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(54) Title: HOST CELL SPECIFIC BINDING MOLECULES CAPABLE OF NEUTRALIZING VIRUSES AND USES THEREOF

(57) Abstract: The present invention provides human binding molecules specifically binding to a host cell protein and having virus neutralizing activity, nucleic acid molecules encoding the human binding molecules, compositions comprising the human binding molecules and methods of identifying or producing the human binding molecules. The human binding molecules can be used in the diagnosis, prophylaxis and/or treatment of viral infections.



WO 2006/120230 A2

TITLE OF THE INVENTION

Host cell specific binding molecules capable of neutralizing viruses and uses thereof

FIELD OF THE INVENTION

The invention relates to medicine. In particular the invention relates to the diagnosis, prophylaxis and/or treatment of viral infections.

BACKGROUND OF THE INVENTION

Several viruses assemble their core proteins and genomic material in the cytoplasm of a host cell and exit the cell by budding from the plasma membrane. Studies of these viruses, e.g. HIV-1 viruses, have shown that in addition to proteins encoded by the virus, host cell proteins can be found in viruses. While some of these proteins may be taken into the viruses simply because of their proximity to the viral assembly and budding sites, other host cell proteins are likely to be included in viruses as a result of their interaction with viral proteins during assembly and release. Additionally, some host cell proteins may be incorporated to provide a function for the virus during the infection process. Host cell proteins have been found on the surface or the interior of the viruses. Despite their detection on or in viruses, the role and function of host cell proteins in the viral assembly process is poorly understood and still highly speculative.

A variety of agents are presently used to combat viral infection. These agents include anti-viral compounds, compounds suitable for active immunization such as vaccines and compounds suitable for passive immunization such as neutralizing immunoglobulins. The latter group is often

focused on neutralizing immunoglobulins that act through specific binding to viral proteins or cell surface receptors involved in viral entry. Due to their binding specificity, such immunoglobulins are however only suitable in the prophylaxis and/or treatment of specific viral diseases and are not broadly applicable in the treatment of viral diseases.

Neutralization of viruses has been described for immunoglobulins directed against virus-incorporated host cell derived proteins. For instance HIV-1 has been shown to incorporate the host cell derived protein Intercellular Adhesion Molecule-1 (ICAM-1) and an antibody against ICAM-1 has been shown to neutralize ICAM-1 expressing HIV-1 virions (see Rizzuto and Sodroski, 1997). Disadvantageously, ICAM-1 is also localized on the surface of uninfected host cells, so protection and/or treatment of a viral infection with the immunoglobulin against ICAM-1 may, due to interaction of the immunoglobulin with uninfected host cells, result in serious and unwanted side effects. A further disadvantage of the anti-ICAM-1 immunoglobulin is that its neutralizing activity is, similar to the viral protein-specific and cell surface receptor-specific immunoglobulins, virus (HIV-1) specific. Accordingly, there is an urgent need for immunoglobulins that do not have the above-described disadvantages.

The present invention provides such immunoglobulins. The immunoglobulins found are capable of binding an intracellular host cell protein which is incorporated into viruses or expressed at the cell surface. Due to the intracellular localization of the protein in uninfected host cells no unwanted side effects occur upon administration of the immunoglobulins. A further advantage of the immunoglobulins is that they are not dependent on specific viral identification and that they interact with a host cell protein that is

commonly involved in the viral assembly and budding process and consequently is capable of neutralizing several distinct viruses.

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DESCRIPTION OF THE FIGURES

Figure 1 shows the titration of anti-WNV monoclonal antibody CR4354L4328 in a murine WNV challenge model. From top to bottom titration of anti-WNV monoclonal antibody CR4354L4328 using doses of 0.03, 0.01, 0.003, and 0.001 mg/kg and titration with a control antibody at a concentration of 10 mg/kg are shown. On the X-axis days are shown and on the Y-axis the survival probability (%) is represented.

Figure 2 shows the titration of anti-WNV monoclonal antibody CR4348 in a murine WNV challenge model. From top to bottom titration of anti-WNV monoclonal antibody CR4348 using doses of 0.1, 0.03, 0.01, 0.003, and 0.001 mg/kg and titration with a control antibody at a concentration of 10 mg/kg are shown. On the X-axis days are shown and on the Y-axis the survival probability (%) is represented.

Figure 3 shows the ELISA binding of dilutions of the optimised antibodies CR4354L4261, CR4354L4267, CR4354L4328, CR4354L4335, CR4354L4383 and the parent antibody CR4354 to WNV. On the Y-axis the absorbance (OD) at 492 nm is shown and on the X-axis the amount of antibody in µg/ml is shown.

Figure 4 shows neutralisation data of CR4348 (closed circles) and CR4354L4328 (open circles) of the flaviviruses yellow fever virus, Japanese encephalitis virus, St. Louis encephalitis virus and dengue virus 2 in a plaque reduction assay.

Figure 5 shows an immunoblot with from left to right mycNDUFV-1 cell lysate and lysate immunoprecipitated with CR4348, CR4361, CR4374 or CR4354L4328.

DESCRIPTION OF THE INVENTION

Here below follow definitions of terms as used in the invention.

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DEFINITIONSAmino acid sequence

The term "amino acid sequence" as used herein refers to
10 naturally occurring or synthetic molecules and to a peptide, oligopeptide, polypeptide or protein sequence.

Binding molecule

As used herein the term "binding molecule" refers to an intact
15 immunoglobulin including monoclonal antibodies, such as chimeric, humanized or human monoclonal antibodies, or to an antigen-binding and/or variable domain comprising fragment of an immunoglobulin that competes with the intact immunoglobulin for specific binding to the binding partner of the
20 immunoglobulin, e.g. a host cell protein. Regardless of structure, the antigen-binding fragment binds with the same antigen that is recognized by the intact immunoglobulin. An antigen-binding fragment can comprise a peptide or polypeptide comprising an amino acid sequence of at least 2 contiguous
25 amino acid residues, at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 30 contiguous amino acid residues, at least
30 35 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70

contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least 200 contiguous amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of the binding molecule.

The term "binding molecule", as used herein includes all immunoglobulin classes and subclasses known in the art. Depending on the amino acid sequence of the constant domain of their heavy chains, binding molecules can be divided into the five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4.

Antigen-binding fragments include, *inter alia*, Fab, F(ab'), F(ab')₂, Fv, dAb, Fd, complementarity determining region (CDR) fragments, single-chain antibodies (scFv), bivalent single-chain antibodies, single-chain phage antibodies, diabodies, triabodies, tetrabodies, (poly)peptides that contain at least a fragment of an immunoglobulin that is sufficient to confer specific antigen binding to the (poly)peptide, etc. The above fragments may be produced synthetically or by enzymatic or chemical cleavage of intact immunoglobulins or they may be genetically engineered by recombinant DNA techniques. The methods of production are well known in the art and are described, for example, in *Antibodies: A Laboratory Manual*, Edited by: E. Harlow and D. Lane (1988), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, which is incorporated herein by reference. A binding molecule or antigen-binding fragment thereof may have

one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or they may be different.

The binding molecule can be a naked or unconjugated binding molecule but can also be part of an immunoconjugate. A naked or unconjugated binding molecule is intended to refer to a binding molecule that is not conjugated, operatively linked or otherwise physically or functionally associated with an effector moiety or tag, such as *inter alia* a toxic substance, a radioactive substance, a liposome, an enzyme. It will be understood that naked or unconjugated binding molecules do not exclude binding molecules that have been stabilized, multimerized, humanized or in any other way manipulated, other than by the attachment of an effector moiety or tag.

Accordingly, all post-translationally modified naked and unconjugated binding molecules are included herewith, including where the modifications are made in the natural binding molecule-producing cell environment, by a recombinant binding molecule-producing cell, and are introduced by the hand of man after initial binding molecule preparation. Of course, the term naked or unconjugated binding molecule does not exclude the ability of the binding molecule to form functional associations with effector cells and/or molecules after administration to the body, as some of such interactions are necessary in order to exert a biological effect. The lack of associated effector group or tag is therefore applied in definition to the naked or unconjugated binding molecule *in vitro*, not *in vivo*.

30 Biological sample

As used herein, the term "biological sample" encompasses a variety of sample types, including blood and other liquid

samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures, or cells derived thereof and the progeny thereof. The term also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides. The term encompasses various kinds of clinical samples obtained from any species, and also includes cells in culture, cell supernatants and cell lysates.

Complementarity determining regions (CDR)

The term "complementarity determining regions" as used herein means sequences within the variable regions of binding molecules, such as immunoglobulins, that usually contribute to a large extent to the antigen binding site which is complementary in shape and charge distribution to the epitope recognized on the antigen. The CDR regions can be specific for linear epitopes, discontinuous epitopes, or conformational epitopes of proteins or protein fragments, either as present on the protein in its native conformation or, in some cases, as present on the proteins as denatured, e.g., by solubilization in SDS. Epitopes may also consist of posttranslational modifications of proteins.

Deletion

The term "deletion", as used herein, denotes a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent as compared to the parent, often the naturally occurring, molecule.

Expression-regulating nucleic acid sequence

The term "expression-regulating nucleic acid sequence" as used herein refers to polynucleotide sequences necessary for and/or affecting the expression of an operably linked coding sequence in a particular host organism. The expression-regulating nucleic acid sequences, such as *inter alia* appropriate transcription initiation, termination, promoter, enhancer sequences; repressor or activator sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., ribosome binding sites); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion, can be any nucleic acid sequence showing activity in the host organism of choice and can be derived from genes encoding proteins, which are either homologous or heterologous to the host organism. The identification and employment of expression-regulating sequences is routine to the person skilled in the art.

Functional variant

The term "functional variant", as used herein, refers to a binding molecule that comprises a nucleotide and/or amino acid sequence that is altered by one or more nucleotides and/or amino acids compared to the nucleotide and/or amino acid sequences of the parental binding molecule and that is still capable of competing for binding to the binding partner, e.g. an intracellular host cell protein, with the parental binding molecule. In other words, the modifications in the amino acid and/or nucleotide sequence of the parental binding molecule do not significantly affect or alter the binding characteristics of the binding molecule encoded by the nucleotide sequence or containing the amino acid sequence, i.e. the binding molecule

is still able to recognize and bind its target. The functional variant may have conservative sequence modifications including nucleotide and amino acid substitutions, additions and deletions. These modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis and random PCR-mediated mutagenesis, and may comprise natural as well as non-natural nucleotides and amino acids.

Conservative amino acid substitutions include the ones in which the amino acid residue is replaced with an amino acid residue having similar structural or chemical properties. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cystine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). It will be clear to the skilled artisan that other classifications of amino acid residue families than the one used above can also be employed. Furthermore, a variant may have non-conservative amino acid substitutions, e.g., replacement of an amino acid with an amino acid residue having different structural or chemical properties. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing immunological activity may be found using computer programs well known in the art.

A mutation in a nucleotide sequence can be a single alteration made at a locus (a point mutation), such as transition or transversion mutations, or alternatively, multiple nucleotides may be inserted, deleted or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleotide sequence. The mutations may be performed by any suitable method known in the art.

