Form P.8

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

(To be lodged in duplicate)

PUBLICATION PARTICULARS AND ABSTRACT
(Section 32(3)(a) - Regulations 22(1)(g) and 31)

REFERENCE : P31183ZA00

OFFICIAL APPLICATION NO.	LODGING DATE	ACCEPTANCE DATE
21 01 2005/04522	22/23 02 June 2005	43 8.2.06

INTERNATIONAL CLASSIFICATION

51 C07K

NOT FOR PUBLICATION

CLASSIFIED BY :

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EARLIEST PRIORITY CLAIMED	COUNTRY	NUMBER	DATE
NOTE : The country must be indicated by its International Abbreviation - see Schedule 4 of the Regulations.	33 GB	31 0228832.2	32 10 December 2002

TITLE OF INVENTION

54 ANTIBODY ("11C7") ANTI NOGO AND ITS PHARMACEUTICAL USE

57 ABSTRACT (NOT MORE THAN 150 WORDS)

NUMBER OF PAGES 138

FOR ABSTRACT SEE THE NEXT SHEET

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 June 2004 (24.06.2004)

PCT

(10) International Publication Number
WO 2004/052932 A2

(51) International Patent Classification[7]: C07K 16/00

(21) International Application Number: PCT/EP2003/013960

(22) International Filing Date: 9 December 2003 (09.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0228832.2 10 December 2002 (10.12.2002) GB

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LT, LU, LV, MA, MD, MK, MN, MX, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SE, SG, SK, SY, TJ, TM, TN, TR, TT, UA, US, UZ, VC, VN, YU, ZA, ZW.

(84) Designated States (*regional*): Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOGO A BINDING MOLECULES AND PHARMACEUTICAL USE THEREOF

(57) Abstract: This invention relates to molecules, such as for example monoclonal antibodies or Fab fragments thereof, which are capable of binding to the human NogoA polypeptide or human NiG or human NiG- or human NogoA_623-640 with a dissociation constant < 1000nM; polynucleotides encoding such a binding molecule; an expression vector comprising such polynucleotides; the use of such a binding molecule in the treatment of nerve repair; a pharmaceutical composition comprising such a binding molecule; and to a method of treatment of diseases associated with nerve repair.

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Nogo A Binding Molecules And Pharmaceutical Use Thereof

This invention relates to NogoA binding molecules, such as for example monoclonal antibodies or Fab fragments thereof.

Neuronal regeneration following injury in the adult central nervous system (CNS) is limited due to the presence of the inhibitory myelin environment that ensheaths axons and formation of scar tissue. In the last few years important insights have been gained into the molecular understanding why the CNS is unable to spontaneously repair itself following injury. Inhibitory molecules in the myelin are the major impediment for the axonal regeneration, particularly immediately after the injury. So far NogoA, Myelin-Associated Glycoprotein (MAG) and myelin-oligodendrocyte glycoprotein (OMgp) have been characterised as potent inhibitors of neurite outgrowth. In addition, myelin also contains other inhibitory components, such as, chondroitin sulphate proteoglycans. Nogo-A is a member of the reticulon protein family and it has at least two biologically active and pharmacologically distinct domains termed Amino-Nogo and Nogo-66. While the receptor site for the former is not known so far, Nogo-66 inhibits neuronal growth in vitro and in vivo via the neuronal receptor NgR. In addition to Nogo-66, MAG and OMgp also bind to the NgR with high affinity and inhibit neurite outgrowth.

Potential new research approaches currently pursued for enhancement of nerve repair include digestion of scar tissue using an enzyme chondroitinase ABC, bridging techniques using Olfactory ensheathing cells and stem cells and protein growth factors to boost neuronal growth. Blocking actions of neurite outgrowth inhibitors by modulation of intracellular signalling mediators such as Rho , a membrane-bound guanosine trisphosphatase (GTPase), which appears to be a key link in the inhibition of axonal growth. Cyclic adenosine monophosphate (cAMP) which can overcome myelin associated inhibition in vitro and induce regeneration in vivo. Use of peptide inhibitor of the NgR receptor (NEP 1-40) to induce neuronal regrowth and functional recovery in rats following spinal injury.

In addition to the use of the approaches described above, attention has also focused upon the use of certain monoclonal antibodies to neutralize neurite growth inhibitory molecules of the central and peripheral nervous system, in particular to neutralize the neurite growth inhibitory activity of NogoA. Thus it has been shown that the monoclonal antibody IN-1 or the

IN-1 Fab fragment thereof induce neurite outgrowth in vitro and enhance sprouting and regeneration in vivo (Schwab ME et al. (1996) Physiol. Rev. 76, 319-370). Testing different domains of the NogoA for neurite growth inhibitory acitvity have delineated several inhibitory domains in the molecule (Chen et al. (2000) Nature 403, 434-439; GrandPre eta l. (2000) Nature 403, 439-444; Prinjha et al. (2000) Nature 403, 383-384; see also detailed analysis in Example 1).

Natural immunoglobulins or antibodies comprise a generally Y- shaped multimeric molecule having an antigen-binding site at the end of each upper arm. The remainder of the structure, in particular the stem of the Y mediates effector functions associated with the immunoglobulins. Antibodies consists of a 2 heavy and 2 light chains. Both heavy and light chains comprise a variable domain and a constant part. An antigen binding site consists of the variable domain of a heavy chain associated with the variable domain of a light chain. The variable domains of the heavy and light chains have the same general structure. More particularly, the antigen binding characteristics of an antibody are essentially determined by 3 specific regions in the variable domain of the heavy and light chains which are called hypervariable regions or complementarity determining regions (CDRs). These 3 hypervariable regions alternate with 4 framework regions (FRs) whose sequences are relatively conserved and which are not directly involved in binding. The CDRs form loops and are held in close proximity by the framework regions which largely adopt a β-sheet conformation. The CDRs of a heavy chain together with the CDRs of the associated light chain essentially constitute the antigen binding site of the antibody molecule. The determination as to what constitutes an FR or a CDR region is usually made by comparing the amino acid sequence of a number of antibodies raised in the same species. The general rules for identifying the CDR and FR regions are general knowledge of a man skilled in the art and can for example be found in the webside (http://www.bioinf.org.uk/abs/).

It has now surprisingly been found that a novel monoclonal mouse antibody (hereinafter called "11C7") raised against a polypeptide fragment of rat NogoA (SEQ ID NO: 1) and of the IgG1 type has better properties than the NogoA antibodies of the prior art especially with regard to the binding affinity to NogoA of different species including the homo sapiens and with regard to its higher NogoA neurite outgrowth neutralizing activity at a given antibody concentration. Moreover it is now possible to construct other NogoA binding molecules having the same hypervariable regions as the said antibody.

Accordingly, the invention provides binding molecules to a particular region or epitope of NogoA (hereinafter referred to as "the Binding Molecules of the invention" or simply "Binding Molecules"). Preferably the Binding Molecules of the invention bind to human NogoA_623-640 (orthologous fragment against which 11C7 was raised; = SEQ ID NO: 6), human Nig-D20 (orthologous to the smallest fragment of NogoA with neurite outgrowth inhibitory activity, SEQ ID NO: 24), human NogoA (SEQ ID NO: 5) or human NiG (which is the most potent neurite outgrowth inhibitory fragment of NogoA and starts at amino acid No. 186 and ends at amino acid No. 1004 of human NogoA, = SEQ ID NO: 5) with a dissociation constant (Kd) < 1000nM, more preferably with a Kd < 100 nM, most preferably with a Kd < 10 nM. The binding reaction may be shown by standard methods (qualitative assays) including, for example, the ELISA method described in Example 6 and the biosensor affinity method described in the example 7. In addition, the binding to human NogoA and almost more importantly the efficiency may be shown in a neurite outgrowth assay, e.g. as described below.

Thus, in a further preferred embodiment the Binding Molecules (at a concentration of 1 mg/ml, more preferably at 0.1 mg/ml even more preferably at 0.01 mg/ml culture medium) enhance the number of neurites of rat cerebellar granule cells on a substrate of rat spinal cord protein extract by at least 20%, preferably 50%, most preferred 100% compared to the number of neurites of rat cerebellar granule cells which are treated with a control antibody that does not bind to the human NogoA, human NiG, human Nig-D20 or NogoA_623-640 polypeptide (i.e. that has a dissociation constant > 1000 nM).

In a further preferred embodiment the Binding Molecules of the invention comprises at least one antigen binding site, said antigen binding site comprising in sequence, the hypervariable regions CDR1-11C7, CDR2-11C7 and CDR3-11C7; said CDR1-11C7 having the amino acid sequence SEQ ID NO: 8, said CDR2-11C7 having the amino acid sequence SEQ ID NO: 9, and said CDR3-11C7 having the amino acid sequence SEQ ID NO: 10; and direct equivalents thereof.

In a further aspect of the invention, the Binding Molecule of the invention comprises at least one antigen binding site, said antigen binding site comprising either

a) in sequence the hypervariable regions CDR1-11C7, CDR2-11C7 and CDR3-11C7; said CDR1-11C7 having the amino acid sequence of SEQ ID NO: 8, said CDR2-11C7 having the amino acid sequence of SEQ ID NO: 9, and said CDR3-11C7 having the amino acid sequence SEQ ID NO: 10; or
b) in sequence the hypervariable regions CDR1'-11C7, CDR2'-11C7 and CDR3'-11C7, said CDR1'-11C7 having the amino acid sequence of SEQ ID NO: 11, said CDR2'-11C7 having the amino acid sequence of SEQ ID NO: 12, and said CDR3'-11C7 having the amino acid sequence of SEQ ID NO: 13; or
c) direct equivalents thereof.

In a further aspect of the invention, the Binding Molecule of the invention comprises at least
a) a first domain comprising in sequence the hypervariable regions CDR1-11C7, CDR2-11C7 and CDR3-11C7; said CDR1-11C7 having the amino acid sequence of SEQ ID NO: 8, said CDR2-11C7 having the amino acid sequence of SEQ ID NO: 9, and said CDR3-11C7 having the amino acid sequence SEQ ID NO: 10; and
b) a second domain comprising in sequence the hypervariable regions CDR1'-11C7, CDR2'-11C7 and CDR3'-11C7, said CDR1'-11C7 having the amino acid sequence of SEQ ID NO: 11, said CDR2'-11C7 having the amino acid sequence of SEQ ID NO: 12, and said CDR3'-11C7 having the amino acid sequence of SEQ ID NO: 13; or
c) direct equivalents thereof.

Moreover, the invention also provides the following Binding Molecule of the invention, which comprises at least one antigen binding site comprising
a) either the variable part of the heavy chain of 11C7 (SEQ ID NO: 2); or
b) the variable part of the light chain of 11C7 (SEQ ID NO: 3), or direct equivalents thereof.

When the antigen binding site comprises both the first and second domains, these may be located on the same polypeptide molecule or, preferably, each domain may be on a different chain, the first domain being part of an immunoglobulin heavy chain or fragment thereof and the second domain being part of an immunoglobulin light chain or fragment thereof.

Examples of Binding Molecules of the invention include antibodies as produced by B-cells or hybridomas and chimeric or humanized antibodies or any fragment thereof, e.g. $F(ab')_2$; and Fab fragments, as well as single chain or single domain antibodies.

A single chain antibody consists of the variable domains of an antibody heavy and light chains covalently bound by a peptide linker usually consisting of from 10 to 30 amino acids, preferably from 15 to 25 amino acids. Therefore, such a structure does not include the constant part of the heavy and light chains and it is believed that the small peptide spacer should be less antigenic than a whole constant part. By "chimeric antibody" is meant an antibody in which the constant regions of heavy or light chains or both are of human origin while the variable domains of both heavy and light chains are of non- human (e.g. murine) origin. By "humanized antibody" is meant an antibody in which the hypervariable regions (CDRs) are of non-human (e.g. murine) origin, while all or substantially all the other parts of the immunoglobulin e.g. the constant regions and the highly conserved parts of the variable domains, i.e. the framework regions, are of human origin. A humanized antibody may however retain a few amino acids of the murine sequence in the parts of the framework regions adjacent to the hypervariable regions.

Hypervariable regions may be associated with any kind of framework regions, preferably of murine or human origin. Suitable framework regions are described in "Sequences of proteins of immunological interest", Kabat E.A. et al, US department of health and human services, Public health service, National Institute of Health. Preferably the constant part of a human heavy chain of the Binding Molecules may be of the IgG4 type, including subtypes, preferably the constant part of a human light chain may be of the κ or λ type, more preferably of the κ type.

Monoclonal antibodies raised against a protein naturally found in all humans may be developed in a non-human system e. g. in mice. As a direct consequence of this, a xenogenic antibody as produced by a hybridoma, when administered to humans, elicits an undesirable immune response, which is predominantly mediated by the constant part of the xenogenic immunoglobulin. This clearly limits the use of such antibodies as they cannot be administered over a prolonged period of time. Therefore it is particularly preferred to use single chain, single domain, chimeric or humanized antibodies which are not likely to elicit a substantial allogenic response when administered to humans.

In view of the foregoing, a more preferred Binding Molecule of the invention is selected from a chimeric antibody, which comprises at least

a) one immunoglobulin heavy chain or fragment thereof which comprises (i) a variable domain comprising in sequence the hypervariable regions CDR1-11C7, CDR2-11C7 and CDR3-11C7 and (ii) the constant part or fragment thereof of a human heavy chain; said CDR1-11C7 having the amino acid sequence (SEQ ID NO: 8), said CDR2-11C7 having the amino acid sequence (SEQ ID NO: 9), and said CDR3-11C7 having the amino acid sequence (SEQ ID NO: 10), and

b) one immunoglobulin light chain or fragment thereof which comprises (i) a variable domain comprising in sequence the hypervariable regions CDR1'-11C7, CDR2'-11C7 and CDR3'-11C7 and (ii) the constant part or fragment thereof of a human light chain; said CDR1'-11C7 having the amino acid sequence (SEQ ID NO: 11), said CDR2'-11C7 having the amino acid sequence (SEQ ID NO: 12), and said CDR3'-11C7 having the amino acid sequence (SEQ ID NO: 13); or

direct equivalents thereof.

Alternatively, a Binding Molecule of the invention may be selected from a single chain binding molecule which comprises an antigen binding site comprising

a) a first domain comprising in sequence the hypervariable CDR1-11C7, CDR2-11C7 and CDR3-11C7; said CDR1-11C7 having the amino acid sequence (SEQ ID NO: 8), said CDR2-11C7 having the amino acid sequence (SEQ ID NO: 9), and said CDR3-11C7 having the amino acid sequence (SEQ ID NO: 10); and

b) a second domain comprising in sequence the hypervariable CDR1'-11C7, CDR2'-11C7 and CDR3'-11C7; said CDR1'-11C7 having the amino acid sequence (SEQ ID NO: 11), said CDR2'-11C7 having the amino acid sequence (SEQ ID NO: 12), and said CDR3'-11C7 having the amino acid sequence (SEQ ID NO: 13); and

c) a peptide linker which is bound either to the N- terminal extremity of the first domain and to the C-terminal extremity of the second domain or to the C-terminal extremity of the first domain and to the N-terminal extremity of second domain;

or direct equivalents thereof.

As it is well known, minor changes in an amino acid sequence such as deletion, addition or substitution of one or several amino acids may lead to an allelic form of the original protein which has substantially identical properties. Thus, by the term "direct equivalents thereof" is meant either any single domain Binding Molecule of the invention (molecule X)

(i) in which each of the hypervariable regions CDR1, CDR2, and CDR3 of the Binding Molecule is at least 50 or 80% homologous, preferably at least 90% homologous, more preferably at least 95, 96, 97, 98, 99% homologous to the equivalent hypervariable regions of CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9) and CDR3-11C7 (SEQ ID NO: 10), whereas CDR1 is equivalent to CDR1-11C7, CDR2 is equivalent to CDR2-11C7, CDR3 is equivalent to CDR3-11C7; and

(ii) which is capable of binding to the human NogoA, human NiG, human NiG-D20, or human NogoA_623-640, preferably with a dissociation constant (Kd) < 1000nM, more preferably with a Kd < 100 nM, most preferably with a Kd < 10 nM, or

any binding molecule of the invention having at least two domains per binding site (molecule X')

(iii) in which each of the hypervariable regions CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is at least 50 or 80% homologous, preferably at least 90% homologous, more preferably at least 95, 96, 97, 98, 99% identical to the equivalent hypervariable regions of CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9), CDR3-11C7 (SEQ ID NO: 10), CDR1'-11C7 (SEQ ID NO: 11), CDR2'-11C7 (SEQ ID NO: 12), and CDR3'-11C7 (SEQ ID NO: 13), whereas CDR1 is equivalent to CDR1-11C7, CDR2 is equivalent to CDR2-11C7, CDR3 is equivalent to CDR3-11C7, CDR1' is equivalent to CDR1'-11C7, CDR2' is equivalent to CDR2'-11C7, CDR3' is equivalent to CDR3'-11C7; and

(iv) which is capable of binding the human NogoA, human NiG, human NiG-D20, or human NogoA_623-640, preferably with a dissociation constant (Kd) < 1000nM, more preferably with a Kd < 100 nM, most preferably with a Kd < 10 nM.

Thus further embodiments of the inventions are for example a Binding Molecule which is capable of binding to the human NogoA, human NiG, human NiG-D20, or human NogoA_623-640 with a dissociation constant < 1000nM and comprises at least one antigen binding site, said antigen binding site comprising either

- in sequence the hypervariable regions CDR1, CDR2, and CDR3, of which each of the hypervariable regions are at least 50%, preferably 80, 90, 95, 96, 97, 98, 99% homologous to their equivalent hypervariable regions CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9) and CDR3-11C7 (SEQ ID NO: 10); or
- in sequence the hypervariable regions CDR1', CDR2', and CDR3', of which each of the hypervariable regions are at least 50%, preferably 80, 90, 95, 96, 97, 98, 99%

homologous to their equivalent hypervariable regions CDR1 '-11C7 (SEQ ID NO: 11), CDR2'-11C7 (SEQ ID NO: 12) and CDR3'-11C7 (SEQ ID N O: 13).

Furthermore, a Binding Molecule which is capable of binding the human NogoA, human NiG, human NiG-D20, or human NogoA_623-640 with a dissociation constant < 1000nM and comprises

- a first antigen binding site comprising in sequence the hypervariable regions CDR1, CDR2, and CDR3, of which each of the hypervariable regions are at least 50%, preferably 80, 90, 95, 96, 97, 98, 99% homologous to their equivalent hypervariable regions CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9) and CDR3-11C7 (SEQ ID NO: 10); and
- a second antigen binding site comprising in sequence the hypervariable regions CDR1', CDR2', and CDR3', of which each of the hypervariable regions are at least 50%, preferably 80, 90, 95, 96, 97, 98, 99% homologous to their equivalent hypervariable regions CDR1'-11C7 (SEQ ID NO: 11), CDR2'-11C7 (SEQ ID NO: 12) and CDR3'-11C7 (SEQ ID NO: 13).

This dissociation constant may be conveniently tested in various as- says including, for example, the biosensor affinity method described in the example 7. In addition, the binding and functional effect of the Binding Molecules may be shown in a bioassay, e.g. as described below.

The constant part of a human heavy chain may be of the γ1; γ2; γ3 ; γ4; α1; α2; δ or ε type, preferably of the γ type, more preferably of the γ4; type, whereas the constant part of a human light chain may be of the κ or λ type (which includes the λ1; λ2; and λ3 subtypes) but is preferably of the κ type. The amino acid sequence of all these co nstant parts are given in Kabat et al (Supra).

Conjugates of the binding molecules of the invention, e. g. enzyme or toxin or radioisotope conjugates, are also included within the scope of the invention.

