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(71) Applicant: HADASIT MEDICAL RESEARCH SERVICES AND DEVELOPMENT LTD. [IL/IL]; P.O. Box 12000, 9112001 Jerusalem (IL).

(72) Inventor: SAFADI, Rifaat; 3 Nitufa Street, POB 4825, 17501 Nazareth Elit (IL).

(74) Agents: FRIEDMAN, Nathalie et al.; Webb & Co., P.O. Box 2189, 7612100 Rehovot (IL).

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(54) Title: INHIBITORS OF NEUROLIGIN 4 - NEUREXIN 1-BETA PROTEIN-PROTEIN INTERACTION FOR TREATMENT OF LIVER DISORDERS

(57) Abstract: Method for treating, attenuating and/or preventing progression of a liver disorder in a subject, the method including administering a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1-beta (Nrx1b) protein-protein interaction; and compositions including the agent.

INHIBITORS OF NEUROLIGIN 4 – NEUREXIN 1-BETA PROTEIN-PROTEIN INTERACTION FOR TREATMENT OF LIVER DISORDERS

FIELD OF THE INVENTION

5 The present invention relates to antibodies and recombinant proteins capable of interfering with, inhibiting and/or preventing neuroligin-4 (NLGn4)-neurexin 1 β (Nrx1b) protein-protein interaction, and to the use thereof for the treatment and/or attenuation of liver disorders.

10 **BACKGROUND OF THE INVENTION**

 The normal liver is composed of hepatocytes and non-parenchymal cells, which include Kupffer cells, sinusoidal endothelial cells, and myofibroblasts known as Hepatic Stellate Cells (HSCs). HSCs are considered involved in the pathogenesis of liver fibrosis from any etiology, including NASH-related hepatic fibrosis and hepatocellular carcinoma. In normal liver, HSCs are in a quiescent state and serve to store retinoids (vitamin A). Quiescent stellate cells represent 5-8% of the total number of liver cells. When the liver is damaged, HSCs change into an activated state characterized by contractions, loss of lipid droplets, enhanced proliferation, cell migration and cellular adhesion. HSCs are also unequivocally the main cells involved in the production of excessive ECM seen in liver fibrosis. Since activated HSCs themselves secrete inflammatory chemokines, a vicious cycle is formed, whereby fibrogenic and inflammatory cells stimulate each other and perpetuate a process of liver damage and repair.

 Natural killer (NK) cells are a key component of the innate immune system, and play a critical role in the early stages of the immune response against tumor cells, as well as those infected by viral and microbial pathogens.

 In humans, two NK-cell subsets have been characterized according to the cell-surface density of CD56 and expression of CD16. CD56^{dim}CD16^{bright} NK cells (hereinafter CD56^{dim}) compose approximately 90% of circulating NK cells; CD56^{bright}CD16^{dim} NK cells (hereinafter CD56^{bright}) constitute approximately 10%.

CD56^{bright} NK cells proliferate and produce interferon in response to stimulation with interleukin-12 (IL-12), whereas CD56^{dim} NK cells are more cytolytic and produce significant amounts of cytokine when their activating receptors are engaged.

In a paper on which the inventor is a principle investigator, it was found that, as
5 opposed to CD8 immune cells, NK cells have anti-fibrotic activity through HSC killing (Melhem et al., J. Hepatology; 2006; 45: 60-71). It has also been reported that the function of NK cells decreases when the liver disease progresses into cirrhosis, suggesting that attenuating NK function is a prerequisite for the progression of the disease (Seki et al.; Clin Dev Immunol.; 2011; Article ID 868345).

10 Human neuroligin-4 (NLG4, NLGn4, NLGn4X) is a family member of neuronal cell surface proteins called the Neuroligins. FIG. 1 illustrates the neuroligins and their interactions. Members of this family are membrane-anchored proteins acting as ligands for beta-neurexins and are thought to be involved in the formation and remodeling of central nervous system synapses. The encoded protein interacts with discs, large
15 (Drosophila) homolog 4 (DLG4). Mutations in this gene have been associated with autism and Asperger syndrome. High levels of NLGn4 has been reported heart tissue and lower levels in liver, skeletal muscle and pancreas.

Neurexin (NRXN) is a presynaptic protein that helps to glue together neurons at the synapse. Neurexins are located mostly on the presynaptic membrane and contain a
20 single transmembrane domain. The extracellular domain interacts with proteins in the synaptic cleft, most notably neuroligin, while the intracellular cytoplasmic portion interacts with proteins associated with exocytosis.

Non-alcoholic fatty-liver disease (NAFLD) is one of the most prevalent liver diseases in western countries. The full pathophysiology of NAFLD is still unknown. Both
25 obesity and insulin resistance are considered to play a strong role in the disease process. Indeed, the rising rates of obesity and diabetes mellitus correlate with the increasing incidence of NAFLD, which is the hepatic and early manifestation of metabolic syndrome. Estimates suggest that about 20% to 30% of adults in developed countries have excess fat accumulation in the liver, 50% among people with diabetes, and about
30 80% in the obese and morbidly obese individuals.

Non-alcoholic steatohepatitis (NASH) is the most severe form of NAFLD, and can progress to more severe forms of liver disease, including fibrosis, cirrhosis, and hepatocellular carcinoma.

5 The disease begins with the aberrant accumulation of triglycerides in the liver, resulting in simple steatosis; most patients who develop steatosis are stable and further disease does not develop. However, some individuals progress to NASH, the severe form of NAFLD. In NASH, up to 20% of patients' progress into cirrhosis. The clinical implications of NAFLD are derived mostly from its potential to progress to cirrhosis and liver failure.

10 There is an unmet medical need for compositions and methods for treating NAFLD and preventing the progression to cirrhosis and hepatocellular carcinoma. Nowhere in the art has it been suggested that treating, attenuating and/or preventing progression of liver disorders can be achieved by administering an agent capable of interfering with, inhibiting and/or preventing NLGn4-Nrx1b protein-protein interaction.

15

SUMMARY OF THE INVENTION

The present invention stems, in part, from the surprising finding that liver disorders can be treated and/or attenuated by administering to a subject in need thereof an agent capable of interfering, inhibiting and/or preventing NLGn4-Nrx1b protein-protein interaction. The agent may be an anti-NLGn4 antibody, an anti-Nrx1b antibody,
20 recombinant NLGn4, recombinant Nrx1b or any fragment, analog or derivative thereof capable of interfering, inhibiting and/or preventing the NLGn4-Nrx1b interaction.

According to some embodiments, the invention provides a method of treating, attenuating and/or preventing progression of a liver disorder in a subject, the method
25 comprising administering to the subject in need thereof a therapeutically effective amount of an agent capable of interfering, inhibiting and/or preventing with neuroigin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein interaction; thereby treating, attenuating and/or preventing progression of the liver disorder.

According to some embodiments, the invention provides a composition for use
30 in the treatment, attenuation and/or prevention of progression of a liver disorder, the

composition comprising a therapeutically effective amount of an agent capable of interfering, inhibiting and/or preventing with neuroligin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein interaction. According to some embodiments, the composition is suitable for administration to a subject suffering from a liver disorder. According to some
5 embodiments, the composition is suitable for administration to an immune cell population of the subject.

According to some embodiments, the agent comprises recombinant NLGn4. According to some embodiments, the recombinant NLGn4 is soluble (rsNLGn4); wherein the rsNLGn4 comprises the extracellular domain of NLGn4 as set forth in SEQ
10 ID NO: 7 or a fragment, derivative or analog thereof. According to some embodiments, the rsNLGn4 consists essentially of SEQ ID NO: 7 or a fragment, derivative or analog thereof. According to some embodiments, the recombinant NLGn4 comprises Gln42-Ser676 of NP_065793. According to some embodiments, the recombinant NLGn4 consists essentially of Gln42-Ser676 of NP_065793.

15 According to some embodiments, the rsNLGn4 competes with endogenous NLGn4 for binding to Nrx1b.

According to some embodiments, the rsNLGn4 is devoid of the intracellular domain and/or the transmembrane domain of NLGn4.

According to some embodiments, the agent comprises a fusion protein
20 comprising rsNLGn4 and an immunoglobulin molecule. According to some embodiments, the agent comprises a fusion protein comprising rsNLGn4 and an Fc domain of an immunoglobulin molecule, also referred to herein as rsNLGn4-Fc fusion protein and rsNLGn4-Fc chimera. According to some embodiments, the Fc domain may include part or the entire CH1 domain, CH2 domain, CH3 domain and hinge region of
25 IgG1, IgG2, IgG3 and IgG4. According to some embodiments, the Fc domain may be devoid of the CH1 domain. According to some embodiments, the Fc domain comprises Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain consists essentially of Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain may be linked to rsNLGn4 through a linker. According to some
30 embodiments, the linker may include the amino acid sequence set forth in SEQ ID NO: 13 (IEGRMD).

According to some embodiments, the agent is a chimeric protein formed from NLGn4 polypeptides or fragments fused with a second polypeptide to form a soluble NLGn4. According to some embodiments, the agent comprises a DNA sequences, which combine two partial DNA sequences, one sequence encoding soluble fragments of NLGn4 (i.e. the DNA sequence encoding the extracellular domain of NLGn4) and the other partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE. These DNA sequences may subsequently be expressed in target cells using expression vectors as known in the art, thereby obtaining endogenous expression of recombinant proteins having the extracellular domain of NLGn4 joined to the Fc fragment of an immunoglobulin molecule.

According to some embodiments, the agent comprises recombinant NrX1b. According to some embodiments, the recombinant NrX1b is soluble (rsNrX1b); wherein the rsNrX1b comprises the extracellular domain of NrX1b as set forth in SEQ ID NO: 10 or a fragment, derivative or analog thereof. According to some embodiments, the recombinant NrX1b comprises Ala51-Ser363 of NP_620072. According to some embodiments, the recombinant NrX1b consists essentially of Ala51-Ser363 of NP_620072. According to some embodiments, the rsNrX1b consists essentially of SEQ ID NO: 10 or a fragment, derivative or analog thereof.

According to some embodiments, the rsNrX1b competes with endogenous NrX1b for binding to NLGn4.

According to some embodiments, the rsNrX1b is devoid of the intracellular domain and/or the transmembrane domain of NrX1b.

According to some embodiments, NLGn4 is encoded by the sequence set forth in SEQ ID NO: 1.

According to some embodiments, the agent comprises a fusion protein comprising rsNrX1b and an immunoglobulin molecule. According to some embodiments, the agent comprises a fusion protein comprising rsNrX1b and an Fc domain of an immunoglobulin molecule, also referred to herein as rsNrX1b-Fc fusion protein and rsNrX1b-Fc chimera. According to some embodiments, the Fc domain may include part or all of the CH1 domain, the CH2 domain, the CH3 domain and hinge region of IgG1,

IgG2, IgG3 and IgG4. According to some embodiments, the Fc domain may be devoid of the CH1 domain. According to some embodiments, the Fc domain may include all domains of the Fc domain apart from CH1. According to some embodiments, the Fc domain comprises Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain consists essentially of Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain may be linked to rsNrX1b through a linker. According to some embodiments, the linker may have the amino acid sequence set forth in SEQ ID NO: 13 (IEGRMD).

According to some embodiments, the agent is a chimeric protein formed from NrX1b polypeptides or fragments fused with a second polypeptide to form a soluble NrX1. According to some embodiments, the agent comprises a DNA sequences, which combine two partial DNA sequences, one sequence encoding soluble fragments of NrX1b (i.e. the DNA sequence encoding the extracellular domain of NrX1b) and the other partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE. These DNA sequences may subsequently be expressed in target cells using expression vectors as known in the art, thereby obtaining endogenous expression of recombinant proteins having the extracellular domain of NrX1b joined to the Fc fragment of an immunoglobulin molecule.

According to some embodiments, NrX1b is encoded by the sequence set forth in SEQ ID NO: 6.

According to some embodiments, the agent is an antibody to human NLGn4 and/or its receptor NrX1b.

According to some embodiments, the antibody is a monoclonal antibody, according to additional embodiments the antibody is a humanized monoclonal antibody. According to some embodiments, the humanized monoclonal antibody is an antibody directed to human NLGn4.

According to some embodiments, the antibody interferes with the binding of NLGn4 to NrX1b. According to some embodiments, the antibody is capable of reducing NLGn4 protein levels.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to the interaction domain of NLGn4, the interaction domain mediating the interaction between NLGn4 to NrX1b.

5 According to some embodiments, the anti-NLGn4 antibody or a fragment, derivative or analog thereof is capable of binding to amino acids 359-364, set forth in SEQ ID NO: 2 (QGEFLN), of the human NLGN4 protein.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid E361 of human NLGn4.

10 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid L363 of human NLGn4.

15 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 261-270, set forth in SEQ ID NO: 3 (SLLTLSHYSE), of human NLGn4.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid H267 of human NLGn4.

20 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 461-470, set forth in SEQ ID NO: 4 (AQYGSPTYFY), of human NLGn4.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid Y463 of human NLGn4.

25 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 265-275, set forth in SEQ ID NO: 5 (LSHYSEGLFQK), of human NLGn4.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid E270 of human NLGn4.

5 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising an amino acid selected from the group consisting of E361, L363, H267, Y463, E270 or any combination thereof. Each possibility is a separate embodiment of the invention.

According to some embodiments, antibody is an antibody to human NrX1b.

10 According to some embodiments, the NrX1b monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to the interaction domain of NrX1b, the interaction domain mediating the interaction between NLGn4 to NrX1b.

15 According to some embodiments, the anti-NrX1b antibody or a fragment, derivative or analog thereof is capable of binding to amino acids 103-109, set forth in SEQ ID NO: 7 (LLADTPV) and/or amino acids 234-239 as set forth in SEQ ID NO: 7 (CSVVDD), of the human NrX1 protein. Each possibility is a separate embodiment of the invention.

20 According to some embodiments, the liver disorder is selected from the group consisting of: non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, hepatitis, liver adenoma, insulin hypersensitivity, liver cancer and any combination thereof. Each possibility is a separate embodiment of the invention.

25 According to some embodiments, the liver disorder is characterized by NLGn4 overexpression. According to some embodiments, NLGn4 overexpression comprises a 2, 3, 4, 5-10 fold or more increase in NLGn4 expression relative to the expression level obtained in normal subjects. Each possibility is a separate embodiment of the invention. According to some embodiments, the overexpression attenuates NK cell activity, inhibits the expression of NLGn4 and modulates and/or activates the function of the NK cell.

30 According to some embodiments, the liver disorder is NAFLD and/or hepatocellular carcinoma. According to some embodiments, the liver disorder is NAFLD. According to some embodiments, the liver disorder is hepatocellular carcinoma.

According to some embodiments, administering to the subject a therapeutically effective amount of a humanized, monoclonal antibody to NLGn4 and/or its receptor Nrxb1b comprises administering the composition to an immune cell population of the subject.

5 According to some embodiments, administering a therapeutically effective amount of a humanized, monoclonal antibody to NLGn4 to a subject reduces the NLGn4 protein levels in the subject's NK cells. According to some embodiments, reducing NLGn4 protein levels in NK cells reduces the activity of hepatic stellate cells (also referred to herein as HSCs). According to some embodiments, reducing NLGn4 protein
10 levels increases apoptosis of the hepatic stellate cells.