10 Host

The term "host", as used herein, is intended to refer to an organism or a cell into which a vector such as a cloning vector or an expression vector has been introduced. The organism or cell can be prokaryotic or eukaryotic. It should be understood that this term is intended to refer not only to the particular subject organism or cell, but to the progeny of such an organism or cell as well. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent organism or cell, but are still included within the scope of the term "host" as used herein.

Human

25 The term "human", when applied to binding molecules as defined herein, refers to molecules that are either directly derived from a human or based upon a human sequence. When a binding molecule is derived from or based on a human sequence and subsequently modified, it is still to be considered human as used throughout the specification. In other words, the term human, when applied to binding molecules is intended to include binding molecules having variable and constant regions

derived from human germline immunoglobulin sequences or based on variable or constant regions occurring in a human or human lymphocyte and modified in some form. Thus, the human binding molecules may include amino acid residues not encoded by human germline immunoglobulin sequences, comprise substitutions and/or deletions (e.g., mutations introduced by for instance random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). "Based on" as used herein refers to the situation that a nucleic acid sequence may be exactly copied from a template, or with minor mutations, such as by error-prone PCR methods, or synthetically made matching the template exactly or with minor modifications. Semi-synthetic molecules based on human sequences are also considered to be human as used herein.

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Insertion

The term "insertion", also known as the term "addition", denotes a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the parent sequence.

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Isolated

The term "isolated", when applied to binding molecules as defined herein, refers to binding molecules that are substantially free of other proteins or polypeptides, particularly free of other binding molecules having different antigenic specificities, and are also substantially free of other cellular material and/or chemicals. For example, when the binding molecules are recombinantly produced, they are preferably substantially free of culture medium, and when the binding molecules are produced by chemical synthesis, they are

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preferably substantially free of chemical precursors or other chemicals, *i.e.*, they are separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. The term "isolated" when applied to nucleic acid molecules encoding binding molecules as defined herein, is intended to refer to nucleic acid molecules in which the nucleotide sequences encoding the binding molecules are free of other nucleotide sequences, particularly nucleotide sequences encoding binding molecules that bind binding partners other than host cell proteins. Furthermore, the term "isolated" refers to nucleic acid molecules that are substantially separated from other cellular components that naturally accompany the native nucleic acid molecule in its natural host, *e.g.*, ribosomes, polymerases, or genomic sequences with which it is naturally associated. Moreover, "isolated" nucleic acid molecules, such as cDNA molecules, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

Monoclonal antibody

The term "monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to an antibody displaying a single binding specificity that has variable and constant regions derived from or based on human germline immunoglobulin sequences or derived from completely synthetic sequences. The method of preparing the monoclonal antibody is not relevant.

Naturally occurring

The term "naturally occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

10 Nucleic acid molecule

The term "nucleic acid molecule" as used in the present invention refers to a polymeric form of nucleotides and includes both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either type of nucleotide. The term also includes single- and double-stranded forms of DNA. In addition, a polynucleotide may include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages. The nucleic acid molecules may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analogue, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages

(e.g., alpha anomeric nucleic acids, etc.). The above term is also intended to include any topological conformation, including single-stranded, double-stranded, partially duplexed, triplex, hairpinned, circular and padlocked conformations. Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. A reference to a nucleic acid sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid molecule having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence. The complementary strand is also useful, e.g., for anti-sense therapy, hybridisation probes and PCR primers.

Operably linked

The term "operably linked" refers to two or more nucleic acid sequence elements that are usually physically linked and are in a functional relationship with each other. For instance, a promoter is operably linked to a coding sequence, if the promoter is able to initiate or regulate the transcription or expression of a coding sequence, in which case the coding sequence should be understood as being "under the control of" the promoter.

Pharmaceutically acceptable excipient

By "pharmaceutically acceptable excipient" is meant any inert substance that is combined with an active molecule such as a drug, agent, or binding molecule for preparing an agreeable or convenient dosage form. The "pharmaceutically acceptable

excipient" is an excipient that is non-toxic to recipients at the dosages and concentrations employed, and is compatible with other ingredients of the formulation comprising the drug, agent or binding molecule.

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Specifically Binding

The term "specifically binding", as used herein, in reference to the interaction of a binding molecule, e.g. an antibody, and its binding partner, e.g. an antigen, means that the
10 interaction is dependent upon the presence of a particular structure, e.g. an antigenic determinant or epitope, on the binding partner. In other words, the antibody preferentially binds or recognizes the binding partner even when the binding partner is present in a mixture of other molecules or
15 organisms. The binding may be mediated by covalent or non-covalent interactions or a combination of both. In yet other words, the term "specifically binding" means immunospecifically binding to an antigen or a fragment thereof and not immunospecifically binding to other antigens. A
20 binding molecule that immunospecifically binds to an antigen may bind to other peptides or polypeptides with lower affinity as determined by, e.g., radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), BIACORE, or other assays known in the art. Binding molecules or fragments thereof that
25 immunospecifically bind to an antigen may be cross-reactive with related antigens. Preferably, binding molecules or fragments thereof that immunospecifically bind to an antigen do not cross-react with other antigens.

Substitutions

A "substitution", as used herein, denotes the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

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Therapeutically effective amount

The term "therapeutically effective amount" refers to an amount of the binding molecule as defined herein that is effective for preventing, ameliorating and/or treating a condition resulting from a viral infection.

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Treatment

The term "treatment" refers to therapeutic treatment as well as prophylactic or preventative measures to cure or halt or at least retard disease progress. Those in need of treatment include those already inflicted with a condition resulting from a viral infection as well as those in which a viral infection is to be prevented. Subjects partially or totally recovered from a viral infection might also be in need of treatment. Prevention encompasses inhibiting or reducing the spread of a virus or inhibiting or reducing the onset, development or progression of one or more of the symptoms associated with a viral infection.

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25 Vector

The term "vector" denotes a nucleic acid molecule into which a second nucleic acid molecule can be inserted for introduction into a host where it will be replicated, and in some cases expressed. In other words, a vector is capable of transporting a nucleic acid molecule to which it has been linked. Cloning as well as expression vectors are contemplated by the term "vector", as used herein. Vectors include, but are not limited

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to, plasmids, cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC) and vectors derived from bacteriophages or plant or animal (including human) viruses. Vectors comprise an origin of replication recognized by the proposed host and in case of expression vectors, promoter and other regulatory regions recognized by the host. A vector containing a second nucleic acid molecule is introduced into a cell by transformation, transfection, or by making use of viral entry mechanisms. Certain vectors are capable of autonomous replication in a host into which they are introduced (e.g., vectors having a bacterial origin of replication can replicate in bacteria). Other vectors can be integrated into the genome of a host upon introduction into the host, and thereby are replicated along with the host genome.

SUMMARY OF THE INVENTION

The invention provides binding molecules capable of specifically binding to a host cell protein and capable of neutralizing viruses. The invention also pertains to nucleic acid molecules encoding at least the binding region of the binding molecules. The invention further provides for the use of the binding molecules of the invention in the prophylaxis and/or treatment of a subject having, or at risk of developing, a viral infection. Besides that, the invention pertains to the use of the binding molecules of the invention in the diagnosis/detection of a viral infection.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect the present invention encompasses binding molecules capable of specifically binding to a host cell protein. Preferably, the host cell protein is an intracellular

host cell protein, meaning that the host cell protein is normally (e.g. in an uninfected host cell) not exposed on the outside of the host cell such as e.g. a cell surface receptor. In an aspect the host cell protein is a mammalian, preferably a human, host cell protein. In normal (e.g. uninfected) host cells the intracellular host cell protein may have a cytoplasmic localization (is a cytoplasmic protein). Alternatively, it may also be localized on or inside host cell organelles including, but not limited to, mitochondria, the Golgi apparatus, nucleus, vacuoles, vesicles and/or the endoplasmic reticulum. After infection of the host cell with a virus the intracellular host cell protein may travel to the cell membrane and may become localized/incorporated in or on the cell surface of the host cell. In other words, after infection of the host cell with a virus the intracellular host cell protein may become displayed on the surface of the host cell. The localization of the host cell protein in normal (e.g. uninfected) cells compared to infected cells may therefore differ. The binding molecules of the invention may be capable of specifically binding to a host cell, once the intracellular host cell protein is exposed in or on the surface of the cell. Preferably, the binding molecules of the invention may also bind a fragment of a host cell protein, said fragment at least comprising an antigenic determinant recognized by the binding molecules of the invention. An "antigenic determinant" as used herein is a moiety, such as a (poly)peptide, protein, glycoprotein, analogue or fragment thereof, that is capable of binding to a binding molecule of the invention with sufficiently high affinity to form a detectable antigen-binding molecule complex.

In a preferred embodiment the intracellular host cell protein recognised by the binding molecules of the invention

is Fas-associated factor-1 (FAF-1; for nucleotide sequence see SEQ ID NO:1 (nucleotides 278-2230 encode the protein) and for amino acid sequence see SEQ ID NO:2; see also Genbank No. BC067100) or NADH-dehydrogenase (ubiquinone) flavoprotein-1

5 (NDUFV-1; for nucleotide sequence see SEQ ID NO:3 (nucleotides 34-1428 encode the protein) and for amino acid sequence see SEQ ID NO:4; see also Genbank No. BC015645.2). In an embodiment of the invention, naturally-occurring truncated or secreted forms, naturally-occurring variant forms (e.g.,

10 alternatively spliced forms) and naturally-occurring allelic variants of FAF-1 or NDUFV-1, particularly the FAF-1 or NDUFV-1 comprising an amino acid sequence as shown in SEQ ID NO:2 and SEQ ID NO:4, respectively, are also a part of the present invention. A variant form of NDUFV-1 is shown under Genbank

15 No. BC008146.1. This variant form has an amino acid sequence that is identical to SEQ ID NO:4, with the proviso that it lacks amino acids 16-24 of SEQ ID NO:4. Other variant forms of NDUFV-1 can be found under Genbank Nos. S67973.1, CR605492.1, and BC007619.1. A variant form of FAF-1 is shown under Genbank

20 No. NM_131917. This variant form has an amino acid sequence that is identical to SEQ ID NO:2, with the proviso that as a result of the lack of an internal coding sequence it lacks amino acids 189-344 (but has the same N- and C-termini as compared to the full-length form of FAF-1). The amino acids

25 189-344 have been replaced by the amino acids FSSR in this variant form. Binding molecules of the invention are preferably also capable of specifically binding to the variants and alternative forms of FAF-1 or NDUFV-1, as long as the modifications in the variants and alternative forms do not

30 abolish the binding of the binding molecules to them. FAF-1 or NDUFV-1 variants may comprise an amino acid sequence having a homology of at least 95%, preferably at least 97%, more

preferably at least 98%, and even more preferably at least 99% with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, respectively. Molecular biological techniques to make variants and forms of FAF-1 or NDUFV-1 recombinantly and/or purify them
5 from a (natural) source are well within the reach of the person skilled in the art and are also contemplated herein.

In a further embodiment the invention provides a pharmaceutical composition comprising FAF-1 and/or NDUFV-1 and a pharmaceutically acceptable excipient. Further disclosure of
10 elements concerning pharmaceutical compositions in general is given below. The host cell proteins described herein may be used as vaccine for the prophylaxis and/or treatment of viral diseases including, but not limited to, flavivirus infections such as WNV infections.