"Polypeptide", if not otherwise specified herein, includes any peptide or protein comprising amino acids joined to each other by peptide bonds, having an amino acid sequence starting at the N-terminal extremity and ending at the C-terminal extremity. Preferably the

polypeptide of the present invention is a monoclonal antibody, more preferred is a chimeric (also called V-grafted) or humanised (also called CDR-grafted) monoclonal antibody. The humanised (CDR-grafted) monoclonal antibody may or may not include further mutations introduced into the framework (FR) sequences of the acceptor antibody.

A functional derivative of a polypeptide as used herein includes a molecule having a qualitative biological activity in common with a polypeptide to the present invention, i.e. having the ability to bind to the human NogoA, human NiG, human NiG-D20, or human NogoA_623-640. A functional derivative includes fragments and peptide analogs of a polpypeptide according to the present invention. Fragments comprise regions within the sequence of a polypeptide according to the present invention, e.g. of a specified sequence. The term "derivative" is used to define amino acid sequence variants, and covalent modifications of a polypeptide according to the present invention. e.g. of a specified sequence. The functional derivatives of a polypeptide according to the present invention, e.g. of a specified sequence, e.g. of the hypervariable region of the light and the heavy chain, preferably have at least about 65%, more preferably at least about 75%, even more preferably at least about 85%, most preferably at least about 95, 96, 97, 98, 99% overall sequence homology with the amino acid sequence of a polypeptide according to the present invention, e.g. of a specified sequence, and substantially retain the ability to bind the human NogoA, human NiG, human NiG-D20, or human NogoA_623-640.

The term "covalent modification" includes modifications of a polypeptide according to the present invention, e.g. of a specified sequence; or a fragment thereof with an organic proteinaceous or non-proteinaceous derivatizing agent, fusions to heterologous polypeptide sequences, and post-translational modifications. Covalent modified polypeptides, e.g. of a specified sequence, still have the ability bind to the human NogoA, human NiG, human NiG-D20, or human NogoA_623-640 by crosslinking. Covalent modifications are traditionally introduced by reacting targeted amino acid residues with an organic derivatizing agent that is capable of reacting with selected sides or terminal residues, or by harnessing mechanisms of post-translational modifications that function in selected recombinant host cells. Certain post-translational modifications are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deaminated under mildly acidic conditions. Other post-translational

modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl, tyrosine or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains, see e.g. T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 (1983). Covalent modifications e.g. include fusion proteins comprising a polypeptide according to the present invention, e.g. of a specified sequence and their amino acid sequence variants, such as immunoadhesins, and N-terminal fusions to heterologous signal sequences.

"Homology" with respect to a native polypeptide and its functional derivative is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues of a corresponding native polypeptide, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity or homology. Methods and computer programs for the alignment are well known.

"Amino acid(s)" refer to all naturally occurring L-α-amino acids, e.g. and including D-amino acids. The amino acids are identified by either the well known single-letter or three-letter designations.

The term "amino acid sequence variant" refers to molecules with some differences in their amino acid sequences as compared to a polypeptide according to the present invention, e.g. of a specified sequence. Amino acid sequence variants of a polypeptide according to the present invention, e.g. of a specified sequence, still have the ability to bind to human NogoA or human NiG or more preferably to NogoA_623-640. Substitutional variants are those that have at least one amino acid residue removed and a different amino acid inserted in its place at the same position in a polypeptide according to the present invention, e.g. of a specified sequence. These substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule. Insertional variants are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a polypeptide according to the present invention, e.g. of a specified sequence. Immediately adjacent to an amino acid means connected to either the α-carboxy or α-amino functional group of the amino acid. Deletional variants are those with one or more amino acids in a polypeptide

according to the present invention, e.g. of a specified sequence, removed. Ordinarily, deletional variants will have one or two amino acids deleted in a particular region of the molecule.

A binding molecule of the invention may be produced by recombinant DNA techniques. In view of this, one or more DNA molecules encoding the binding molecule must be constructed, placed under appropriate control sequences and transferred into a suitable host organism for expression.

In a very general manner, there are accordingly provided

(i) DNA molecules encoding a single domain Binding Molecule of the invention, a single chain Binding Molecule of the invention, a heavy or light chain or fragments thereof of a Binding Molecule of the invention; and

(ii) the use of the DNA molecules of the invention for the production of a Binding Molecule of the invention by recombinant means.

The present state of the art is such that the skilled man will be able to synthesize the DNA molecules of the invention given the information provided herein i.e. the amino acid sequences of the hypervariable regions and the DNA sequences coding for them. A method for constructing a variable domain gene is for example described in EP 239 400 and may be briefly summarized as follows: A gene encoding a variable domain of a monoclonal antibody of whatever specificity is cloned. The DNA segments encoding the framework and hypervariable regions are determined and the DNA segments encoding the hypervariable regions are removed so that the DNA segments encoding the framework regions are fused together with suitable restriction sites at the junctions. The restriction sites may be generated at the appropriate positions by mutagenesis of the DNA molecule by standard procedures. Double stranded synthetic CDR cassettes are prepared by DNA synthesis according to the sequences given CDR1-11C7, CDR2-11C7, CDR3-11C7, CDR1'-11C7, CDR2'-11C7 and CDR3'-11C7 above. These cassettes are provided with sticky ends so that they can be ligated at the junctions to the framework by standard protocol for achieving a DNA molecule encoding an immunoglobulin variable domain.

Furthermore, it is not necessary to have access to the mRNA from a producing hybridoma cell line in order to obtain a DNA construct coding for the monoclonal antibodies of the

invention. Thus PCT application WO 90/07861 gives full instructions for the production of a monoclonal antibody by recombinant DNA techniques given only written information as to the nucleotide sequence of the gene.

The method comprises the synthesis of a number of oligonucleotides, their amplification by the PCR method, and their splicing to give the desired DNA sequence.

Expression vectors comprising a suitable promoter or genes encoding heavy and light chain constant parts are publicly available. Thus, once a DNA molecule of the invention is prepared it may be conveniently transferred in an appropriate expression vector.

DNA molecules encoding single chain antibodies may also be prepared by standard methods, for example, as described in WO 88/1649.

In a particular embodiment of the invention, the recombinant means for the production of some of the Binding Molecules of the invention includes first and second DNA constructs as described below:

The first DNA construct encodes a heavy chain or fragment thereof and comprises

a) a first part which encodes a variable domain comprising alternatively framework and hypervariable regions, said hypervariable regions comprising in sequence DNA-CDR1-11C7 (SEQ ID NO: 15), DNA-CDR2-11C7 (SEQ ID NO: 16) and DNA-CDR3-11C7 (SEQ ID NO: 17); this first part starting with a codon encoding the first amino acid of the variable domain and ending with a codon encoding the last amino acid of the variable domain, and
b) a second part encoding a heavy chain constant part or fragment thereof which starts with a codon encoding the first amino acid of the constant part of the heavy chain and ends with a codon encoding the last amino acid of the constant part or fragment thereof, followed by a non-sense codon.

Preferably, the second part encodes the constant part of a human heavy chain, more preferably the constant part of the human γ4 chain. This second part may be a DNA fragment of genomic origin (comprising introns) or a cDNA fragment (without introns).

The second DNA construct encodes a light chain or fragment thereof and comprises

a) a first part which encodes a variable domain comprising alternatively framework and hypervariable regions; said hypervariable regions comprising in sequence DNA-CDR1'-11C7 (SEQ ID NO: 17), DNA-CDR2'-11C7 (SEQ ID NO: 18) and DNA-CDR3'-11C7 (SEQ ID NO: 19), this first part starting with a codon encoding the first amino acid of the variable domain and ending with a codon encoding the last amino acid of the variable domain, and

b) a second part encoding a light chain constant part or fragment thereof which starts with a codon encoding the first amino acid of the constant part of the light chain and ends with a codon encoding the last amino acid of the constant part or fragment thereof followed by a non-sense codon.

Preferably, the second part encodes the constant part of a human light chain, more preferably the constant part of the human κ chain.

The first or second DNA construct advantageously comprises a third part which is located upstream of the first part and which encodes part of a leader peptide; this third part starting with the codon encoding the first amino acid and ending with the last amino acid of the leader peptide. This peptide is required for secretion of the chains by the host organism in which they are expressed and is subsequently removed by the host organism. Preferably, the third part of the first DNA construct encodes a leader peptide having an amino acid sequence substantially identical to the amino acid sequence of the heavy chain leader sequence as shown in SEQ ID NO: 21 (starting with the amino acid at position -19 and ending with the amino acid at position –1). Also preferably, the third part of the second DNA construct encodes a leader peptide having an amino acid sequence as shown in SEQ ID NO: 23 (light chain, starting with the amino acid at position -18 and ending with the amino acid at position –1).

Each of the DNA constructs are placed under the control of suitable control sequences, in particular under the control of a suitable promoter. Any kind of promoter may be used, provided that it is adapted to the host organism in which the DNA constructs will be transferred for expression. However, if expression is to take place in a mammalian cell, it is particularly preferred to use the promoter of an immunoglobulin gene.

The desired antibody may be produced in a cell culture or in a transgenic animal. A suitable transgenic animal may be obtained according to standard methods which include micro injecting into eggs the first and second DNA constructs placed under suitable control sequences transferring the so prepared eggs into appropriate pseudo- pregnant females and selecting a descendant expressing the desired antibody.

When the antibody chains have to be produced in a cell culture, the DNA constructs must first be inserted into either a single expression vector or into two separate but compatible expression vectors, the latter possibility being preferred.

Accordingly, the invention also provides an expression vector able to replicate in a prokaryotic or eukaryotic cell line which comprises at least one of the DNA constructs above described.

Each expression vector containing a DNA construct is then transferred into a suitable host organism. When the DNA constructs are separately inserted on two expression vectors, they may be transferred separately, i.e. one type of vector per cell, or co- transferred, this latter possibility being preferred. A suitable host organism may be a bacterium, a yeast or a mammalian cell line, this latter being preferred. More preferably, the mammalian cell line is of lymphoid origin e.g. a myeloma, hybridoma or a normal immortalized B-cell, but does not express any endogeneous antibody heavy or light chain.

It is also preferred that the host organism contains a large number of copies of the vectors per cell. If the host organism is a mammalian cell line, this desirable goal may be reached by amplifying the number of copies according to standard methods. Amplification methods usually consist of selecting for increased resistance to a drug, said resistance being encoded by the expression vector.

In another aspect of the invention, there is provided a process for producing a multi-chain binding molecule of the invention, which comprises (i) culturing an organism which is transformed with the first and second DNA constructs of the invention and (ii) recovering an active binding molecule of the invention from the culture.

Alternatively, the heavy and light chains may be separately recovered and reconstituted into an active binding molecule after in vitro refolding. Reconstitution methods are well-known in the art; Examples of methods are in particular provided in EP 120 674 or in EP 125 023. Therefore a process may also comprise

(i) culturing a first organism which is transformed with a first DNA construct of the invention and recovering said heavy chain or fragment thereof from the culture and

(ii) culturing a second organism which is transformed with a second DNA construct of the invention and recovering said light chain or fragment thereof from the culture and

(iii) reconstituting in vitro an active binding molecule of the invention from the heavy chain or fragment thereof obtained in (i) and the light chain or fragment thereof obtained in (ii).

In a similar manner, there is also provided a process for producing a single chain or single domain binding molecule of the invention which comprises

(i) culturing an organism which is transformed with a DNA construct respectively encoding a single chain or single domain binding molecule of the invention and

(ii) recovering said molecule from the culture.

The binding molecules of the invention exhibit very good nerve repair activity as shown, for example, in the granule cell neurite outgrowth model.

1. Granule cell neurite outgrowth assay (*in vitro)*

Neurite outgrowth from dissociated cerebellar granule cells are determined as described (Niederöst et al. (1999) J.Neurosci. 19: 8979-8989). Briefly, cerebella are removed from decapitated postnatal day 5 – 7 rats and dissociated by trypsin treatment. To reduce fibroblast contamination, the cells are preplated onto bacterial dishes. 75'000 cells are then cultured per well in 4–well Greiner tissue culture (Huber & Co AG,Rheinach, Basel) dishes (well surface: 1 cm2) in medium (Neurobasal with B27 serum replacement, Invitrogen). Culture dishes are coated with poly-L-lysine (Sigma). Chaps extracted proteins from total spinal cord homogenates of adult rats (Spillmann et al. (1998) J. Biol. Chem. 273: 19283-19293) is coated at protein concentrations of 0.5 till 8 µg per well over night at 4°C and washed. The binding molecules of the invention are then pre-incubated for 30 min on the test substrate and removed before the cells are added. Cerebellar granule cells are added and incubated for 24 hours. To stop the experiment, 2 ml of 4 % buffered formaldehyde is

slowly added to the culture dishes. Cultures are then stained by immunofluorescence for the growth-associated protein GAP-43 and with Hoechst for cell nuclei (Granule cells are stained with Hoechst in order to see if all the cells have neurites (neurite visualised with anti-GAP-43)). Three pictures are taken randomly at a defined distance of the upper, lower and lateral edge of each well with a 40x objectif on a Zeiss Axiophot Fluorescence Microscope. All the neurites in a field are counted on number-coded, randomly arranged photographs. The response (outgrowth of the granule cell neurites) is dose-dependent in the range of about 0.1 – 10 µg total protein per well (the specific activities of a given preparation vary within this range).

Enhancement of neurite outgrowth of cerebellar granule cell in the non-permissive environment of the above prepared spinal cord extract by preincubation with a binding molecule of the invention may be observed. E.g. a typical profile for the neutralizing effect of the mouse 11C7-IgG1 antibody in the granule cell neurite outgrowth model is given below:

Assay 1:

rat myelin coated at 1µg per well	Neurites per field	Percentage
no antibody	80,5	100 %
+mouse IgG	86,5	108 %
11C7 250 µg/ml	160	199 %

Assay 2:

rat myelin (prep. 2) coated at 8 ug per well	Neurites per field	Percentage
no antibody	20	100 %
+mouse IgG	17,3	86,5 %
11C7 250 µg/ml	31	155 %
11C7 75µg/ml	26	130 %
11C7 7,5 µg/ml	26	130 %

The neutralizing activity of the molecules of the invention may also be estimated by measuring the regenerative sprouting and neurite outgrowth in the *in vivo* spinal cord injury model as follows:

2. Spinal cord injury model (*in vivo*)

Adult Lewis rats are injured microsurgically by transecting the dorsal half of the spinal cord bilaterally at the level of the 8th thoracic vertebra. Laminectomy, anesthesia and surgery are

described in Schnell and Schwab 1993 (Eur.J. Neurosci. 5: 1156 – 1171). Controls or binding molecules of the invention are applied in two different ways: either by implanting 10^6 freshly harvested hybridoma cells into one side of the cerebral cortex (grafted animals) or, alternatively, by an implanted intraventricular canula linked to a subcutaneously implanted 2ml Alzet (Alza Corporation, Palo Alto) pump (pump animals). – *Hybridoma grafted animals*: Rats are immunosuppressed for 7 – 10 days with cyclosporin A and sacrificed by transcardial perfusion with 4% buffered formalin 14 days after injury. – *Pump animals*: Binding molecules of the invention (e.g. at 3.3 mg/ml for mouse 11C7) are filled into 2 ml pumps delivering 0.5 µl/h into the lateral ventricle for 2 weeks. Pumps are implanted at the time of the spinal cord lesion, and rats are sacrificed 2 weeks later.

Neuroanatomical tracing: The motor and sensory corticospinal tract is traced by injecting the anterograde tracer biotin dextran amine (BDA) into the cortex of the side opposite to the pump or the graft. BDA is transported to the spinal cord within 10 – 14 days and visualized using diaminobenzidine (DAB) as a substrate as described in Brösamle et al., (2000 J.Neurosci. 20: 8061-8068).

Evalutation of anatomical results: Two methods of evaluation are used: a semi-quantitative and a quantitative one. *Semi-quantitative estimation of intensity of sprouting and regeneration:* Complete sagittal section series of number-coded, randomly mixed animals are evaluated for the presence and density of regenerating sprouts rostral to the lesion using the following definitions: regenerative sprouts are fibers emanating from the transected CST; they are long, irregular in their course, much less branched than the normal grey matter collaterals, and they growth towards and ventrally or laterally around the lesion. Regenerative sprouts often end in a growth cone which can be small and bulbouse or large and branched. Density of sprouting is rated on a scale of 0 – 3 for each animal. – *Long distance regeneration:* fibers that can be followed through the lesion into the caudal spinal cord are considered long-distance regenerating fibers. Their maximal distance from the lesion site can be measured, but is often a minimal distance as some unlesioned fibers from the small ventral funiculus CST are often present; their branches mix with those of regenerating axons and make distinction difficult.

Fiber counts (quantitative assay): A line positioned at -0.5 mm rostral to the end of the transected CST is posed on alternating sections of the grey matter, and all intersections with

CST fibers (normal collaterals or sprouts) are counted. Similar lines are positioned caudal to the lesion at a distance of +0.5, +2 and +5 mm from the lesion center. Intersecting fibers are counted and the 3 levels are added to a sum reflecting CST fibers in the caudal spinal cord. These caudal fibers are divided by the number of fibers -0.5 mm rostral to the CST end to obtain a ratio.

Two weeks after a spinal cord injury destroying about 40 % of the spinal cord segment T8, mainly in the dorsal half, including both main CSTs: tracing of the CST in control animals show a moderate degree of reactive sprouting of the tract. This phenomenon corresponds to the spontaneous sprouting in response to injury well known in the literature. Injured rats being treated with the binding molecules of the invention or with pumps delivering the binding molecules of the invention may show an enhanced sprouting at the lesion site and regeneration of damaged axons neurite outgrowth of damaged neurites.

Therefore the invention also provides

(i) the use of the binding molecules of the invention in the nerve repair of a mammalian nervous system, in particular human nervous system,

(ii) a method of repairing nerves of a mammalian nervous system, in particular human nervous system which comprises administering an effective amount of the binding molecules of the invention to a patient in need of such treatment, or

(iii) a pharmaceutical composition for nerve repair of a mammalian nervous system, in particular human nervous system which comprises the binding molecules of the invention and a pharmaceutically acceptable carrier or diluent.

In particular, the binding molecules of the invention are useful for axonal regeneration and improved sprouting after nerve fiber damage. Thus the molecules of the invention have a wide utility in particular for human subjects. For example the binding molecule of the invention are useful in the treatment of various diseases of the peripheral (PNS) and central (CNS) nervous system, i.e. more particularly in neurodegenerative diseases such as Alzheimer disease, Parkinson disease, Amyotrophic lateral sclerosis (ALS), Lewy like pathologies or other dementia in general, diseases following cranial, cerebral or spinal trauma, stroke or a demyeliating disease. Such demyelinating diseases include, but are not

limited to, multiple sclerosis, monophasic demyelination, encephalomyelitis, multifocal leukoencephalopathy, panencephalitis, Marchiafava-Bignami disease, pontine myelmolysis, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, Spongy degeneration, Alexander's disease, Canavan's disease, metachromatic leukodystrophy and Krabbe's disease. In one example, administration of the binding molecules of the invention can be used to treat a demyelinating disease associated with NogoA protein. In another example, cells which express the binding molecules of the invention may be transplanted to a site spinal cord injury to facilitate axonal growth throughout the injured site. Such transplanted cells would provide a means for restoring spinal cord function following injury or trauma. Such cells could include olfactory ensheathing cells and stem cells of different lineages of fetal nerve or tissue grafts.