According to some embodiments, there is provided a humanized monoclonal antibody reactive with a human NLGN4. According to some embodiments, the human NLGN4 protein is a polypeptide sequence comprising SEQ ID NO: 1.

15 According to some embodiments, the anti-NLGn4 monoclonal antibody or a fragment, derivative or analog thereof is capable of binding to amino acids 359-364, set forth in SEQ ID NO: 2 (QGEFLN), of the human NLGN4 protein.

According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid E361 of human NLGN4.

20 According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid L363 of human NLGN4.

25 According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 261-270, set forth in SEQ ID NO: 3 (SLLTLSHYSE), of human NLGN4.

According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid H267 of human NLGN4.

According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 461-470, set forth in SEQ ID NO: 4 (AQYGSPTYFY), of human NLGn4.

5 According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid Y463 of human NLGn4.

According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 265-275, set forth in SEQ ID NO: 5 (LSHYSEGLFQK), of human NLGn4.

10 According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to an amino acid selected from the group consisting of E361, L363, H267, Y463, E270 or any combination thereof, of human NLGn4.

15 According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of reducing human NLGn4 protein levels.

According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of interfering with, inhibiting and/or preventing binding of NLGn4 to Nr1b.

20 According to some embodiments, there is provided a composition comprising a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing NLGn4–Nr1b protein-protein interaction and a pharmaceutically acceptable carrier. According to some embodiments, the composition is for the use of treating a liver disorder. According to some embodiments, the liver disorder is selected from the group
25 consisting of: non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, hepatitis, liver adenoma, insulin hypersensitivity, liver cancer and any combination thereof. Each possibility is a separate embodiment of the invention. According to some embodiments, the liver disorder is NAFLD and/or hepatocellular carcinoma. According to some embodiments, the liver disorder is NAFLD. According to
30 some embodiments, the liver disorder is hepatocellular carcinoma.

According to some embodiments, the agent comprises recombinant NLGn4. According to some embodiments, the recombinant NLGn4 is soluble (rsNLGn4); wherein the rsNLGn4 comprises the extracellular domain of NLGn4 or a fragment, derivative or analog thereof.

5 According to some embodiments, the extracellular domain of NLGn4 consists of SEQ ID NO: 7. According to some embodiments, the rsNLGn4 consists of SEQ ID NO: 7, or a fragment, derivative or analog thereof.

 According to some embodiments, the recombinant NLGn4 comprises Gln42-Ser676 of NP_065793. According to some embodiments, the recombinant NLGn4
10 consists essentially of Gln42-Ser676 of NP_065793.

 According to some embodiments, the rsNLGn4 competes with endogenous NLGn4 for binding to NrX1b.

 According to some embodiments, the rsNLGn4 is devoid of the intracellular domain and/or the transmembrane domain of NLGn4.

15 According to some embodiments, the agent comprises a fusion protein comprising rsNLGn4 and an immunoglobulin molecule. According to some
 embodiments, the agent comprises a fusion protein comprising rsNLGn4 and an Fc domain of an immunoglobulin molecule. According to some
 embodiments, the Fc domain may include part or the entire CH1 domain, CH2 domain, CH3 domain and hinge
20 region of IgG1, IgG2, IgG3 and IgG4. According to some embodiments, the Fc domain may be devoid of the CH1 domain. According to some
 embodiments, the Fc domain comprises Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain consists essentially of
 Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain may be linked to rsNLGn4 through a linker. According to
25 some embodiments, the linker may include the amino acid sequence set forth in SEQ ID NO: 13 (IEGRMD).

 According to some embodiments, the agent is a chimeric protein formed from NLGn4 polypeptides or fragments fused with a second polypeptide to form a soluble
 NLGn4. According to some embodiments, the agent comprises a DNA sequences, which
30 combine two partial DNA sequences, one sequence encoding soluble fragments

of NLGn4 (i.e. the DNA sequence encoding the extracellular domain of NLGn4) and the other partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE. These DNA sequences may subsequently be expressed in target cells using expression vectors as
5 known in the art, thereby obtaining endogenous expression of recombinant proteins having the extracellular domain of NLGn4 joined to the Fc fragment of an immunoglobulin molecule.

According to some embodiments, NLGn4 is encoded by the sequence set forth in SEQ ID NO: 1.

10 According to some embodiments, the agent comprises recombinant NrX1b. According to some embodiments, the recombinant NrX1b is soluble (rsNrX1b); wherein the rsNrX1b comprises the extracellular domain of NrX1b or a fragment, derivative or analog thereof capable of binding NLGn4.

According to some embodiments, the recombinant NrX1b comprises Ala51-
15 Ser363 of NP_620072. According to some embodiments, the recombinant NrX1b consists essentially of Ala51-Ser363 of NP_620072.

According to some embodiments, the extracellular domain of NrX1b consists of SEQ ID NO: 10. According to some embodiments, the rsNrX1b consists of SEQ ID NO: 10, or a fragment, derivative or analog thereof.

20 According to some embodiments, the rsNrX1b competes with endogenous NrX1b for binding to NLGn4.

According to some embodiments, the rsNrX1b is devoid of the intracellular domain and/or the transmembrane domain of NrX1b.

According to some embodiments, NrX1b is encoded by the sequence set forth in
25 SEQ ID NO: 6.

According to some embodiments, the agent comprises a fusion protein comprising rsNrX1b and an immunoglobulin molecule. According to some embodiments, the agent comprises a fusion protein comprising rsNrX1b and an Fc domain of an immunoglobulin molecule. According to some embodiments, the Fc domain may include

part or the entire CH1 domain, the CH2 domain, the CH3 domain and hinge region of IgG1, IgG2, IgG3 and IgG4. According to some embodiments, the Fc domain may be devoid of the CH1 domain. According to some embodiments, the Fc domain may include all domains of the Fc domain apart from CH1. According to some embodiments, the Fc domain comprises Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain consists essentially of Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain may be linked to rsNrxb1 through a linker. According to some embodiments, the linker may have the amino acid sequence set forth in SEQ ID NO: 13 (IEGRMD).

10 According to some embodiments, the agent is a chimeric protein formed from Nrxb1 polypeptides or fragments fused with a second polypeptide to form a soluble Nrxb1. According to some embodiments, the agent comprises a DNA sequences, which combine two partial DNA sequences, one sequence encoding soluble fragments of Nrxb1 (i.e. the DNA sequence encoding the extracellular domain of Nrxb1) and the other partial
15 sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE. These DNA sequences may subsequently be expressed in target cells using expression vectors as known in the art, thereby obtaining endogenous expression of recombinant proteins having the extracellular domain of Nrxb1 joined to the Fc fragment of an immunoglobulin molecule.

20 According to some embodiments, the agent comprises recombinant NLGN4 and recombinant Nrxb1. According to some embodiments, the recombinant NLGN4 and Nrxb1 are soluble (rsNLGN4 and rsNrxb1, respectively); wherein the rsNLGN4 comprises the extracellular domain of NLGN4 or a fragment, derivative or analog thereof capable of binding NLGN4; and wherein the rsNrxb1 comprises the extracellular domain
25 of Nrxb1 or a fragment, derivative or analog thereof capable of binding NLGN4.

According to some embodiments, the agent is an antibody to NLGN4. According to some embodiments, the antibody is a monoclonal antibody. According to some embodiments, the agent is an antibody to Nrxb1. According to some embodiments, the antibody is a monoclonal antibody. According to some embodiments, the antibody is a
30 humanized antibody. According to some embodiments, there is provided a composition comprising a therapeutically effective amount of monoclonal antibodies to NLGN4 and to Nrxb1.

According to some embodiments, the composition is for the use in treating, attenuating and/or preventing progression of a liver disorder in a subject.

According to some embodiments, the composition further comprises a GLUT4 antagonist. According to some embodiments, NLGn4 expression is regulated by a specific type of ionotropic glutamate receptor N-methyl-D-aspartate (NMDA or GLUT4 receptor; NMDAR). According to some embodiments, NLGn4 is linked to NMDR and both localize and bind PSD-95; a post synaptic density protein (PSD) According to some embodiments, the composition comprises an NMDAR antagonist selected from the group consisting of: Ketamin, Amantadine, Phencyclidine, Nitrous oxide, Dextromethorphan (and dextrorphan), Memantine, Ethanol, Riluzole, Xenon, HU-211, Lead (Pb²⁺), Conantokins, and Huperzine A.

According to an alternative embodiment, administering an N-methyl D aspartate receptor (NMDAR) agonist can increase NMDAR-mediated NLGn4 expression and as a result attenuate NK cell activity. Non-limiting examples of NMDAR agonists are Aminocyclopropanecarboxylic acid, D-Cycloserine, cis-2,3-Piperidinedicarboxylic acid, L-aspartate, L-alanine, Quinolate, Homocysteate, D-serine, and ACPL.

According to some embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

According to some embodiments, there is provided method of treating, attenuating and/or preventing progression of a liver disorder in a subject, the method comprising administering to the subject in need thereof a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein-protein interaction; thereby treating, attenuating and/or preventing progression of the liver disorder.

According to some embodiments, there is provided composition for use in treating, attenuating and/or preventing progression of a liver disorder in a subject, the composition comprising a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein-protein interaction.

According to some embodiments, there is provided composition comprising a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein-protein interaction for use in treating, attenuating and/or preventing progression of a liver disorder.

5 According to some embodiments, there is provided an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein-protein interaction for use in preparation of a composition for treating, attenuating and/or preventing progression of a liver disorder.

 According to some embodiments, the agent comprises recombinant soluble
10 NLGn4 (rsNLGn4); wherein the rsNLGn4 comprises the extracellular domain of NLGn4 or a fragment, derivative or analog thereof. According to some embodiments, the extracellular domain of NLGn4 consists of SEQ ID NO: 7 or a fragment, derivative or analog thereof. According to some embodiments, the rsNLGn4 competes with endogenous NLGn4 for binding to Nrx1b. According to some embodiments, the
15 rsNLGn4 is devoid of the intracellular domain and/or transmembrane domain of NLGn4. According to some embodiments, NLGn4 is encoded by the sequence set forth in SEQ ID NO: 1. According to some embodiments, the agent comprises a fusion protein comprising the Fc portion of an immunoglobulin molecule and SEQ ID NO: 7 or a fragment thereof.

20 According to some embodiments, the therapeutic agent comprises recombinant soluble Nrx1b (rsNrx1b); wherein the rsNrx1b comprises the extracellular domain of Nrx1b or a fragment, derivative or analog thereof capable of binding the NLGn4. According to some embodiments, the extracellular domain of Nrx1b consists of SEQ ID NO: 10. According to some embodiments, the rsNrx1b consists of SEQ ID NO: 10, or a
25 fragment, derivative or analog thereof. According to some embodiments, the rsNrx1b competes with endogenous Nrx1b for binding to NLGn4. According to some embodiments, the rsNrx1b is devoid of the intracellular domain and/or the transmembrane domain of Nrx1b. According to some embodiments, the Nrx1b is encoded by the sequence set forth in SEQ ID NO: 6. According to some embodiments,
30 the agent comprises a fusion protein comprising the Fc portion of an immunoglobulin molecule and SEQ ID NO: 10 or a fragment thereof.

According to some embodiments, the therapeutic agent comprises an anti-NLGn4 antibody, or a fragment, derivative or analog thereof, comprising at least the antigen-binding portion thereof. According to some embodiments, the antibody is a humanized monoclonal anti-NLGn4 antibody. According to some embodiments, the anti-NLGn4 antibody is capable of binding specifically to an interaction domain of human NLGn4.

5 According to some embodiments, the anti-NLGn4 antibody is capable of binding specifically to amino acids 359-364 of the human NLGN4 protein. According to some embodiments, the anti-NLGn4 antibody is capable of binding to an epitope within residues 261-270 of the human NLGn4, as set forth in SEQ ID NO: 3 (SLLTSLSHYSE).

10 According to some embodiments, the anti-NLGn4 antibody is capable of binding to an epitope within residues 265-275, as set forth in SEQ ID NO: 5 (LSHYSEGLFQK), of the human NLGn4. According to some embodiments, the anti-NLGn4 antibody is capable of binding to an epitope within residues 461-470 of the human NLGn4, as set forth in SEQ ID NO: 4 (AQYGSPTYFY).

15 According to some embodiments, the liver disorder is selected from the group consisting of: non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, hepatitis, liver adenoma, insulin hypersensitivity, liver cancer and any combination thereof. According to some embodiments, the liver disorder is NAFLD. According to some embodiments, the liver disorder is hepatocellular carcinoma.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a schematic representation of the Neuroligins and their interactions.

FIG. 2 shows NLGn4 expression in NK cells obtained from NASH patients with hepatic fibrosis. Isolated NK cells from healthy and NASH cirrhotic cases were assessed by DNA spotted Microarrays.

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FIG. 3. Shows NLGn4 expression levels in a leptin-deficient fatty liver disease mouse model as compared control mice.

FIG. 4A shows serum ALT levels in homozygous NLG4^{-/-} mice as compared to WT, upon induction of fibrosis.

FIG. 4B shows alpha-SMA levels in homozygous NLG4^{-/-} mice as compared to WT, upon induction of fibrosis.

FIG. 5A shows NLGn4 protein expression levels in NK cells isolated from NAFLD patients and incubated with a hepatic stellate cell line (LX2) in the absence or
5 in the presence of increasing concentrations of an anti-NLGn4 antibody.

FIG. 5B shows CD107a levels in NK cells isolated from NAFLD patients and incubated with a hepatic stellate cell line in the absence or in the presence of increasing concentrations of an anti-NLGn4 antibody.

FIG. 5C shows alpha-SMA intensities (LX2 activity) in LX2 cells, incubated
10 with NK cells isolated from NAFLD patients in the absence or in the presence of increasing concentrations of an anti-NLGn4 antibody.

FIG. 6A shows CD107a levels in NK cells isolated from mice induced with carbon tetrachloride to cause acute hepatic fibrosis (or left untreated) and either injected with an anti-NLGn4 antibody or left untreated.

FIG. 6B shows serum ALT levels in WT mice induced with carbon tetrachloride
15 to cause acute hepatic fibrosis (or left untreated) and either injected with an anti-NLGn4 antibody or left untreated.

FIG. 6C shows alpha-SMA levels in WT mice induced with carbon tetrachloride to cause acute hepatic fibrosis (or left untreated) and either injected with an anti-NLGn4
20 antibody or left untreated.

FIG. 7 shows α -feto-protein levels in mice induced for hepatocellular carcinoma by injection of human hepatoma cells (Hep3B) co-cultured with either wt or NLGn4 ^{-/-} NK cells.

FIG. 8A shows CD107a levels in NK cells isolated from NAFLD patients and
25 incubated with a hepatic stellate cell line in the absence or in the presence of recombinant NrX1b (4nM, 10nM).

FIG. 8B shows alpha-SMA intensities (LX2 activity) in LX2 cells, incubated with NK cells isolated from NAFLD patients in the absence or in the presence of recombinant NrX1b (4nM, 10nM).

FIG. 9A shows serum ALT levels in WT mice induced with carbon tetrachloride to cause acute hepatic fibrosis (or left untreated) and either injected with a recombinant Nr_x1b or left untreated.