15 The present invention also encompasses binding molecules capable of specifically binding to a fragment of FAF-1 or NDUFV-1 or any of the variants or forms described above. The fragment should at least comprise the antigenic determinant of FAF-1 or NDUFV-1 recognised by the respective binding
20 molecules.

In an aspect of the invention the intracellular host cell protein can be incorporated into a viral membrane. Incorporation can take place inside the host cell, e.g. in the cytoplasm, but may also take place inside or on the surface of
25 one of the host cells organelles. Alternatively, incorporation can also take place near, in or on the host cell surface, e.g. when after infection of the host cell with a virus the intracellular host cell protein is displayed on the surface of the host cell. In other words, the host cell protein may be
30 incorporated into the viral membrane of newly formed viruses after infection of a host cell with a virus. Therefore, the intracellular host cell protein may also be present in or on

viruses, virus-like particles (VLP) or other material at least comprising a viral membrane or parts thereof comprising the host cell protein. Consequently, the binding molecules of the invention may be capable of specifically binding to viruses,
5 virus-like particles or the other material at least comprising a viral membrane or parts thereof comprising the host cell protein. The viruses comprising the host cell protein may be in activated or inactivated/attenuated form. Methods for inactivating/attenuating viruses are well known in the art and
10 include, but are not limited to, heat inactivation, inactivation by UV irradiation, and inactivation by gamma irradiation. As used herein, "virus-like particle" refers to a virus particle that assembles into intact enveloped viral structures. A virus-like particle does however not contain
15 genetic information sufficient to replicate. Virus-like particles have essentially a similar physical appearance as the wild-type virus, *i.e.* they are morphologically and antigenically essentially similar to authentic virions. The virus-like particles as used herein may comprise, next to the
20 intracellular host cell protein, wild-type viral amino acid sequences. The virus-like particles may also include functional copies of certain genes. Furthermore, the virus-like particles may also include foreign nucleic acid. The virus-like particles can be naturally or non-naturally
25 occurring viral particles. They may lack functional copies of certain genes of the wild-type virus, and this may result in the virus-like particle being incapable of some function that is characteristic of the wild-type virus, such as replication and/or cell-cell movement. The missing functional copies of
30 the genes can be provided by the genome of a host cell or on a plasmid present in the host cell, thereby restoring the function of the wild-type virus to the virus-like particle

when in the host cell. Preferably, virus-like particles display the same cellular tropism as the wild-type virus. The virus-like particle may be non-infectious, but is preferably infectious. The term "infectious" as used herein means the capacity of the virus-like particle to complete the initial steps of viral cycle that lead to cell entry. In an embodiment of the invention the virus-like particle self assembles. In another embodiment the virus-like particles are pseudoviruses. Pseudoviruses and their production are well known to the skilled person. Preferably, the pseudoviruses as used herein comprise the intracellular host cell protein on their surface, e.g. incorporated into their viral membrane. Virus-like particles can be produced in suitable host cells such as *inter alia* mammalian cells. They can be produced intracellularly and/or extracellularly and can be harvested, isolated and/or purified as intact virus-like particles by means known to the skilled person such as *inter alia* affinity chromatography, gel filtration chromatography, ion-exchange chromatography, and/or density gradient sedimentation. A virus-like particle as herein described, particularly one that comprises an intracellular host cell protein, e.g. FAF-1 and/or NDUFV-1, incorporated into its viral membrane is also part of the present invention.

In an embodiment the virus-like particle is derived from an enveloped virus, preferably a member of the flavivirus genus such as WNV. In an embodiment the WNV-derived virus-like particle also comprises WNV E-protein. In another embodiment, this virus-like particle further comprises WNV M-protein. By a "WNV E- and M-protein" is meant an envelope and membrane protein, respectively, from any WNV strain. Preferably, the WNV E- and M-protein are derived from a same WNV strain.

In a further aspect the binding molecules of the invention have virus neutralizing activity. Preferably, the binding molecules of the invention have virus neutralizing activity against viruses of more than one genus. Preferably, they neutralize viruses of at least two different genera, more preferably at least three different genera, even more preferably at least four different genera, and in particular at least five or more different genera. The viruses may be non-enveloped viruses, but preferably they are enveloped viruses including, but not limited to, Herpesviridae (e.g. Herpes Simplex Virus type 1, 2, 6, 7, 8, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus), Poxviridae (e.g. Smallpox virus), Retroviridae (e.g. HIV1, HIV2, HTLV1, HTLV2), Paramyxoviridae (e.g. Mumps virus, measles virus, respiratory syncytial virus, parainfluenza virus), HepaDNAviridae (e.g. Hepatitis B virus), Orthomyxoviridae (e.g. Influenza virus A or B), Togaviridae (e.g. Rubella), Flaviviridae (e.g. Yellow fever virus, Dengue virus, West-Nile Virus, Hepatitis C virus, Japanese encephalitis virus, Venezuela encephalitis virus) Rhabdoviridae (e.g. Rabies virus), Arenaviridae (e.g. Lassa virus, lymphocytic choriomeningitis virus) and Coronaviridae (e.g. SARS virus, metapneumoniae virus). Moreover, they may also neutralize viruses not mentioned above, including, but not limited to, Sindbis virus, poliovirus, human papilloma virus, adeno-associated virus, coxsackivirus, enterovirus, Hepatitis A virus, tick-borne encephalitis virus, astrovirus, Ebola virus, Marburg virus, La Crosse virus, California encephalitis virus, Hantaan virus, Crimean-Congo virus, Rift Valley fever, Argentine hemorrhagic fever virus, Bolivian hemorrhagic fever virus, Colorado tick fever, JC virus, BK virus, human adenovirus, and human parvovirus. Preferably, the binding molecules of the invention have a broad virus

neutralizing activity and are capable of neutralizing different, preferably all, viruses within a genus. Preferably, they neutralize different, preferably all, strains of a given virus.

5 In a specific aspect the binding molecules of the invention have flavivirus neutralizing activity, preferably West Nile virus neutralizing activity. The genus flavivirus is a member of the Flaviviridae family. Flaviviruses are small spherical enveloped positive-strand RNA viruses. The
10 flavivirus genus comprises more than 80 highly related viruses including several human pathogens such as *inter alia* yellow fever virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, tick-borne encephalitis virus, and dengue virus. Other viruses
15 belonging to the genus flavivirus can *inter alia* be found in Kuno *et al.* (1998) which is incorporated by reference herein. Preferably, the binding molecules of the invention are capable of neutralizing both WNV lineage I variants such as *inter alia* strain 385-99 and WNV lineage II variants such as *inter alia*
20 strain H-442. In the most preferred embodiment the binding molecules of the invention are capable of neutralizing essentially all WNV variants and strains currently known. In an embodiment, the binding molecules of the invention also neutralize at least one other flavivirus including, but not
25 limited to, yellow fever virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, tick-borne encephalitis virus, and dengue virus, e.g. dengue virus 1, 2, 3, 4. In addition to members of the flavivirus genera, viral members of the pestivirus en
30 hepacivirus such as Hepatitis C virus may be neutralized. Like flavivirus, the Hepatitis C virus assembly and budding takes place in the ER.

In a further aspect the binding molecules of the invention also have rabies virus neutralizing activity. In an aspect of the invention they are also capable of specifically binding to rabies virus. Rabies virus is member of the

5 Lyssavirus genus. In total, the Lyssavirus genus includes eleven genotypes: rabies virus (genotype 1), Lagos bat virus (genotype 2), Mokola virus (genotype 3), Duvenhage virus (genotype 4), European bat lyssavirus 1 (genotype 5), European bat lyssavirus 2 (genotype 6), Australian bat lyssavirus

10 (genotype 7), Aravan virus (genotype 8), Khujand virus (genotype 9), Irkut virus (genotype 10) and West Caucasian virus (genotype 11). Besides binding to rabies virus, the binding molecules of the invention may also be capable of binding to other genotypes of the Lyssavirus genus.

15 Preferably, the binding molecules may also be capable of neutralizing other genotypes of the Lyssavirus genus. Furthermore, the binding molecules of the invention may be capable of binding to and/or neutralizing viruses, other than Lyssaviruses, of the rhabdovirus family. This family includes,

20 but is not limited to, the genera ephemerovirus, lyssavirus, rhabdovirus and vesiculovirus.

In an embodiment the binding molecules of the invention are human binding molecules. The binding molecules of the invention can be intact immunoglobulin molecules such as

25 polyclonal or monoclonal antibodies or the binding molecules can be antigen-binding fragments including, but not limited to, Fab, F(ab'), F(ab')₂, Fv, dAb, Fd, complementarity determining region (CDR) fragments, single-chain antibodies (scFv), bivalent single-chain antibodies, single-chain phage

30 antibodies, diabodies, triabodies, tetrabodies, and (poly)peptides that contain at least a fragment of an immunoglobulin that is sufficient to confer specific antigen

binding to the intracellular host cell protein or fragment thereof. In a preferred embodiment the human binding molecules having virus neutralizing activity are administered in IgG1, IgA or IgM format.

5 The binding molecules of the invention can be used in non-isolated or isolated form. Furthermore, the binding molecules of the invention can be used alone or in a mixture comprising at least one binding molecule (or variant or fragment thereof) of the invention. In other words, the
10 binding molecules can be used in combination, e.g., as a pharmaceutical composition comprising two or more binding molecules of the invention, variants or fragments thereof. For example, binding molecules having different, but complementary activities can be combined in a single therapy to achieve a
15 desired prophylactic, therapeutic or diagnostic effect, but alternatively, binding molecules having identical activities can also be combined in a single therapy to achieve a desired prophylactic, therapeutic or diagnostic effect. The mixture may further comprise at least one other therapeutic agent.
20 Preferably, the therapeutic agent is useful in the prophylaxis and/or treatment of a viral infection (e.g. WNV infection).

Typically, binding molecules according to the invention can bind to their binding partners, i.e. the intracellular host cell proteins or fragments thereof, with an affinity
25 constant (K_d -value) that is lower than $0.2 \cdot 10^{-4}$ M, $1.0 \cdot 10^{-5}$ M, $1.0 \cdot 10^{-6}$ M, $1.0 \cdot 10^{-7}$ M, preferably lower than $1.0 \cdot 10^{-8}$ M, more preferably lower than $1.0 \cdot 10^{-9}$ M, more preferably lower than $1.0 \cdot 10^{-10}$ M, even more preferably lower than $1.0 \cdot 10^{-11}$ M, and in particular lower than $1.0 \cdot 10^{-12}$ M. The affinity constants can
30 vary for antibody isotypes. For example, affinity binding for an IgM isotype refers to a binding affinity of at least about $1.0 \cdot 10^{-7}$ M. Affinity constants can for instance be measured

using surface plasmon resonance, *i.e.* an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using
5 the BIACORE system (Pharmacia Biosensor AB, Uppsala, Sweden).