In addition, the Binding Molecules of the invention are useful for the treatment of degenerative ocular disorders which may directly or indirectly involve the degeneration of retinal or corneal cells including ischemic retinopathies in general, anterior ischemic optic neuropathy, all forms of optic neuritis, age-related macular degeneration, diabetic retinopathy, cystoid macular edema (CME), retinitis pigmentosa, Stargardt's disease, Best's vitelliform retinal degeneration, Leber's congenital amaurosis and other hereditary retinal degenerations, pathologic myopia, retinopathy of prematurity,and Leber's hereditary optic neuropathy, the after effects of corneal transplantation or of refractive corneal surgery, and herpes keratitis.

Furthermore, it was shown that NogoA plays a role in psychiatric conditions, in particular schizophrenia and depression. Hence, the binding molecules of the invention are useful for the treatment of psychiatric conditions, in particular schizophrenia and depression.

The Binding Molecules of the invention can be provided alone, or in combination, or in sequential combination with other agents. For example, the binding molecules of the invention can be administered in combination with anti-inflammatorv agents such as but not limited to corticosteroids following stroke or spinal cord injury as a means for blocking further neuronal damage and inhibition of axonal regeneration, Neurotrophic factors such as NGF, BDNF or other drugs for neurodegenerative diseases such as Exelon™ or Levodopa. As used herein, two agents are said to be administered in combination when the two agents are

administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

For the treatment of psychiatric conditions, in particular schizophrenia or depression, the Binding Molecules of the invention can be provided alone or in combination in particular with other agents selected from the group consisting of (a) anti-epileptic drugs selected from barbiturates and derivatives thereof, benzodiazepines, carboxamides, hydantoins, succinimides, valproic acid and other fatty acid derivates and other anti-epileptic drugs, (b) conventional antipsychotics, (c) atypical antipsychotics and (d) antidepressants.

The term "barbiturates and derivatives thereof" as used herein includes, but is not limited to Phenobarbital and primidon. The term "benzodiazepines" as used herein includes, but is not limited to clonazepam, diazepam and lorazepam. The term "carboxamides" as used herein includes, but is not limited to carbamazepine, oxcarbazepine and 10-hydroxy-10,11-dihydrocarbamazepine. The term "hydantoins" as used herein includes, but is not limited to phenytoin. The term "succinimides" as used herein includes, but is not limited to ethosuximide and mesuximide. The term "valproic acid and other fatty acid derivates" as used herein includes, but is not limited to valproic acid sodium salt, tiagabine hydrochloride monohydrate and vigrabatrine. The term "other anti-epileptic drugs" as used herein includes, but is not limited to levetiracetam, lamotrigine, gabapentin and felbamate.

The term "conventional antipsychotics" as used herein includes, but is not limited to haloperidol and fluphenazine.

The term "atypical antipsychotics" as used herein relates to clozaril, risperidone, olanzapine, quetiapine, ziprasidone and aripiprazol.

The term "antidepressants" as used herein includes, but is not limited to selective serotonin reuptake inhibitors (SSRI's), or selective serotonin and norepinephrine reuptake inhibitors (SNRI-s). An SSRI's suitable for the present invention can be selected from fluoxetine, fuvoxamine, sertraline, paroxetine, citalopram and escitalopram.

The structure of the active ingredients identified by code nos., generic or trade names may be taken from the actual edition of the standard compendium "The Merck Index" or from

databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference. Any person skilled in the art is fully enabled to identify the active ingredients and, based on these references, likewise enabled to manufacture and test the pharmaceutical indications and properties in standard test models, both *in vitro* and *in vivo*.

For the indications mentioned above, the appropriate dosage will, of course, vary depending upon, for example, the particular molecule of the invention to be employed, the mode of administration and the nature and severity of the condition being treated. The Binding Molecules of the invention are conveniently administered by pumps or injected as therapeutics at the lesioned site, e.g. they can be administered directly into the CNS intracranially or into the spine intrathecally to the lesioned site.

Pharmaceutical compositions of the invention may be manufactured in conventional manner. E.g. a composition according to the invention comprising the molecules of the invention is preferably provided in lyophilized form. For immediate administration it is dissolved in a suitable aqueous carrier, for example sterile water for injection or sterile buffered physiological saline.

To aid in making up suitable compositions, the binding molecules of the invention and optionally a second drug enhancing the effect of the Binding Molecules of the invention, may be packaged separately within the same container, with instructions for mixing or concomitant administration. Optional second drug candidates are provided above.

The synergistic effect of a combination of the binding molecules of the invention and growth factors such as NGF may be demonstrated in vivo by the spinal cord injury model described above.

Brief description of the drawing

Figure 1: Sequence Comparison: Sequence comparison of the NiG from different species, showing the immunogenic peptide sequence for the 11C7 mAb.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention.

In the following examples all temperatures are in degree Celsius (°C).

The monoclonal antibody of attention in the Examples is a Binding Molecule according to the present invention comprising the variable part of the light chain (SEQ ID NO: 3) and the variable part of the heavy chain (SEQ ID NO: 2).

The following abbreviations are used:

ELISA	enzyme linked immuno-sorbant assay
FACS	fluorescence activated cell sorting
FITC	fluorescein isothiocyanate
FBS	foetal bovine serum
HCMV	human cytomegalovirus promoter
IgG	immunoglobulin isotype G
MAb	monoclonal antibody
PBS	phosphate-buffered saline
PCR	polymerase chain reaction

Example 1: NiG-D20 (SEQ ID NO: 24) is one of the neurite outgrowth inhibitory fragments of NogoA

Methods:

a) Rat Nogo-A deletion library: Deletion constructs are made using internal restriction sites, by ExonucleaseIII/Mung Bean Nuclease treatment and by PCR with rat Nogo-A-specific primers on rat Nogo- (method as in WO00/31235): rat Nogo-A (aa 1-1163; DNA as shown hereafter related to the amino acids of rat NogoA (SEQ ID NO: 26), e.g. aa 1-1163 means that the cDNA construct encodes for polypeptide which starts at the amino acid1 and ends at amino acid 1163 of the rat polypeptide sequence of NogoA), rat Nogo-B (aa 1-172 + 976-1163), rat Nogo-C (Nogo-C N-terminal 11 aa + aa 976-1163), rat Nogo-66 (aa 1019-1083), rat GST-Nogo-66 (aa 1026-1091), rat NiR-G (aa 1-979), rat NiR (1-172), rat NiR-D1 (aa 1-31), rat NiR-D2 (aa 59-172), rat NiR-D3 (aa 1-31 + 59-172), rat EST-Nogo1 (aa 762-1163), rat NiG (aa 174-979), rat NiG-D1 (aa174-909), rat NiG-D2 (aa 174-865), rat NiG-D3 (a a 172-723), rat NiG-D4 (aa 172-646), rat NiG-D5 (aa 293-647), rat NiG-D6 (aa 763-975), rat NiG-D7 (aa 174-235 + 294-979), rat NiG-D8 (aa 218-653), rat NiG-D9 (aa 172-259 + 646-974), rat NiG-D10 (aa 293-979), rat NiG-D11 (aa 209-268), rat NiG-D12 (aa 198-233), rat NiG-D13 (aa 174-216), rat NiG-D14 (aa 174-260), rat NiG-D15 (aa 174-190 + 493-979), rat NiG—D16 (aa 174-190 + 621-979), rat NiG-D17 (aa 174-190 + 259-979), rat NiG-D18 (aa 174-190 + 263-979), rat NiG-D19 (aa 763-865), rat NiG-D20 (aa 544-725), rat NiG-D21 (aa 812-918), rat NiG-D22 (aa 866-975), rat NiG-D23 (aa 914-975), rat NiG-D24 (aa 544-685), rat NiG-D25 (aa 614-725), rat NiG-D26 (aa 544-613), rat NiG-D27 (aa 581-648), rat NiG-D28 (aa 614-685), rat NiG-D29 (aa 648-725), rat NiG-D30 (aa 682-725), rat NiG-D31 (aa 544-580), rat NiG-D32 (aa 581-613), rat NiG-D33 (aa 614-648), rat NiG-D34 (aa 648-685), rat NiG-D35 (aa 260-556), rat NiG-D36 (aa 260-415). NiR-G and NiR-a are derived from Nogo-A-pET28 by restriction enzyme digestions. NiG is derived from NiR-G by restriction digestion and MungBean Nuclease treatment. NiG-D1, -D3, -D4, -D5, -D7, -D8, -D9, -D10 derived from NiG-pET28 by restriction enzyme digestions. NiG-D15, -D16, -D17, -D18 derived from NiG-pET28 by Exonuclease III digestion. NiR-b, NiR-D1, -D2, -D3 derived by PCR with NiR—a-pET28 as a template. NiG-D2, -D6, -D11, -D12, -D13, -D14, -D19, -D20, -D21, -D22, -D23, -D24, -D25, -D26, -D27, -D28, -D29, -D30, -D31, -D32, -D33, -D34, -D35, -D36 derived by PCR using NiG-pET28 as a template. All constructs subcloned into pET28. pET28 used for all the constructs mentioned aboved. pGEX-6P used for GST-Nogo66 and pET26 for periplasmic expression of rat NiG. Human GST-Nogo-66 (aa 1055-1120 of human Nogo-A)

is cloned by PCR on human NogoA DNA (SEQ ID NO: 4) as a template. Deletion constructs are then cloned into pET28 vector (Novagen), pGEX-6P (Amersham Pharmacia Biotech) and pET26 vector (Novagen). Human GST-Nogo-66 corresponds to the GST-nogo protein published by GrandPré et al. (supra). Synthetic rat peptide 4 EELVQKYSNSALGHVNSTIKELRRL (SEQ ID NO: 27) corresponds to the human peptide 4 (Human peptide 4 has been shown to be the inhibitory region of the Nogo-66 domain (GrandPré et al., 2000)). The orthologous rat peptide has a single mismatch C->S (see peptide 4 sequence in GrandPré et al., 2000, supra). Synthetic Pro/Ser-rich peptide PSSPPPSSPPPSSPPPS (SEQ ID NO: 28) as well as rat peptide 4 have been produced and HPLC-purified by Primm SA. Human NogoA_623-640 (SEQ ID NO: 6) is synthesised and purified by Research Genetics Inc.

b) Generation of human Nogo-A expression constructs (pRK7-hNogo-A): A human cDNA library constructed in lambda gt10 (Clontech) is screened with duplicate filter sets using standard procedures. Fragments of human Nogo-A are amplified by PCR from human whole brain cDNA (Clontech) using a standard protocol and subsequently cloned into pBluescript, digested and isolated, or used as screening probes directly. A 400bp XhoI/SmaI fragment is used as 5' probe, the 3' probe is amplified with primers CA-NA-2F: 5'-AAG CAC CAT TGA ATT CTG CAG TTC C-3' (SEQ ID NO: 29) and CA-NA-3R: 5'-AAC TGC AGT ACT GAG CTC CTC CAT CTG C-3' (SEQ ID NO: 30). Positive clones are isolated, subcloned and sequence confirmed. To obtain a full length human Nogo-A cDNA, overlapping clones are assembled using an unique EcoRI restriction site in the human Nogo-A sequence and subcloned into Bluescript vector, named Pbsnogoa. To obtain pRK7–hNogo-A, the full length cDNA was inserted into the eukaryotic expression vector pRK-7 by directional cloning.

c) Generation of human NiG (hNiG) expression plasmids (pET28a-hNiG) for bacterial production: A hNiG encoding DNA fragment is subcloned into BamHI/XhoI of pET28a (Novagen), after PCR amplification of the respective coding region from Pbsnogoa, in frame with the N-terminal His- and T7-tag for bacterial expression, using primer sets: forward 5'-GTC GCG GAT CCA TGG AGA CCC TTT TTG CTC TTC-3' (SEQ ID NO: 31); reverse 5'-GTT CTC GAG TTA TGA AGT TTT ACT CAG-3' (SEQ ID NO: 32). The final plasmid is termed pET28a-hNiG. hNiG was then expressed in E.coli BL21 pRP by induction with 1 mM Isopropyl-beta-D-thiogalactopyranoside (IPGT).

d) Generation of mouse NiG-exon3 (mNiG-exon3) expression plasmid: The region encoding mouse exon 3 is amplified from mouse genome BAC template with primers : forward 5'-GTG CGG ATC CAT GGA TTT GAA GGA GCA GC-3' (SEQ ID NO: 33); reverse 5'-GTT TCT CGA GTG AAG TTT TAT TCA GCT C-3' (SEQ ID NO: 34) and subcloned into the BamHI/XhoI cloning sites of pET28a. The final plasmid construct is named pET28a-mNiG-exon3.

Cloning of monkey NiG: PolyA RNA is isolated from frozen monkey brain tissue and cDNA are synthesised using an oligo dT primer. Two overlapping fragments covering the 5' and the 3' region of the cDNA are amplified by PCR using sequence-specific primers and a proof-reading enzyme. The primers are designed using the known sequence of the human NiG cDNA. For amplification of the 5' fragment the primers are 5'-TCCACCCCGGCCGCGCCCAA-3' (SEQ ID NO: 35) and 5'-AATGATGGGCAAAGCTGTGCTG-3' (SEQ ID NO: 36), for the 3'-fragment 5'-GGTACAAAGATTGCTTATGAAACA-3' (SEQ ID NO: 37) and 5'-AGCAGGGCCAAGGCAATGTAGG-3' (SEQ ID NO: 38). The two fragments are then subcloned and for each fragment at least 4 independent clones were sequenced. The full length cDNA is assembled by overlapping PCR using the primers mentioned above and the resulting product is cloned and sequenced again.

e) Production of recombinant NogoNiG proteins and the Nogo-A-deletion library as defined above: The bacterial Nogo-A-deletion library is expressed in Escherichia coli. Proteins are extracted either by repeated sonication in sonication buffer (20 mM Tris, 50 mM NaH_2PO_4, 100 mM NaCl, pH 8.0) with 0.75 mg/ml Lysozyme, by solubilisation with B-Per™ (Pierce) or with 8 M urea. NiG expressed with pelB-leader is obtained from the periplasmic space according to the Novagen protocol for periplasmic protein purification. Supernatants of pET28-constructs are purified using the Co^{2+}-Talon™ Metal Affinity Resin (Clontech) in a batch procedure. 8 M urea and B-Per™ solubilised lysates are brought to non-denaturing conditions by increasingly substituting the buffer with sonication buffer during the resin-batch procedure. Proteins are eluted with 250 mM imidazole in sonication buffer on a gravity column (BioRad). NiG proteins are further purified by gel filtration on Superdex 200 HiLoad 16/60. Supernatants of pGEX-6P constructs are purified with G-sepharose column in a batch procedure according to manufacturer indications (Amersham Pharmacia). Cleavage of GST-Nogo-66 is done by incubating solubilised GST-Nogo-66 with PreScission protease and

subsequent HPLC purification. Gel electroelution is performed by preparative SDS-PAGE of IMAC-purified recombinant Nogo and elution with BioRad Electro-Eluter into 50 mM Tris, pH 7.4, 100 mM NaCl, 0.2% (w/v) CHAPS for 1 hr at 250 mA and followed by 30 s of reversed electrode polarities. Protein concentrations of chromatography-purified proteins are determined using Pierce Coomassie Stain and BSA as standard protein. Protein concentrations of gel eluted proteins are estimated based on band intensity of silver-stained gels (Merril CR, Dunau ML, Goldman D (1981) A rapid sensitive silver stain for polypeptides in polyacrylamide gels. Analyt.Biochem. 110:201-207) with BSA as a standard.

f) Production of recombinant NogoA fragments in CHO cells: A 3119 bp fragment resulting from a partial HincII digest of rat Nogo-A cDNA, NiR-G, is cloned into pSecTag2 expression vectors (Invitrogen, Groningen, The Netherlands). Transfection of pNiR-G into CHO cells results in intracellular, cytoplasmic expression of NiR-G. Stable NiR-G CHO cell lines are selected with 250 µg/ml Zeocin (Invitrogen). Recombinant NiR-G from cell lysate is purified over a Ni^{2+}-NTA column (Qiagen AG, Basel, Switzerland). Rat NiG-D20 and Nogo-66 are cloned into pAPtag5 vector by PCR. Transfection of pNiG-D20-AP into CHO cells results in NiG-δ20-AP that was secreted into the culture supernatant. Stable pNiG-D20-AP and pNogo-66-AP cell lines were selected with 250 µg/ml Zeocin (Invitrogen). Both cell lines are adapted to serum-free medium (Gibco) conditions and grown in a cell-line chamber (Integra). Supernatants are tenfold concentrated prior to use, and the concentration of fusion protein is assessed as described elsewhere (Flanagan JG, Leder P (1990) The kit ligand: a cell surface molecule altered in steel mutant fibroblasts. Cell 63:185-194).

g) 3T3 fibroblast and CHO spreading assays: The 3T3 spreading assays are performed as described previously (Spillmann AA, Bandtlow CE, Lottspeich F, Keller F, Schwab ME (1998) Identification and characterization of a bovine neurite growth inhibitor (bNI-220). J.Biol.Chem. 273:19283-19293). CHO spreading assays are performed essentially the same way as for 3T3 fibroblasts. Briefly, CHO cells are split 1:2. 24 hrs later they are trypsinised in PBS-EDTA for 30 s and ~8'000 CHO cells are plated onto culture dishes precoated with 5, 1, 0.5 and 0.2 µg/well NiG or Nogo-66. After 30-45 min the cells are fixed with 4% (w/v) PFA, 5% (w/v) sucrose and then analysed as described Spillmann et al, supra). ~100 cells are counted per well with light microscopy; criterion of spreaded cells: (a) attachement to the dish AND (b) extended morphology indicative for lamellipodia; under light microscopy the cells appear darker and larger than not spreaded, round cells; non-spreaded cells are

considered those cells that are (a) not attached to the dish OR (b) attached to the dish, but small, rounded, without detectable lamellipodia protruding on the dish. The ratio between spreaded and not spreaded cells defines the degree of non-permissiveness of the substratum.

h) PC12 Neurite outgrowth assays: PC12 neurite outgrowth assays are performed as described previously (Rubin BP, Spillmann AA, Bandtlow CE, Keller F, Schwab ME (1995) Inhibition of PC-12 cell attachment and neurite outgrowth by detergent solubilized CNS myelin proteins. Europ. J. Neurosci. 7: 2524-2529). PC12 cells (a PC12 cell clone able to grow independently of laminin obtained from Moses Chao, New York) are primed for two days with 50-100 ng/ml NGF (Harlan Bioproducts, Indianapolis) to DMEM, 5% foetal calf serum, 10% horse serum, 100 U/ml Penicillin and 0.5 mg/ml Streptomycin (Pen-Strep from Gibco-BRL). PC12 cells are detachde mechanically, trypsinised for 5 minutes with 0.05% trypsin (Sigma) in HBSS (Gibco) and plated at a density of 3,000-5,000 cells/cm2 in culture medium with 100 ng/ml NGF. Assays were stopped after 24 hrs by adding 4% (w/v) PFA, 5% (w/v) sucrose in PBS, pH8. Cell culture dishes were coated for PC12 cells the same way as for 3T3 cells.

i) Retinal ganglion cell stripe assays: The retinal ganglion cell stripe assay is performed according to Vielmetter (see Vielmetter J, Stolze B, Bonhoeffer F, Stuermer CA (1990) In vitro assay to test differential substrate affinities of growing axons and migratory cells. Exp. Brain Res. 81:283-287) with modifications (see Schmalfeldt M, Bandtlow CE, Dours-Zimmermann MT, Winterhalter KH, Zimmermann DR (2000) Brain derived versican V2 is a potent inhibitor of axonal growth. J. Cell Sci. 113:807-816). Explants are evaluated after fixation with 4% (w/v) PFA, 0.1% (v/v) glutaraldehyde in PBS for 10 min at RT. For immunostainings, fixed explants are blocked for 1 hr at RT with RNO-blocking solution (0.5% (w/v) BSA, 0.3% (w/v) TopBlock (Juro Supply), 0.1% (w/v) NaN_3 in PBS), permeabilised for 10 min with 0.05% (v/v) Tx-100 in RNO-blocking solution, frozen for one minute at –20 °C and incubated with primary antibodies (AS Bianca for NiR, AS Laura for Nogo-A, NiR-G, NiG, NiG-D3 and NiG-D20, Novagen mAb anti-T7 for Nogo-C and beta-Gal control protein). After washing with PBS, FITC- and TRITC (FITC: Fluorescein-IsoThioCyanate: TRITC: Tetramethyl Rhodamine IsoThiocyanate)-conjugated antibodies (Jackson ImmunoResearch

Laboratories) are added (1:150) to the explants. The samples are coverslipped in 50% (v/v) glycerol, 25 mM $NaHCO_3$, 40 mM NaCl, 1% (w/v) p-Phenylendiamine (Sigma).