FIG. 9B shows alpha-SMA levels in WT mice induced with carbon tetrachloride to cause acute hepatic fibrosis (or left untreated) and either injected with recombinant Nr_x1b or left untreated.

DETAILED DESCRIPTION

In the following description, various aspects of the invention will be described. For the purpose of explanation, specific details are set forth in order to provide a thorough understanding of the invention. However, it will also be apparent to one skilled in the art that the invention may be practiced without specific details being presented herein. Furthermore, well-known features may be omitted or simplified in order not to obscure the invention.

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

The following are terms which are used throughout the description and which should be understood in accordance with the various embodiments to mean as follows:

The terms "administration" and "administering" refer to providing or giving a subject a therapeutic agent (e.g. a recombinant polypeptide), by any effective route. Exemplary routes of administration include, but are not limited to, injection or infusion (such as subcutaneous, intramuscular, intradermal, intraperitoneal, intrathecal, intravenous, intracerebroventricular, intrastriatal, intracranial and into the spinal cord), oral, intraductal, sublingual, rectal, transdermal, intranasal, vaginal and inhalation routes. Each possibility is a separate embodiment.

The terms "peptide", "polypeptide" and "protein" as used herein are intended to encompass any amino acid sequence including modified sequences, such as but not limited to modified NLGn4 and/or NrX1b. The terms "peptide" and "polypeptide" are specifically intended to cover naturally occurring proteins, as well as those, which are recombiantly or synthetically produced. "Peptides" of the invention also include modified peptides (with amino acid substitutions, both conservative and non-conservative as described below) that have the same or improved activity as a wild-type or unmodified peptide. "Salts" of the peptides of the invention contemplated by the invention are physiologically and pharmaceutically acceptable organic and inorganic salts.

Isolated polynucleotide sequences comprising at least one sequence encoding a peptide, peptide analog, conjugate or fusion protein of NLGn4 or NrX1b are also included in the scope of the present invention. According to some embodiments, the polynucleotide sequence encoding the peptide or peptide analog is translationally linked to another polynucleotide sequence such as an RNA or DNA molecule and is recombiantly expressed within target cells. According to other embodiments, the polynucleotide sequence is part of a recombinant viral or bacterial vector.

The term "analog" refers to a molecule, which has the amino acid sequence according to the invention except for one or more amino acid changes. Analogs according to the present invention may include peptidomimetics. "Peptidomimetic" refers to a peptide modified in such a way that it includes at least one non-coded residue or non-peptidic bond. Such modifications include, e.g., alkylation and more specific methylation of one or more residues, insertion of or replacement of natural amino acid by non-natural amino acids, replacement of an amide bond with another covalent bond. A peptidomimetic according to the present invention may optionally comprise at least one bond, which is an amide-replacement bond such as urea bond, carbamate bond, sulfonamide bond, hydrazine bond, or any other covalent bond. The design of appropriate "analogs" may be computer assisted. Analogs are included in the invention as long as they remain pharmaceutically acceptable.

The amino acids used in this invention are those, which are available commercially or are available by routine synthetic methods. Certain residues may require

special methods for incorporation into the peptide, and either sequential, divergent or convergent synthetic approaches to the peptide sequence are useful in this invention. Natural coded amino acids and their derivatives are represented by three-letter codes according to IUPAC conventions. When there is no indication, the L isomer was used.

5 The D isomers are indicated by "D" before the residue abbreviation.

Conservative substitutions of amino acids as known to those skilled in the art are within the scope of the present invention. Conservative amino acid substitutions include replacement of one amino acid with another having the same type of functional group or side chain, e.g., aliphatic, aromatic, positively charged, negatively charged. These
10 substitutions may enhance oral bioavailability, penetration into the islets, targeting to specific beta cell populations, immunogenicity, and the like. One of skill will recognize that individual substitutions, deletions or additions to a peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration
15 results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

- 20 1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
25 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The peptides of the present invention may be produced by any method known in the art, including recombinant and synthetic methods. Synthetic methods include

exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation, or classical solution synthesis. Solid phase peptide synthesis procedures are well known to one skilled in the art and described, for example by John Morrow Stewart and Janis Dillaha Young, Solid Phase Polypeptide Syntheses (2nd Ed., Pierce Chemical Company, 5 1984). In some embodiments, synthetic peptides are purified by preparative high performance liquid chromatography (Creighton T. (1983) Proteins, structures and molecular principles. WH Freeman and Co. N.Y.) The peptide sequence may be confirmed by amino acid sequencing using methods known to one skilled in the art.

In some embodiments, recombinant protein techniques are used to generate the 10 peptide of the present invention. In some embodiments, recombinant protein techniques are used for generation of relatively long polypeptides (typically longer than 18 amino acids) or nucleic acid sequences or viral or bacterial vectors for vaccine formulation. Recombinant techniques are described for example by Bitter et al., (1987) Methods in Enzymol. 153:516-544, Studier et al. (1990) Methods in Enzymol. 185:60-89, Brisson et 15 al. (1984) Nature 310:511-514, Takamatsu et al. (1987) EMBO J. 6:307-311, Coruzzi et al. (1984) EMBO J. 3:1671-1680 and Brogli et al., (1984) Science 224:838-843, Gurley et al. (1986) Mol. Cell. Biol. 6:559-565 and Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463.

The terms "recombinant" and "recombinant protein" refer, according to some 20 embodiments, to proteins having sequence that is not naturally occurring. The term recombinant includes proteins that have been altered by addition, substitution, or deletion of a portion of a natural nucleic acid molecule or protein. According to some embodiments, the term recombinant refers to proteins that have been altered so as to generate soluble forms of NLGn4 and/or NRx1b.

25 As used herein, the term "soluble" refers to a polypeptide that is not bound to the cell membrane. Usually, a receptor is in soluble form when its amino acid sequence lacks the transmembrane domain. In this context, a form will be soluble if using conventional assays known to one of skill in the art most of this form can be detected in fractions that are not associated with the membrane, e.g., in cellular supernatants or serum.

30 In the present invention, "Fc domain" means the constant domain of an antibody. It is preferably the Fc domain of human IgG1, but is not limited thereto. In addition to

human IgG1, IgG2, IgG3 and IgG4 may also be used, and the Fc domain may comprise. Preferably, it may comprise the CH2 domain, the CH3 domain and the hinge region, excluding the CH1 domain.

As used herein, the term "NLGn4-Fc fusion protein" refers to a product resulting from linkage of the entire or a part of the NLGn4 protein with immunoglobulin Fc region (or part thereof). As used herein, the term "rsNLGn4-Fc fusion protein" refers to a product resulting from linkage of the extracellular domain of NLGn4 protein with immunoglobulin Fc region (or part thereof). The linkage may be by enzymatic action, or resulting from expression of two polypeptides into a single polypeptide by gene manipulation. In the NLGn4-Fc and rsNLGn4-Fc fusion proteins, the NLGn4 protein (or part thereof) and the immunoglobulin Fc region may be directly linked with each other, or linked via a peptide linker, but is not limited thereto.

As used herein, the term "Nrx1b-Fc fusion protein" refers to a product resulting from linkage of the entire or a part of the Nrx1b protein with immunoglobulin Fc region (or part thereof). As used herein, the term "rsNrx1b-Fc fusion protein" refers to a product resulting from linkage of the extracellular domain of Nrx1b protein with immunoglobulin Fc region (or part thereof). The linkage may be by enzymatic action, or resulting from expression of two polypeptides into a single polypeptide by gene manipulation. In the Nrx1b-Fc and rsNrx1b fusion proteins, the Nrx1b protein (or part thereof) and the immunoglobulin Fc region may be directly linked with each other, or linked via a peptide linker, but is not limited thereto.

As used herein, the term "immunoglobulin (Ig) Fc region" refers to a part of immunoglobulin that contains the heavy-chain constant region 2 (CH2), the heavy-chain constant region 3 (CH3), and a hinge region, excluding the variable regions of the heavy and light chains, the heavy-chain constant region 1 (CH1) and the light-chain constant region 1 (CL1) of the immunoglobulin. The immunoglobulin Fc region of the present invention includes a native amino acid sequence, and a sequence derivative thereof. In addition, the immunoglobulin Fc region may be an Fc region that is derived from IgG, IgM, IgE, IgA or IgD, or that is made by combinations thereof or hybrids thereof. Preferably, it is derived from IgG, which is known to enhance the half-life of binding proteins. More preferably, it is derived from IgG1, but is not limited thereto.

The Ig combinations, as used herein, refer to polypeptides encoding single-chain immunoglobulin Fc regions of the same origin are linked to a single-chain polypeptide of a different origin to form a dimer or multimer. That is, a dimer or multimer may be formed from two or more fragments selected from the group consisting of IgG Fc, IgA
5 Fc, IgM Fc, IgD Fc, and IgE Fc fragments.

Ig hybrids, as used herein, refer to sequences encoding two or more immunoglobulin Fc regions of different origin are present in a single-chain immunoglobulin Fc region. In the present invention, various types of hybrids are possible. That is, domain hybrids may be composed of one to four domains selected from
10 the group consisting of CH1, CH2, CH3 and CH4 of IgG Fc, IgM Fc, IgA Fc, IgE Fc and IgD Fc, and may include the hinge region. On the other hand, IgG is also divided into IgG1, IgG2, IgG3 and IgG4 subclasses, and the present invention includes combinations and hybrids thereof.

The term "sequence identity/similarity" refers to the degree of similarity between
15 two or more nucleic acid sequences, or two or more amino acid sequences, and is measured as percentage identity; the higher the percentage, the more identical the sequences are. Sequence similarity can be measured in terms of percentage similarity (which takes into account conservative amino acid substitutions); the higher the percentage, the more similar the sequences are. Homologs or orthologs of nucleic acid
20 or amino acid sequences possess a relatively high degree of sequence identity/similarity when aligned using standard methods.

The terms "liver disorder", "liver disease" and "hepatic disease" are used interchangeably and refer to diseases and disorders that cause the liver to function improperly or to stop functioning.

25 The terms "subject" and "patient" are interchangeable and as used herein refer to any individual suffering from a liver disorder.

The term "treatment" as used herein refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

The terms "composition" and "pharmaceutical composition" are used interchangeably and as used herein refer to any composition comprising at least one chemical or biological agent, for example an antibody.

The term "pharmaceutically acceptable carrier" refer to any carrier conventional
5 used in the production of pharmaceutical compositions. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, Pa., 15th Edition, 1975, describes compositions and formulations suitable for pharmaceutical delivery of the compositions disclosed herein.

The terms "Neuroigin 4", "Neuroigin 4X", "NLGn4" and "NLG4" are
10 interchangeable and as used herein refer to the protein product of the NLGn4 gene e.g., NP_001269075.1, NP_001269074.1, NP_851849.1 and NP_065793.1.

The terms "anti-NLGn4 antibody", "an antibody which recognizes NLGn4", "an antibody against NLGn4" and "an antibody to NLGn4" are interchangeable, and as used
15 herein refer to an antibody that binds to the NLGn4 protein with sufficient affinity and specificity. According to some embodiments, the term refer to an antibody capable of interfering, inhibiting and/or preventing with NLGn4 binding to NrX1b.

The terms "neurexin-1-beta", "NrX1b" and "NrXn1" are interchangeable and as used herein refer to the protein product of the NrX1b gene e.g.,
NP_001129131.1 NP_004792.1 NP_620072.1.

20 The terms "anti-NrX1b antibody", "an antibody which recognizes NrX1b ", "an antibody against NrX1b " and "an antibody to NrX1b " are interchangeable, and as used herein refer to an antibody that binds to the NrX1b protein with sufficient affinity and specificity. According to some embodiments, the term refer to an antibody capable of interfering with, inhibiting and/or preventing NrX1b binding to NLGn4.

25 It should be understood that, according to the present invention, anti-human-NLGn4 antibodies and/or anti-human-NrX1b antibodies may bind to NLGn4 and/or NrX1b both in-vivo, i.e. bind NLGn4/NrX1b in a patient's NK cells, and/or bind NLGn4/NrX1b in NK cells in-vitro, i.e. bind NLGn4/NrX1b in NK cells extracted from the patient's body, the NK cells later administered to the patient.

The term "antigen" as used herein refers to a molecule or a portion of a molecule capable of eliciting antibody formation and being bound by an antibody. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding
5 antibody and not with the multitude of other antibodies, which may be evoked by other antigens. According to some embodiments, the antigen is an NLGn4 protein or a fragment thereof. According to some embodiments, the antigen is an Nrxb protein or a fragment thereof.

The term "antigenic determinant" or "epitope" as used herein refers to the region
10 of an antigen molecule that specifically reacts with a particular antibody. Peptide sequences derived from an epitope can be used, alone or in conjunction with a carrier moiety, applying methods known in the art, to immunize animals and to produce polyclonal or monoclonal antibodies. Isolated peptides derived from an epitope may be used in diagnostic methods to detect antibodies and as therapeutic agents when inhibition
15 of antibodies is required.

Antibodies, or immunoglobulins, comprise two heavy chains linked together by disulfide bonds and two light chains, each light chain being linked to a respective heavy chain by disulfide bonds in a "Y" shaped configuration. Proteolytic digestion of an antibody yields Fv (Fragment variable) and Fc (Fragment crystalline) domains. The
20 antigen binding domains, Fab, include regions where the polypeptide sequence varies. The term F(ab')₂ represents two Fab' arms linked together by disulfide bonds. The central axis of the antibody is termed the Fc fragment. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains (C_H). Each light chain has a variable domain (V_L) at one end and a constant domain (C_L) at its other end, the light
25 chain variable domain being aligned with the variable domain of the heavy chain and the light chain constant domain being aligned with the first constant domain of the heavy chain (CH1). The variable domains of each pair of light and heavy chains form the antigen-binding site. The domains on the light and heavy chains have the same general structure and each domain comprises four framework regions, whose sequences are
30 relatively conserved, joined by three hyper-variable domains known as complementarity determining regions (CDRs 1-3). These domains contribute specificity and affinity of the antigen-binding site. The isotype of the heavy chain (gamma, alpha, delta, epsilon or mu)

determines immunoglobulin class (IgG, IgA, IgD, IgE or IgM, respectively). The light chain is either of two isotypes (kappa, κ or lambda, λ) found in all antibody classes.

The term "antibody" is used in the broadest sense and includes monoclonal antibodies (including full length or intact monoclonal antibodies), polyclonal antibodies, 5 multivalent antibodies, multi-specific antibodies (e.g., bi-specific antibodies), and antibody fragments long enough to exhibit the desired biological activity.

The antibody according to the present invention is a molecule comprising at least the antigen-binding portion of an antibody. Antibody or antibodies according to the invention include intact antibodies, such as polyclonal antibodies or monoclonal 10 antibodies (mAbs), as well as proteolytic fragments thereof, such as the Fab or F(ab')₂ fragments. Single chain antibodies also fall within the scope of the present invention.