The binding molecules according to the invention may bind to the intracellular host cell protein or fragment thereof in soluble form such as for instance in a sample or may bind to the intracellular host cell protein or a fragment thereof
10 bound or attached to a carrier or substrate, *e.g.*, microtiter plates, membranes and beads, etc. Carriers or substrates may be made of glass, plastic (*e.g.*, polystyrene), polysaccharides, nylon, nitrocellulose, or Teflon, etc. The surface of such supports may be solid or porous and of any
15 convenient shape. Furthermore, the binding molecules may bind to the intracellular host cell protein in purified/isolated or non-purified/non-isolated form. Consequently, the binding molecules may bind to the intracellular host cell protein when it is incorporated into the host cell and/or viral membrane,
20 such as in an infected host cell, a virus or a virus-like particle.

The binding molecules of the invention are capable of neutralizing virus infectivity. This may be achieved by targeting viral specific events wherein the intracellular host
25 cell proteins are involved including, but not limited to, virus attachment to cell membranes and penetration in cells, virus uncoating, virus nucleic acid synthesis, viral protein synthesis and maturation, assembly and release of infectious particles, targeting the membrane of infected cells resulting
30 in a signal that leads to no replication or a lower replication rate of the virus. Neutralization assays for different viruses are known in the art. WNV, yellow fever

virus, Japanese encephalitis virus, St. Louis encephalitis virus, dengue virus and rabies virus neutralizing activity can for instance be measured as described herein. Alternative WNV neutralization assays are described in for instance Gollins and Porterfield (1986).

In a preferred embodiment, the binding molecules according to the invention comprise at least a CDR3 region, preferably a heavy chain CDR3 region, comprising the amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:6. In an embodiment the binding molecules of the invention may comprise two, three, four, five or even all six CDR regions of the binding molecules of the invention. The heavy chain CDR1 region, heavy chain CDR2 region, light chain CDR1 region, light chain CDR2 region and light chain CDR3 region of the binding molecules of the invention are shown in Table 9. CDR regions are according to Kabat *et al.* (1991) as described in Sequences of Proteins of Immunological Interest.

In yet another embodiment, the binding molecules according to the invention comprise a heavy chain comprising the variable heavy chain of the amino acid sequence selected from the group consisting of SEQ ID NO:18 and SEQ ID NO:20. In a further embodiment, the binding molecules according to the invention comprise a light chain comprising the variable light chain of the amino acid sequence selected from the group consisting of SEQ ID NO:22 and SEQ ID NO:24. Table 8 specifies, next to the heavy chain CDR3 region, the nucleotide and amino acid sequence of the heavy and light chains (and their variable regions) of the single chain antibodies.

In yet a further embodiment the binding molecules of the invention comprise a heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs 30

and 32, and/or comprise a light chain comprising the amino acid sequence selected from the group consisting of SEQ ID Nos 34 and 36.

In another embodiment the binding molecules of the invention comprise a heavy chain CDR1 region, heavy chain CDR2 region, heavy chain CDR3 region, light chain CDR1 region, light chain CDR2 region and light chain CDR3 region as shown in SEQ ID Nos 12, 13, 6, 143, 144 and 145, respectively. In yet another embodiment the binding molecules of the invention comprise a heavy chain comprising the variable heavy chain of the amino acid sequence of SEQ ID NO:20 and/or comprise a light chain comprising the variable light chain of the amino acid sequence of SEQ ID NO:74, *i.e.* amino acids 1-113 of SEQ ID NO:74. In yet a further embodiment the binding molecules of the invention comprise a heavy chain comprising the amino acid sequence of SEQ ID NO 32, and/or comprise a light chain comprising the amino acid sequence of SEQ ID NO:74.

Another aspect of the invention includes functional variants of the binding molecules as defined herein. Molecules are considered to be functional variants of a binding molecule according to the invention, if the variants are capable of competing for specifically binding to the intracellular host cell proteins or fragment thereof with the parent binding molecules. The host cell protein can be incorporated into the viral membrane. In other words, when the functional variants are still capable of binding to the intracellular host cell proteins or fragment thereof. Furthermore, molecules are considered to be functional variants of a binding molecule according to the invention, if they have virus neutralizing activity (*e.g.* WNV neutralizing activity). Functional variants include, but are not limited to, derivatives that are substantially similar in primary structural sequence, but

which contain e.g. *in vitro* or *in vivo* modifications, chemical and/or biochemical, that are not found in the parental binding molecule. Such modifications include *inter alia* acetylation, acylation, covalent attachment of a nucleotide or nucleotide
5 derivative, covalent attachment of a lipid or lipid derivative, cross-linking, disulfide bond formation, glycosylation, hydroxylation, methylation, oxidation, pegylation, proteolytic processing, phosphorylation, and the like.

10 Alternatively, functional variants can be binding molecules as defined in the present invention comprising an amino acid sequence containing substitutions, insertions, deletions or combinations thereof of one or more amino acids compared to the amino acid sequences of the parental binding
15 molecules. Furthermore, functional variants can comprise truncations of the amino acid sequence at either or both the amino or carboxyl termini. Functional variants according to the invention may have the same or different, either higher or lower, binding affinities compared to the parental binding
20 molecule but are still capable of binding to the intracellular host cell proteins or fragment thereof. For instance, functional variants according to the invention may have increased or decreased binding affinities for the intracellular host cell proteins or fragment thereof compared
25 to the parental binding molecules. Preferably, the amino acid sequences of the variable regions, including, but not limited to, framework regions, hypervariable regions, in particular the CDR3 regions, are modified. Generally, the light chain and the heavy chain variable regions comprise three hypervariable
30 regions, comprising three CDRs, and more conserved regions, the so-called framework regions (FRs). The hypervariable regions comprise amino acid residues from CDRs and amino acid

residues from hypervariable loops. Functional variants intended to fall within the scope of the present invention have at least about 50% to about 99%, preferably at least about 60% to about 99%, more preferably at least about 70% to about 99%, even more preferably at least about 80% to about 99%, most preferably at least about 90% to about 99%, in particular at least about 95% to about 99%, and in particular at least about 97% to about 99% amino acid sequence homology with the parental human binding molecules as defined herein.

Computer algorithms such as *inter alia* Gap or Bestfit known to a person skilled in the art can be used to optimally align amino acid sequences to be compared and to define similar or identical amino acid residues. Functional variants can be obtained by altering the parental binding molecules or parts thereof by general molecular biology methods known in the art including, but not limited to, error-prone PCR, oligonucleotide-directed mutagenesis, site-directed mutagenesis and heavy or light chain shuffling. Preferably, the functional variants of the invention have virus neutralizing activity. This neutralizing activity may either be identical, or be higher or lower compared to the parental binding molecules. Furthermore, preferably the functional variants have neutralizing activity against more than one genus, preferably against viruses of at least two, three, four, five, six, etc, different genera. Henceforth, when the term (human) binding molecule is used, this also encompasses functional variants of the (human) binding molecule.

In yet a further aspect, the invention includes immunoconjugates, *i.e.* molecules comprising at least one binding molecule as defined herein and further comprising at least one tag, such as *inter alia* a detectable moiety/agent. Also contemplated in the present invention are mixtures of

immunoconjugates according to the invention or mixtures of at least one immunoconjugates according to the invention and another molecule, such as a therapeutic agent or another binding molecule or immunoconjugate. In a further embodiment, the immunoconjugates of the invention may comprise more than one tag. These tags can be the same or distinct from each other and can be joined/conjugated non-covalently to the binding molecules. The tag(s) can also be joined/conjugated directly to the human binding molecules through covalent bonding. Alternatively, the tag(s) can be joined/conjugated to the binding molecules by means of one or more linking compounds. Techniques for conjugating tags to binding molecules are well known to the skilled artisan.

The tags of the immunoconjugates of the present invention may be therapeutic agents, but preferably they are detectable moieties/agents. The tags may also be toxins, such as botulinum toxin or functional parts thereof. Immunoconjugates comprising a detectable agent can be used diagnostically to, for example, assess if a subject has been infected with a virus or monitor the development or progression of a virus infection as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. However, they may also be used for other detection and/or analytical and/or diagnostic purposes such as detection of viruses or virus-like particles comprising the intracellular host cell protein or host cells that have been infected and as a consequence thereof display the intracellular host cell protein on their surface. Detectable moieties/agents include, but are not limited to, enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals, and non-radioactive paramagnetic metal ions. The tags used to

label the binding molecules for detection and/or analytical and/or diagnostic purposes depend on the specific detection/analysis/diagnosis techniques and/or methods used such as *inter alia* immunohistochemical staining of (tissue) samples, flow cytometric detection, scanning laser cytometric detection, fluorescent immunoassays, enzyme-linked immunosorbent assays (ELISA's), radioimmunoassays (RIA's), bioassays (e.g., neutralization assays), Western blotting applications, etc. Suitable labels for the detection/analysis/diagnosis techniques and/or methods known in the art are well within the reach of the skilled artisan.

Furthermore, the human binding molecules or immunoconjugates of the invention can also be attached to solid supports, which are particularly useful for *in vitro* immunoassays or purification of the intracellular host cell protein or fragment thereof, host cells displaying the intracellular host cell protein on their surface, or viruses or virus-like particles having the intracellular host cell protein incorporated into the viral membrane. Such solid supports might be porous or nonporous, planar or non-planar. The binding molecules of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. Examples include, but are not limited to, the hexa-histidine tag, the hemagglutinin (HA) tag, the myc tag or the flag tag. Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate. In another aspect the binding molecules of the invention may be conjugated/attached to one or more antigens. Preferably, these antigens are antigens which are recognized by the immune system of a subject to which the binding molecule-antigen conjugate is administered. The antigens may be identical, but may also differ from each other. Conjugation methods for

attaching the antigens and binding molecules are well known in the art and include, but are not limited to, the use of cross-linking agents. The binding molecules of the invention will bind to the intracellular host cell protein and the antigens
5 attached to the binding molecules will initiate a powerful T-cell attack on the conjugate, which will eventually lead to the destruction of the structure to which the intracellular host cell protein is bound or wherein it is incorporated.

Next to producing immunoconjugates chemically by
10 conjugating, directly or indirectly, via for instance a linker, the immunoconjugates can be produced as fusion proteins comprising the binding molecules of the invention and a suitable tag. Fusion proteins can be produced by methods known in the art such as, e.g., recombinantly by constructing
15 nucleic acid molecules comprising nucleotide sequences encoding the binding molecules in frame with nucleotide sequences encoding the suitable tag(s) and then expressing the nucleic acid molecules.

It is another aspect of the present invention to provide
20 a nucleic acid molecule encoding at least a binding molecule or immunoconjugate according to the invention. Such nucleic acid molecules can be used as intermediates for cloning purposes, e.g. in the process of affinity maturation as described above. In a preferred embodiment, the nucleic acid
25 molecules are isolated or purified.

The skilled man will appreciate that functional variants of these nucleic acid molecules are also intended to be a part of the present invention. Functional variants are nucleic acid sequences that can be directly translated, using the standard
30 genetic code, to provide an amino acid sequence identical to that translated from the parental nucleic acid molecules.

Preferably, the nucleic acid molecules encode binding molecules comprising a CDR3 region, preferably a heavy chain CDR3 region, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:6. In a
5 further embodiment the nucleic acid molecules encode binding molecules comprising two, three, four, five or even all six CDR regions of the binding molecules of the invention.