Results:

a) Two regions in the N-terminal part of Nogo-A are inhibitory for spreading of 3T3 fibroblasts: In order to identify the regions of Nogo-A responsible for the inhibition of 3T3 fibroblast spreading, a library of 50 Nogo deletion constructs is made and recombinant proteins are expressed in bacteria (see method 1a). The apparent EC_{50} for inhibition of 3T3 fibroblast spreading was approximately 400-500 ng/0.1ml Nogo-A coated overnight per cm^2 of culture dish (~4 pmol/cm^2). Treatment of Nogo-A or its fragments with 8 M urea results in a strong decrease of inhibitory activity, indicating that conformation is important. The analysis of Nogo fragments in the fibroblast spreading assay reveals that at least two stretches of the Nogo-A protein mediate inhibition of the spreading of freshly plated fibroblasts, namely NiR-D2 (aa 59-172) and NiG-D20 (aa 544-725). All the fragments derived from the NiG-region displaying inhibitory activity (e.g. NiG-D4 and NiG-D8) partially overlap with NiG-D20. Minor inhibitory activity at high protein concentration is seen for NiG-D19 within the NiG-D6 region. Nogo-C, Nogo-66 and rat Peptide 4 (shown to be the inhibitory region of Nogo-66 by GrandPré et al., 2000) are not inhibitory for fibroblast spreading. These data show that the anti-spreading activity of Nogo-A on 3T3 fibroblasts resides in two defined stretches located at the N-terminus (NiR-D2) and within the Nogo-A-specific part (NiG-D20) of the protein. Non-specific physico-chemical properties (acidity of the fragments, structural effects due to proline and serine residues) are not responsible for this effect. The C-terminal RTN domain is *not* involved in the inhibition of fibroblast spreading.

b) NiG-D20 Region of Nogo-A is inhibitory for neurite outgrowth: To determine whether the fragments of Nogo-A that are non-permissive for cell spreading are also inhibitory for neurite outgrowth, a series of bacterially produced Nogo-A fragments as well as eukaryotically produced Nogo-AP chimeras in different neuronal assays are tested. In the stripe assay (method 1), neurites avoid laminin/Nogo-A coated stripes, growing on the laminin-only stripes, whereas stripes coated with laminin/beta-Galactosidase are not circumvented. Full-length Nogo-A is strongly non-permissive for retinal ganglion cell (RGC) neurite outgrowth, while the N-terminal part (NiR) had only marginal effects. Nogo-C activity is indistinguishable from the control protein beta-Galactosidase. The Nogo-A-specific region NiG-D20 appears to

contain the main region responsible for the non-permissive activity on RGC neurite outgrowth; the growth cones stop when encountering NiG—D20-coated stripes. The nonpermissive effect is concentration-dependent. At lower Nogo-A concentrations the number of crossing fibers increased. No obvious difference is observed between nasal and temporal RGC neurites concerning their responsiveness to Nogo-A regions. A laminin-independent, NGF-responsive clone of PC12 cells is primed with 50 ng/ml NGF for 24 hrs and then plated onto dishes coated with bacterially produced Nogo fragments at 0.1-3 $\mu g/cm^2$. Neurite outgrowth is scored one day later. The Nogo-A-specific region (NiG) and its fragment NiG-δ20 strongly inhibited PC12 neurite outgrowth. In contrast, the N-terminal fragment NiR has only minor activity, detectable only at high protein concentration. Nogo-C and Nogo-66 are inactive.

Example 2: Presence of binding site(s) for NiR-G and NiG-D20 on 3T3 fibroblasts and rat cortical brain membranes:

Methods:

a) Radioactive labelling and binding experiments: IMAC-purified NiG-D20 is iodinated by ANAWA Trading SA (Wangen, Switzerland) (2,030 Ci/mmol) using Lactoperoxidase and purified by reverse-phase HPLC. Membranes from rat brain cortex are prepared as described (Olpe HR, Karlsson G, Pozza MF, Brugger F, Steinmann M, Van Riezen H, Fagg G, Hall RG, Froestl W, Bittiger H (1990) CGP 35348: a centrally active blocker of GABAB receptors. Eur.J.Pharmacol. 187:27-38). Binding is performed for 1 hr at RT essentially as described (Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, McMaster G, Angst C, Bittiger H, Froestl W, Bettler B (1997) Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. Nature 386:239-246.) using 1.5 ml tubes preincubated for 2 hrs with 1% (w/v) bovine serum albumin to reduce non-specific binding. Membrane homogenates in HEPES buffer pH 7.4 (125 mM NaCl, 5 mM KCl, 0.6 mM $MgCl_2$, 1.8 mM $CaCl_2$, 20 mM HEPES, 6 mM dextrose) containing protease inhibitors (Rôche Diagnostics, Mannheim, FRG) are incubated with 1 1.3 nM iodinated NiG-D20 in the absence or presence of increasing concentrations of unlabelled NiG-D20.

b) Flow cytometry: Flow cytometry and cell sorting are performed on a Cytomation MoFlo high-speed cell sorter (Fort Collins, Colorado). The flow cytometer is equipped with an argon-ion/UV Enterprise II laser tuned to 488 nm with 130 mW of power. Fluorescein (FITC)

fluorescence is collected through a 530/40 nm bandpass filter. For analysis 3T3 fibroblasts are detached with Cell Dissociation Buffer (Gibco). The pre-formed complex used to detect binding of NiR-G to 3T3 fibroblasts is prepared as follows: NiR-G and anti-Myc antibody (9E10) are incubated at a 1:1 molar ratio for 30 min at 4 ºC. Next, FITC conjugated $F(ab)_2$ Goat Anti Mouse IgG is added and incubated for additional 30 min at 4 ºC . The resulting molar ratio of the trimeric complex is 1:1:0.5. The complex is added to $1x10^6$ 3T3 fibroblasts in a final volume of 0.1 ml, incubated for 2 hrs at 4 ºC, washed, and analysed by flow cytometry.

Results:

Presence of binding site(s) for Nogo-A-specific active fragments on 3T3 fibroblasts and rat cortical brain membranes: Since the NiR-D2 and NiG-D20 regions of Nogo-A are inhibitory for cell spreading and neurite outgrowth despite the absence of Nogo-66 and independently of NgR, the presence of a separate, Nogo-A-specific receptor has to be postulated. Thus binding studies are performed of multimerised, myc-tagged and IMAC-purified NiR-G to living 3T3 fibroblasts that are analysed by flow cytometry. Ab-complexed NiR-G is binding efficiently to 3T3 cells as seen by a fluorescence shift of over 90% of the 3T3 cells. In contrast, 3T3 cells are not labelled after incubation with the 9E10 primary mouse anti-myc mAb complexed with a FITC-conjugated secondary $F(ab)_2$ goat anti-mouse IgG nor with the secondary Ab alone. To test binding of NiG-D20 to rat cortical membranes, [^{125}I]-labelled NiG-D20 in a radioligand binding assay is used. At a concentration of 1.3 nM of [^{125}I]-NiG-D20, evidence for a specific NiG-D20 binding sites on brain membranes as shown by a concentration-dependent competition of radioligand binding by unlabelled NiG-D20 is found. These results show that aminoterminal fragments of Nogo-A can bind to the surface of 3T3 cells and to rat cortical membranes, demonstrating the presence of membrane-bound, Nogo-A-specific binding sites or receptor(s).

Example 3: Generation of mouse 11C7-IgG1

Mice (C3H- and C57BI6/J-strains) are immunised subcutaneously with the synthetic peptide SYDSIKLEPENPPPYEEA (= rat NogoA_623-640; SEQ ID NO: 1), corresponding to a particular epitope in NiG-D20. This epitope is highly conserved in human, cynomologus monkey and mouse NiG-D20 Nogo-A specific region and starts at amino acid 623 and ends

at amino acid 640 of the human NogoA amino acid sequence (SEQ ID NO: 5) (See also sequence alignment: Figure 1).

mAb 11C7 has been obtained out of a fusion of rat NogoA_623-640 with the carrier protein Key hole limped hemagglutinin (KLH) immunised mice. Monoclonal antibodies have been screened by ELISA on rat NogoA_623-640-KLH, rat NogoA_623-640 free peptide and a nonrelated peptide-KLH. In a further screen, the mAbs have been tested by ELISA on NiR-G versus b-Galactosidase, both expressed as his-tagged proteins and purified by metal affinity chromatography . Subsequently, the mAbs have been tested for recognition of Nogo-A on Western blot of oligodendrocyte and brain lysates (rat origin). Antibodies are tested for recognition of the protein in immunocytochemistry of rat Nogo-A-transfected CHO or COS cells and of endogenous Nogo-A of rat oligodendrocytes (permeabilised cells). They have also been tested for surface binding to living rat oligodednrocytes. Species crossreactivity is tested on recombinant NiG of rat, mouse, human and bovine origin by ELISA and on endogenous rat, mouse, human and monkey Nogo-A by Western blot of tissue or cell extracts.

Western blot analysis: SDS-PAGE and Westernblotting are performed as described earlier (Huber AB, Weinmann O, Brosamle C, Oertle T, Schwab ME (2002) Patterns of Nogo mRNA and protein expression in the developing and adult rat and after CNS lesions. J. Neurosci. 22: 3553-3567), blocking is done with 3% (w/v) Top Block (Juro Supply, Lucerne, Switzerland). Antibodies are diluted as follows: Purified monoclonal 11C7 or hybridoma supernatants 1:150. Secondary antibodies are HRP-conjugate anti-mouse ((Pierce; 1:5000,) 1:50,000). Hybridisation with the 11C7 antibody is carried out over night at 4°C. For detection the ECL detection reagents from Amersham Pharmacia are used.

Results:

The 11C7 mAb identifies the 190 kD Nogo-A band on a Western blot of oligodendrocyte cell culture homogenate. 11C7 also identifies human NiG, Cynomolgus NiG cell lysate and rat NiG-D20 in western blots. 11C7 mAb is characterised as a IgG1 isotype (IsoStrip Kit, Roche).

Example 4: Characterisation of the mouse 11C7 mAb

Immunocytochemistry: Optic nerve oligodendrocytes are prepared as described (Schwab, Caroni, 1988, Neuron). Three to five day-old cultures grown on poly-L-lysine coated coverslips are washed twice with PBS, fixed in 4% (w/v) paraformaldehyde (PFA), 5% (w/v) sucrose in PBS for 15 min at room temperature (RT) and non-specific binding is blocked with 10% (v/v) FCS. Cells were then incubated with mouse 11C7 (1:100). Secondary antibodies are goat-anti-mouse TRITC (Jackson ImmunoResearch Laboratories). For cell surface staining, two day-old rat optic nerve cultures are incubated with monoclonal antibody in medium for 25 min at RT. Secondary alkaline phosphatase conjugated antibodies (Milan Analytica, Lausanne) are used at 1:7,500 in 0.1 M maleic acid with 1% (w/v) blocking reagent (1 hr). The cultures are washed twice with maleic acid buffer, once with alkaline phosphatase buffer (0.1 M Tris-HCl pH 9.5, 0.1 M NaCl, 5 mM $MgCl_2$) and the staining is developed for 3 hrs at room temperature with 0.175 mg/ml BCIP (Sigma) and 0.338 mg/ml NBT (Sigma) in alkaline phosphatase buffer.

NogoA_623-640 epitope of Nogo-A present at the cell surface of cultured oligodendrocytes: Living cultures of oligodendrocytes incubated with mouse 11C7 mAb stain the differentiated oligodendrocyte cell bodies and their radial processes. The control mouse IgG and the antibodies against the intracellular protein CNPase do not stain the living cells. Pre-incubation of mouse 11C7 with the corresponding immunogenic peptide (= rat NogoA_623-640 SED ID NO: 1) reduces staining to background levels (competitive assay). Cell surface staining is present on all major and small processes and on the cell body. Thus, the Nogo-A specific part of the molecule recognised by mouse 11C7 mAb is exposed to the extracellular space on the plasma membrane of oligodendrocytes.

Production and Purification of mouse 11C7 mAb: A 10-L glas bioreactor is used for continuous-mode cultivation of the hybridoma clone producing the mouse 11C7 mAb. The bioreactor is equipped with a marine impeller placed in a center tube for gentle agitation, a spin filter for cell retention, and coiled silicone tubing for bubble-free aeration. The hybridoma cells are cultivated in our RPMI based serum free medium. The medium is inoculated with cells at 3.7 x 10^5/ml. After 28 hours continuous medium flow through the bioreactor is started with a rate of 0.5 fermentor volumes /day (5 liters/day). Another 24 hours later the flow rate is increased to its final level of 1 fermentor volume/day (10 liters/day). After 1 week the culture reaches a steady state with 11 x 10^5 cells/ml and the process is continued for another week. The titer of the mouse 11C7 mAb is determined daily

by HPLC. A total of 150 liters culture supernatant is harvested from the bioreactor, sterile filtered for removal of cells and cell debris. 150 L culture supernatant are concentrated to about 6 L using a Pellikon tangential flow device (Millipore ; 10 kDa cut-off). The concentrated supernatant is purified in 3 runs over a 220 ml bed volume column of Protein A Sepharose CI-4B (Pharmacia ; 11 cm bed height). Briefly, the culture supernatant after pH correction to 8.1 is loaded at 4 ml/min and the column washed to base-line at 8 ml/min using 100 mM Na_2HPO_4, pH 8.1. Bound material is finally eluted at 8 ml/min using 50 mM NaH_2PO_4, pH 3.0, 140 mM NaCl and immediately neutralized (pH 7.0) with 5 N NaOH and sterile filtered. Absorbance is monitored at 280 nm. Portion of the purified material are eventually further concentrated by ultrafiltration and/or dialyzed against PBS. All the buffers used in the purification are filtered on a 10 kDa ULTRASETTE™ tangential flow device (Filtron Technology Corporation) in order to remove possible endotoxin contaminations. For the same reason the Protein A resin is extensively washed with 20% ethanol and all tubings/pumps treated with 0.1 M NaOH prior to use. Protein concentration is measured spectrophotometrically at 280 nm using a reference absorption of 1.35 for 1 mg/ml. Purity is routinely assessed by SDS-PAGE under reducing conditions using 4-20% Novex gradient gels. Endotoxin content is measured by the classical Limulus Amoebocyte Lysate (LAL) reaction according to the manufacturer instructions (Endotell AG, Allschwil, Switzerland).

Generation of F_{ab} fragments: A portion of mouse 11C7 mAb is extensively dialyzed against 100 mM Na-actetate, pH 5.5, 2 mM EDTA and adjusted to a concentration of 6 mg/ml. F_{ab} fragments are generated by papain digestion (1:200 w/w ratio) in the presence of 0.25 mM cysteine. The reaction is allowed to proceed for 16 hours at 37 °C and then stopped by the addition of the specific papain inhibitor E64 (N-[N-(L-3-trans-carboxirane- 2-carbonyl)-L-leucyl]-agmatine) in large excess (10 μM). The digested antibody is then passed over a column of protein A Sepharose Fast Flow in order to remove intact material and Fc fragments. The F_{ab} fraction is extensively dialysed against PBS and concentrated to about 3 mg/ml. (Papain and E64 are from Roche Molecular Biochemicals).

HPLC, Mass Spectrometry and N-terminal amino acid sequencing of V_L and V_H regions:

a) Reduction and Alkylation: Purified, dried 11C7 antibody are dissolved in 40 µl of 8M urea, 0.4M NH_4HCO_3, pH 8.3. 60 ug DTT (Calbiochem), pre-dissolved in 10 ul of the same buffer as the protein, are added. Reduction is performed at 50°C for 30 min under argon (100 fold molar excess of DTT over protein thiols). After reduction, the sample is cooled

to room temperature. 304 ug of iodoacetamide (Sigma Ultra, I-1149) dissolved in the same buffer as the protein is added. Carboxamidomethylation is carried out at room temperature for 15 min in the dark. 1 µl β-mercaptoethanol is added to quench the reaction.

b) Isolation of Heavy- and Light-Chain: Carboxamidomethylated heavy and light chains of antibody are isolated by Reverse Phase High Pressure Liquid Chromatography (RP-HPLC) on a Hewlett Packard 1090M HPLC System with DR5 pumping system and diode-array UV detector. The conditions for chromatography are: PerSeptive Biosystems Poros 2.1x100 mm column packed with R1/H material; flow is 0.5 ml/min; solvents: (A) 0.1% TFA in water and (B) 0.09% TFA / acetonitril/water 9:1; gradient 25-70% B in 8 minutes at 80°C; detection at 218 / 280 nm.

c) LC-ESI-MS: Mass spectrometry is carried out using a Q-Tof (Micromass, Manchester, UK) quadrupole time-of-flight hybrid tandem mass spectrometer equipped with a Micromass Z-type electrospray ionization source (ESI). Acquisition mass range is typically m/z 500-2000. Data are recorded and processed using MassLynx software. Calibration of the 500-2500 m/z scale is achieved by using the multiple-charged ion peaks of horse heart myoglobin (MW 16951.5).

d) HPLC-MS of heavy and light chain: Separation of reduced and carboxamidomethylated heavy and light chain is performed on a HP1100 HPLC system (Hewlett Packard, Palo-Alto, CA, USA) employing a 1mmx150mm LC Packings column packed with Perseptive Biosystems POROS R1/H. The column is held at 60°C. Sample volumes of 10 µl are injected onto the column using a CTC PAL autosampler (CTC, Zwingen, Switzerland) fitted with a Valco model C6UW HPLC valve (Valco, Houston, TX, USA) and a 10 µl injection loop. HPLC was controlled by MassLynx software (Micromass, Manchester, UK). UV detection is at 214 nm. Eluent A is water containing 0.05% TFA. Eluent B is a 1:9 mixture of water : acetonitrile containing 0.045% TFA. A gradient from 20% B to 90% B is run in 20 minutes at 80 °C. The flow rate is typically 60 µl/min. The total flow from the LC system is introduced into the UV detection cell, then the ESI source without any splitting. The HPLC system is controlled and the signal from the UV detector is processed using MassLynx software (Micromass, Manchester, UK). The following 5 signals are detected:

Table 1:

Measured:	**Signal interpretation**
A= 50959.0 Da	H-Chain with carboxamidomethyl-cysteine (CAMCys)*
B= 51119.5 Da	Signal A+162 Da (= hexose)**

C= 51086.0 Da	Signal A+ 127 (Lys), H-Chain with CAMCys*
D= 51251.0 Da	Signal C+1 62 Da (= hexose)**
E= 24464.8 Da	L-Chain with CAMCys
	*There are two types of H-chain present, one with and one without Lys at the C-terminal end. The ratio of both forms is approximately 50 : 50%. **Both types of H-chains have two corresponding glycosylated forms (+162)

d) N-terminal amino acid sequencing of V_L and V_H regions: Collected H+L chains peaks form HPLC are used for sequence analysis. Amino acid sequences are determined on a Hewlett Packard G1000A N–terminal Protein Sequencing System. The system performs automated Edman chemistry on protein samples retained on miniature adsorptive biphasic columns. An optimized chemistry method (double couple 3.0) is used to enhance chemical efficiency; minimize lags and herewith extend sequence analysis to about 50 residues. Analysis of PTH-amino acids is performed on an on-line Hewlett Packard HP1090 HPLC System equipped with a ternary pumping system and a narrowbore (2.1mm x 25cm) PTH column.