"Antibody fragments" comprise only a portion of an intact antibody, generally including an antigen-binding site of the intact antibody and thus retaining the ability to bind antigen. Examples of antibody fragments encompassed by the present definition 15 include: (i) the Fab fragment, having VL, CL, VH and CH1 domains; (ii) the Fab' fragment, which is a Fab fragment having one or more cysteine residues at the C-terminus of the CH1 domain; (iii) the Fd fragment having VH and CH1 domains; (iv) the Fd' fragment having VH and CH1 domains and one or more cysteine residues at the C-terminus of the CH1 domain; (v) the Fv fragment having the VL and VH domains of a 20 single arm of an antibody; (vi) the dAb fragment (Ward et al., Nature 1989, 341, 544-546) which consists of a VH domain; (vii) isolated CDR regions; (viii) F(ab')₂ fragments, a bivalent fragment including two Fab' fragments linked by a disulphide bridge at the hinge region; (ix) single chain antibody molecules (e.g. single chain Fv; scFv) (Bird et al., Science 1988, 242, 423-426; and Huston et al., PNAS (USA) 1988, 85,5879-5883); 25 (x) "diabodies" with two antigen binding sites, comprising a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (see, e.g., EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 1993, 90, 6444-6448); (xi) "linear antibodies" comprising a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a 30 pair of antigen binding regions (Zapata et al. Protein Eng., 1995, 8, 1057-1062; and U.S. Pat. No. 5,641,870).

Single chain antibodies can be single chain composite polypeptides having antigen binding capabilities and comprising amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain i.e. linked V_H-V_L or single chain Fv (scFv).

5 The term "neutralizing antibody" as used herein refers to a molecule having an antigen-binding site to a specific receptor or ligand target capable of reducing or inhibiting (blocking) activity or signaling through a receptor, as determined by in-vivo or in-vitro assays, as per the specification.

10 The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different
15 determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. mAbs may be obtained by methods known to those skilled in the art. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method
20 first described by Kohler et al., Nature 1975, 256, 495, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature 1991, 352, 624-628 or Marks et al., J. Mol. Biol., 1991, 222:581-597, for example.

25 The mAbs of the present invention may be of any immunoglobulin class including IgG, IgM, IgE, IgA. A hybridoma producing a mAb may be cultivated *in-vitro* or *in-vivo*. High titers of mAbs can be obtained by *in-vivo* production where cells from the individual hybridomas are injected intra-peritoneally (i.p.) into pristine-primed Balb/c mice to produce ascites fluid containing high concentrations of the desired mAbs.
30 mAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture

supernatants, using column chromatography methods well known to those of skill in the art.

The terms "human antibody" and "humanized antibody" are interchangeable and as used herein refer to an antibody that possesses an amino acid sequence, which
5 corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein.

The terms "molecule having the antigen-binding portion of an antibody" and "antigen-binding-fragments" as used herein is intended to include not only intact immunoglobulin molecules of any isotype and generated by any animal cell line or
10 microorganism, but also the antigen-binding reactive fraction thereof, including, but not limited to, the Fab fragment, the Fab' fragment, the F(ab')₂ fragment, the variable portion of the heavy and/or light chains thereof, Fab mini-antibodies (see WO 93/15210, US patent application 08/256,790, WO 96/13583, US patent application 08/817,788, WO 96/37621, US patent application 08/999,554, the entire contents of which are
15 incorporated herein by reference), dimeric bispecific mini-antibodies (see Muller et al., 1998) and single-chain antibodies incorporating such reactive fraction, as well as any other type of molecule in which such antibody reactive fraction has been physically inserted. Such molecules may be provided by any known technique, including, but not limited to, enzymatic cleavage, peptide synthesis or recombinant techniques.

The invention also provides conservative amino acid variants of the antibody
20 molecules according to the invention. Variants according to the invention also may be made that conserve the overall molecular structure of the encoded proteins. Given the properties of the individual amino acids comprising the disclosed protein products, some rational substitutions will be recognized by the skilled worker. Amino acid substitutions,
25 *i.e.* "conservative substitutions," may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

The term "antibody analog" as used herein refers to an antibody derived from another antibody by one or more conservative amino acid substitutions.

The term "antibody variant" as used herein refers to any molecule comprising the antibody of the present invention. For example, fusion proteins in which the antibody or an antigen-binding-fragment thereof is linked to another chemical entity is considered an antibody variant.

5 The phrase "capable of inhibiting or blocking the interaction" as used herein refers to the capability of an agent, for example an antibody, to interact with a at least one target, for example a cell's membrane-bound receptor, in such a way that the target becomes less accessible, preferably inaccessible, to binding by at least one other agent, for example the receptor's natural antigen.

10 Antibodies according to the invention can be obtained by administering NLGn4 or Nr1b peptides, analogs, or cells expressing these, to an animal, preferably a nonhuman, using routine protocols. For preparation of mAbs, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C.,
15 Nature 256: 495-497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); Cole et al., pg. 77-96 in Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc. (1985).

Besides the conventional method of raising antibodies in vivo, antibodies can be generated in vitro using phage display technology. Such a production of recombinant antibodies is much faster compared to conventional antibody production and they can be
20 generated against an enormous number of antigens. Moreover, affinity maturation (i.e., increasing the affinity and specificity) of recombinant antibodies is very simple and relatively fast. Finally, large numbers of different antibodies against a specific antigen can be generated in one selection procedure. To generate recombinant mAbs one can use various methods all based on display libraries to generate a large pool of antibodies with
25 different antigen recognition sites. Such a library can be made in several ways: One can generate a synthetic repertoire by cloning synthetic CDR regions in a pool of H chain germline genes and thus generating a large antibody repertoire, from which recombinant antibody fragments with various specificities can be selected. One can use the lymphocyte pool of humans as starting material for the construction of an antibody
30 library. It is possible to construct naive repertoires of human IgM antibodies and thus create a human library of large diversity.

Non-human antibodies may be humanized by any methods known in the art. In one method, the non-human CDRs are inserted into a human antibody or consensus antibody FR sequence. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity.

5 Anti-idiotypic antibodies specifically immunoreactive with an antibody of the invention are also comprehended.

Techniques for the production of single-chain antibodies can be adapted to produce single-chain antibodies to polypeptides or polynucleotides of this invention. Also, transgenic mice, or other organisms such as other mammals, can be used to express
10 humanized antibodies specific to the polypeptides or polynucleotides of the invention.

The invention also provides conservative amino acid variants of the peptides and antibody molecules according to the invention. Variants according to the invention also may be made that conserve the overall molecular structure of the encoded proteins or peptides. Given the properties of the individual amino acids comprising the disclosed
15 protein products, some rational substitutions will be recognized by the skilled worker. Amino acid substitutions, i.e. "conservative substitutions," may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

A humanized antibody, typically has a human FR grafted with non-human CDRs.
20 Thus, a humanized antibody has one or more amino acid sequence introduced into it from a source, which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-
25 327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies wherein substantially less than an intact human V domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human
30 antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human VH and VL domains to be used in making the humanized antibodies is very important for reducing immunogenicity. According to the so-called "best-fit" method, the sequence of the V domain of a rodent antibody is screened against the entire library of known human -domain sequences. The human sequence, which is closest to that of the rodent, is then accepted as the human FR for the humanized antibody (Sims et al., J. Immunol., 151:2296 (1993); Chothia et al., J. Mol. Biol., 196:901 (1987)). Another method uses a particular FR derived from the consensus sequence of all human antibodies of a particular subgroup of H or L chains. It is further important that antibodies be humanized with retention of high specificity and affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

According to some aspects, the present invention provides methods and compositions for treating liver disorders by activating attenuated natural killer (NK) cells and thereby reducing Hepatic stellate cell (HCSs) induced fibrosis and/or hepatocellular carcinoma.

According to some aspects, the present invention provides a composition for use in treating liver disorders, the composition comprising an agent capable of activating

attenuated natural killer (NK) cells, thereby reducing Hepatic stellate cell (HCSs) induced fibrosis and/or hepatocellular carcinoma.

According to some embodiments, the method includes administering to the subject in need thereof a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1β (Nrx1b) protein-protein interaction; thereby treating, attenuating and/or preventing progression of the liver disorder. According to some embodiments, the composition comprises a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1β (Nrx1b) protein-protein interaction. According to some embodiments, the composition is suitable for administration to a subject.

According to some embodiments, the agent comprises recombinant soluble NLGn4 (rsNLGn4).

According to some embodiments, the recombinant soluble human NLGn4 may function as a decoy competing with endogenous NLGn4 for binding to Nrx1b.

According to some embodiments, the recombinant NLGn4 comprises Gln42-Ser676 of NP_065793. According to some embodiments, the recombinant NLGn4 consists essentially of Gln42-Ser676 of NP_065793.

According to some embodiments, the rsNLGn4 comprises the extracellular domain of NLGn4, the extracellular domain of NLGn4 having the polypeptide sequence set forth in SEQ ID: 7.

SEQ ID NO: 7:

QAQYPVVNTNYGKIRGLRTPLPNEILGPVEQYLGVPYASPPTGERRFQPPEPP
 SSWTGIRNTTQFAAVCPQHLDERSLLHDMLPIWFTANLDTLMTYVQDQNECL
 25 YLNIYVPTEDDIHDQNSKKPVMVYIHGGSYMEGTGNMIDGSILASYGNVIVIT
 INYRLGILGFLSTGDQAAKGNYGLLDQIQALRWIEENVGAFGGDPKRVTIFGS
 GAGASCVSLTLTSHYSEGLFQKAI IQSGTALSSWAVNYQPAKYTRILADKVGC
 NMLDITDMVECLRNKNYKELIQQTITPATYHIAFGPVIDGDVIPDDPQILMEQ
 GEFLNYDIMLGVNQGEGLKFVDGIVDNEGDVTPNDFDFSVSNFVDNLYGYPEG
 30 KDTLRETIKFMYPDWADKENPETRRKTLVALFTDHQWVAPAVATADLHAQYGS

PTYFYAFYHHCQSEMKPSWADSAHGDEVVYVFGIPMIGPTELFSCNFSKNDVM
 LSAVVMTYWTNFAKTGDPNQVPVQDTKFIHTKPNRFEEVAWSKYNPQDQLYLH
 IGLKPRVRDHYRATKVAFWLELVPHLHNLNEIFQYVSTTTKVPPDMTSFPYG
 TRRSPAKIWPTTKRPAITPANNPKHSDPHKTGPEDTTVLIETKRDYSTELS

5 According to some embodiments, the rsNLGn4 consists of SEQ ID NO: 7, or a fragment, derivative or analog thereof. According to some embodiments, the rsNLGn4 has at least 70%, at least 80%, at least 90%, at least 95% or at least 98% sequence similarity to SEQ ID NO: 7. Each possibility is separate embodiment.

10 According to some embodiments, the rsNLGn4 is devoid of the intracellular domain of NLGn4 set forth in SEQ ID NO: 8:

SEQ ID NO: 8:

YKKDKRRHETHRRPSPQRNTTNDIAHIQNEEIMSLQMKQLEHDHECESLQAHD
 TLRLTCLPPDYTLTLRRSPDDIPLMTPNTITMIPTLTLTGMQPLHTFNTFSGGQN
 STNLPHGHSTTRV

15 According to some embodiments, the rsNLGn4 is devoid of the transmembrane domain of NLGn4 set forth in SEQ ID NO: 9:

SEQ ID NO: 9:

VTIAVGASLLFLNILAFAALY

20 According to some embodiments, the agent comprises recombinant soluble NrX1b (rsNrX1b).

 According to some embodiments, the recombinant NrX1b comprises Ala51-Ser363 of NP_620072. According to some embodiments, the recombinant NrX1b consists essentially of Ala51-Ser363 of NP_620072.

25 According to some embodiments, recombinant soluble human NrX1b may function as a decoy competing with endogenous NrX1b for binding to NLGn4.

According to some embodiments, the rsNrx1b comprises the extracellular domain of Nrx1b, the extracellular domain of Nrx1b having the polypeptide sequence set forth in SEQ ID: 10.

SEQ ID NO: 10:

5 ASSLGAHHIHHFHGSSKHHSVP IAIYRSPASLRGGHAGTTYI FSKGGGQITYK
 WPPNDRPSTRADRLAIGFSTVQKEAVLVRVDSSSGLGDYLELHIHQGKIGVKF
 NVGTDDIAIEESNAI INDGKYHVVRFTRSGGNATLQVDSWPVIERYPAGRQLT
 IFNSQATII IGGKEQGQPFQGLSGLYYNGLKVLNMAAENDANIAIVGNVRLV
 GEVPSMTTESTATAMQSEMSTS IMETTTTLATSTARRGKPPTKEPISQTTDD
 10 ILVASAECPSDDEDIDPCEPSSGGLANPTRAGGREPYPGSAEVIRESS

According to some embodiments, the rsNrx1b consists of SEQ ID NO: 10, or a fragment, derivative or analog thereof. According to some embodiments, the rsNrx1b has at least 70%, at least 80%, at least 90%, at least 95% or at least 98% sequence similarity to SEQ ID NO: 10. Each possibility is separate embodiment.

15 According to some embodiments, the rsNrx1b is devoid of the intracellular domain of Nrx1b set forth in SEQ ID NO: 11:

SEQ ID NO: 11:

MYKYRNRDEGSYHVDESRNYI SNSAQSN GAVVKEKQPSSAKSSNKKNKKNKDKE
 YYV

20 According to some embodiments, the rsNLGn4 is devoid of the transmembrane domain of NLGn4 set forth in SEQ ID NO: 12:

SEQ ID NO: 12:

STTGMVVGIVAAAALCILILLYA

25 According to some embodiments, the agent comprises a combination of recombinant soluble NLGn4 and recombinant soluble Nrx1b (rsNrx1b).

According to some embodiments, the liver disorder is selected from the group consisting of: non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis

(NASH), cirrhosis, hepatitis, liver adenoma, insulin hypersensitivity, liver cancer and any combination thereof.

According to some embodiments, of the agent comprises an anti-NLGn4 antibody.

5 According to some embodiments, administering a therapeutically effective amount of the agent includes contacting an immune cell, such as an NK cell and/or a CD56^{dim} NK cell subset, with the therapeutically effective amount of the agent.

According to some embodiments, the antibody is a monoclonal antibody. According to some embodiments, the antibody is a humanized antibody. According to
10 some embodiments, the antibody does not cross the blood-brain barrier (BBB).

According to some embodiments, the antibody is an anti-NLGn4 monoclonal antibody or a fragment, derivative or analog thereof.