In another embodiment, the nucleic acid molecules encode binding molecules comprising a heavy chain comprising the
10 variable heavy chain of the amino acid sequence selected from the group consisting of SEQ ID NO:18 and SEQ ID NO:20. In another embodiment the nucleic acid molecules encode binding molecules comprising a light chain comprising the variable light chain of the amino acid sequence selected from the group
15 consisting of SEQ ID NO:22 and SEQ ID NO:24.

In yet a further embodiment the nucleic acid molecules encode binding molecules comprising a heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID Nos 30 and 32, and/or comprising a light chain
20 comprising the amino acid sequence selected from the group consisting of SEQ ID Nos 34 and 36.

In another embodiment the nucleic acid molecules encode binding molecules comprising a heavy chain CDR1 region, heavy chain CDR2 region, heavy chain CDR3 region, light chain CDR1
25 region, light chain CDR2 region and light chain CDR3 region as shown in SEQ ID Nos 12, 13, 6, 143, 144 and 145, respectively. In yet another embodiment the nucleic acid molecules encode binding molecules comprising a heavy chain comprising the variable heavy chain of the amino acid sequence of SEQ ID
30 NO:20 and/or comprising a light chain comprising the variable light chain of the amino acid sequence of SEQ ID NO:74, *i.e.* amino acids 1-113 of SEQ ID NO:74. In yet a further embodiment

the nucleic acid molecules encode binding molecules comprising a heavy chain comprising the amino acid sequence of SEQ ID NO 32, and/or comprising a light chain comprising the amino acid sequence of SEQ ID NO:74.

5 It is another aspect of the invention to provide vectors, *i.e.* nucleic acid constructs, comprising one or more nucleic acid molecules according to the present invention. Vectors can be derived from plasmids such as *inter alia* F, R1, RP1, Col, pBR322, TOL, Ti, etc; cosmids; phages such as lambda,
10 lambdoid, M13, Mu, P1, P22, Q β , T-even, T-odd, T2, T4, T7, etc; plant viruses. Vectors can be used for cloning and/or for expression of the binding molecules of the invention and might even be used for gene therapy purposes. Vectors comprising one or more nucleic acid molecules according to the invention
15 operably linked to one or more expression-regulating nucleic acid molecules are also covered by the present invention. The choice of the vector is dependent on the recombinant procedures followed and the host used. Introduction of vectors in host cells can be effected by *inter alia* calcium phosphate
20 transfection, virus infection, DEAE-dextran mediated transfection, lipofectamin transfection or electroporation. Vectors may be autonomously replicating or may replicate together with the chromosome into which they have been integrated. Preferably, the vectors contain one or more
25 selection markers. The choice of the markers may depend on the host cells of choice, although this is not critical to the invention as is well known to persons skilled in the art. They include, but are not limited to, kanamycin, neomycin, puromycin, hygromycin, zeocin, thymidine kinase gene from
30 herpes simplex virus (HSV-TK), dihydrofolate reductase gene from mouse (dhfr). Vectors comprising one or more nucleic acid molecules encoding the human binding molecules as described

above operably linked to one or more nucleic acid molecules encoding proteins or peptides that can be used to isolate the human binding molecules are also covered by the invention. These proteins or peptides include, but are not limited to, glutathione-S-transferase, maltose binding protein, metal-binding polyhistidine, green fluorescent protein, luciferase and beta-galactosidase.

Hosts containing one or more copies of the vectors mentioned above are an additional subject of the present invention. Preferably, the hosts are host cells. Host cells include, but are not limited to, cells of mammalian, plant, insect, fungal or bacterial origin. Bacterial cells include, but are not limited to, cells from Gram-positive bacteria such as several species of the genera *Bacillus*, *Streptomyces* and *Staphylococcus* or cells of Gram-negative bacteria such as several species of the genera *Escherichia*, such as *E. coli*, and *Pseudomonas*. In the group of fungal cells preferably yeast cells are used. Expression in yeast can be achieved by using yeast strains such as *inter alia* *Pichia pastoris*, *Saccharomyces cerevisiae* and *Hansenula polymorpha*. Furthermore, insect cells such as cells from *Drosophila* and Sf9 can be used as host cells. Besides that, the host cells can be plant cells such as *inter alia* cells from crop plants such as forestry plants, or cells from plants providing food and raw materials such as cereal plants, or medicinal plants, or cells from ornamentals, or cells from flower bulb crops. Transformed (transgenic) plants or plant cells are produced by known methods, for example, *Agrobacterium*-mediated gene transfer, transformation of leaf discs, protoplast transformation by polyethylene glycol-induced DNA transfer, electroporation, sonication, microinjection or bolistic gene transfer. Additionally, a suitable expression system can be a

baculovirus system. Expression systems using mammalian cells such as Chinese Hamster Ovary (CHO) cells, COS cells, BHK cells or Bowes melanoma cells are preferred in the present invention. Mammalian cells provide expressed proteins with

5 posttranslational modifications that are most similar to natural molecules of mammalian origin. Since the present invention deals with molecules that may have to be administered to humans, a completely human expression system would be particularly preferred. Therefore, even more

10 preferably, the host cells are human cells. Examples of human cells are *inter alia* HeLa, 911, AT1080, A549, 293 and HEK293T cells. In preferred embodiments, the human producer cells comprise at least a functional part of a nucleic acid sequence encoding an adenovirus E1 region in expressible format. In

15 even more preferred embodiments, said host cells are derived from a human retina and immortalized with nucleic acids comprising adenoviral E1 sequences, such as 911 cells or the cell line deposited at the European Collection of Cell Cultures (ECACC), CAMR, Salisbury, Wiltshire SP4 OJG, Great

20 Britain on 29 February 1996 under number 96022940 and marketed under the trademark PER.C6® (PER.C6 is a registered trademark of Crucell Holland B.V.). For the purposes of this application "PER.C6" refers to cells deposited under number 96022940 or ancestors, passages up-stream or downstream as well as

25 descendants from ancestors of deposited cells, as well as derivatives of any of the foregoing. Production of recombinant proteins in host cells can be performed according to methods well known in the art. The use of the cells marketed under the trademark PER.C6® as a production platform for proteins of

30 interest has been described in WO 00/63403 the disclosure of which is incorporated herein by reference in its entirety.

A method of producing a binding molecule or an immunoconjugate according to the invention is an additional part of the invention. The method comprises the steps of a) culturing a host according to the invention under conditions conducive to the expression of the binding molecule or immunoconjugate, and b) optionally, recovering the expressed binding molecule or immunoconjugate. The expressed binding molecules or immunoconjugates can be recovered from the cell free extract, but preferably they are recovered from the culture medium. The above method of producing can also be used to make functional variants of the binding molecules and immunoconjugates of the present invention. Methods to recover proteins, such as binding molecules, from cell free extracts or culture medium are well known to the man skilled in the art. Binding molecules or immunoconjugates as obtainable by the above-described method are also a part of the present invention.

Alternatively, next to the expression in hosts, such as host cells, the binding molecules and immunoconjugates of the invention can be produced synthetically by conventional peptide synthesizers or in cell-free translation systems using RNA nucleic acid derived from DNA molecules according to the invention. Binding molecules and immunoconjugates as obtainable by the above-described synthetic production methods or cell-free translation systems are also a part of the present invention.

In yet another embodiment, binding molecules of the present invention can also be produced in transgenic, non-human, mammals such as *inter alia* rabbits, goats or cows, and secreted into for instance the milk thereof.

In yet another alternative embodiment, binding molecules according to the present invention, preferably human binding

molecules specifically binding to the intracellular host cell protein or fragment thereof, may be generated by transgenic non-human mammals, such as for instance transgenic mice or rabbits, that express human immunoglobulin genes. Preferably, the transgenic non-human mammals have a genome comprising a human heavy chain transgene and a human light chain transgene encoding all or a portion of the human binding molecules as described above. The transgenic non-human mammals can be immunized with a purified or enriched preparation of the intracellular host cell protein or fragment thereof. Protocols for immunizing non-human mammals are well established in the art. See Using Antibodies: A Laboratory Manual, Edited by: E. Harlow, D. Lane (1998), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York and Current Protocols in Immunology, Edited by: J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober (2001), John Wiley & Sons Inc., New York, the disclosures of which are incorporated herein by reference. Immunization protocols often include multiple immunizations, either with or without adjuvants such as Freund's complete adjuvant and Freund's incomplete adjuvant, but may also include naked DNA immunizations. In another embodiment, the human binding molecules are produced by B-cells or plasma cells derived from the transgenic animals. In yet another embodiment, the human binding molecules are produced by hybridomas, which are prepared by fusion of B-cells obtained from the above-described transgenic non-human mammals to immortalized cells. B-cells, plasma cells and hybridomas as obtainable from the above described transgenic non-human mammals and human binding molecules as obtainable from the above-described transgenic non-human mammals, B-cells, plasma cells and hybridomas are also a part of the present invention.

The intracellular host cell proteins FAF-1 and/or NDUFV-1 may also be produced and purified according to the methods and with the vectors and host cells as described above.

Alternatively, they may also be produced and/or purified from
5 a "native" host cell, e.g. a host cell normally expressing the intracellular host cell protein. Expression in a "native" host cell may be increased by substituting the natural promoter of proteins and/or by adding, substituting and/or mutating expression enhancing elements of proteins. The elements and
10 methods to increase expression are known to the skilled artisan.

In a further aspect, the invention provides a method of identifying binding molecules according to the invention such as human binding molecules, e.g. monoclonal antibodies or
15 fragments thereof, specifically binding to a host cell protein or nucleic acid molecules encoding such binding molecules and comprises the steps of a) contacting a collection of binding molecules on the surface of replicable genetic packages with a virus, virus-like particle or fragment thereof under
20 conditions conducive to binding, b) selecting at least once for a replicable genetic package binding to the virus, virus-like particle or fragment thereof, and c) separating and recovering the replicable genetic package binding to the virus, virus-like particle or fragment thereof from replicable
25 genetic packages that do not bind. The host cell protein may be an intracellular host cell protein.

A replicable genetic package as used herein can be prokaryotic or eukaryotic and includes cells, spores, yeasts, bacteria, viruses, (bacterio)phage, ribosomes and polysomes. A
30 preferred replicable genetic package is a phage. The binding molecules, such as for instance single chain Fvs, are displayed on the replicable genetic package, i.e. they are

attached to a group or molecule located at an exterior surface of the replicable genetic package. The replicable genetic package is a screenable unit comprising a binding molecule to be screened linked to a nucleic acid molecule encoding the binding molecule. The nucleic acid molecule should be replicable either *in vivo* (e.g., as a vector) or *in vitro* (e.g., by PCR, transcription and translation). *In vivo* replication can be autonomous (as for a cell), with the assistance of host factors (as for a virus) or with the assistance of both host and helper virus (as for a phagemid). Replicable genetic packages displaying a collection of binding molecules is formed by introducing nucleic acid molecules encoding exogenous binding molecules to be displayed into the genomes of the replicable genetic packages to form fusion proteins with endogenous proteins that are normally expressed from the outer surface of the replicable genetic packages. Expression of the fusion proteins, transport to the outer surface and assembly results in display of exogenous binding molecules from the outer surface of the replicable genetic packages.