Results:

From mass analysis homogeneous heavy and light chain of mouse 11C7-IgG1 are determined. The H-chain is single glycosylated and there are two forms with a difference on the C-terminal Lysine. Total mass analysis of heavy and light chain shows a single mass for both chains. HPLC chromatography of mouse 11C7-IgG1 shows a single peak. After HPLC purification followed by reduction and alkylation pure heavy and light chain are available. N-terminal sequence degradation is performed on light-chain and heavy-chain. 45 to 55 amino acids from the N-terminal sequence of L-chain and H-chain are identified by sequence degradation.

Light Chain

1 5 10 15 20 25 30

▼ ▼ ▼ ▼ ▼ ▼ ▼

DVLLTQTPLTLSITIGQPASISC KSSQSLL

31 35 40 45 50 55 60

▼ ▼ ▼ ▼ ▼ ▼ ▼

HSDGKTYLNWLLQRPGQ

Heavy Chain

1 5 10 15 20 25 30

▼ ▼ ▼ ▼ ▼ ▼ ▼

EVKLLESGGGLVQPGGSLKLSCVVSGFDFR

31 35 40 45 50 55 60

▼ ▼ ▼ ▼ ▼ ▼ ▼

RNWMSWVRQAPGKGLEWIGEINPD

Example 5: Cloning of the heavy and light chain genes of mouse 11C7 mAb

Total RNA is prepared from 10^7 hybridoma cells (clone 11C7) using TriPure reagent (Roche diagnostics, Germany, Cat.# 1667157) according to the manufacturers instructions. For cDNA synthesis, mRNA is isolated from above prepared total RNA using Oligotex Resin (Qiagen, Germany, cat. # 70022).

cDNA is generated by reverse transcription using the following conditions: 2 µl mRNA, 2 µl 10 x reverse transcription buffer, 2 µl $(dT)_{20}$ primer (10 µM), 0.5 µl RNasin (Promega, 40 U/ml), 2 µl dNTPs (5 mM each), 1 µl Omniscript™ reverse transcriptase (Qiagen, Cat # 205110), 10.5 µl ddH_2O, Reaction:1hr at 37°C. For PCR amplification of cDNA encoding for the V_H and V_L the proofreading enzyme ProofStart™ DNA polymerase is used.

PCR of light and heavy chain: Reaction mix: 2 µl cDNA , 5 µl 10 x reaction buffer, 3 µl dNTPs (5 mM each), 2 µl 5'primer (10 µM) (see Table 2), 2µl 3'primer (10 µM) (see Table 2), 1 µl ProofStart (Qiagen, Cat # 202203), 36 µl ddH_2O. PCR conditions: 95°C/5 min, (95°C/40 sec, 53°C/1 min, 72°C 1 min) x 35, 72°C/ 10 min. The resulting PCR products are ligated directly into pCRbluntTOPO (Invitrogen). The ligation mix is transfected into TOP 10 cells (Invitrogen) and several clones are picked. The nucleotide sequences of the variable part of the heavy chain of the 11C7 mAb (V-H, SEQ ID NO: 43) and of the light chain of the 11C7 mAb (V-L, SEQ ID NO: 44) cDNas are determined on an ABI sequencer. The subsequent amino acid sequence of V-H and V-L are shown in SEQ ID NO: 2 (V-H) and SEQ ID NO: 3 (V-L). Primers used for PCR amplification of the V_H and V_l cDNAs; all primers are synthesized by MWG Biotech, Germany.

Table2:

Primer	Sequence	SEQ ID

		NO:
5'-V_L leader	A ATATGAGTCCTGCCCAGTTCCTGTTTC	39
3'-C_K	T TAGGAATTCCTAACACTCTCCCCTGTTGAAG	40
5'-V_H leader	A ATATGGATTTTGGGCTGATTTTTTTTATTG	41
3'-C_H hinge	A ATTGGGCAACGTTGCAGGTGACG	42

Example 6: Binding of 11C7 and Fab to Nogo-A domains using ELISA

Greiner 96 well PS plates (#655161) are coated with 0.4-2ug/ml Nogo protein fragments in PBS (100ul/well) covered and incubated 4 hours at room temperature. Plates are flicked and refilled with 200ul/well blocking buffer (PBS+2% BSA), covered and incubated. 1h at RT or overnight at 4 °C, then washed 4 times with water and PBS. Different concentrations of mouse 11C7 mAb or 11C7 Fab are diluted in PBS +2% BSA (100 ul/well), and incubated 2h at RT or overnight at 4 °C. Wash step is repeated and Goat anti-mouse IgG conjugated with horse radish peroxidase (HRP) at a dilution of 1:5000 (ICN #55550) in PBS/0.1%BSA /0.1%Nonidet 40 (100 ul/well) is added and incubated. 2h at RT or overnight at 4 °C and wash step is repeated. HRP reaction is started by adding 100 ul/well BM blue POD (Roche #1484281) and incubated in the dark at RT for 15 minutes . H2SO4 50ul/well 1M is added to stop HRP substrate reaction and the optical density is determinated using a microplate reader (Packard Spectra Count) set to 450nm.

The mouse 11C7 mAb binds to human NiG, rat NiG, mouse NiG, rat NiG-D20 and peptide 472 at very low concentrations of 0.02 to 2.5 nM. Binding to human NiG, rat NiG, mouse NiG at very low concentration is confirmed by the very high affinity (Kd 0.1 – 0.44nM Biosensor affinity measurements) and is consistent with the fact that 472 peptide with the exception of 2-3 amino acids is identical in human compared to rat and mouse equivalent region. The specificity of the binding is indicated by the fact that the mouse 11C7 mAb does not show any binding at all to rat NiG-D6 and Nogo-66 fragments over the same concentration range. The Fab monovalent fragment bound to human NiG and rat NiG-D20 at concentrations 0.025 to 25nM and showed no binding to rat NiG-D6 and Nogo-66 fragments over the same concentration range. The Kd measured by Biosensor was 7.14 nM for human NiG.

Example 7: Biosensor affinity measurements for mouse 11C7-IgG1 and Fab to Nogo-A domains

The affinity of the mouse 11C7 mAb and of the 11C7 Fab are measured by surface plasmon resonance (SPR) using a BIAcore 2000 optical biosensor (Biacore, Uppsala, Sweden) according to the manufacture's instructions (see Figure 2). Recombinant human, mouse, and rat NIG are covalently attached to three separate flow cells of a CM5 sensor chip using amine-coupling chemistry. Briefly; the carboxymethlyladed dextran matrix is activated by injecting 35ul of a solution containing 0.025M NHS and 0.1M EDC. For the immobilization on the sensor chip the recombinant mouse, human, and rat NIG are diluted in 0.01M citrate buffer at a pH varying between 3.5 and 4.5 and injected at a flow rate of 5ul/min to achieve coupling levels allowing affinity measurements. The deactivation of the remaining NHS-ester group is performed by injection of 35ul of 1M ethanolamine hydrochloride (pH 8.5). The surface of the sensor chip is regenerated by injecting 5ul 0.1M HCl. For the measurement of the affinity the antibodies are injected at different concentration, ranging from 0.50nM to 100nM at a flow rate of 200 ul/min. After each injection the sensor chip surface is regenerated with the injection of 10 ul 0.1M HCl without loss of binding activity on the surface. The kinetic constants, *ka* and *kd* and the affinity constants KA and KD are evaluated using the BIAevaluations 3.0 software supplied by the manufacturer.

Affinity measurement in BIAcore: The kinietc and the affinity binding constants of the mouse 11C7 mAb and the 11C7 derived monovalent Fab fragment to recombinat NogoA are measured in real time using surface plasmon resonance (SPR) technology (Biacore). For this analysis recombinant human, mouse and rat NIGs are coupled on three independent sensor chip surfaces and different concentrations of the antibodies are injected. Kinetic parameters of the binding interactions are derived from the sensorgrams by non-linear curve fitting. The affinity constants at equilibrium of mouse 11C7-IgG1 are KD= 0.1nM, KD= 0.4nM and KD= 0.19nM for human, rat, and mouse NIG respectively (table 3). For the 11C7 derived Fab fragment the affinity constant to human NIG is KD= 7.14nM. The lower affinity of the Fab fragment results from a decrease of both kinetic constants, association and dissociation (*ka, kd).* Lower affinity of the Fab fragment compared to the complete antibody is probably related to the avidity effect, which is lacking in the monomeric Fab.

Table 3:

11C7	Ka (1/Ms)	kd (1/s)	KA (M^{-1})	KD (M)
Human IgG	4.48×10^{5}	4.6×10^{-5}	9.73×10^{9}	1.03×10^{-10}
Rat IgG	8.76×10^{5}	3.89×10^{-4}	2.25×10^{9}	4.44×10^{-10}
Mouse IgG	5.52×10^{5}	1.06×10^{-4}	5.2×10^{9}	1.92×10^{-10}
11C7 Fab	Ka (1/Ms)	kd (1/s)	KA (M^{-1})	KD (M)
Human IgG	7.29×10^{4}	5.28×10^{-4}	1.4×10^{8}	7.14×10^{-9}

Claims:

1.) A binding molecule which is capable of binding to the human NogoA polypeptide (SEQ ID NO: 5) or human NiG (SEQ ID NO: 7) or human NiG-D20 (SEQ ID NO: 24) or human NogoA_623-640 (SEQ ID NO: 6) with a dissociation constant < 1000nM.

2.) A binding molecule which is capable of binding to the human NogoA polypeptide (SEQ ID NO: 5) or human NiG (SEQ ID NO: 7) or human NiG-D20 (SEQ ID NO: 24) or human NogoA_623-640 (SEQ ID NO: 6) with a dissociation constant < 1000nM and comprises at least one antigen binding site, said antigen binding site comprising either

- in sequence the hypervariable regions CDR1, CDR2, and CDR3, of which each of the hypervariable regions are at least 50% homologous to their equivalent hypervariable regions CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9) and CDR3-11C7 (SEQ ID NO: 10); or
- in sequence the hypervariable regions CDR1', CDR2', and CDR3', of which each of the hypervariable regions are at least 50% homologous to their equivalent hypervariable regions CDR1'-11C7 (SEQ ID NO: 11), CDR2'-11C7 (SEQ ID NO: 12) and CDR3'-11C7 (SEQ ID NO: 13).

3.) A binding molecule which is capable of binding to the human NogoA polypeptide (SEQ ID NO: 5) or human NiG (SEQ ID NO: 7) or human NiG-D20 (SEQ ID NO: 24) or human NogoA_623-640 (SEQ ID NO: 6) with a dissociation constant < 1000nM and comprises

- a first antigen binding site comprising in sequence the hypervariable regions CDR1, CDR2, and CDR3, of which each of the hypervariable regions are at least 50% homologous to their equivalent hypervariable regions CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9) and CDR3-11C7 (SEQ ID NO: 10); and
- a second antigen binding site comprising in sequence the hypervariable regions CDR1', CDR2', and CDR3', of which each of the hypervariable regions are at least 50% homologous to their equivalent hypervariable regions CDR1'-11C7 (SEQ ID NO: 11), CDR2'-11C7 (SEQ ID NO: 12) and CDR3'-11C7 (SEQ ID NO: 13).

4.) A binding molecule which comprises at least one antigen binding site, said antigen binding site comprising either

- in sequence the hypervariable regions CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9) and CDR3-11C7 (SEQ ID NO: 10); or
- in sequence the hypervariable regions CDR1'-11C7 (SEQ ID NO: 11), CDR2'-11C7 (SEQ ID NO: 12) and CDR3'-11C7 (SEQ ID NO: 13); or
- direct equivalents thereof.

5.) A binding molecule comprising

- a first antigen binding site comprising in sequence the hypervariable regions CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9) and CDR3-11C7 (SEQ ID NO: 10); and
- a second antigen binding site comprising in sequence the hypervariable regions CDR1'-11C7 (SEQ ID NO: 11), CDR2'-11C7 (SEQ ID NO: 12) and CDR3'-11C7 (SEQ ID NO: 13); or
- direct equivalents thereof.

6.) The binding molecule according to claims 1 to 5 which comprises at least

- one immunoglobulin heavy chain or fragment thereof which comprises (i) a variable domain comprising in sequence the hypervariable regions regions CDR1-11C7 (SEQ ID NO: 8), CDR2-11C7 (SEQ ID NO: 9) and CDR3-11C7 (SEQ ID NO: 10) and (ii) the constant part or fragment thereof of a human heavy chain; and
- one immunoglobulin light chain or fragment thereof which comprises (i) a variable domain comprising in sequence the hypervariable regions CDR1'-11C7 (SEQ ID NO: 11), CDR2'-11C7 (SEQ ID NO: 12) and CDR3'-11C7 (SEQ ID NO: 13) and (ii) the constant part or fragment thereof of a human light chain; or
- direct equivalents thereof.

7. The binding molecule according to claim 6 in which the constant part or fragment thereof of the human heavy chain is of the γ4 type and the constant part or fragment thereof of the human light chain is of the κ type.

8. The binding molecule according to claims 1 to 7, which is a chimeric or humanised monoclonal antibody.

9. A binding molecule comprising polypeptide sequences as shown in SEQ ID NO: 2 and SEQ ID NO: 3.

10. A polynucleotide comprising polynucleotides encoding a binding molecule according to any of claims 1 to 9.

11. A polynucleotide comprising either

- polynucleotide sequences as shown in SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16; or
- polynucleotide sequences as shown in SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19.

12. An expression vector comprising polynucleotides according to any one of claims 10 or 11.

13. An expression system comprising a polynucleotide according to any one of claims 10 or 11, wherein said expression system or part thereof is capable of producing a polypeptide of any one of claims 1 to 9, when said expression system or part thereof is present in a compatible host cell.

14. An isolated host cell which comprises an expression system according to claim 13.

15. The use of a binding molecule according to any one of claims 1 to 9 as a pharmaceutical.

16. The use of a binding molecule according to any one of claims 1 to 9 in the manufacture of a pharmaceutical for the treatment of nerve repair.

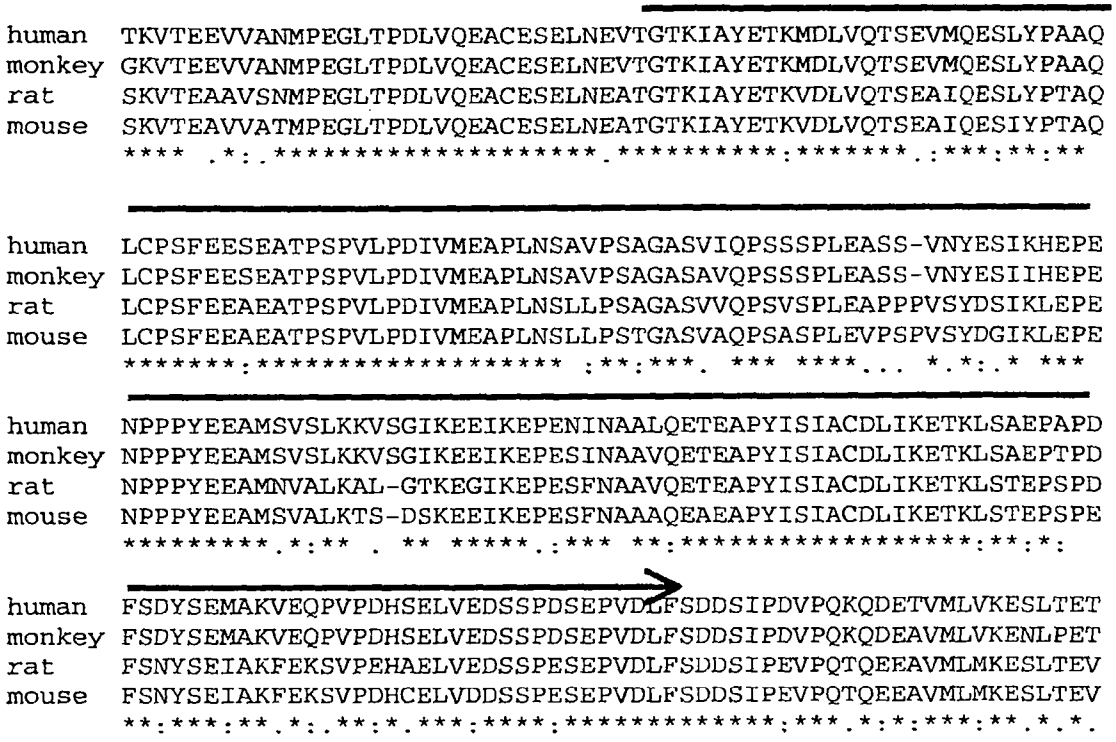
17. A pharmaceutical composition comprising a binding molecule according to any one of claims 1 to 9 in association with at least one pharmaceutically acceptable carrier or diluent.

18. A binding molecule according to any one of claims 1 to 9, for use in the treatment of diseases associated with nerve repair.