According to some embodiments, the polypeptide sequence of the NLGn4 is set forth in SEQ ID NO: 1:

15 MSRPQGLLWLPPLLETPVCVMLNSNVLLWLTALAIKFTLIDSQAQYPVNTNYGKIRGL
RTPLPNEILGPVEQYLGVPYASPTGERRFQPPEPPSSWTGIRNTTQFAAVCPQHLDE
RSLLDMLPIWFTANLDTLMTYVQDQNEDECLYLNIYVPTEDDIHDQNSKKPVMVYIHG
GSYMEGTGNMIDGSI LASYGNVIVITINRYRLGILGFLSTGDQAAKGNYGLLDQIQALR
WIEENVGAFGGDPKRVTIFGSGAGASCVSLTSLSHYSEGLFQKAI IQSGTALSSWAVN
20 YQPAKYTRILADKVGCMMLDTTDMVECLRKNKYKELIQQTITPATYHIAFGPVIDGDV
IPDDPQIILMEQGEFLNYDIMLGVNQGEGLKFVDGIVDNEGDVTPNDFDFSVSNFVDNL
YGYPEGKDTLRET IKFMYTDWADKENPETRRKTLVALFTDHQWVAPAVATADLHAQYG
SPTYFYAFYHHCQSEMKPSWADSAHGDEVYVFGIPMIGPTELFSCNFSKNDVMLSAV
VMTYWTNFAKTGDPNPQVPQDTKFIHTKPNRFEEVAWSKYNPKDQLYLHIGLKPRVRD
25 HYRATKVAFWLELVPHLHNLNEIFQYVSTTTKVPPDMTSFPYGTRRSPAKIWPTTKR
PAITPANNPKHSDPHKTGPEDTTVLIETKRDYSTELESVTIAVGASLLFLNILAFAAL
YYKKDKRRHETHRRPSPQRNTTNDIAHIQNEEIMSLQMKQLEHDHECESLQAHDTLRL
TCPPDYTLTLRRSPDDIPLMTPNTITMIPNTLTGMQPLHTFNTFSGGQNSTNLPHGHS
30 TTRV

According to some embodiments, the NLGn4 monoclonal antibody, or a fragment, derivative or analog thereof is capable of binding to the interaction domain of NLGn4, mediating the interaction between NLGn4 and NrX1b.

According to some embodiments, the anti-NLGn4 monoclonal antibody or a fragment, derivative or analog thereof is capable of binding to amino acids 359-364 of the human NLGN4 protein as set forth in SEQ ID NO: 2 (QGEFLN).

5 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid E361 of human NLGn4.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid L363 of human NLGn4.

10 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 261-270, as set forth in SEQ ID NO: 3 (SLLTLSHYSE), of human NLGn4.

15 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid H267 of human NLGn4.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 461-470, as set forth in SEQ ID NO: 4 (AQYGSPTYFY), of human NLGn4.

20 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid Y463 of human NLGn4.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope within residues 265-275, as set forth in SEQ ID NO: 5 (LSHYSEGLFQK), of human NLGn4.

25 According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising the amino acid E270 of human NLGn4.

According to some embodiments, the NLGn4 antibody, or a fragment, derivative or analog thereof is capable of binding to an epitope comprising an amino acid selected

from the group consisting of E361, L363, H267, Y463, E270 or any combination thereof. Each possibility is a separate embodiment.

According to some embodiments, the antibody is an anti-Nrx1b antibody or a fragment, derivative or analog thereof. According to some embodiments, the Nrx1b antibody, or a fragment, derivative or analog thereof is capable of binding to the interaction domain of Nrx1b, mediating the interaction between NLGn4 to Nrx1b.

According to some embodiments, the polypeptide sequence of the Nrx1b is set forth in SEQ ID NO: 6:

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10  MGTALLQRGGCFLLCLSLLLLGCWAEELGSGLEFPGAEGQWTRFPKWNACCESEMSFQL
    KTRSARGLVLYFDDEGFCDLELILTRGGRLQLSFSIFCAEPATLLADTPVNDGAWHS
    VRIRRQFRNTTLFIDQVEAKWVEVKSKRRDMTVFSGLFVGGGLPPELRAAALKLTLASV
    REREPFKGWIRDVRVNSSQVLPVDSGEVKLDDEPPNSGGGSPCEAGEEGEGGVCLNGG
    VCSVVDDQAVCDCSRGTGFRGKDCSQEIKFGLQCVLVLLHDNDQGKYCCINTAKPLTE
15  KDNNVEGLAHLMMGDQGKSKGKEYIATFKGSEYFCYDLSQNP IQSSSDEITLSFKTL
    QRNGLMLHTGKSADYVNLALKNGAVSLVINLGSGAFEALVEPVNGKFNDNAWHDVKVT
    RNLRQHSGIGHAMVNKLHCSVTISVDGILTTTGYTQEDYTMLGSDDFFYVGGSPSTAD
    LPGSPVSNFMGCLKEVVYKNNDVRLELSRLAKQGDPMKIHGVAFAKENVATLDPIT
    TFETPESFISLPKWNAKKTGSISFDFRTTEPNGLILFESHGKPRHQKDAKHPQMIKVDF
20  FAIEMLDGHLVLLDMGSGTIKIKALLKKVNDGEWYHVDFQRDGRSGTISVNTLRTPY
    TAPGESEILDLDDELYLGGLPENKAGLVFPTEVWVTALLNYGYVGCIRDLFIDGQSKDI
    RQMAEVQSTAGVKPSCSKETAKPCLSNPCKNNGMCRDGNRYVCDCSGTGYLGRSCER
    EATVLSYDGSFMFKIQLPVVMHTEAEDVSLRFRSQRAYGILMATTSRDSADTLRLELD
    AGRVKLTVNLDCIRINCNSKGPETLFAGYNLNDNEWHTVRVVRGKSLKLTVDQQA
    MTGQMAGDHTRLEFHNIETGIITERRYLSSVPSNFIHGLQSLTFNGMAYIDLCKNGDI
25  DYCELNARFGFRNI IADPVTFKTKSSYVALATLQAYTSMHLFFQFKTSLDGLILYNS
    GDGNDFIVVELVKGYLHYVFDLNGANLIKGSSENKPLNDNQWHNVMSRDTSNLHTVK
    IDTKITTTQITAGARNLDLKSPLYIGGVAKETYKSLPKLVHAKEGFQGCCLASVDLNGRL
    PDLISDALFCNGQIERGCEGPSTTCQEDSCSNQGVCLQQWDGFSCDCSMTSFSGPLCN
    DPGTTYIFSKGGGQITYKWPPNDRPSTRADRLAIGFSTVQKEAVLVRVDSSSGLGDYL
30  ELHIHQGKIGVKFNVGTDDIAIEESNAIINDGKYHVVRFTRSGGNATLQVDSWPVIER
    YPAGNNDNERLAIARQRIPIYRLGRVVDEWLLDKGRQLTIFNSQATIIIGGKEQGQPFQ
    GQLSGLYNGLKVLNMAAENDANIAIVGNVRLVGEVPSMTTESTATAMQSEMSTSIM
    ETTTTLATSTARRGKPPTKEPISQTTDDILVASAECPSDDEDIDPCEPSSGGLANPTR
    AGGREPYPGSAEVIRESSTTGMVGVIVAAAALCILILLYAMYKYRNRDEGSYHVDES
35  RNYISNSAQSNQAVVKEKQPSAKSSNKNKKNKDKEYYV
    
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According to some embodiments, the anti-Nrx1b monoclonal antibody or a fragment, derivative or analog thereof is capable of binding to amino acids 103-109 as set forth in SEQ ID NO: 7 (LLADTPV) and/or amino acids 234-239 as set forth in SEQ ID NO: 8 (CSVVDD) of the human Nrx1b protein. Each possibility is a separate embodiment of the invention.

According to some embodiments, the liver disorders is Non-alcoholic fatty liver disease (NAFLD), and Non-alcoholic steatohepatitis (NASH). Additionally or alternatively as the liver disorder is cirrhosis, hepatitis, liver adenoma, insulin resistance, liver cancer, or any NK related inflammatory or neoplastic disorder well. The clinical
5 implications of NAFLD are derived mostly from its potential to progress to Non-alcoholic steatohepatitis, cirrhosis and liver failure. In accordance, the invention, addresses the long felt need to attenuate the progression of NAFLD into cirrhosis and liver failure by inhibiting, attenuating and/or preventing NLGn4-Nrx1b protein-protein interaction and thereby modulating the cytotoxic activity of NK cells. According to some
10 embodiments, the invention provides a method for modulating the activity of a natural killer (NK) cell.

According to some embodiments, the NK cells are liver NK cells, which are attenuated in patients having a liver disorder. According to yet another embodiment, the liver disorder is characterized by overexpression of NLGn4 protein. Such overexpression
15 can attenuate NK cell activity.

According to some embodiments and without being bound to any theory, reducing NLGn4 protein levels and/or interfering with the binding of NLGn4 to Nrx1b, modulates the function of the NK cell for example by activating the NK cell and/or the CD56^{dim} NK cell subset. As a result of NK activation, the activity of hepatic stellate cells
20 (HSCs) and hence fibrosis is reduced. In addition, and according to yet another embodiment, modulating and/or activating the NK cells increases the apoptosis of the HSCs.

According to yet another embodiment there is provided a method for modulating the activity of a natural killer (NK) cell and/or treating, preventing and/or attenuating a
25 liver disorder by administering to a patient a composition comprising an anti-NLGn4 antibody and/or an anti-Nrx1b antibody.

According to some embodiments, the composition may further include a GLUT4 antagonist. Such antagonist can according to the present invention inhibit GLUT4 mediated NLGn4 expression. The antagonist can be selected from the group comprising
30 Ketamine, Amantadine, Phencyclidine, Nitrous oxide, Dextromethorphan (and

dextrorphan), Memantine, Ethanol, Riluzole (used in ALS), Xenon, HU-211 (also a cannabinoid), Lead (Pb²⁺), Conantokins, and Huperzine A.

Without being bound to any theory, reducing NLGn4 protein levels and/or inhibiting NLGn4-Nrx1b binding can, according to the present invention, enhance the cytotoxicity of the NK cells and/or specific NK cell subpopulations. According to certain
5 embodiments, and without being bound to any theory, enhancing the cytotoxicity comprises enhancing the expression of CD107a (marker of NK cell functional activity) on said NK cell.

In certain liver disorders, NK cell function can be attenuated. According to the
10 present invention and without being bound by any theory, such attenuation can be a result of NLGn4 overexpression. In accordance, reducing NLGn4 protein levels modulates and/or activates the function of attenuated NK cell. In turn, activating the NK cell may reduce HSC activity and/or increase their apoptosis.

As further demonstrated herein below, it has now surprisingly been shown that
15 administration of anti-NLGn4 antibodies, increases NK cell activity and attenuates HSC activation, both *in-vitro* and *in-vivo*. These results show that anti-NLGn4 antibodies can serve as therapeutically agents in the treatment of liver disorders such as but not limited to NAFLD and preventing its progression to cirrhosis.

According to some embodiments, there is provided a composition comprising a
20 therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein-protein interaction and a pharmaceutically acceptable carrier.

According to some embodiments, the recombinant NLGn4 comprises Gln42-Ser676 of NP_065793. According to some embodiments, the recombinant NLGn4
25 consists essentially of Gln42-Ser676 of NP_065793.

According to some embodiments, the agent comprises recombinant soluble NLGn4 (rsNLGn4); wherein the rsNLGn4 comprises the extracellular domain of NLGn4 or a fragment, derivative or analog thereof.

According to some embodiments, the extracellular domain of NLGn4 consists of SEQ ID NO: 7. According to some embodiments, the rsNLGn4 consists of SEQ ID NO: 7, or a fragment, derivative or analog thereof.

According to some embodiments, the rsNLGn4 competes with endogenous
5 NLGn4 for binding to NrX1b.

According to some embodiments, the rsNLGn4 is devoid of the intracellular domain and/or the transmembrane domain of NLGn4.

According to some embodiments, the agent comprises a fusion protein comprising rsNLGn4 and an immunoglobulin molecule. According to some
10 embodiments, the agent comprises a fusion protein comprising rsNLGn4 and an Fc domain of an immunoglobulin molecule. According to some embodiments, the Fc domain may include part or the entire CH1 domain, CH2 domain, CH3 domain and hinge region of IgG1, IgG2, IgG3 and IgG4. According to some embodiments, the Fc domain may be devoid of the CH1 domain. According to some embodiments, the Fc domain
15 comprises Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain consists essentially of Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain may be linked to rsNLGn4 through a linker. According to some embodiments, the linker may have the amino acid sequence set forth in SEQ ID NO: 13 (IEGRMD).

According to some embodiments, the agent is a chimeric protein formed from
20 NLGn4 polypeptides or fragments fused with a second polypeptide to form a soluble NLGn4. According to some embodiments, the agent comprises a DNA sequences, which combine two partial DNA sequences, one sequence encoding soluble fragments of NLGn4 (i.e. the DNA sequence encoding the extracellular domain of NLGn4) and the
25 other partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE. These DNA sequences may subsequently be expressed in target cells using expression vectors as known in the art, thereby obtaining endogenous expression of recombinant proteins having the extracellular domain of NLGn4 joined to the Fc fragment of an
30 immunoglobulin molecule.

According to some embodiments, the NLGn4 is encoded by the sequence set forth in SEQ ID NO: 1.

According to some embodiments, the recombinant NrX1b comprises Ala51-Ser363 of NP_620072. According to some embodiments, the recombinant NrX1b
5 consists essentially of Ala51-Ser363 of NP_620072.

According to some embodiments, the agent comprises recombinant soluble NrX1b (rsNrX1b); wherein the rsNrX1b comprises the extracellular domain of NrX1b or a fragment, derivative or analog thereof capable of binding NLGn4.

According to some embodiments, the extracellular domain of NrX1b consists of
10 SEQ ID NO: 10. According to some embodiments, the rsNrX1b consists of SEQ ID NO: 10, or a fragment, derivative or analog thereof.

According to some embodiments, the rsNrX1b competes with endogenous NrX1b for binding to NLGn4.

According to some embodiments, the rsNrX1b is devoid of the intracellular
15 domain and/or the transmembrane domain of NrX1b.

According to some embodiments, NrX1b is encoded by the sequence set forth in SEQ ID NO: 6.

According to some embodiments, the agent comprises a fusion protein comprising rsNrX1b and an immunoglobulin molecule. According to some embodiments,
20 the agent comprises a fusion protein comprising rsNrX1b and an Fc domain of an immunoglobulin molecule, also referred to herein as rsNrX1b-Fc fusion protein. According to some embodiments, the Fc domain may include part or the entire CH1 domain, the CH2 domain, the CH3 domain and hinge region of IgG1, IgG2, IgG3 and IgG4. According to some embodiments, the Fc domain may be devoid of the CH1
25 domain. According to some embodiments, the Fc domain may include all domains of the Fc domain apart from CH1. According to some embodiments, the Fc domain comprises Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain consists essentially of Pro100-Lys330 of Human IgG1. According to some embodiments, the Fc domain may be linked to rsNrX1b through a linker. According to some

embodiments, the linker may have the amino acid sequence set forth in SEQ ID NO: 13 (IEGRMD).

According to some embodiments, the agent is a chimeric protein formed from NrX1b polypeptides or fragments fused with a second polypeptide to form a soluble NrX1.

5 According to some embodiments, the agent comprises a DNA sequences, which combine two partial DNA sequences, one sequence encoding soluble fragments of NrX1b (i.e. the DNA sequence encoding the extracellular domain of NrX1b) and the other partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulin IgG, IgA, IgM, or IgE. These DNA sequences may
10 subsequently be expressed in target cells using expression vectors as known in the art, thereby obtaining endogenous expression of recombinant proteins having the extracellular domain of NrX1b joined to the Fc fragment of an immunoglobulin molecule.

According to some embodiments, the agent comprises a combination of recombinant soluble NLGn4 and recombinant soluble NrX1b (rsNrX1b).