In one embodiment the selection step in the method according to the present invention is performed in the presence of virus that is inactivated. The inactivation of the virus may be performed by viral inactivation methods well known to the skilled artisan. Methods to test if a virus is still infective or partly or completely inactivated are well known to the person skilled in the art. The virus used in the above method may be non-isolated, e.g. present in serum and/or blood of an infected individual. The virus used may also be isolated either before or after inactivation. Purification may be performed by means of well-known purification methods suitable for viruses such as for instance centrifugation

through a glycerol cushion. The virus contains the (intracellular) host cell protein incorporated into its viral membrane.

Alternatively, the selection step may be performed in the presence of a fragment of the virus such as a fragment comprising the viral membrane or virus-like particles having the (intracellular) host cell protein incorporated into their membranes. The inactivated virus, virus-like particle or fragment thereof may be immobilized to a suitable material before use. In a specific embodiment the selection can be performed on different materials comprising the (intracellular) host cell proteins. For instance, the first selection round can be performed on (inactivated) virus, while the second selection round can be performed on WNV-like particles. Alternatively, the first selection round can be performed on host cells displaying the (intracellular) host cell protein on their surface, while the second selection round can be performed on (inactivated) virus. Of course, other combinations are also suitable. For instance, the first and second selection round can be performed on identical material such as WNV-like particles. Different materials comprising the (intracellular) host cell protein can also be used during one selection/panning step.

In yet a further aspect, the invention provides a method of obtaining a binding molecule specifically binding to a host cell protein or a nucleic acid molecule encoding such a binding molecule specifically binding to a host cell protein, wherein the method comprises the steps of a) performing the above described method of identifying binding molecules, and b) isolating from the recovered replicable genetic package the binding molecule and/or the nucleic acid molecule encoding the binding molecule. The collection of binding molecules on the

surface of replicable genetic packages can be a collection of scFvs or Fabs. Once a new scFv or Fab has been established or identified with the above mentioned method of identifying binding molecules or nucleic acid molecules encoding the binding molecules, the DNA encoding the scFv or Fab can be isolated from the bacteria or phages and combined with standard molecular biological techniques to make constructs encoding bivalent scFvs or complete human immunoglobulins of a desired specificity (e.g. IgG, IgA or IgM). These constructs can be transfected into suitable cell lines and complete human monoclonal antibodies can be produced (see Huls *et al.*, 1999; Boel *et al.*, 2000).

As mentioned before the preferred replicable genetic package is a phage. Phage display methods for identifying and obtaining (human) binding molecules, e.g. monoclonal antibodies, are by now well-established methods known by the person skilled in the art. They are e.g. described in US Patent Number 5,696,108; Burton and Barbas, 1994; de Kruif *et al.*, 1995b; and Phage Display: A Laboratory Manual. Edited by: CF Barbas, DR Burton, JK Scott and GJ Silverman (2001), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. All these references are herewith incorporated herein in their entirety. For the construction of phage display libraries, collections of human monoclonal antibody heavy and light chain variable region genes are expressed on the surface of bacteriophage, preferably filamentous bacteriophage, particles, in for example single-chain Fv (scFv) or in Fab format (see de Kruif *et al.*, 1995b). Large libraries of antibody fragment-expressing phages typically contain more than 1.0×10^9 antibody specificities and may be assembled from the immunoglobulin V-regions expressed in the B-lymphocytes of immunized or non-immunized individuals. In a specific

embodiment of the invention the phage library of binding molecules, preferably scFv phage library, is prepared from RNA isolated from cells obtained from a subject that has been vaccinated or exposed to a virus. RNA can be isolated from
5 *inter alia* bone marrow or peripheral blood, preferably peripheral blood lymphocytes. The subject can be an animal vaccinated or exposed to a virus, but is preferably a human subject which has been vaccinated or has been exposed to a virus. Preferably the human subject has recovered from the
10 viral infection. In an embodiment of the invention the virus is a flavivirus such as WNV.

Alternatively, phage display libraries may be constructed from immunoglobulin variable regions that have been partially assembled *in vitro* to introduce additional antibody diversity
15 in the library (semi-synthetic libraries). For example, *in vitro* assembled variable regions contain stretches of synthetically produced, randomized or partially randomized DNA in those regions of the molecules that are important for antibody specificity, e.g. CDR regions. Host cell protein
20 specific phage antibodies can be selected from the library by for instance immobilizing target antigens such as viruses, virus-like particles, host cells or host cell proteins, e.g. intracellular host cell proteins, on a solid phase and subsequently exposing the target antigens to a phage library
25 to allow binding of phages expressing antibody fragments specific for the solid phase-bound antigen(s). Non-bound phages are removed by washing and bound phages eluted from the solid phase for infection of *E.coli* bacteria and subsequent propagation. Multiple rounds of selection and propagation are
30 usually required to sufficiently enrich for phages binding specifically to the target antigen(s). If desired, before exposing the phage library to target antigens the phage

library can first be subtracted by exposing the phage library to non-target antigens bound to a solid phase. Phages may also be selected for binding to complex antigens such as complex mixtures of host cell proteins or (poly)peptides, (expression) host cells expressing one or more host cell proteins or (poly)peptides, virus-like particles comprising host cell proteins, or whole inactivated viruses. Antigen specific phage antibodies can be selected from the library by incubating a solid phase with bound thereon virus-like particles with the phage antibody library to let for example the scFv or Fab part of the phage bind to the virus-like particle. Of course, in an alternative embodiment selections and subtractions may also be performed in solution. After incubation and several washes to remove unbound and loosely attached phages, the phages that have bound with their scFv or Fab part to the virus-like particle are eluted and used to infect *E. coli* to allow amplification of the new specificity. Generally, one or more selection rounds are required to separate the phages of interest from the large excess of non-binding phages.

Alternatively, the host cell proteins can be expressed in expression host cells, e.g. PER.C6® cells, and these cells can be used for selection of phage antibodies specific for the proteins or (poly)peptides. A phage display method using (expression) host cells can be extended and improved by subtracting non-relevant binders during screening by addition of an excess of host cells comprising no target molecules or non-target molecules that are similar, but not identical, to the target, and thereby strongly enhance the chance of finding relevant binding molecules. Of course, the subtraction may also be performed before or after the screening with material comprising the host cell protein or fragment thereof. In an embodiment the selection can take place on host cells that

have been infected with a virus or a virus-like particle. These cells display the normally intracellular located host cell protein after infection on their surface. Subtraction may be performed before, during, or after the selection and may be performed with host cells that have not been infected and thus comprise the intracellular host cell protein in its original cellular location, *i.e.* inside the host cell. The subtraction process is referred to as the Mabstract® process (Mabstract® is a registered trademark of Crucell Holland B.V., see also US Patent Number 6,265,150 which is incorporated herein by reference).

In yet another aspect the invention provides a method of obtaining a binding molecule potentially having neutralizing activity against a virus and being capable of specifically binding to a host cell protein, wherein the method comprises the steps of a) performing the method of obtaining a binding molecule specifically binding to the host cell protein or a nucleic acid molecule encoding such a binding molecule specifically binding to the host cell protein as described above, and b) verifying if the binding molecule isolated has neutralizing activity against a virus. Preferably, the host cell protein is an intracellular host cell protein. In an embodiment the methods described above are performed with a flavivirus such as WNV. In a preferred embodiment of the method it is verified if the binding molecule isolated has neutralizing activity against viruses of at least two different genera. More preferably, it is verified if the binding molecules are broadly neutralizing binding molecules and are able to neutralize many different genera. Assays for verifying if a binding molecule has neutralizing activity for a specific virus are well known in the art.

In a further aspect the invention pertains to a host cell protein specific binding molecule being obtainable by the methods as described above. Preferably, the binding molecule has neutralizing activity against at least one virus.

5 In yet a further aspect, the invention provides compositions comprising at least one binding molecule, at least one functional variant thereof, at least one immunoconjugate according to the invention or a combination thereof. The invention also pertains to compositions
10 comprising at least two binding molecules, preferably human binding molecules, at least one of which may be a binding molecule of the invention. Preferably, both binding molecules have virus neutralizing activity. The activity may be against the same virus but also against different viruses. In an
15 embodiment the compositions comprising two or more binding molecules having virus neutralizing activity exhibit synergistic virus neutralizing activity. In an embodiment the binding molecules acting synergistically in neutralizing one virus may also be capable of neutralizing other viruses
20 synergistically. As used herein, the term "synergistic" means that the combined effect of the binding molecules when used in combination is greater than their additive effects when used individually. A way of calculating synergy is by means of the combination index. The concept of the combination index (CI)
25 has been described by Chou and Talalay, 1984. In an alternative embodiment the compositions comprise two or more binding molecules having different modes of action. In an embodiment the binding molecules of the invention have flavivirus, e.g. WNV, neutralizing activity. In addition to
30 that, the compositions may comprise *inter alia* stabilizing molecules, such as albumin or polyethylene glycol, or salts. Preferably, the salts used are salts that retain the desired

biological activity of the binding molecules and do not impart any undesired toxicological effects. If necessary, the human binding molecules of the invention may be coated in or on a material to protect them from the action of acids or other
5 natural or non-natural conditions that may inactivate the binding molecules.

In yet a further aspect, the invention provides compositions comprising at least one nucleic acid molecule as defined in the present invention. The compositions may
10 comprise aqueous solutions such as aqueous solutions containing salts (e.g., NaCl or salts as described above), detergents (e.g., SDS) and/or other suitable components.

Furthermore, the present invention pertains to pharmaceutical compositions comprising at least one binding
15 molecule (or functional fragment or variant thereof), at least one immunoconjugate according to the invention, at least one composition according to the invention, or combinations thereof. The pharmaceutical composition of the invention further comprises at least one pharmaceutically acceptable
20 excipient. A pharmaceutical composition according to the invention can further comprise at least one other therapeutic, prophylactic and/or diagnostic agent. Preferably, the pharmaceutical composition comprises at least one other prophylactic and/or therapeutic agent. Preferably, said
25 further therapeutic and/or prophylactic agents are agents capable of preventing and/or treating an infection and/or a condition resulting from a virus, e.g. a flavivirus such as WNV. Therapeutic and/or prophylactic agents include, but are not limited to, anti-viral agents. Such agents can be binding
30 molecules, small molecules, organic or inorganic compounds, enzymes, polynucleotide sequences etc. Agents capable of preventing and/or treating a viral infection and/or a

condition resulting from a virus that are in the experimental phase might also be used as other therapeutic and/or prophylactic agents useful in the present invention.

The binding molecules or pharmaceutical compositions of the invention can be tested in suitable animal model systems prior to use in humans. Such animal model systems include, but are not limited to, a murine model, a hamster model, and a geese model system.

Typically, pharmaceutical compositions must be sterile and stable under the conditions of manufacture and storage. The binding molecules, immunoconjugates, nucleic acid molecules or compositions of the present invention can be in powder form for reconstitution in the appropriate pharmaceutically acceptable excipient before or at the time of delivery. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Alternatively, the binding molecules, immunoconjugates, nucleic acid molecules or compositions of the present invention can be in solution and the appropriate pharmaceutically acceptable excipient can be added and/or mixed before or at the time of delivery to provide a unit dosage injectable form. Preferably, the pharmaceutically acceptable excipient used in the present invention is suitable to high drug concentration, can maintain proper fluidity and, if necessary, can delay absorption.