19. The use according to claim 15 or 16, substantially as herein described and exemplified.

20. A pharmaceutical composition according to claim 17, substantially as herein described and exemplified.

Figure 1: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mouse SKVTEAVVATMPEGLTPDLVQEACESELNEATGTKIAYETKVDLVQTSEAIQESIYPTAQ
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Arg Asn Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
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Trp Ile Gly Glu Ile Asn Pro Asp Ser Ser Lys Ile Asn Tyr Thr Pro
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Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser
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            20                  25                  30

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        35                  40                  45

ctg gag gag ctg gag gtg ctg gag agg aag ccc gcc gcc ggg ctg tcc      192
Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly Leu Ser
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gcg gcc cca gtg ccc acc gcc cct gcc gcc ggc gcg ccc ctg atg gac      240
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Ser Lys Glu Val Ser Glu Lys Ala Lys Thr Leu Leu Ile Asp Arg Asp
        275                 280                 285

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    290                 295                 300

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305                 310                 315                 320

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gaa agt aaa gtg gat aaa aaa tgt ttt gca gat agc ctt gag caa act      1296
Glu Ser Lys Val Asp Lys Lys Cys Phe Ala Asp Ser Leu Glu Gln Thr
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aat cac gaa aaa gat agt gag agt agt aat gat gat act tct ttc ccc      1344
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820 825 830

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            900                 905                 910

aca gaa ttg ccc cat gac ctt tct ttg aag aac ata caa ccc aaa gtt     2784
Thr Glu Leu Pro His Asp Leu Ser Leu Lys Asn Ile Gln Pro Lys Val
        915                 920                 925

gaa gag aaa atc agt ttc tca gat gac ttt tct aaa aat ggg tct gct     2832
Glu Glu Lys Ile Ser Phe Ser Asp Asp Phe Ser Lys Asn Gly Ser Ala
    930                 935                 940

aca tca aag gtg ctc tta ttg cct cca gat gtt tct gct ttg gcc act     2880
Thr Ser Lys Val Leu Leu Leu Pro Pro Asp Val Ser Ala Leu Ala Thr
945                 950                 955                 960

caa gca gag ata gag agc ata gtt aaa ccc aaa gtt ctt gtg aaa gaa     2928
Gln Ala Glu Ile Glu Ser Ile Val Lys Pro Lys Val Leu Val Lys Glu
                965                 970                 975
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gct gag aaa aaa ctt cct tcc gat aca gaa aaa gag gac aga tca cca 2976
Ala Glu Lys Lys Leu Pro Ser Asp Thr Glu Lys Glu Asp Arg Ser Pro
980 985 990

tct gct ata ttt tca gca gag ctg agt aaa act tca gtt gtt gac ctc 3024
Ser Ala Ile Phe Ser Ala Glu Leu Ser Lys Thr Ser Val Val Asp Leu
995 1000 1005

ctg tac tgg aga gac att aag aag act gga gtg gtg ttt ggt gcc 3069
Leu Tyr Trp Arg Asp Ile Lys Lys Thr Gly Val Val Phe Gly Ala
1010 1015 1020

agc cta ttc ctg ctg ctt tca ttg aca gta ttc agc att gtg agc 3114
Ser Leu Phe Leu Leu Leu Ser Leu Thr Val Phe Ser Ile Val Ser
1025 1030 1035

gta aca gcc tac att gcc ttg gcc ctg ctc tct gtg acc atc agc 3159
Val Thr Ala Tyr Ile Ala Leu Ala Leu Leu Ser Val Thr Ile Ser
1040 1045 1050

ttt agg ata tac aag ggt gtg atc caa gct atc cag aaa tca gat 3204
Phe Arg Ile Tyr Lys Gly Val Ile Gln Ala Ile Gln Lys Ser Asp
1055 1060 1065

gaa ggc cac cca ttc agg gca tat ctg gaa tct gaa gtt gct ata 3249
Glu Gly His Pro Phe Arg Ala Tyr Leu Glu Ser Glu Val Ala Ile
1070 1075 1080

tct gag gag ttg gtt cag aag tac agt aat tct gct ctt ggt cat 3294
Ser Glu Glu Leu Val Gln Lys Tyr Ser Asn Ser Ala Leu Gly His
1085 1090 1095

gtg aac tgc acg ata aag gaa ctc agg cgc ctc ttc tta gtt gat 3339
Val Asn Cys Thr Ile Lys Glu Leu Arg Arg Leu Phe Leu Val Asp
1100 1105 1110

gat tta gtt gat tct ctg aag ttt gca gtg ttg atg tgg gta ttt 3384

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Asp Leu  Val Asp Ser Leu Lys  Phe Ala Val Leu Met  Trp Val Phe
    1115                 1120                 1125

acc tat  gtt ggt gcc ttg ttt  aat ggt ctg aca cta  ctg att ttg      3429
Thr Tyr  Val Gly Ala Leu Phe  Asn Gly Leu Thr Leu  Leu Ile Leu
    1130                 1135                 1140

gct ctc  att tca ctc ttc agt  gtt cct gtt att tat  gaa cgg cat      3474
Ala Leu  Ile Ser Leu Phe Ser  Val Pro Val Ile Tyr  Glu Arg His
    1145                 1150                 1155

cag gca  cag ata gat cat tat  cta gga ctt gca aat  aag aat gtt      3519
Gln Ala  Gln Ile Asp His Tyr  Leu Gly Leu Ala Asn  Lys Asn Val
    1160                 1165                 1170

aaa gat  gct atg gct aaa atc  caa gca aaa atc cct  gga ttg aag      3564
Lys Asp  Ala Met Ala Lys Ile  Gln Ala Lys Ile Pro  Gly Leu Lys
    1175                 1180                 1185

cgc aaa  gct gaa tga aaacgcccaa aataattagt aggagttcat ctttaaaggg   3619
Arg Lys  Ala Glu
    1190

gatattcatt tgattatacg ggggagggtc agggaagaac gaaccttgac gttgcagtgc   3679

agtttcacag atcgttgtta gatctttatt tttagccatg cactgttgtg aggaaaaatt   3739

acctgtcttg actgccatgt gttcatcatc ttaagtattg taagctgcta tgtatggatt   3799

taaaccgtaa tcatatcttt ttcctatctg aggcactggt ggaataaaaa acctgtatat   3859

tttactttgt tgcagatagt cttgccgcat cttggcaagt tgcagagatg gtggagctag   3919
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<210> 5

<211> 1192

<212> PRT

<213> Homo sapiens

<400> 5

Met Glu Asp Leu Asp Gln Ser Pro Leu Val Ser Ser Ser Asp Ser Pro
1 5 10 15

Pro Arg Pro Gln Pro Ala Phe Lys Tyr Gln Phe Val Arg Glu Pro Glu
20 25 30

Asp Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Asp Glu Asp Glu Asp
35 40 45

Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly Leu Ser
50 55 60

Ala Ala Pro Val Pro Thr Ala Pro Ala Ala Gly Ala Pro Leu Met Asp
65 70 75 80

Phe Gly Asn Asp Phe Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala
85 90 95

Ala Pro Pro Val Ala Pro Glu Arg Gln Pro Ser Trp Asp Pro Ser Pro
100 105 110

Val Ser Ser Thr Val Pro Ala Pro Ser Pro Leu Ser Ala Ala Ala Val
115 120 125

Ser Pro Ser Lys Leu Pro Glu Asp Asp Glu Pro Pro Ala Arg Pro Pro
130 135 140

Pro Pro Pro Pro Ala Ser Val Ser Pro Gln Ala Glu Pro Val Trp Thr
145 150 155 160

Pro Pro Ala Pro Ala Pro Ala Ala Pro Pro Ser Thr Pro Ala Ala Pro
165 170 175

Lys Arg Arg Gly Ser Ser Gly Ser Val Asp Glu Thr Leu Phe Ala Leu
180 185 190

Pro Ala Ala Ser Glu Pro Val Ile Arg Ser Ser Ala Glu Asn Met Asp
195 200 205

Leu Lys Glu Gln Pro Gly Asn Thr Ile Ser Ala Gly Gln Glu Asp Phe
210 215 220

Pro Ser Val Leu Leu Glu Thr Ala Ala Ser Leu Pro Ser Leu Ser Pro
225 230 235 240

Leu Ser Ala Ala Ser Phe Lys Glu His Glu Tyr Leu Gly Asn Leu Ser
245 250 255

Thr Val Leu Pro Thr Glu Gly Thr Leu Gln Glu Asn Val Ser Glu Ala
260 265 270

Ser Lys Glu Val Ser Glu Lys Ala Lys Thr Leu Leu Ile Asp Arg Asp
275 280 285

Leu Thr Glu Phe Ser Glu Leu Glu Tyr Ser Glu Met Gly Ser Ser Phe
290 295 300

Ser Val Ser Pro Lys Ala Glu Ser Ala Val Ile Val Ala Asn Pro Arg
305 310 315 320

Glu Glu Ile Ile Val Lys Asn Lys Asp Glu Glu Glu Lys Leu Val Ser
325 330 335

Asn Asn Ile Leu His Asn Gln Gln Glu Leu Pro Thr Ala Leu Thr Lys
340 345 350

Leu Val Lys Glu Asp Glu Val Val Ser Ser Glu Lys Ala Lys Asp Ser
355 360 365

Phe Asn Glu Lys Arg Val Ala Val Glu Ala Pro Met Arg Glu Glu Tyr
370 375 380

Ala Asp Phe Lys Pro Phe Glu Arg Val Trp Glu Val Lys Asp Ser Lys
385 390 395 400

Glu Asp Ser Asp Met Leu Ala Ala Gly Gly Lys Ile Glu Ser Asn Leu

405 410 415

Glu Ser Lys Val Asp Lys Lys Cys Phe Ala Asp Ser Leu Glu Gln Thr
420 425 430

Asn His Glu Lys Asp Ser Glu Ser Ser Asn Asp Asp Thr Ser Phe Pro
435 440 445

Ser Thr Pro Glu Gly Ile Lys Asp Arg Ser Gly Ala Tyr Ile Thr Cys
450 455 460

Ala Pro Phe Asn Pro Ala Ala Thr Glu Ser Ile Ala Thr Asn Ile Phe
465 470 475 480

Pro Leu Leu Gly Asp Pro Thr Ser Glu Asn Lys Thr Asp Glu Lys Lys
485 490 495

Ile Glu Glu Lys Lys Ala Gln Ile Val Thr Glu Lys Asn Thr Ser Thr
500 505 510

Lys Thr Ser Asn Pro Phe Leu Val Ala Ala Gln Asp Ser Glu Thr Asp
515 520 525

Tyr Val Thr Thr Asp Asn Leu Thr Lys Val Thr Glu Glu Val Val Ala
530 535 540

Asn Met Pro Glu Gly Leu Thr Pro Asp Leu Val Gln Glu Ala Cys Glu
545 550 555 560

Ser Glu Leu Asn Glu Val Thr Gly Thr Lys Ile Ala Tyr Glu Thr Lys
565 570 575

Met Asp Leu Val Gln Thr Ser Glu Val Met Gln Glu Ser Leu Tyr Pro
580 585 590

Ala Ala Gln Leu Cys Pro Ser Phe Glu Glu Ser Glu Ala Thr Pro Ser
595 600 605

Pro Val Leu Pro Asp Ile Val Met Glu Ala Pro Leu Asn Ser Ala Val
610 615 620

Pro Ser Ala Gly Ala Ser Val Ile Gln Pro Ser Ser Ser Pro Leu Glu
625 630 635 640

Ala Ser Ser Val Asn Tyr Glu Ser Ile Lys His Glu Pro Glu Asn Pro
645 650 655

Pro Pro Tyr Glu Glu Ala Met Ser Val Ser Leu Lys Lys Val Ser Gly
660 665 670

Ile Lys Glu Glu Ile Lys Glu Pro Glu Asn Ile Asn Ala Ala Leu Gln
675 680 685

Glu Thr Glu Ala Pro Tyr Ile Ser Ile Ala Cys Asp Leu Ile Lys Glu
690 695 700

Thr Lys Leu Ser Ala Glu Pro Ala Pro Asp Phe Ser Asp Tyr Ser Glu
705 710 715 720

Met Ala Lys Val Glu Gln Pro Val Pro Asp His Ser Glu Leu Val Glu
725 730 735

Asp Ser Ser Pro Asp Ser Glu Pro Val Asp Leu Phe Ser Asp Asp Ser
740 745 750

Ile Pro Asp Val Pro Gln Lys Gln Asp Glu Thr Val Met Leu Val Lys
755 760 765

Glu Ser Leu Thr Glu Thr Ser Phe Glu Ser Met Ile Glu Tyr Glu Asn
770 775 780

Lys Glu Lys Leu Ser Ala Leu Pro Pro Glu Gly Gly Lys Pro Tyr Leu
785 790 795 800

Glu Ser Phe Lys Leu Ser Leu Asp Asn Thr Lys Asp Thr Leu Leu Pro
805 810 815

Asp Glu Val Ser Thr Leu Ser Lys Lys Glu Lys Ile Pro Leu Gln Met
820 825 830

Glu Glu Leu Ser Thr Ala Val Tyr Ser Asn Asp Asp Leu Phe Ile Ser
835 840 845

Lys Glu Ala Gln Ile Arg Glu Thr Glu Thr Phe Ser Asp Ser Ser Pro
850 855 860

Ile Glu Ile Ile Asp Glu Phe Pro Thr Leu Ile Ser Ser Lys Thr Asp
865 870 875 880

Ser Phe Ser Lys Leu Ala Arg Glu Tyr Thr Asp Leu Glu Val Ser His
885 890 895

Lys Ser Glu Ile Ala Asn Ala Pro Asp Gly Ala Gly Ser Leu Pro Cys
900 905 910

Thr Glu Leu Pro His Asp Leu Ser Leu Lys Asn Ile Gln Pro Lys Val
915 920 925

Glu Glu Lys Ile Ser Phe Ser Asp Asp Phe Ser Lys Asn Gly Ser Ala
930 935 940

Thr Ser Lys Val Leu Leu Leu Pro Pro Asp Val Ser Ala Leu Ala Thr
945 950 955 960

Gln Ala Glu Ile Glu Ser Ile Val Lys Pro Lys Val Leu Val Lys Glu
965 970 975

Ala Glu Lys Lys Leu Pro Ser Asp Thr Glu Lys Glu Asp Arg Ser Pro
980 985 990

Ser Ala Ile Phe Ser Ala Glu Leu Ser Lys Thr Ser Val Val Asp Leu

995 1000 1005

Leu Tyr Trp Arg Asp Ile Lys Lys Thr Gly Val Val Phe Gly Ala
1010 1015 1020

Ser Leu Phe Leu Leu Leu Ser Leu Thr Val Phe Ser Ile Val Ser
1025 1030 1035

Val Thr Ala Tyr Ile Ala Leu Ala Leu Leu Ser Val Thr Ile Ser
1040 1045 1050

Phe Arg Ile Tyr Lys Gly Val Ile Gln Ala Ile Gln Lys Ser Asp
1055 1060 1065

Glu Gly His Pro Phe Arg Ala Tyr Leu Glu Ser Glu Val Ala Ile
1070 1075 1080

Ser Glu Glu Leu Val Gln Lys Tyr Ser Asn Ser Ala Leu Gly His
1085 1090 1095

Val Asn Cys Thr Ile Lys Glu Leu Arg Arg Leu Phe Leu Val Asp
1100 1105 1110

Asp Leu Val Asp Ser Leu Lys Phe Ala Val Leu Met Trp Val Phe
1115 1120 1125

Thr Tyr Val Gly Ala Leu Phe Asn Gly Leu Thr Leu Leu Ile Leu
1130 1135 1140

Ala Leu Ile Ser Leu Phe Ser Val Pro Val Ile Tyr Glu Arg His
1145 1150 1155

Gln Ala Gln Ile Asp His Tyr Leu Gly Leu Ala Asn Lys Asn Val
1160 1165 1170

Lys Asp Ala Met Ala Lys Ile Gln Ala Lys Ile Pro Gly Leu Lys
1175 1180 1185

Arg Lys Ala Glu
1190

<210> 6

<211> 18

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1)..(18)

<223> Human NogoA_623-640

<400> 6

Asn Tyr Glu Ser Ile Lys His Glu Pro Glu Asn Pro Pro Pro Tyr Glu
1 5 10 15

Glu Ala

<210> 7

<211> 819

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1).. (819)

<223> human_ Nig

<400> 7

Asp Glu Thr Leu Phe Ala Leu Pro Ala Ala Ser Glu Pro Val Ile Arg
1 5 10 15

Ser Ser Ala Glu Asn Met Asp Leu Lys Glu Gln Pro Gly Asn Thr Ile
20 25 30

Ser Ala Gly Gln Glu Asp Phe Pro Ser Val Leu Leu Glu Thr Ala Ala
35 40 45

Ser Leu Pro Ser Leu Ser Pro Leu Ser Ala Ala Ser Phe Lys Glu His
50 55 60

Glu Tyr Leu Gly Asn Leu Ser Thr Val Leu Pro Thr Glu Gly Thr Leu
65 70 75 80

Gln Glu Asn Val Ser Glu Ala Ser Lys Glu Val Ser Glu Lys Ala Lys
85 90 95

Thr Leu Leu Ile Asp Arg Asp Leu Thr Glu Phe Ser Glu Leu Glu Tyr
100 105 110

Ser Glu Met Gly Ser Ser Phe Ser Val Ser Pro Lys Ala Glu Ser Ala
115 120 125

Val Ile Val Ala Asn Pro Arg Glu Glu Ile Ile Val Lys Asn Lys Asp
130 135 140

Glu Glu Glu Lys Leu Val Ser Asn Asn Ile Leu His Asn Gln Gln Glu
145 150 155 160

Leu Pro Thr Ala Leu Thr Lys Leu Val Lys Glu Asp Glu Val Val Ser
165 170 175

Ser Glu Lys Ala Lys Asp Ser Phe Asn Glu Lys Arg Val Ala Val Glu
180 185 190

Ala Pro Met Arg Glu Glu Tyr Ala Asp Phe Lys Pro Phe Glu Arg Val
195 200 205

Trp Glu Val Lys Asp Ser Lys Glu Asp Ser Asp Met Leu Ala Ala Gly
210 215 220

Gly Lys Ile Glu Ser Asn Leu Glu Ser Lys Val Asp Lys Lys Cys Phe
225 230 235 240

Ala Asp Ser Leu Glu Gln Thr Asn His Glu Lys Asp Ser Glu Ser Ser
245 250 255

Asn Asp Asp Thr Ser Phe Pro Ser Thr Pro Glu Gly Ile Lys Asp Arg
260 265 270

Ser Gly Ala Tyr Ile Thr Cys Ala Pro Phe Asn Pro Ala Ala Thr Glu
275 280 285

Ser Ile Ala Thr Asn Ile Phe Pro Leu Leu Gly Asp Pro Thr Ser Glu
290 295 300

Asn Lys Thr Asp Glu Lys Lys Ile Glu Glu Lys Lys Ala Gln Ile Val

305 310 315 320

Thr Glu Lys Asn Thr Ser Thr Lys Thr Ser Asn Pro Phe Leu Val Ala
325 330 335

Ala Gln Asp Ser Glu Thr Asp Tyr Val Thr Thr Asp Asn Leu Thr Lys
340 345 350

Val Thr Glu Glu Val Val Ala Asn Met Pro Glu Gly Leu Thr Pro Asp
355 360 365

Leu Val Gln Glu Ala Cys Glu Ser Glu Leu Asn Glu Val Thr Gly Thr
370 375 380

Lys Ile Ala Tyr Glu Thr Lys Met Asp Leu Val Gln Thr Ser Glu Val
385 390 395 400

Met Gln Glu Ser Leu Tyr Pro Ala Ala Gln Leu Cys Pro Ser Phe Glu
405 410 415

Glu Ser Glu Ala Thr Pro Ser Pro Val Leu Pro Asp Ile Val Met Glu
420 425 430

Ala Pro Leu Asn Ser Ala Val Pro Ser Ala Gly Ala Ser Val Ile Gln
435 440 445

Pro Ser Ser Ser Pro Leu Glu Ala Ser Ser Val Asn Tyr Glu Ser Ile
450 455 460

Lys His Glu Pro Glu Asn Pro Pro Pro Tyr Glu Glu Ala Met Ser Val
465 470 475 480

Ser Leu Lys Lys Val Ser Gly Ile Lys Glu Glu Ile Lys Glu Pro Glu
485 490 495

Asn Ile Asn Ala Ala Leu Gln Glu Thr Glu Ala Pro Tyr Ile Ser Ile
500 505 510

Ala Cys Asp Leu Ile Lys Glu Thr Lys Leu Ser Ala Glu Pro Ala Pro
515 520 525

Asp Phe Ser Asp Tyr Ser Glu Met Ala Lys Val Glu Gln Pro Val Pro
530 535 540

Asp His Ser Glu Leu Val Glu Asp Ser Ser Pro Asp Ser Glu Pro Val
545 550 555 560

Asp Leu Phe Ser Asp Asp Ser Ile Pro Asp Val Pro Gln Lys Gln Asp
565 570 575

Glu Thr Val Met Leu Val Lys Glu Ser Leu Thr Glu Thr Ser Phe Glu
580 585 590

Ser Met Ile Glu Tyr Glu Asn Lys Glu Lys Leu Ser Ala Leu Pro Pro
595 600 605

Glu Gly Gly Lys Pro Tyr Leu Glu Ser Phe Lys Leu Ser Leu Asp Asn
610 615 620

Thr Lys Asp Thr Leu Leu Pro Asp Glu Val Ser Thr Leu Ser Lys Lys
625 630 635 640

Glu Lys Ile Pro Leu Gln Met Glu Glu Leu Ser Thr Ala Val Tyr Ser
645 650 655

Asn Asp Asp Leu Phe Ile Ser Lys Glu Ala Gln Ile Arg Glu Thr Glu
660 665 670

Thr Phe Ser Asp Ser Ser Pro Ile Glu Ile Ile Asp Glu Phe Pro Thr
675 680 685

Leu Ile Ser Ser Lys Thr Asp Ser Phe Ser Lys Leu Ala Arg Glu Tyr
690 695 700

Thr Asp Leu Glu Val Ser His Lys Ser Glu Ile Ala Asn Ala Pro Asp
705 710 715 720

Gly Ala Gly Ser Leu Pro Cys Thr Glu Leu Pro His Asp Leu Ser Leu
725 730 735

Lys Asn Ile Gln Pro Lys Val Glu Glu Lys Ile Ser Phe Ser Asp Asp
740 745 750

Phe Ser Lys Asn Gly Ser Ala Thr Ser Lys Val Leu Leu Leu Pro Pro
755 760 765

Asp Val Ser Ala Leu Ala Thr Gln Ala Glu Ile Glu Ser Ile Val Lys
770 775 780

Pro Lys Val Leu Val Lys Glu Ala Glu Lys Lys Leu Pro Ser Asp Thr
785 790 795 800

Glu Lys Glu Asp Arg Ser Pro Ser Ala Ile Phe Ser Ala Glu Leu Ser
805 810 815

Lys Thr Ser

<210> 8

<211> 10

<212> PRT

<213> Mus musculus

<220>

<221> BINDING

<222> (1)..(10)

