Another feature shared by Groups II and III is the vector of claim 24 harboring the polynucleotide of claim 23.

-----please see continuation on next extra sheet-----

Continuation of: Box No. III Observations where unity of invention is lacking

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2010/0196390 A1 to Lin et al. (hereditary 'Lin').

Lin discloses [claim 1] an antibody or an antigen-binding fragment that specifically binds to tropomyosin receptor kinase B (TrkB) (para [0012] "This invention relates to agonist antibodies that selectively interact with and activate TrkB function"; para [0016] "the invention provides an antibody which specifically binds TrkB").

with the same binding specificity as that of a second antibody (para [0029] "the invention provides an humanized agonist anti-TrkB antibody which comprises an antibody which binds to TrkB . . . wherein the humanized antibody overlaps the same epitope of TrkB that is recognized by monoclonal antibody 38B8"; para [0154] "Yet another method which can be used to characterize a TrkB agonist antibody is to use competition assays with other antibodies known to bind to the same antigen, i.e., various fragments on TrkB, to determine if the TrkB agonist antibody binds to the same epitope as other antibodies. Competition assays are well known to those of skill in the art"; para [0238] "It is understood that the compositions can comprise more than one TrkB agonist antibody (e.g., a mixture of TrkB agonist antibodies that recognize different epitopes of TrkB). Other exemplary compositions comprise more than one TrkB agonist antibodies that recognize the same epitope(s), or different species of TrkB agonist antibodies that bind to different epitopes of TrkB."), wherein the second antibody comprises heavy chain CDRs 1-3 and light chain CDRs 1-3 sequences (para [0016] "the invention provides an antibody which specifically binds TrkB and comprises a light chain having the amino acid sequence . . . a heavy chain having the amino acid sequence"; para [0017] "an antibody which specifically binds TrkB and comprises a VH CDR1 . . . a VH CDR2 . . . and/or a VH CDR3"; para [0018] "an antibody comprising a VL CDR1 . . . a CDR2 . . . and/or CDR3").

Lin discloses [claim 20] a pharmaceutical composition comprising a therapeutically effective amount of the antibody or antigen-binding fragment and a pharmaceutically acceptable carrier (para [0031] "The invention also provides a pharmaceutical composition comprising a humanized TrkB antibody as described herein"; para [0114] "Therapeutic formulations of the TrkB agonist antibody used in accordance with the present invention are prepared for storage by mixing an antibody or peptide having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers"; para [0159] "This invention encompasses compositions, including pharmaceutical compositions, comprising antibodies described herein . . . compositions comprise one or more antibodies promote TrkB activity . . . These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients including buffers, which are well known in the art").

Lin discloses [claim 21] a bispecific compound comprising the antibody or antigen-binding fragment (para [0128] "The antibodies useful in the present invention can encompass . . . bispecific antibodies"; para [0255] "a fusion antibody or immunoadhesin may be made that comprises all or a portion of a TrkB antibody of the invention linked to another polypeptide. . . . In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful . . . if one wants to create a bispecific antibody").

Lin discloses [claim 22] a kit comprising an antibody or antigen-binding fragment (para [0241] "The invention also provides kits for use in the instant methods. Kits of the invention include one or more containers comprising a TrkB agonist antibody (such as a humanized antibody) or peptide described herein and instructions for use in accordance with any of the methods of the invention described herein").

Lin discloses [claim 23] a polynucleotide encoding the variable region of the heavy chain and/or light chain of the antibody or antigen-binding fragment (para [0140] "If desired, the TrkB agonist antibody (monoclonal or polyclonal) of interest may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use"; para [0218] "In some embodiments, the composition comprises an expression vector comprising a polynucleotide encoding the antibody as described herein").

Lin discloses [claim 24] a vector harboring the polynucleotide (para [0140] "If desired, the TrkB agonist antibody (monoclonal or polyclonal) of interest may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use"; para [0218] "In some embodiments, the composition comprises an expression vector comprising a polynucleotide encoding the antibody as described herein").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

The inventions listed as Groups I+, II, and III therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.