The choice of the optimal route of administration of the pharmaceutical compositions will be influenced by several factors including the physico-chemical properties of the

active molecules within the compositions, the urgency of the clinical situation and the relationship of the plasma concentrations of the active molecules to the desired therapeutic effect. For instance, if necessary, the binding molecules of the invention can be prepared with carriers that will protect them against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can *inter alia* be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Furthermore, it may be necessary to coat the binding molecules with, or co-administer the binding molecules with, a material or compound that prevents the inactivation of the human binding molecules. For example, the binding molecules may be administered to a subject in an appropriate carrier, for example, liposomes or a diluent.

The routes of administration can be divided into two main categories, oral and parenteral administration. The preferred administration route is intravenous.

Oral dosage forms can be formulated *inter alia* as tablets, troches, lozenges, aqueous or oily suspensions, dispersable powders or granules, emulsions, hard capsules, soft gelatin capsules, syrups or elixirs, pills, dragees, liquids, gels, or slurries. These formulations can contain pharmaceutically excipients including, but not limited to, inert diluents, granulating and disintegrating agents, binding agents, lubricating agents, preservatives, colouring, flavouring or sweetening agents, vegetable or mineral oils, wetting agents, and thickening agents.

The pharmaceutical compositions of the present invention can also be formulated for parenteral administration.

Formulations for parenteral administration can be *inter alia* in the form of aqueous or non-aqueous isotonic sterile non-toxic injection or infusion solutions or suspensions. The solutions or suspensions may comprise agents that are non-
5 toxic to recipients at the dosages and concentrations employed such as 1,3-butanediol, Ringer's solution, Hank's solution, isotonic sodium chloride solution, oils, fatty acids, local anaesthetic agents, preservatives, buffers, viscosity or solubility increasing agents, water-soluble antioxidants, oil-
10 soluble antioxidants, and metal chelating agents.

In a further aspect, the binding molecules (functional fragments and variants thereof), immunoconjugates, compositions, or pharmaceutical compositions of the invention can be used as a medicament. So, a method of treatment and/or
15 prevention of a viral infection using the binding molecules, immunoconjugates, compositions, or pharmaceutical compositions of the invention is another part of the present invention. The above-mentioned molecules can *inter alia* be used in the diagnosis, prophylaxis, treatment, or combination thereof, of
20 one or more conditions resulting from a virus or viral infection. Preferably, they are broadly applicable to prevent, detect and/or treat several viral infections caused by viruses of different genera and/or strains, including, but not limited to, Herpesviridae (e.g. Herpes Simplex Virus type 1, 2, 6, 7,
25 8, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus), Poxviridae (e.g. Smallpox virus), Retroviridae (e.g. HIV1, HIV2, HTLV1, HTLV2), Paramyxoviridae (e.g. Mumps virus, measles virus, respiratory syncytial virus, parainfluenza virus), HepaDNAviridae (e.g. Hepatitis B virus),
30 Orthomyxoviridae (e.g. Influenza virus A or B), Togaviridae (e.g. Rubella), Flaviviridae (e.g. Yellow fever virus, Dengue virus, West-Nile Virus, Hepatitis C virus, Japanese

encephalitis virus, Venezuela encephalitis virus)
Rhabdoviridae (e.g. Rabies virus), Arenaviridae (e.g. Lassa
virus, lymphocytic choriomeningitis virus) and Coronaviridae
(e.g. SARS virus, metapneumoniae virus). Moreover, they may
5 also be applicable to prevent, detect and/or treat several
viral infections caused by viruses not mentioned above
including, but not limited to, Sindbis virus, poliovirus,
human papilloma virus, adeno-associated virus, coxsackivirus,
enterovirus, hepatitis A virus, tick-borne encephalitis virus,
10 astrovirus, Ebola virus, Marburg virus, La Crosse virus,
California encephalitis virus, Hantaan virus, Crimean-Congo
virus, Rift Valley fever, Argentine hemorrhagic fever virus,
Bolivian hemorrhagic fever virus, Colorado tick fever, JC
virus, BK virus, human adenovirus, and human parvovirus. In
15 other words, the binding molecules of the invention are broad-
spectrum anti-viral binding molecules. They are suitable for
treatment of yet untreated patients suffering from a condition
resulting from a virus and patients who have been or are
treated from a condition resulting from a virus or a viral
20 infection. They protect against further infection by a virus
for approximately 1 month and/or will retard the onset or
progress of the symptoms associated with a virus. They may
also be used in post-exposure prophylaxis, when there is a
chance of infection but symptoms are absent. They may also be
25 used as prophylaxis in the transplant of infected organs or in
other patient populations at high risk of exposure and
progression to disease due to *inter alia* age or immune status.
In a specific embodiment the compounds and compositions are
used to treat and/or prevent a flavivirus, e.g. WNV, infection
30 and/or a rabies virus infection.

The above-mentioned molecules or compositions may be
employed in conjunction with other molecules useful in

diagnosis, prophylaxis and/or treatment. They can be used *in vitro*, *ex vivo* or *in vivo*. For instance, the binding molecules, immunoconjugates or pharmaceutical compositions of the invention can be co-administered with a vaccine against the intracellular host cell proteins. Alternatively, the vaccine may also be administered before or after administration of the molecules of the invention. Instead of a vaccine, other anti-viral agents can also be employed in conjunction with the binding molecules of the present invention. In an aspect the invention is also directed to the use of an intracellular host cell protein, preferably FAF-1 and/or NDUFV-1, as a vaccine suitable for preventing and/or treating a viral infection, e.g. a flavivirus infection such as WNV infection.

The molecules are typically formulated in the compositions and pharmaceutical compositions of the invention in a therapeutically or diagnostically effective amount. Alternatively, they may be formulated and administered separately. For instance the other molecules such as the anti-viral compounds may be applied systemically, while the binding molecules of the invention may be applied intrathecally, intraventricularly or intradermally.

Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic response). A suitable dosage range may for instance be 0.1-100 mg/kg body weight, preferably 0.5-15 mg/kg body weight. Furthermore, for example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. The molecules and compositions according to the present invention are preferably sterile. Methods to render these molecules and compositions sterile are

well known in the art. The other molecules useful in diagnosis, prophylaxis and/or treatment can be administered in a similar dosage regimen as proposed for the binding molecules of the invention. If the other molecules are administered
5 separately, they may be administered to a patient prior to (e.g., 2 minutes, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 7
10 days, 2 weeks, 4 weeks or 6 weeks before), concomitantly with, or subsequent to (e.g., 2 minutes, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 2 days, 3 days, 4 days, 5
15 days, 7 days, 2 weeks, 4 weeks or 6 weeks after) the administration of one or more of the human binding molecules or pharmaceutical compositions of the invention. The exact dosing regimen is usually sorted out during clinical trials in human patients.

20 Human binding molecules and pharmaceutical compositions comprising the human binding molecules are particularly useful, and often preferred, when to be administered to human beings as *in vivo* therapeutic agents, since recipient immune response to the administered antibody will often be
25 substantially less than that occasioned by administration of a monoclonal murine, chimeric or humanized binding molecule.

In another aspect, the invention concerns the use of the (human) binding molecules (functional fragments and variants thereof), immunoconjugates, nucleic acid molecules,
30 compositions or pharmaceutical compositions according to the invention in the preparation of a medicament for the

diagnosis, prophylaxis, treatment, or combination thereof, of a viral infection and/or a condition resulting thereof.

Next to that, kits comprising at least one binding molecule (functional fragments and variants thereof), at least one immunoconjugate, at least one nucleic acid molecule, at least one composition, at least one pharmaceutical composition, at least one vector, at least one host according to the invention or a combination thereof are also a part of the present invention. Optionally, the above-described components of the kits of the invention are packed in suitable containers and labelled for diagnosis, prophylaxis and/or treatment of the indicated conditions. The above-mentioned components may be stored in unit or multi-dose containers as an aqueous, preferably sterile, solution or as a lyophilized, preferably sterile, formulation for reconstitution. The containers may be formed from a variety of materials such as glass or plastic and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The kit may further comprise more containers comprising a pharmaceutically acceptable buffer. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, culture medium for one or more of the suitable hosts and, possibly, even at least one other therapeutic, prophylactic or diagnostic agent. Associated with the kits can be instructions customarily included in commercial packages of therapeutic, prophylactic or diagnostic products, that contain information about for example the indications, usage, dosage, manufacture, administration, contraindications and/or warnings concerning the use of such therapeutic, prophylactic or diagnostic products.

The invention further pertains to a method of detecting an intracellular host cell protein such as FAF-1 and/or NDUFV-1 in a sample, wherein the method comprises the steps of a) contacting a sample with a diagnostically effective amount of a binding molecule (functional fragments and variants thereof) or an immunoconjugate according to the invention, and b) determining whether the binding molecule or immunoconjugate specifically binds to a molecule of the sample. The sample may be a biological sample including, but not limited to blood, serum, urine, tissue or other biological material from (potentially) infected subjects, or a non-biological sample such as water, drink, etc. The (potentially) infected subjects may be human subjects, but also animals that are suspected as carriers of a virus might be tested for the presence of the virus using the human binding molecules or immunoconjugates of the invention. By detecting the intracellular host cell proteins a viral infection (infected host cell and/or virus particle) may be detected. The sample may first be manipulated to make it more suitable for the method of detection.

Manipulation means *inter alia* treating the sample suspected to contain and/or containing a virus in such a way that the virus will disintegrate into antigenic components such as proteins, (poly)peptides or other antigenic fragments. Preferably, the human binding molecules or immunoconjugates of the invention are contacted with the sample under conditions which allow the formation of an immunological complex between the human binding molecules and intracellular host cell protein or antigenic components thereof that may be present in the sample. The formation of an immunological complex, if any, indicating the presence of a virus in the sample, is then detected and measured by suitable means. Such methods include, *inter alia*, homogeneous and heterogeneous binding

immunoassays, such as radio-immunoassays (RIA), ELISA, immunofluorescence, immunohistochemistry, FACS, BIACORE and Western blot analyses.

Preferred assay techniques, especially for large-scale
5 clinical screening of patient sera and blood and blood-derived products are ELISA and Western blot techniques. ELISA tests are particularly preferred. For use as reagents in these assays, the binding molecules or immunoconjugates of the invention are conveniently bonded to the inside surface of
10 microtiter wells. The binding molecules or immunoconjugates of the invention may be directly bonded to the microtiter well. However, maximum binding of the binding molecules or immunoconjugates of the invention to the wells might be accomplished by pre-treating the wells with polylysine prior
15 to the addition of the binding molecules or immunoconjugates of the invention. Furthermore, the binding molecules or immunoconjugates of the invention may be covalently attached by known means to the wells. Generally, the binding molecules or immunoconjugates are used between 0.01 to 100 µg/ml for
20 coating, although higher as well as lower amounts may also be used. Samples are then added to the wells coated with the binding molecules or immunoconjugates of the invention.