<223> hypervariable part of heavy chain of 11C7

<400> 8

Gly Phe Asp Phe Arg Arg Asn Trp Met Ser
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<210> 9

<211> 17

<212> PRT

<213> Mus musculus

<220>

<221> BINDING

<222> (1)..(17)

<223> hypervariable part of heavy chain of 11C7

<400> 9

Glu Ile Asn Pro Asp Ser Ser Lys Ile Asn Tyr Thr Pro Ser Leu Lys
1 5 10 15

Asp

<210> 10

<211> 9

<212> PRT

<213> Mus musculus

<220>

<221> BINDING

<222> (1)..(9)

<223> hypervariable part of heavy chain of 11C7

<400> 10

Pro Val Trp Met Tyr Ala Met Asp Tyr
1 5

<210> 11

<211> 16

<212> PRT

<213> Mus musculus

<220>

<221> BINDING

<222> (1)..(16)

<223> hypervariable part of light chain of 11C7

<400> 11

Lys Ser Ser Gln Ser Leu Leu His Ser Asp Gly Lys Thr Tyr Leu Asn
1 5 10 15

<210> 12

<211> 7

<212> PRT

<213> Mus musculus

<220>

<221> BINDING

<222> (1)..(7)

<223> hypervariable part of light chain of 11C7

<400> 12

Leu Val Ser Lys Leu Asp Ser
1 5

<210> 13

<211> 9

<212> PRT

<213> Mus musculus

<220>

<221> BINDING

<222> (1)..(9)

<223> hypervariable part of light chain of 11C7

<400> 13

Trp Gln Gly Thr His Phe Pro Gln Thr
1 5

<210> 14

<211> 30

<212> DNA

<213> Mus musculus

<220>

<221> misc_binding

<222> (1)..(30)

<223> DNA-CDR1-11C7

<400> 14
ggattcgatt ttagaagaaa ttggatgagt 30

<210> 15

<211> 51

<212> DNA

<213> Mus musculus

<220>

<221> misc_binding

<222> (1)..(51)

<223> DNA-CDR2-11C7

<400> 15
gaaattaatc cagatagcag taagataaac tatacgccat ctctaaagga t 51

<210> 16

<211> 27

<212> DNA

<213> Mus musculus

<220>

<221> misc_binding

<222> (1)..(27)

<223> DNA-CDR3-11C7

<400> 16
ccggtctgga tgtatgctat ggactac 27

<210> 17

<211> 48

<212> DNA

<213> Mus musculus

<220>

<221> misc_binding

<222> (1)..(48)

<223> DNA-CDR'1-11C7

<400> 17
aagtcaagtc agagcctctt gcatagtgat ggaaagacat atttgaat 48

<210> 18

<211> 21

<212> DNA

<213> Mus musculus

<220>

<221> misc_binding

<222> (1)..(21)

<223> DNA-CDR'2-11C7

<400> 18
ctggtgtcta aactggactc t 21

<210> 19

<211> 27

<212> DNA

<213> Mus musculus

<220>

<221> misc_binding

<222> (1)..(27)

<223> DNA-CDR'3-11C7

<400> 19
tggcaaggta cacattttcc tcagacg 27

<210> 20

<211> 54

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(54)

<223> leader sequence for heavy chain of 11C7

<400> 20

atg gat ttt ggg ctg att ttt ttt att gtt ggt ctt tta aaa ggg gtc 48
Met Asp Phe Gly Leu Ile Phe Phe Ile Val Gly Leu Leu Lys Gly Val
1 5 10 15

cag tgt 54
Gln Cys

<210> 21

<211> 18

<212> PRT

<213> Mus musculus

<400> 21

Met Asp Phe Gly Leu Ile Phe Phe Ile Val Gly Leu Leu Lys Gly Val
1 5 10 15

Gln Cys

<210> 22

<211> 57

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(57)

<223> leader sequence for 11C7-light chain

<400> 22
atg agt cct gcc cag ttc ctg ttt ctg tta gtg ct c tgg att cgg gaa 48
Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu Trp Ile Arg Glu
1 5 10 15

acc agc ggt 57
Thr Ser Gly

<210> 23

<211> 19

<212> PRT

<213> Mus musculus

<400> 23

Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu Trp Ile Arg Glu
1 5 10 15

Thr Ser Gly

<210> 24

<211> 181

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1)..(181)

<223> human Nig-D20

<400> 24

Gly Thr Lys Ile Ala Tyr Glu Thr Lys Met Asp Leu Val Gln Thr Ser
1 5 10 15

Glu Val Met Gln Glu Ser Leu Tyr Pro Ala Ala Gln Leu Cys Pro Ser
20 25 30

Phe Glu Glu Ser Glu Ala Thr Pro Ser Pro Val Leu Pro Asp Ile Val
35 40 45

Met Glu Ala Pro Leu Asn Ser Ala Val Pro Ser Ala Gly Ala Ser Val
50 55 60

Ile Gln Pro Ser Ser Ser Pro Leu Glu Ala Ser Ser Val Asn Tyr Glu
65 70 75 80

Ser Ile Lys His Glu Pro Glu Asn Pro Pro Pro Tyr Glu Glu Ala Met
85 90 95

Ser Val Ser Leu Lys Lys Val Ser Gly Ile Lys Glu Glu Ile Lys Glu
100 105 110

Pro Glu Asn Ile Asn Ala Ala Leu Gln Glu Thr Glu Ala Pro Tyr Ile
115 120 125

Ser Ile Ala Cys Asp Leu Ile Lys Glu Thr Lys Leu Ser Ala Glu Pro
130 135 140

Ala Pro Asp Phe Ser Asp Tyr Ser Glu Met Ala Lys Val Glu Gln Pro
145 150 155 160

Val Pro Asp His Ser Glu Leu Val Glu Asp Ser Ser Pro Asp Ser Glu
165 170 175

Pro Val Asp Leu Phe
180

<210> 25

<211> 3492

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> (1)..(3492)

<223> rat NogoA

<400> 25

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Met Glu Asp Ile Asp Gln Ser Ser Leu Val Ser Ser Ser Thr Asp Ser
1               5                   10                  15

ccg ccc cgg cct ccg ccc gcc ttc aag tac cag ttc gtg acg gag ccc       96
Pro Pro Arg Pro Pro Pro Ala Phe Lys Tyr Gln Phe Val Thr Glu Pro
            20                  25                  30

gag gac gag gag gac gag gag gag gag gag gac gag gag gag gac gac      144
Glu Asp Glu Glu Asp Glu Glu Glu Glu Glu Asp Glu Glu Glu Asp Asp
        35                  40                  45

gag gac cta gag gaa ctg gag gtg ctg gag agg aag ccc gca gcc ggg      192
Glu Asp Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly
    50                  55                  60

ctg tcc gca gct gcg gtg ccg ccc gcc gcc gcc gcg ccg ctg ctg gac      240
Leu Ser Ala Ala Ala Val Pro Pro Ala Ala Ala Ala Pro Leu Leu Asp
65                  70                  75                  80

ttc agc agc gac tcg gtg ccc ccc gcg ccc cgc ggg ccg ctg ccg gcc      288
Phe Ser Ser Asp Ser Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala
                85                  90                  95

gcg ccc cct gcc gct cct gag agg cag cca tcc tgg gaa cgc agc ccc      336
Ala Pro Pro Ala Ala Pro Glu Arg Gln Pro Ser Trp Glu Arg Ser Pro
            100                 105                 110

gcg gcg ccc gcg cca tcc ctg ccg ccc gct gcc gca gtc ctg ccc tcc      384
Ala Ala Pro Ala Pro Ser Leu Pro Pro Ala Ala Ala Val Leu Pro Ser
        115                 120                 125
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aag ctc cca gag gac gac gag cct ccg gcg agg ccc ccg cct ccg ccg      432
Lys Leu Pro Glu Asp Asp Glu Pro Pro Ala Arg Pro Pro Pro Pro Pro
    130                 135                 140

cca gcc ggc gcg agc ccc ctg gcg gag ccc gcc gcg ccc cct tcc acg      480
Pro Ala Gly Ala Ser Pro Leu Ala Glu Pro Ala Ala Pro Pro Ser Thr
145                 150                 155                 160

ccg gcc gcg ccc aag cgc agg ggc tcc ggc tca gtg gat gag acc ctt      528
Pro Ala Ala Pro Lys Arg Arg Gly Ser Gly Ser Val Asp Glu Thr Leu
                165                 170                 175

ttt gct ctt cct gct gca tct gag cct gtg ata ccc tcc tct gca gaa      576
Phe Ala Leu Pro Ala Ala Ser Glu Pro Val Ile Pro Ser Ser Ala Glu
            180                 185                 190

aaa att atg gat ttg atg gag cag cca ggt aac act gtt tcg tct ggt      624
Lys Ile Met Asp Leu Met Glu Gln Pro Gly Asn Thr Val Ser Ser Gly
        195                 200                 205

caa gag gat ttc cca tct gtc ctg ctt gaa act gct gcc tct ctt cct      672
Gln Glu Asp Phe Pro Ser Val Leu Leu Glu Thr Ala Ala Ser Leu Pro
    210                 215                 220

tct cta tct cct ctc tca act gtt tct ttt aaa gaa cat gga tac ctt      720
Ser Leu Ser Pro Leu Ser Thr Val Ser Phe Lys Glu His Gly Tyr Leu
225                 230                 235                 240

ggt aac tta tca gca gtg tca tcc tca gaa gga aca att gaa gaa act      768
Gly Asn Leu Ser Ala Val Ser Ser Ser Glu Gly Thr Ile Glu Glu Thr
                245                 250                 255

tta aat gaa gct tct aaa gag ttg cca gag agg gca aca aat cca ttt      816
Leu Asn Glu Ala Ser Lys Glu Leu Pro Glu Arg Ala Thr Asn Pro Phe
            260                 265                 270

gta aat aga gat tta gca gaa ttt tca gaa tta gaa tat tca gaa atg      864
```

```
Val Asn Arg Asp Leu Ala Glu Phe Ser Glu Leu Glu Tyr Ser Glu Met
        275                 280                 285

gga tca tct ttt aaa ggc tcc cca aaa gga gag tca gcc ata tta gta      912
Gly Ser Ser Phe Lys Gly Ser Pro Lys Gly Glu Ser Ala Ile Leu Val
    290                 295                 300

gaa aac act aag gaa gaa gta att gtg agg agt aaa gac aaa gag gat      960
Glu Asn Thr Lys Glu Glu Val Ile Val Arg Ser Lys Asp Lys Glu Asp
305                 310                 315                 320

tta gtt tgt agt gca gcc ctt cac agt cca caa gaa tca cct gtg ggt     1008
Leu Val Cys Ser Ala Ala Leu His Ser Pro Gln Glu Ser Pro Val Gly
                325                 330                 335

aaa gaa gac aga gtt gtg tct cca gaa aag aca atg gac att ttt aat     1056
Lys Glu Asp Arg Val Val Ser Pro Glu Lys Thr Met Asp Ile Phe Asn
            340                 345                 350

gaa atg cag atg tca gta gta gca cct gtg agg gaa gag tat gca gac     1104
Glu Met Gln Met Ser Val Val Ala Pro Val Arg Glu Glu Tyr Ala Asp
        355                 360                 365

ttt aag cca ttt gaa caa gca tgg gaa gtg aaa gat act tat gag gga     1152
Phe Lys Pro Phe Glu Gln Ala Trp Glu Val Lys Asp Thr Tyr Glu Gly
    370                 375                 380

agt agg gat gtg ctg gct gct aga gct aat gtg gaa agt aaa gtg gac     1200
Ser Arg Asp Val Leu Ala Ala Arg Ala Asn Val Glu Ser Lys Val Asp
385                 390                 395                 400

aga aaa tgc ttg gaa gat agc ctg gag caa aaa agt ctt ggg aag gat     1248
Arg Lys Cys Leu Glu Asp Ser Leu Glu Gln Lys Ser Leu Gly Lys Asp
                405                 410                 415

agt gaa ggc aga aat gag gat gct tct ttc ccc agt acc cca gaa cct     1296
Ser Glu Gly Arg Asn Glu Asp Ala Ser Phe Pro Ser Thr Pro Glu Pro
```

```
            420                 425                 430

gtg aag gac agc tcc aga gca tat att acc tgt gct tcc ttt acc tca     1344
Val Lys Asp Ser Ser Arg Ala Tyr Ile Thr Cys Ala Ser Phe Thr Ser
        435                 440                 445

gca acc gaa agc acc aca gca aac act ttc cct ttg tta gaa gat cat     1392
Ala Thr Glu Ser Thr Thr Ala Asn Thr Phe Pro Leu Leu Glu Asp His
    450                 455                 460

act tca gaa aat aaa aca gat gaa aaa aaa ata gaa gaa agg aag gcc     1440
Thr Ser Glu Asn Lys Thr Asp Glu Lys Lys Ile Glu Glu Arg Lys Ala
465                 470                 475                 480

caa att ata aca gag aag act agc ccc aaa acg tca aat cct ttc ctt     1488
Gln Ile Ile Thr Glu Lys Thr Ser Pro Lys Thr Ser Asn Pro Phe Leu
                485                 490                 495

gta gca gta cag gat tct gag gca gat tat gtt aca aca gat acc tta     1536
Val Ala Val Gln Asp Ser Glu Ala Asp Tyr Val Thr Thr Asp Thr Leu
            500                 505                 510

tca aag gtg act gag gca gca gtg tca aac atg cct gaa ggt ctg acg     1584
Ser Lys Val Thr Glu Ala Ala Val Ser Asn Met Pro Glu Gly Leu Thr
        515                 520                 525

cca gat tta gtt cag gaa gca tgt gaa agt gaa ctg aat gaa gcc aca     1632
Pro Asp Leu Val Gln Glu Ala Cys Glu Ser Glu Leu Asn Glu Ala Thr
    530                 535                 540

ggt aca aag att gct tat gaa aca aaa gtg gac ttg gtc caa aca tca     1680
Gly Thr Lys Ile Ala Tyr Glu Thr Lys Val Asp Leu Val Gln Thr Ser
545                 550                 555                 560

gaa gct ata caa gaa tca ctt tac ccc aca gca cag ctt tgc cca tca     1728
Glu Ala Ile Gln Glu Ser Leu Tyr Pro Thr Ala Gln Leu Cys Pro Ser
                565                 570                 575
```

```
ttt gag gaa gct gaa gca act ccg tca cca gtt ttg cct gat att gtt     1776
Phe Glu Glu Ala Glu Ala Thr Pro Ser Pro Val Leu Pro Asp Ile Val
            580                 585                 590

atg gaa gca cca tta aat tct ctc ctt cca agc gct ggt gct tct gta     1824
Met Glu Ala Pro Leu Asn Ser Leu Leu Pro Ser Ala Gly Ala Ser Val
        595                 600                 605

gtg cag ccc agt gta tcc cca ctg gaa gca cct cct cca gtt agt tat     1872
Val Gln Pro Ser Val Ser Pro Leu Glu Ala Pro Pro Pro Val Ser Tyr
    610                 615                 620

gac agt ata aag ctt gag cct gaa aac ccc cca cca tat gaa gaa gcc     1920
Asp Ser Ile Lys Leu Glu Pro Glu Asn Pro Pro Pro Tyr Glu Glu Ala
625                 630                 635                 640

atg aat gta gca cta aaa gct ttg gga aca aag gaa gga ata aaa gag     1968
Met Asn Val Ala Leu Lys Ala Leu Gly Thr Lys Glu Gly Ile Lys Glu
                645                 650                 655

cct gaa agt ttt aat gca gct gtt cag gaa aca gaa gct cct tat ata     2016
Pro Glu Ser Phe Asn Ala Ala Val Gln Glu Thr Glu Ala Pro Tyr Ile
            660                 665                 670

tcc att gcg tgt gat tta att aaa gaa aca aag ctc tcc act gag cca     2064
Ser Ile Ala Cys Asp Leu Ile Lys Glu Thr Lys Leu Ser Thr Glu Pro
        675                 680                 685

agt cca gat ttc tct aat tat tca gaa ata gca aaa ttc gag aag tcg     2112
Ser Pro Asp Phe Ser Asn Tyr Ser Glu Ile Ala Lys Phe Glu Lys Ser
    690                 695                 700

gtg ccc gaa cac gct gag cta gtg gag gat tcc tca cct gaa tct gaa     2160
Val Pro Glu His Ala Glu Leu Val Glu Asp Ser Ser Pro Glu Ser Glu
705                 710                 715                 720
```