15 The following examples are presented to provide a more complete understanding of the invention. The specific techniques, conditions, materials, proportions and reported data set forth to illustrate the principles of the invention are exemplary and should not be construed as limiting the scope of the invention.

20 **EXAMPLES**

Example 1: NLGn4 gene is over-expressed in NASH patients with hepatic fibrosis.

Heparinized-blood samples of healthy volunteers and NAFLD patients and/or cirrhotic cases were obtained. Mononuclear cells were isolated by centrifugation over ficoll-hypaque (Pharmacia). After three washes in saline, cells were resuspended in
25 medium of Roswell Park Memorial Institute 1640 with 10% FBS. Human NK cells were isolated from Peripheral blood lymphocytes (PBLs) using a magnetic cell sorting kit (Miltenyi Biotec) according to manufacturer's instructions. RNA was extracted, converted to cDNA and hybridized onto a spotted microarray.

As seen in FIG. 2, a 4-fold increase in NLGn4 gene expression on NASH NK
30 cells was obtained (in the zone of down-and up-expressed genes with a cutoff p-value of

0.015 showed), suggesting a correlation between NLGn4 expression levels and NAFLD/NASH in humans.

Example 2: Leptin-deficient mice (ob/ob) (fatty liver disease model) show increased NLGn4 expression on liver NK cells

5 RNA was extracted from Leptin deficient ob/ob mice (fatty liver disease mouse model), converted into cDNA and the expression level of NLGn4 analyzed by RT-PCR using NLGn4 specific primers. As seen in FIG. 3, the ob/ob mice showed a 4-fold increase in mRNA NLG4 expressions as compared to WT c57bl mice, serving as control. This result shows a correlation between NLGn4 overexpression and fatty liver disease in
10 mice.

Example 3: Homozygous NLG4^{-/-} (KO) mice show decreased inflammation and fibrosis

Naïve Wild Type (WT) and homozygote NLG4^{-/-} mice were induced for fibrosis by i.p injections of carbon tetrachloride 2X/week for 6 weeks and evaluated for serum
15 levels of Alanine transaminase (ALT - biomarker for liver health). As seen from Figure 4A, induction of fibrosis, by carbon tetrachloride injection, was significantly attenuated in the NLG4^{-/-} KO mice as compared to WT. Furthermore, as shown in FIG. 4B, alpha-SMA (marker of HSC activation) levels were lower in the NLG4^{-/-} mice compared to WT, as evaluated by quantitative western blot analysis.

20 These results show the involvement of NLGn4 in HSC activity and in the induction of fibrosis.

Example 4: Incubation of NK cells with anti-NLGn4 antibodies increases NK cell activation and reduces HSC activation in an *in-vitro* assay.

NK cells were isolated form peripheral blood derived from NAFLD patients with
25 advanced fibrosis scoring (histology documentation of F3, F4). Patients without medications and lacking full metabolic syndrome were included. Isolated NK cells were incubated with 4, 8, 10 or 12 μ M of anti-NLG4 (human NLGN4 Antibody, Antigen Affinity-purified Polyclonal Sheep IgG, R&D Systems Catalog # AF5158) for 3 hours prior to incubation with the myofibroblasts LX2- cell line (HSCs) o left untreated

(0). Following 24 hours of NK-LX2 co-culture, harvested cells were analyzed by flow-cytometry for NK NLG4 and CD107a expressions as well as LX2 alpha-SMA intensity.

Incubation of the NK cells with an anti-NLG4 antibody significantly inhibited NLGn4 protein levels (FIG. 5A, $P<0.03$) and increased CD107a expression on the NK
5 cells (FIG. 5B, $P<0.01$) in all tested concentrations. These results show that incubation of NK cells with anti-NLGn4 antibodies increase NK cell activity *in-vitro*.

The increased NK activity was accompanied with increased LX2 killing; as alpha-SMA mean fluorescence intensities decreased with the increase in anti-NLG4 concentrations (Figure 5C, $P<0.01$). This shows that incubation of NK cells with anti-
10 NLGn4 antibodies attenuates HSC activity *in-vitro*.

Example 5: Incubation with anti-NLGn4 antibodies increases NK cell activation and reduces HSC activation in an *in-vivo* assay in mice.

25 WT naïve C57/BL mice (12 weeks) mice were either induced for acute hepatic fibrosis by i.p injections of carbon tetrachloride (3X/week) or left untreated (induced
15 n=10, control n=10). One day after the last injection, the mice were either injected i.p. with NLGn4-antibodies (anti-human NLGN4 Antibody, Antigen Affinity-purified Polyclonal Sheep IgG, R&D Systems Catalog # AF5158) at concentrations of 82 mg/mice or left untreated (5 mice of each of the induced and control mice). 24 hours after injection with the NLGn4 antibodies, the mice were sacrificed and sera were collected
20 for detection of CD107a expression (marker of NK activity), ALT levels biomarker for liver health and livers were harvested for assessment of alpha-SMA expression (marker of HSC activation) using an RT PCR assay.

As seen in FIG. 6A mice induced for acute liver fibrosis that were exposed to the NLGn4 antibody had increased CD107a expression (marker of NK activity), a lesser
25 degree of liver injuries and fibrosis as evidenced by reduced serum ALT levels ($P=0.03$) (FIG. 6B) and alpha-SMC expression (FIG. 6C).

These results show that treatment with anti-NLGn4 antibodies reduces liver injuries and fibrosis *in-vivo* as well as *in-vitro* and show that anti-NLGn4 antibodies can serve as therapeutic agents in the treatment of liver disorders such as but not limited to
30 NAFLD and for preventing progression to cirrhosis.

Example 6: mice induced for hepatocellular carcinoma by injection of human hepatoma cells (Hep3B) co-cultured with NLGn4 -/- NK cells show reduced levels of the cancer marker α -feto-protein

Human hepatoma cells (Hep3B) co-cultured for 24h with either wt NK cells (n=4) or NLGn4 -/- NK cells (n=4) were injected subcutaneously (2×10^6) into C57BI6 mice (13 week old). The mice were subsequently irradiated (5Gry) to induce tumor growth. Subsequently, plasma alpha-fetoprotein (AFP) levels were evaluated. AFP is a biomarker of liver cancers and levels above 500 nanograms/milliliter of AFP in adults is indicative of hepatocellular carcinoma, and metastatic cancers of the liver. As seen from Figure 7, AFP levels were significantly lowered in mice injected with Hep3B cells incubated with NLG4-/- NK cells as compared to mice injected with Hep3B cells incubated with wt NK cells.

These results show the involvement of NLGn4 in NK cell attenuation and hepatoma formation and demonstrate that reducing NLGn4 protein levels inhibit tumor growth.

Example 7: Incubation of NK cells with recombinant Nrxb increases NK cell activation and reduces HSC activation in an *in-vitro* assay.

NK cells were isolated from peripheral blood derived from NAFLD patients with advanced fibrosis scoring (histology documentation of F3, F4). Patients without medications and lacking full metabolic syndrome were included. Isolated NK cells were incubated with 4nM or 10nM recombinant Nrxb-Fc Chimera (Recombinant Human Neurexin 1 β /NXRN1b Fc Chimera, R&D Systems Catalog Number: 5268-NX) including amino acids Ala51-Ser363 of Nrxb (NP_620072) and Pro100-Lys330 of Human IgG1 linked by linker sequence IEGRMD, for 3 hours prior to the NK cells being co-cultured with the hepatic stellate cell line LX2. Following 24 hours of co-culture, harvested cells were analyzed by flow-cytometry for NK CD107a expression as well as LX2 alpha-SMA intensity.

Incubation of the NK cells with recombinant Nrxb significantly increased CD107a expression on the NK cells (FIG. 8A) at both concentrations. These results show that incubation of NK cells with recombinant Nrxb increases NK cell activity *in-vitro*.

The increased NK activity was accompanied with increased LX2 killing; as alpha-SMA mean fluorescence intensities decreased with the increase in NrX1b concentrations (Figure 8B). This shows that co-culturing HSC with NK cells pre-incubated with recombinant NrX1b attenuate HSC activity *in-vitro*.

5 **Example 8: Incubation with recombinant NrX1b increases NK cell activation and reduces HSC activation in an *in-vivo* assay.**

25 WT naïve C57/BL mice (12 weeks) mice were either induced for acute hepatic fibrosis by i.p. injections of carbon tetrachloride (CCl₄ - 3X/week) or left untreated (induced n=12, control n=12). Either one day after the last injection (D9) or the day after
10 each injection (D1, D3 and D7), the mice were injected i.p. with 0.2µg recombinant NrX1b /g body weight or left untreated (3 mice of each of the induced and control mice). 24 hours after the last injection with the recombinant NrX1b-Fc Chimera (Recombinant Human Neurexin 1β/NXRN1b Fc Chimera, R&D Systems Catalog Number: 5268-NX) including amino acids Ala51-Ser363 of NrX1b (NP_620072) and Pro100-Lys330 of
15 Human IgG1 linked by linker sequence IEGRMD, the mice were sacrificed and sera were collected for detection of ALT levels and livers were harvested for assessment of alpha-SMA expression using an RT PCR assay.

As seen in FIG. 9A and 9B mice induced for acute liver fibrosis that were exposed to the recombinant NrX1b had a lesser degree of liver injuries and fibrosis as evidenced
20 by reduced serum ALT levels (FIG. 9A) and reduced alpha-SMC expression (FIG. 9B).

These results show that treatment with recombinant NrX1b reduces liver injuries and fibrosis *in-vivo* as well as *in-vitro* and show that recombinant NrX1b can serve as therapeutic agents in the treatment of liver disorders such as but not limited to NAFLD and for preventing progression to cirrhosis.

25

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such
30 adaptations and modifications should and are intended to be comprehended within the

meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.

5

CLAIMS

1. A composition comprising a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein-protein interaction and a pharmaceutically acceptable carrier for use in treating, attenuating and/or preventing progression of a liver disorder.
5
2. The composition of claim 1, wherein the agent comprises recombinant soluble NLGn4 (rsNLGn4); wherein the rsNLGn4 comprises the extracellular domain of NLGn4 or a fragment, derivative or analog thereof.
- 10 3. The composition of claim 2, wherein the extracellular domain of NLGn4 consists of SEQ ID NO: 7.
4. The composition of claim 2; wherein the rsNLGn4 consists of SEQ ID NO: 7, or a fragment, derivative or analog thereof.
- 15 5. The composition of claim 2, wherein the rsNLGn4 competes with endogenous NLGn4 for binding to Nrx1b.
6. The composition of claim 2, wherein the rsNLGn4 is devoid of the intracellular domain and/or the transmembrane domain of NLGn4.
7. The composition of claim 1, wherein the agent comprises recombinant soluble Nrx1b (rsNrx1b); wherein the rsNrx1b comprises the extracellular domain of Nrx1b or a fragment, derivative or analog thereof capable of binding the NLGn4.
20
8. The composition of claim 7, wherein the extracellular domain of Nrx1b consists of SEQ ID NO: 10.
9. The composition of claim 7, wherein the rsNrx1b consists of SEQ ID NO: 10, or a fragment, derivative or analog thereof.
- 25 10. The composition of claim 7, wherein the rsNrx1b competes with endogenous Nrx1b for binding to NLGn4.

11. The composition of claim 7, wherein the rsNrx1b is devoid of the intracellular domain and/or the transmembrane domain of Nrx1b.
12. The composition of claim 1, wherein NLGn4 is encoded by the sequence set forth in SEQ ID NO: 1.
- 5 13. The composition of claim 1, wherein Nrx1b is encoded by the sequence set forth in SEQ ID NO: 6.
14. The composition of claim 1, a fusion protein comprising the Fc portion of an immunoglobulin molecule and SEQ ID NO: 7 or a fragment thereof.
15. The composition of claim 1, wherein the agent comprises a fusion protein
10 comprising the Fc portion of an immunoglobulin molecule and SEQ ID NO: 10 or a fragment thereof.
16. The composition of claim 1, wherein the therapeutic agent comprises an anti-NLGn4 antibody, or a fragment, derivative or analog thereof, comprising at least the antigen-binding portion thereof; wherein the anti-NLGn4 antibody is capable
15 of binding specifically to an interaction domain of human NLGn4.
17. The composition of claim 16, wherein the anti-NLGn4 antibody is capable of binding specifically to amino acids 359-364 of the human NLGN4 protein.
18. The composition of claim 16, wherein the anti-NLGn4 antibody is capable of binding to an epitope within a sequence selected from the group of: residues 261-
20 270 of the human NLGn4, as set forth in SEQ ID NO: 3 (SLLTLSHYSE); residues 265-275, as set forth in SEQ ID NO: 5 (LSHYSEGLFQK), of the human NLGn4; and residues 461-470 of the human NLGn4, as set forth in SEQ ID NO: 4 (AQYGSPTYFY).
19. The composition of claim 1, wherein the liver disorder is selected from the group
25 consisting of: non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, hepatitis, liver adenoma, insulin hypersensitivity, liver cancer and any combination thereof.
20. The composition of claim 19, wherein the liver disorder is NAFLD.

21. The composition of claim 19, wherein the liver disorder is hepatocellular carcinoma.
22. A composition comprising a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4) – Neurexin 1 β (Nrx1b) protein-protein interaction and a pharmaceutically acceptable carrier wherein the therapeutic agent comprises an anti-NLGn4 antibody, or a fragment, derivative or analog thereof, comprising at least the antigen binding portion thereof.
23. The composition of claim 22, wherein the anti-NLGn4 antibody is capable of binding specifically to amino acids 359-364 of the human NLGN4 protein.
24. The composition of claim 22, wherein the anti-NLGn4 antibody is capable of binding to an epitope within a sequence selected from the group of: residues 261-270 of the human NLGn4, as set forth in SEQ ID NO: 3 (SLLTLSHYSE); residues 265-275, as set forth in SEQ ID NO: 5 (LSHYSEGLFQK), of the human NLGn4; and residues 461-470 of the human NLGn4, as set forth in SEQ ID NO: 4 (AQYGSPTYFY).