```
cca gtt gac tta ttt agt gat gat tcg att cct gaa gtc cca caa aca      2208
Pro Val Asp Leu Phe Ser Asp Asp Ser Ile Pro Glu Val Pro Gln Thr
                725                 730                 735

caa gag gag gct gtg atg ctc atg aag gag agt ctc act gaa gtg tct      2256
Gln Glu Glu Ala Val Met Leu Met Lys Glu Ser Leu Thr Glu Val Ser
            740                 745                 750

gag aca gta gcc cag cac aaa gag gag aga ctt agt gcc tca cct cag      2304
Glu Thr Val Ala Gln His Lys Glu Glu Arg Leu Ser Ala Ser Pro Gln
        755                 760                 765

gag cta gga aag cca tat tta gag tct ttt cag ccc aat tta cat agt      2352
Glu Leu Gly Lys Pro Tyr Leu Glu Ser Phe Gln Pro Asn Leu His Ser
    770                 775                 780

aca aaa gat gct gca tct aat gac att cca aca ttg acc aaa aag gag      2400
Thr Lys Asp Ala Ala Ser Asn Asp Ile Pro Thr Leu Thr Lys Lys Glu
785                 790                 795                 800

aaa att tct ttg caa atg gaa gag ttt aat act gca att tat tca aat      2448
Lys Ile Ser Leu Gln Met Glu Glu Phe Asn Thr Ala Ile Tyr Ser Asn
                805                 810                 815

gat gac tta ctt tct tct aag gaa gac aaa ata aaa gaa agt gaa aca      2496
Asp Asp Leu Leu Ser Ser Lys Glu Asp Lys Ile Lys Glu Ser Glu Thr
            820                 825                 830

ttt tca gat tca tct ccg att gag ata ata gat gaa ttt ccc acg ttt      2544
Phe Ser Asp Ser Ser Pro Ile Glu Ile Ile Asp Glu Phe Pro Thr Phe
        835                 840                 845

gtc agt gct aaa gat gat tct cct aaa tta gcc aaag gag tac act gat      2592
Val Ser Ala Lys Asp Asp Ser Pro Lys Leu Ala Lys Glu Tyr Thr Asp
    850                 855                 860

cta gaa gta tcc gac aaa agt gaa att gct aat atc caa agc ggg gca      2640
```

```
Leu Glu Val Ser Asp Lys Ser Glu Ile Ala Asn Ile Gln Ser Gly Ala
865                 870                 875                 880

gat tca ttg cct tgc tta gaa ttg ccc tgt gac ctt tct ttc aag aat    2688
Asp Ser Leu Pro Cys Leu Glu Leu Pro Cys Asp Leu Ser Phe Lys Asn
                885                 890                 895

ata tat cct aaa gat gaa gta cat gtt tca gat gaa ttc tcc gaa aat    2736
Ile Tyr Pro Lys Asp Glu Val His Val Ser Asp Glu Phe Ser Glu Asn
            900                 905                 910

agg tcc agt gta tct aag gca tcc ata tcg cct tca aat gtc tct gct    2784
Arg Ser Ser Val Ser Lys Ala Ser Ile Ser Pro Ser Asn Val Ser Ala
        915                 920                 925

ttg gaa cct cag aca gaa atg ggc agc ata gtt aaa tcc aaa tca ctt    2832
Leu Glu Pro Gln Thr Glu Met Gly Ser Ile Val Lys Ser Lys Ser Leu
    930                 935                 940

acg aaa gaa gca gag aaa aaa ctt cct tct gac aca gag aaa gag gac    2880
Thr Lys Glu Ala Glu Lys Lys Leu Pro Ser Asp Thr Glu Lys Glu Asp
945                 950                 955                 960

aga tcc ctg tca gct gta ttg tca gca gag ctg agt aaa act tca gtt    2928
Arg Ser Leu Ser Ala Val Leu Ser Ala Glu Leu Ser Lys Thr Ser Val
                965                 970                 975

gtt gac ctc ctc tac tgg aga gac att aag aag act gga gtg gtg ttt    2976
Val Asp Leu Leu Tyr Trp Arg Asp Ile Lys Lys Thr Gly Val Val Phe
            980                 985                 990

ggt gcc agc tta ttc ctg ctg ctg  tct ctg aca gtg ttc  agc att gtc   3024
Gly Ala Ser Leu Phe Leu Leu Leu  Ser Leu Thr Val Phe  Ser Ile Val
        995                 1000                1005

agt gta  acg gcc tac att gcc  ttg gcc ctg ctc tcg  gtg act atc      3069
Ser Val  Thr Ala Tyr Ile Ala  Leu Ala Leu Leu Ser  Val Thr Ile
```

```
    1010                1015                1020

agc ttt  agg ata  tat aag ggc  gtg atc cag gct atc  cag aaa tca      3114
Ser Phe  Arg Ile  Tyr Lys Gly  Val Ile Gln Ala Ile  Gln Lys Ser
    1025                1030                1035

gat gaa  ggc cac  cca ttc agg  gca tat tta gaa tct  gaa gtt gct      3159
Asp Glu  Gly His  Pro Phe Arg  Ala Tyr Leu Glu Ser  Glu Val Ala
    1040                1045                1050

ata tca  gag gaa  ttg gtt cag  aaa tac agt aat tct  gct ctt ggt      3204
Ile Ser  Glu Glu  Leu Val Gln  Lys Tyr Ser Asn Ser  Ala Leu Gly
    1055                1060                1065

cat gtg  aac agc  aca ata aaa  gaa ctg agg cgg ctt  ttc tta gtt      3249
His Val  Asn Ser  Thr Ile Lys  Glu Leu Arg Arg Leu  Phe Leu Val
    1070                1075                1080

gat gat  tta gtt  gat tcc ctg  aag ttt gca gtg ttg  atg tgg gtg      3294
Asp Asp  Leu Val  Asp Ser Leu  Lys Phe Ala Val Leu  Met Trp Val
    1085                1090                1095

ttt act  tat gtt  ggt gcc ttg  ttc aat ggt ctg aca  cta ctg att      3339
Phe Thr  Tyr Val  Gly Ala Leu  Phe Asn Gly Leu Thr  Leu Leu Ile
    1100                1105                1110

tta gct  ctg atc  tca ctc ttc  agt att cct gtt att  tat gaa cgg      3384
Leu Ala  Leu Ile  Ser Leu Phe  Ser Ile Pro Val Ile  Tyr Glu Arg
    1115                1120                1125

cat cag  gtg cag  ata gat cat  tat cta gga ctt gca  aac aag agt      3429
His Gln  Val Gln  Ile Asp His  Tyr Leu Gly Leu Ala  Asn Lys Ser
    1130                1135                1140

gtt aag  gat gcc  atg gcc aaa  atc caa gca aaa atc  cct gga ttg      3474
Val Lys  Asp Ala  Met Ala Lys  Ile Gln Ala Lys Ile  Pro Gly Leu
    1145                1150                1155
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aag cgc aaa gca gat tga 3492
Lys Arg Lys Ala Asp
1160

<210> 26

<211> 1163

<212> PRT

<213> Rattus norvegicus

<400> 26

Met Glu Asp Ile Asp Gln Ser Ser Leu Val Ser Ser Ser Thr Asp Ser
1 5 10 15

Pro Pro Arg Pro Pro Pro Ala Phe Lys Tyr Gln Phe Val Thr Glu Pro
20 25 30

Glu Asp Glu Glu Asp Glu Glu Glu Glu Glu Asp Glu Glu Glu Asp Asp
35 40 45

Glu Asp Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly
50 55 60

Leu Ser Ala Ala Ala Val Pro Pro Ala Ala Ala Ala Pro Leu Leu Asp
65 70 75 80

Phe Ser Ser Asp Ser Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala
85 90 95

Ala Pro Pro Ala Ala Pro Glu Arg Gln Pro Ser Trp Glu Arg Ser Pro
100 105 110

Ala Ala Pro Ala Pro Ser Leu Pro Pro Ala Ala Ala Val Leu Pro Ser
115 120 125

Lys Leu Pro Glu Asp Asp Glu Pro Pro Ala Arg Pro Pro Pro Pro Pro
130 135 140

Pro Ala Gly Ala Ser Pro Leu Ala Glu Pro Ala Ala Pro Pro Ser Thr
145 150 155 160

Pro Ala Ala Pro Lys Arg Arg Gly Ser Gly Ser Val Asp Glu Thr Leu
165 170 175

Phe Ala Leu Pro Ala Ala Ser Glu Pro Val Ile Pro Ser Ser Ala Glu
180 185 190

Lys Ile Met Asp Leu Met Glu Gln Pro Gly Asn Thr Val Ser Ser Gly
195 200 205

Gln Glu Asp Phe Pro Ser Val Leu Leu Glu Thr Ala Ala Ser Leu Pro
210 215 220

```
Ser Leu Ser Pro Leu Ser Thr Val Ser Phe Lys Glu His Gly Tyr Leu
225                 230                 235                 240

Gly Asn Leu Ser Ala Val Ser Ser Ser Glu Gly Thr Ile Glu Glu Thr
                245                 250                 255

Leu Asn Glu Ala Ser Lys Glu Leu Pro Glu Arg Ala Thr Asn Pro Phe
            260                 265                 270

Val Asn Arg Asp Leu Ala Glu Phe Ser Glu Leu Glu Tyr Ser Glu Met
        275                 280                 285

Gly Ser Ser Phe Lys Gly Ser Pro Lys Gly Glu Ser Ala Ile Leu Val
    290                 295                 300

Glu Asn Thr Lys Glu Glu Val Ile Val Arg Ser Lys Asp Lys Glu Asp
305                 310                 315                 320

Leu Val Cys Ser Ala Ala Leu His Ser Pro Gln Glu Ser Pro Val Gly
                325                 330                 335

Lys Glu Asp Arg Val Val Ser Pro Glu Lys Thr Met Asp Ile Phe Asn
            340                 345                 350

Glu Met Gln Met Ser Val Val Ala Pro Val Arg Glu Glu Tyr Ala Asp
        355                 360                 365

Phe Lys Pro Phe Glu Gln Ala Trp Glu Val Lys Asp Thr Tyr Glu Gly
```

370 375 380

Ser Arg Asp Val Leu Ala Ala Arg Ala Asn Val Glu Ser Lys Val Asp
385 390 395 400

Arg Lys Cys Leu Glu Asp Ser Leu Glu Gln Lys Ser Leu Gly Lys Asp
405 410 415

Ser Glu Gly Arg Asn Glu Asp Ala Ser Phe Pro Ser Thr Pro Glu Pro
420 425 430

Val Lys Asp Ser Ser Arg Ala Tyr Ile Thr Cys Ala Ser Phe Thr Ser
435 440 445

Ala Thr Glu Ser Thr Thr Ala Asn Thr Phe Pro Leu Leu Glu Asp His
450 455 460

Thr Ser Glu Asn Lys Thr Asp Glu Lys Lys Ile Glu Glu Arg Lys Ala
465 470 475 480

Gln Ile Ile Thr Glu Lys Thr Ser Pro Lys Thr Ser Asn Pro Phe Leu
485 490 495

Val Ala Val Gln Asp Ser Glu Ala Asp Tyr Val Thr Thr Asp Thr Leu
500 505 510

Ser Lys Val Thr Glu Ala Ala Val Ser Asn Met Pro Glu Gly Leu Thr
515 520 525

Pro Asp Leu Val Gln Glu Ala Cys Glu Ser Glu Leu Asn Glu Ala Thr
530 535 540

Gly Thr Lys Ile Ala Tyr Glu Thr Lys Val Asp Leu Val Gln Thr Ser
545 550 555 560

Glu Ala Ile Gln Glu Ser Leu Tyr Pro Thr Ala Gln Leu Cys Pro Ser
565 570 575

Phe Glu Glu Ala Glu Ala Thr Pro Ser Pro Val Leu Pro Asp Ile Val
580 585 590

Met Glu Ala Pro Leu Asn Ser Leu Leu Pro Ser Ala Gly Ala Ser Val
595 600 605

Val Gln Pro Ser Val Ser Pro Leu Glu Ala Pro Pro Pro Val Ser Tyr
610 615 620

Asp Ser Ile Lys Leu Glu Pro Glu Asn Pro Pro Pro Tyr Glu Glu Ala
625 630 635 640

Met Asn Val Ala Leu Lys Ala Leu Gly Thr Lys Glu Gly Ile Lys Glu
645 650 655

Pro Glu Ser Phe Asn Ala Ala Val Gln Glu Thr Glu Ala Pro Tyr Ile
660 665 670

Ser Ile Ala Cys Asp Leu Ile Lys Glu Thr Lys Leu Ser Thr Glu Pro
675 680 685

Ser Pro Asp Phe Ser Asn Tyr Ser Glu Ile Ala Lys Phe Glu Lys Ser
690 695 700

Val Pro Glu His Ala Glu Leu Val Glu Asp Ser Ser Pro Glu Ser Glu
705 710 715 720

Pro Val Asp Leu Phe Ser Asp Asp Ser Ile Pro Glu Val Pro Gln Thr
725 730 735

Gln Glu Glu Ala Val Met Leu Met Lys Glu Ser Leu Thr Glu Val Ser
740 745 750

Glu Thr Val Ala Gln His Lys Glu Glu Arg Leu Ser Ala Ser Pro Gln
755 760 765

Glu Leu Gly Lys Pro Tyr Leu Glu Ser Phe Gln Pro Asn Leu His Ser
770 775 780

Thr Lys Asp Ala Ala Ser Asn Asp Ile Pro Thr Leu Thr Lys Lys Glu
785 790 795 800

Lys Ile Ser Leu Gln Met Glu Glu Phe Asn Thr Ala Ile Tyr Ser Asn
805 810 815

Asp Asp Leu Leu Ser Ser Lys Glu Asp Lys Ile Lys Glu Ser Glu Thr
820 825 830

Phe Ser Asp Ser Ser Pro Ile Glu Ile Ile Asp Glu Phe Pro Thr Phe
835 840 845

Val Ser Ala Lys Asp Asp Ser Pro Lys Leu Ala Lys Glu Tyr Thr Asp
850 855 860

Leu Glu Val Ser Asp Lys Ser Glu Ile Ala Asn Ile Gln Ser Gly Ala
865 870 875 880

Asp Ser Leu Pro Cys Leu Glu Leu Pro Cys Asp Leu Ser Phe Lys Asn
885 890 895

Ile Tyr Pro Lys Asp Glu Val His Val Ser Asp Glu Phe Ser Glu Asn
900 905 910

Arg Ser Ser Val Ser Lys Ala Ser Ile Ser Pro Ser Asn Val Ser Ala
915 920 925

Leu Glu Pro Gln Thr Glu Met Gly Ser Ile Val Lys Ser Lys Ser Leu
930 935 940

Thr Lys Glu Ala Glu Lys Lys Leu Pro Ser Asp Thr Glu Lys Glu Asp
945 950 955 960

Arg Ser Leu Ser Ala Val Leu Ser Ala Glu Leu Ser Lys Thr Ser Val

965 970 975

Val Asp Leu Leu Tyr Trp Arg Asp Ile Lys Lys Thr Gly Val Val Phe
980 985 990

Gly Ala Ser Leu Phe Leu Leu Leu Ser Leu Thr Val Phe Ser Ile Val
995 1000 1005

Ser Val Thr Ala Tyr Ile Ala Leu Ala Leu Leu Ser Val Thr Ile
1010 1015 1020

Ser Phe Arg Ile Tyr Lys Gly Val Ile Gln Ala Ile Gln Lys Ser
1025 1030 1035

Asp Glu Gly His Pro Phe Arg Ala Tyr Leu Glu Ser Glu Val Ala
1040 1045 1050

Ile Ser Glu Glu Leu Val Gln Lys Tyr Ser Asn Ser Ala Leu Gly
1055 1060 1065

His Val Asn Ser Thr Ile Lys Glu Leu Arg Arg Leu Phe Leu Val
1070 1075 1080

Asp Asp Leu Val Asp Ser Leu Lys Phe Ala Val Leu Met Trp Val
1085 1090 1095

Phe Thr Tyr Val Gly Ala Leu Phe Asn Gly Leu Thr Leu Leu Ile
1100 1105 1110

Leu Ala Leu Ile Ser Leu Phe Ser Ile Pro Val Ile Tyr Glu Arg
1115 1120 1125

His Gln Val Gln Ile Asp His Tyr Leu Gly Leu Ala Asn Lys Ser
1130 1135 1140

Val Lys Asp Ala Met Ala Lys Ile Gln Ala Lys Ile Pro Gly Leu
1145 1150 1155

Lys Arg Lys Ala Asp
1160

<210> 27

<211> 25

<212> PRT

<213> Rattus norvegicus

<220>

<221> PEPTIDE

<222> (1)..(25)

<223> rat PEP4

<400> 27

Glu Glu Leu Val Gln Lys Tyr Ser Asn Ser Ala Leu Gly His Val Asn
1 5 10 15

Ser Thr Ile Lys Glu Leu Arg Arg Leu
20 25

<210> 28

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> PRO/SER rich peptide

<220>

<221> PEPTIDE

<222> (1)..(17)

<223> Synthetic peptide

<400> 28

Pro Ser Ser Pro Pro Pro Ser Ser Pro Pro Pro Ser Ser Pro Pro Pro
1 5 10 15

Ser

<210> 29

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> CA-NA-2F

<220>

<221> primer_bind

<222> (1)..(25)

<223> CA-NA-2F primer

<400> 29
aagcaccatt gaattctgca gttcc 25

<210> 30

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> CA-NA-3R

<220>

<221> primer_bind

<222> (1)..(28)

<223>

<400> 30
aactgcagta ctgagctcct ccatctgc 28

<210> 31

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> forward 5'

<220>

<221> primer_bind

<222> (1)..(33)

<223> forward primer

<400> 31
gtcgcggatc catggagacc ctttttgctc ttc 33

<210> 32

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> reverse 5'

<220>

<221> primer_bind

<222> (1)..(27)

<223> reverse primer

<400> 32
gttctcgagt tatgaagttt tactcag 27

<210> 33

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> forward 5'-1

<220>

<221> primer_bind

<222> (1)..(29)

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<210> 34

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> reverse 5'-1

<220>

<221> primer_bind

<222> (1)..(28)

<223> primer

<400> 34
gtttctcgag tgaagtttta ttcagctc 28

<210> 35

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> 5' primer

<220>

<221> primer_bind

<222> (1)..(20)

<223> primer

<400> 35
tccacccccgg ccgcgcccaa 20

<210> 36

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> 5' primer 2

<220>

<221> primer_bind

<222> (1)..(22)

<223> primer

<400> 36
aatgatgggc aaagctgtgc tg 22

<210> 37

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> 3' primer

<220>

<221> primer_bind

<222> (1)..(24)

<223> primer

<400> 37
ggtacaaaga ttgcttatga aaca 24

<210> 38

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> 3' primer 2

<220>

<221> primer_bind

<222> (1)..(22)

<223> primer

<400> 38
agcagggcca aggcaatgta gg 22

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> 5'-VL leader

<220>

<221> primer_bind

<222> (1)..(28)

<223> primer

<400> 39
aatatgagtc ctgcccagtt cctgtttc 28

<210> 40

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> 3'-Ck

<220>

<221> primer_bind

<222> (1)..(32)

<223> primer

<400> 40
ttaggaattc ctaacactct cccctgttga ag 32

<210> 41

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> 5'-VH leader

<220>

<222> (1)..(31)

<223> primer

<400> 41
aatatggatt ttgggctgat ttttttatt g 31

<210> 42

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> 3'-CH hinge

<220>

<221> primer_bind

<222> (1)..(24)

<223> primer

<400> 42
aattgggcaa cgttgcaggt gacg 24

<210> 43

<211> 663

<212> DNA

<213> Mus musculus

<220>

<221> misc_binding

<222> (1)..(663)

<223> DNA variable part of heavy chain 11C7

<400> 43

atggattttg ggctgatttt ttttattgtt ggtcttttaa aaggggtcca gtgtgaggtg 60

aagcttctcg agtctggagg tggcctggtg cagcctggag gatccctgaa actctcctgt 120

gtagtctcag gattcgattt tagaagaaat tggatgagtt gggtccggca ggctcctggg 180

aaagggctag aatggattgg agaaattaat ccagatagca gtaagataaa ctatacgcca 240

tctctaaagg ataaattcat catctccaga gacaatgcca agaatacgct gtacctgcaa 300

gtgagcacag tgagatctga ggacacagcc ctttattact gtgtgagacc ggtctggatg 360

tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcctcagc caaaacgaca 420

ccccccatctg tctatccact ggcccctgga tctgctgccc aaactaactc catggtgacc 480

ctgggatgcc tggtcaaggg ctatttccct gagccagtga cagtgacctg gaactctgga 540

tccctgtcca gcggtgtgca caccttccca gctgtcctgc agtctgacct ctacactctg 600

agcagctcag tgactgtccc ctccagcacc tggcccagcg agaccgtcac ctgcaacgtt 660

gcc 663

<210> 44

<211> 717

<212> DNA

<213> Mus musculus

<220>

<221> misc_binding

<222> (1)..(717)

<223> variable part of light chain of 11C7

<400> 44

atgagtcctg cccagttcct gtttctgtta gtgctctgga ttcgggaaac cagcggtgat 60

gttctgttga cccagactcc tctcactttg tcgataacca ttggacaacc agcctccatc 120

tcttgcaagt caagtcagag cctcttgcat agtgatggaa agacatattt gaattggttg 180

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