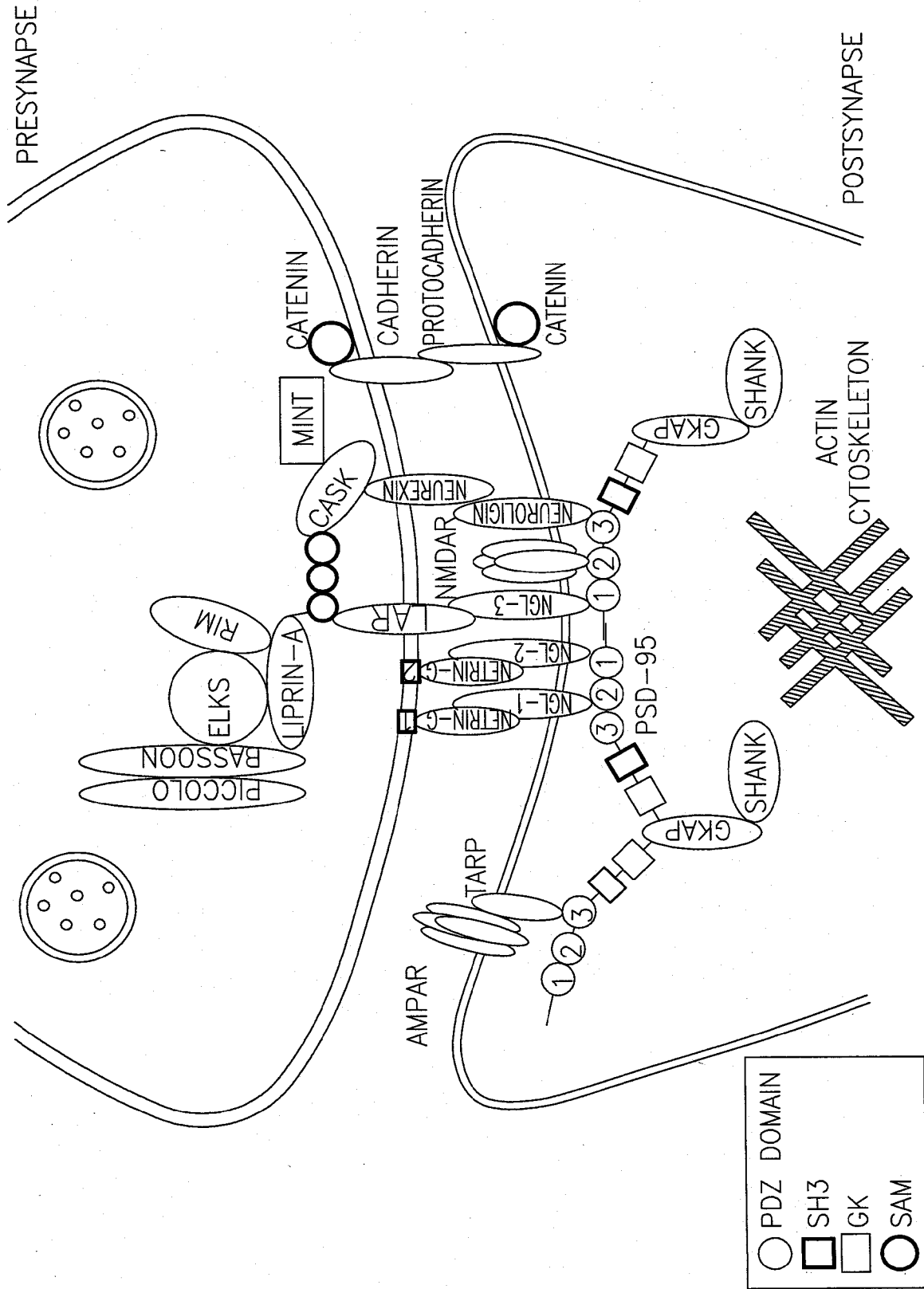


FIG.1

2/7

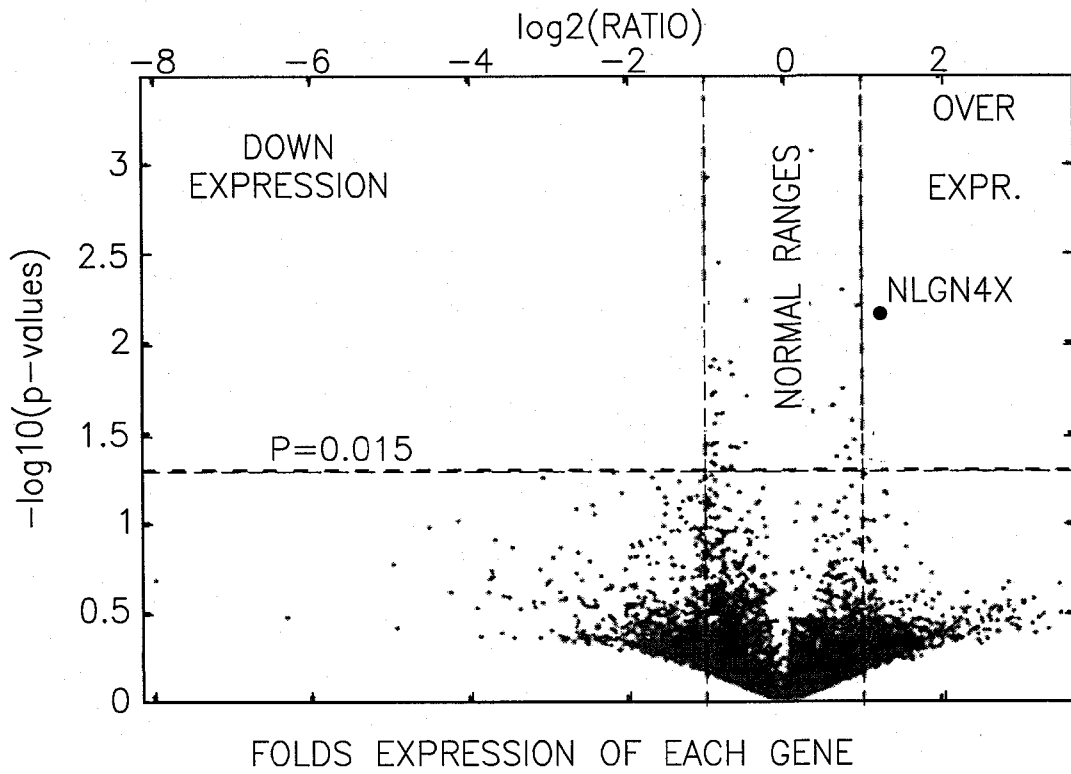


FIG.2

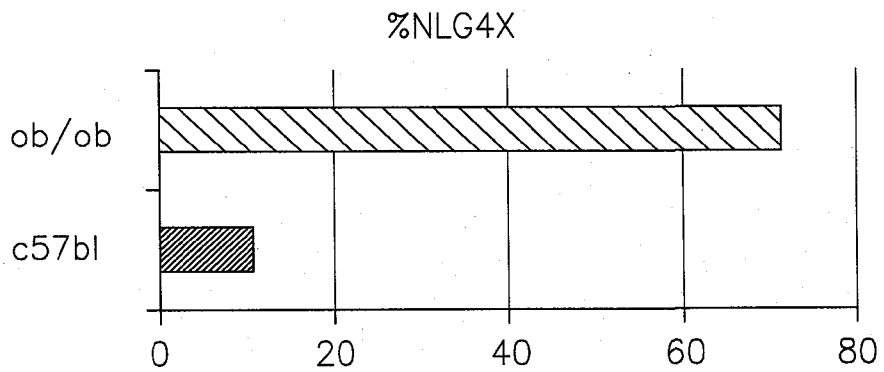


FIG.3

3/7

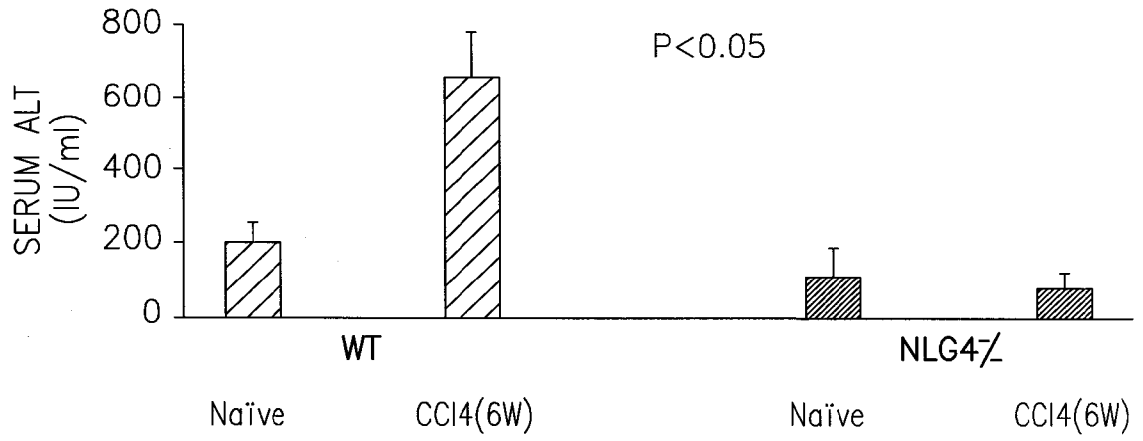


FIG.4A

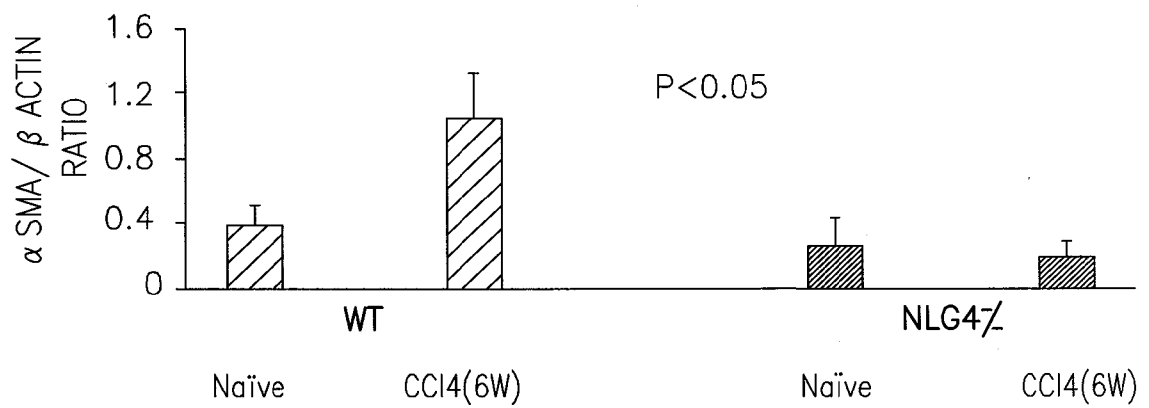


FIG.4B

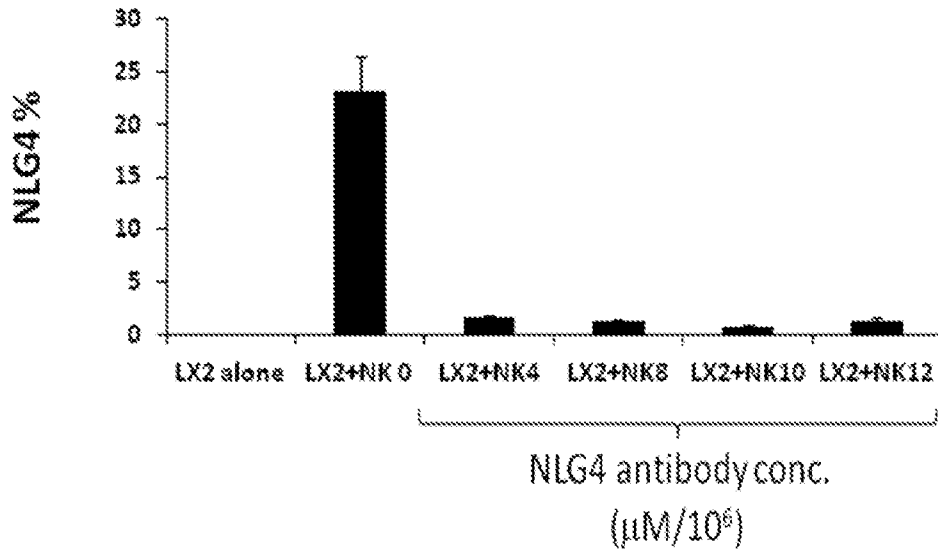


FIG. 5A

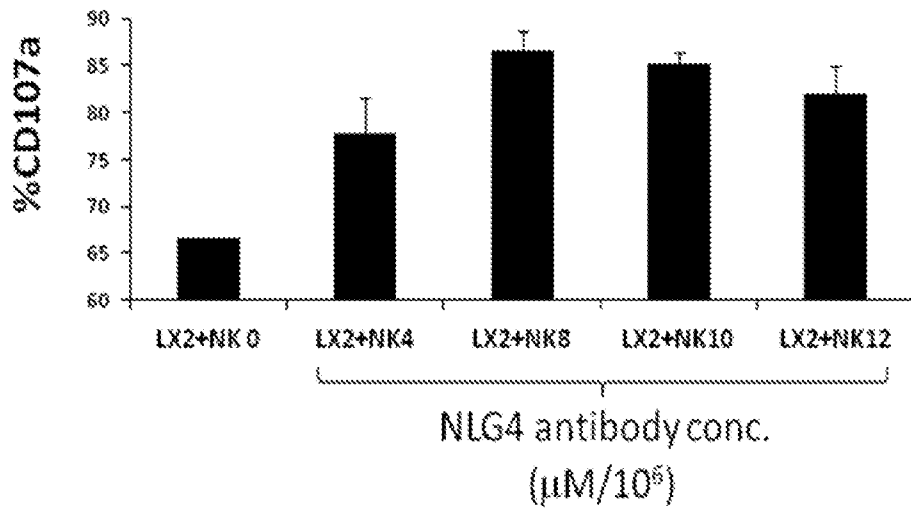


FIG. 5B

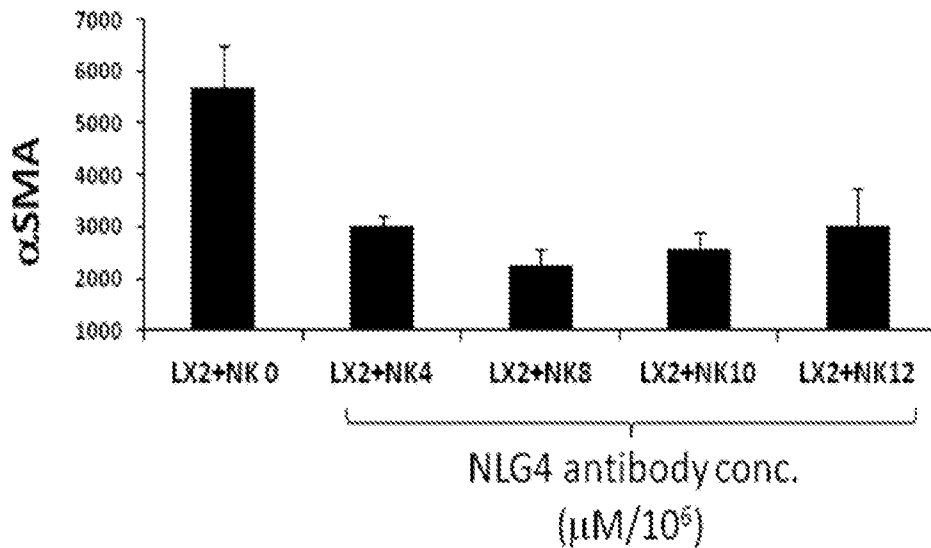


FIG. 5C

FIG 6A

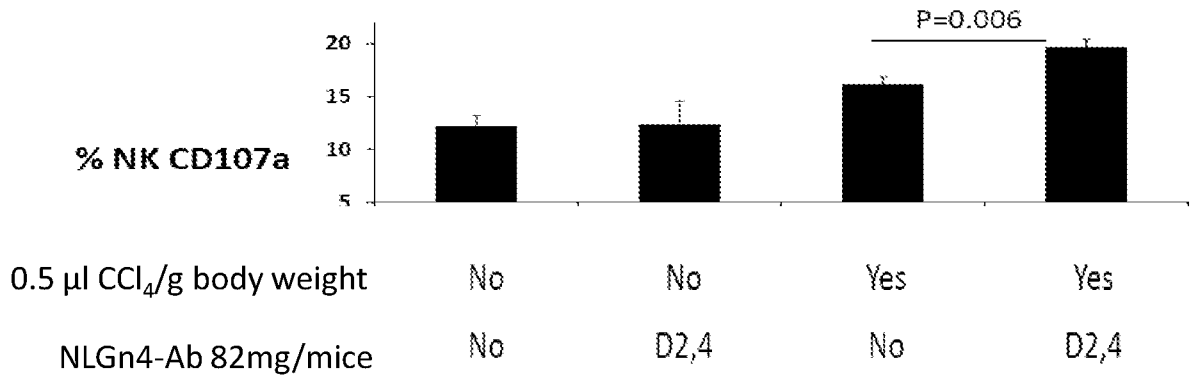


FIG 6B

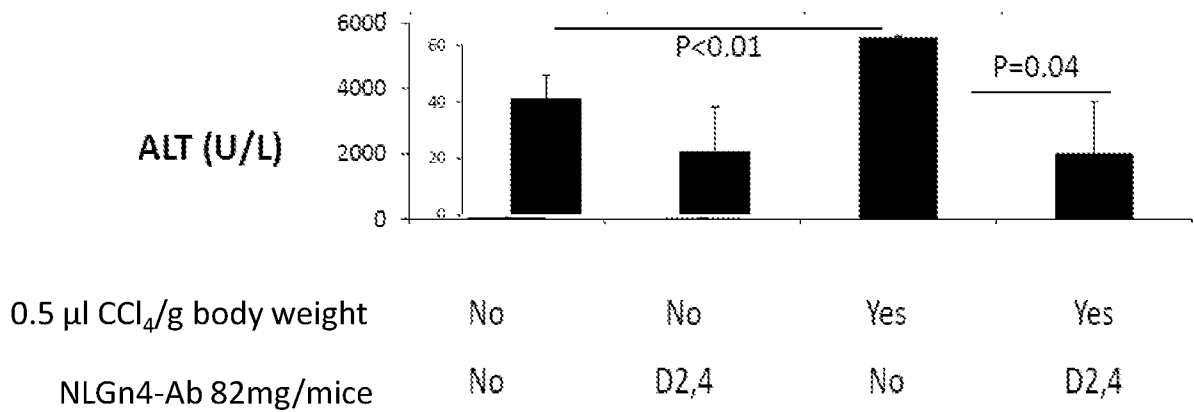


FIG 6C

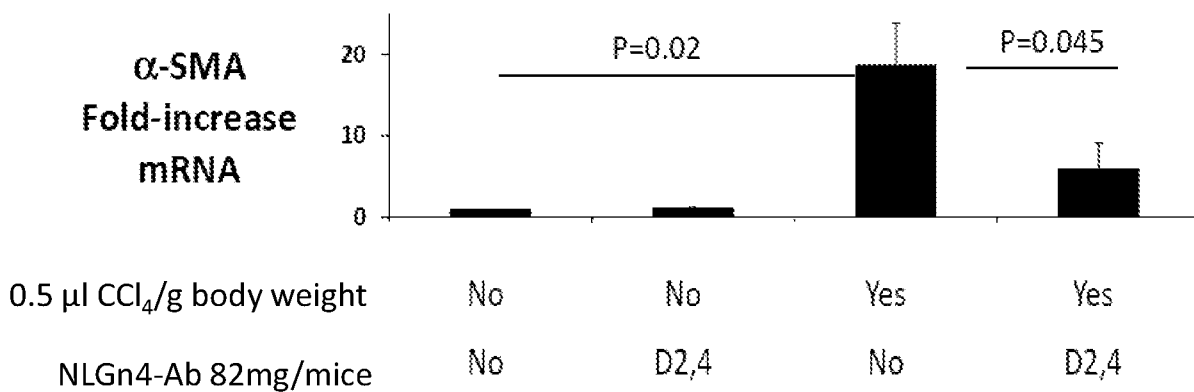


FIG 7

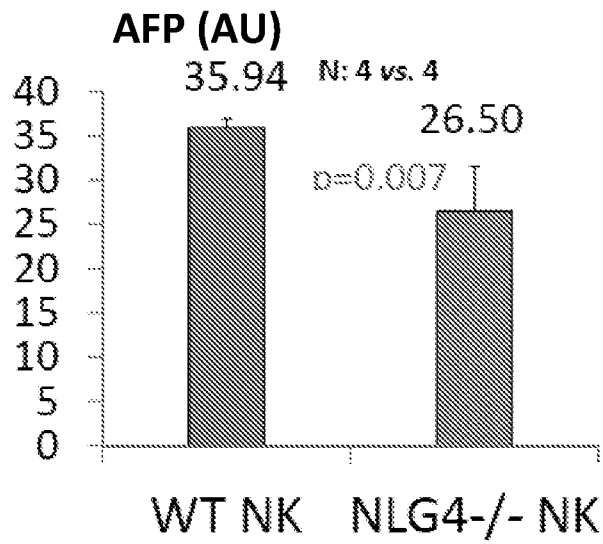


FIG. 8A

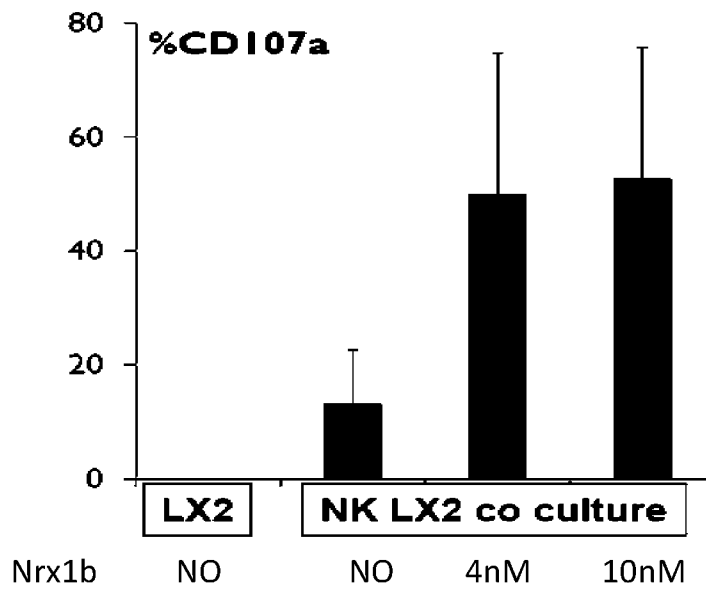


FIG. 8B

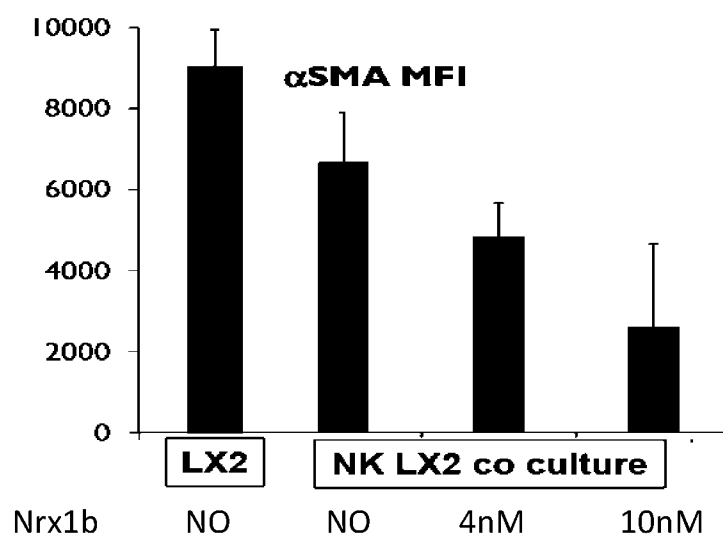


FIG 9A

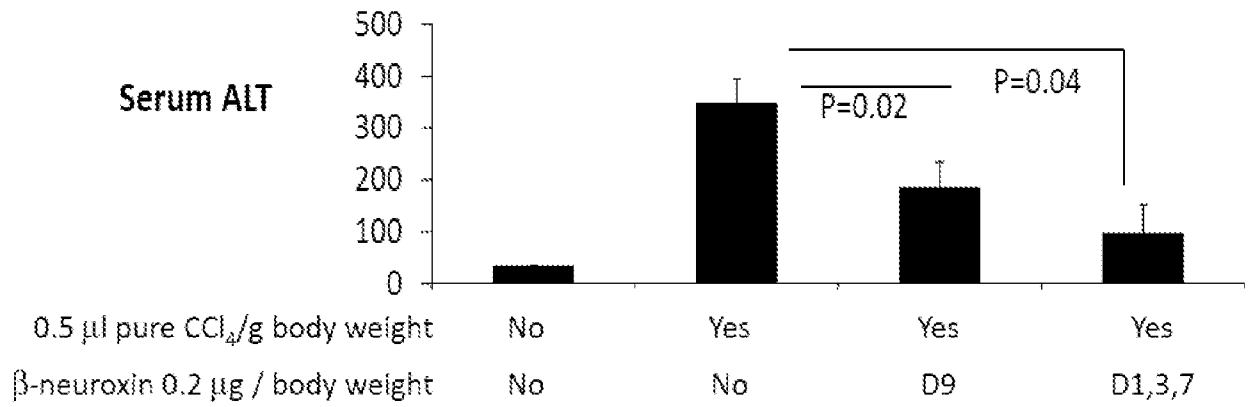
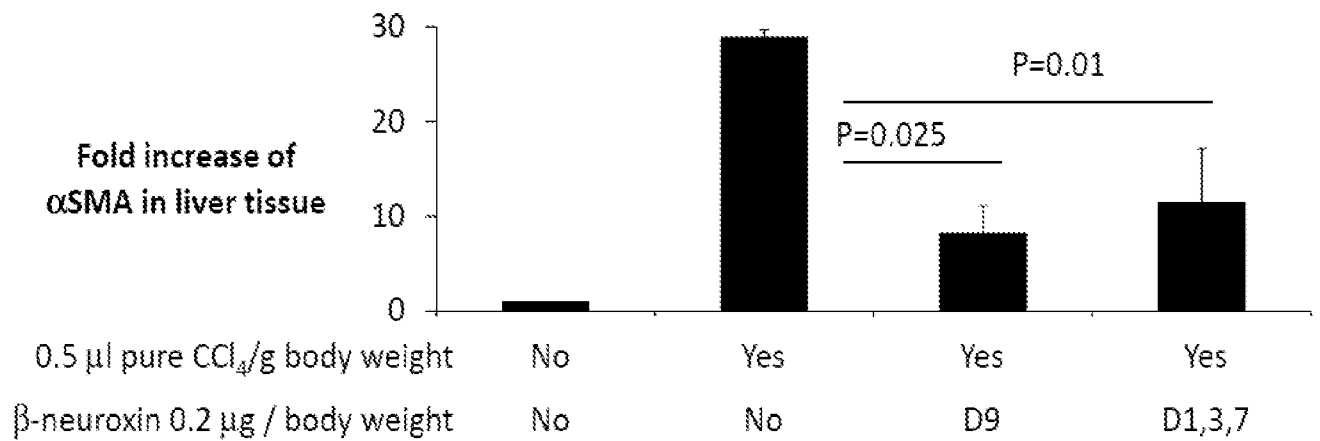


FIG 9B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2016/050351

A. CLASSIFICATION OF SUBJECT MATTER IPC (2016.01) A61K 38/17, A61K 39/395, A61P 1/16		
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) IPC (2016.01) A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Databases consulted: NCBI, THOMSON INNOVATION, CAPLUS, BIOSIS, EMBASE, MEDLINE, REGISTRY, Google Scholar Search terms used: neuroligin-4, neuroligin 4X, NLG4, LGn4, NLGn4X, neurexin-1-beta, Nrx1b, Nrxn1, liver, NAFLD, NASH, Hepatocellular, Hepatic, natural killer, NK		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Amer, J., A. Salhab, and R. Safadi. "P0502: Recombinant neuroligin 4 (NLG4) activates NK cells and attenuates fibrogenesis in nonalcoholic fatty liver disease." <i>Journal of Hepatology</i> 62 (2015): S503. DOI: 10.1016/S0168-8278(15)30710-8 30 Apr 2015 (2015/04/30) abstract	1-15,19-21
X	Abu-Tair, L., et al. "1102 Neuroligin-4 receptor silencing increased human natural killer activity and decreased hepatic stellate cells activation." <i>Journal of Hepatology</i> 58 (2013): S450. DOI: 10.1016/S0168-8278(13)61103-4 30 Apr 2013 (2013/04/30) abstract	1-17,19-22
Y		18,23,24
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> 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	"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	
"E" earlier application or patent but published on or after the international filing date	"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	
"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)	"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	
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 21 Jun 2016	Date of mailing of the international search report 21 Jun 2016	
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Authorized officer HOROWITZ Anat Telephone No. 972-2-5651689	

Box No. 1 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 13ter.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 13ter.1(a)).

on paper or in the form of an image file (Rule 13ter.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:

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:

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.:

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/IL2016/050351

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>Leone, Philippe, et al. "Structural insights into the exquisite selectivity of neurexin/neurologin synaptic interactions." The EMBO journal 29.14 (2010): 2461-2471. DOI 10.1038/emboj.2010.123 Retrieved from the internet: <http://onlinelibrary.wiley.com/doi/10.1038/emboj.2010.123/epdf></p> <p>11 Jun 2010 (2010/06/11) page 2462 right column last paragraph, figure 1A</p>	18,23,24
A	<p>Amer, J., et al. "O155 Homozygous NLG4 (KO) mice showed a decreased inflammatory and fibrotic profile and is associated with NK cells increased killing activity." Journal of Hepatology 60.1 (2014): S64. DOI: 10.1016/S0168-8278(14)60157-4 (A)</p> <p>30 Apr 2014 (2014/04/30) abstract</p>	1-24

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:

Invention/s 1	A composition comprising a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4)-Neurexin 1 beta (Nrx1b) protein-protein interaction and a pharmaceutically acceptable carrier for use in treating, attenuating and/ or preventing progression of a liver disorder, wherein the agent comprises recombinant soluble NLGn4 (rsNLGn4).	Claim/s 1-6,12,14,19-21
Invention/s 2	A composition comprising a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4)-Neurexin 1 beta (Nrx1b) protein-protein interaction and a pharmaceutically acceptable carrier for use in treating, attenuating and/ or preventing progression of a liver disorder, wherein the agent comprises recombinant soluble Nrx1b (rsNrx1b).	Claim/s 1,7-11,13,15,19-21
Invention/s 3	A composition comprising a therapeutically effective amount of an agent capable of interfering with, inhibiting and/or preventing neuroligin 4 (NLGn4)-Neurexin 1 beta (Nrx1b) protein-protein interaction and a pharmaceutically acceptable carrier for use in treating, attenuating and/ or preventing progression of a liver disorder, wherein the agent comprises an anti-NLGn4 antibody, or a fragment, derivative or analog thereof, comprising at least the antigen binding portion thereof.	Claim/s 1,16-24