Furthermore, binding molecules of the invention can be used to identify epitopes of the intracellular host cell
25 proteins such as FAF-1 and/or NDUFV-1. The epitopes can be linear, but also structural and/or conformational. In one embodiment, binding of binding molecules of the invention to a series of overlapping peptides, such as 15-mer peptides, of the host cell protein can be analyzed by means of PEPSCAN
30 analysis (see *inter alia* WO 84/03564, WO 93/09872, Slootstra *et al.*, 1996). The binding of the molecules to each peptide can be tested in a PEPSCAN-based enzyme-linked immunoassay

(ELISA). In another embodiment, a random peptide library comprising peptides from the host cell proteins can be screened for peptides capable of binding to the binding molecules of the invention. In the above assays the use of
5 neutralizing binding molecules may identify one or more neutralizing epitopes. The peptides/epitopes found can be used as vaccines and for the diagnosis of a viral infection.

In a further aspect, the invention provides a method of screening a binding molecule (or a functional fragment or
10 variant thereof) for specific binding to the same epitope of the intracellular host cell protein, such as FAF-1 and/or NDUFV-1, as the epitope bound by a human binding molecule of the invention, wherein the method comprises the steps of a) contacting a binding molecule to be screened, a binding
15 molecule of the invention and material comprising the intracellular host cell protein or antigenic fragment thereof, b) measure if the binding molecule to be screened is capable of competing for specifically binding to the intracellular host cell protein comprising material or antigenic fragment
20 thereof with the binding molecule of the invention. The material comprising the intracellular host cell protein or antigenic fragment thereof may be a host cell displaying the protein on its surface, a virus having the protein incorporated into its viral membrane, a virus-like particle
25 having the protein incorporated into the viral membrane or the protein itself in purified or non-purified form. In a further step it may be determined, if the screened binding molecules that are capable of competing for specifically binding to the intracellular host cell protein or antigenic fragment thereof
30 have neutralizing activity. A binding molecule that is capable of competing for specifically binding to the intracellular host cell protein or an antigenic fragment thereof with the

binding molecule of the invention is another part of the present invention. In the above-described screening method, "specifically binding to the same epitope" also contemplates specific binding to substantially or essentially the same
5 epitope as the epitope bound by the a binding molecule of the invention. The capacity to block, or compete with, the binding of the binding molecules of the invention to the intracellular host cell protein typically indicates that a binding molecule to be screened binds to an epitope or binding site on the
10 intracellular host cell protein that structurally overlaps with the binding site on the intracellular host cell protein that is immunospecifically recognized by the binding molecules of the invention. Alternatively, this can indicate that a binding molecule to be screened binds to an epitope or binding
15 site which is sufficiently proximal to the binding site immunospecifically recognized by the binding molecules of the invention to sterically or otherwise inhibit binding of the binding molecules of the invention to the intracellular host cell protein.

20 In general, competitive inhibition is measured by means of an assay, wherein an antigen composition, *i.e.* a composition comprising the intracellular host cell protein or antigenic fragments thereof, is admixed with reference binding molecules, *i.e.* the binding molecules of the invention, and
25 binding molecules to be screened. Usually, the binding molecules to be screened are present in excess. Protocols based upon ELISAs and Western blotting are suitable for use in such simple competition studies. In certain embodiments, one may pre-mix the reference binding molecules with varying
30 amounts of the binding molecules to be screened (*e.g.*, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90 or 1:100) for a period of time prior to applying to the antigen composition.

In other embodiments, the reference binding molecules and varying amounts of binding molecules to be screened can simply be admixed during exposure to the antigen composition. In yet another embodiment the reference binding molecules or binding
5 molecules to be screened are contacted before the binding molecules to be screened or reference binding molecules, respectively, are contacted with the intracellular host cell protein or antigenic fragment thereof. In any event, by using species or isotype secondary antibodies one will be able to
10 detect only the bound reference binding molecules, the binding of which will be reduced by the presence of a binding molecule to be screened that recognizes substantially the same epitope. In conducting a binding molecule competition study between a reference binding molecule and any binding molecule to be
15 screened (irrespective of species or isotype), one may first label the reference binding molecule with a detectable label, such as, e.g., biotin, an enzymatic, a radioactive or other label to enable subsequent identification. In these cases, one would pre-mix or incubate the labelled reference binding
20 molecules with the binding molecules to be screened at various ratios (e.g., 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90 or 1:100) and (optionally after a suitable period of time) then assay the reactivity of the labelled reference binding molecules and compare this with a control value in
25 which no potentially competing binding molecule was included in the incubation. The assay may again be any one of a range of immunological assays based upon antibody hybridization, and the reference binding molecules would be detected by means of detecting their label, e.g., using streptavidin in the case of
30 biotinylated reference binding molecules or by using a chromogenic substrate in connection with an enzymatic label (such as 3,3',5,5'-tetramethylbenzidine (TMB) substrate with

peroxidase enzyme) or by simply detecting a radioactive label. A binding molecule to be screened that binds to the same epitope as the reference binding molecule will be able to effectively compete for binding and thus will significantly reduce reference binding molecule binding, as evidenced by a reduction in bound label. The reactivity of the (labelled) reference binding molecule in the absence of a completely irrelevant binding molecule would be the control high value. The control low value would be obtained by incubating the labelled reference binding molecule with unlabelled reference binding molecules of exactly the same type, when competition would occur and reduce binding of the labelled reference binding molecule. In a test assay, a significant reduction in labelled reference binding molecule reactivity in the presence of a binding molecule to be screened is indicative of a binding molecule that recognizes the same epitope, *i.e.*, one that "cross-reacts" with the labelled reference binding molecule.

Binding molecules identified by these competition assays ("competitive binding molecules" or "cross-reactive binding molecules") include, but are not limited to, antibodies, antibody fragments and other binding agents that bind to an epitope or binding site bound by the reference binding molecule, *i.e.* a binding molecule of the invention, as well as antibodies, antibody fragments and other binding agents that bind to an epitope or binding site sufficiently proximal to an epitope bound by the reference binding molecule for competitive binding between the binding molecules to be screened and the reference binding molecule to occur. Preferably, competitive binding molecules of the invention will, when present in excess, inhibit specific binding of a reference binding molecule to a selected target species by at

least 10%, preferably by at least 25%, more preferably by at least 50%, and most preferably by at least 75%-90% or even greater. The identification of one or more competitive binding molecules that bind to about, substantially, essentially or at the same epitope as the binding molecules of the invention is a straightforward technical matter. As the identification of competitive binding molecules is determined in comparison to a reference binding molecule, *i.e.* a binding molecule of the invention, it will be understood that actually determining the epitope to which the reference binding molecule and the competitive binding molecule bind is not in any way required in order to identify a competitive binding molecule that binds to the same or substantially the same epitope as the reference binding molecule.

In a further aspect the invention pertains to new leader peptide sequences (also called signal sequences or presequences) and nucleotide sequences encoding the leader peptides. The leader peptides are useful in methods for producing recombinant proteins, *e.g.* immunoglobulin molecules, from a host cell. The entry of almost all secreted polypeptides to the secretory pathway, in both prokaryotes and eukaryotes, is directed by specific leader peptides at the N-terminus of the polypeptide chain. Leader peptides direct nascent polypeptides towards the machinery of the cell that exports polypeptides from the cell into the surrounding medium or, in some cases, into the periplasmic space. The mechanisms by which leader peptides direct nascent polypeptide chains to the secretion pathway and direct the precise and efficient proteolytic cleavage to release mature proteins are incompletely understood. The leader peptide is usually, although not necessarily, located at the N-terminus of the primary translation product and is generally, although not

necessarily, cleaved off the desired polypeptide during the secretion process, to yield the "mature" polypeptide. The secretion of polypeptides is a dynamic and multi-step process involving several elements of the cellular secretory apparatus and specific sequence elements in the signal peptide. It further involves translation, translocation and post-translational processing, and one or more of these steps may not necessarily be completed before another is either initiated or completed. Signal sequences are indispensable for production of phage antibodies, in which antibody fragments such as scFv or Fab are fused to the phage coat proteins such as pIII or pVIII.

Signal sequences are predominantly hydrophobic in nature, a feature which may be important in directing the nascent peptide to the membrane and transfer of secretory proteins across the inner membrane of prokaryotes or the endoplasmic reticulum membranes of eukaryotes. In mammalian cells, leader peptides are recognized by the 54K protein of the signal recognition particle (SRP), which is believed to hold the nascent chain in a translocation competent conformation until it contacts the endoplasmic reticulum membrane. The SRP consists of a 7S RNA and six different polypeptides. The 7S RNA and the 54K leader peptide-binding protein (SRP54) of mammalian SRP exhibit strong sequence similarity to the 4.5S RNA and P48 protein (Ffh) of *Escherichia coli*, which forms the signal recognition particle in bacteria. It is likely that the various sequences found in different signal peptides interact in unique ways with the secretion apparatus. There are no eukaryotic signal sequences available that result in correct protein processing and high protein expression in bacteria or vice versa.

The antibody phage format or scFv fragment format is often not considered a suitable format in assays of functionalities other than binding, e.g. assays for testing neutralizing activity where bivalent binding is required or where functional Fc regions are required. In for instance assays testing neutralizing activity data obtained with phage antibodies or scFv fragments derived from phage antibodies are not representative of the neutralizing activity of the complete immunoglobulin molecules. Therefore, complete immunoglobulin molecules, e.g. IgG1, are needed to test particular specificities for functional activity. To increase the efficiency by which scFv molecules can be reformatted into IgG1 a new vector system making use of new leader peptides was designed. The new vectors and leader peptides were designed to enable direct shuttling of immunoglobulin heavy chain regions (VH region) and immunoglobulin light chain regions (VL region) from bacterial expression vectors, such as phage display vectors, e.g. PDV, pHEN and vectors derived thereof, into an eukaryotic expression system. This approach has a significant time and cost advantage over the standard systems wherein each VH and VL region had to be PCR amplified, cloned and checked for frequent mutations that occur through the amplification process before being shuttled into eukaryotic expression vectors. In short the procedure is as follows. VH and VL fragment repertoires are cloned into prokaryotic expression vectors such as pHEN1 and PDV-C06 using specific restriction enzymes. These sites are chosen to be outside of the V region encoding sequences because there is too much sequence diversity within the V regions for efficient PCR amplification and cloning of V gene repertoires. Typically, the enzyme recognition site at the 5'-end of the VH antibody fragments is located in the coding region of the prokaryotic leader

sequence such as the SfiI site in the PelB leader sequence of pHEN1 or PDV-C06. Therefore, SfiI is one of the available sites for cloning at the 5'-end that is present in all phage display derived scFv clones. When using this site for cloning, the sequence encoded by the SfiI site and the downstream sequence are transferred to the eukaryotic vector; this means that the C-terminal part of the prokaryotic leader is transferred to the eukaryotic vector. In the present invention an eukaryotic leader was designed that is functional when the region encoding the C-terminal part of the prokaryotic leader sequence (e.g. PelB) is transferred into the eukaryotic vector. VL antibody fragment repertoires are cloned using a SalI site that is located within the scFv linker sequence. When this site is used for cloning, the sequence encoded by the SalI site is transferred to the eukaryotic vector and will be part of the eukaryotic leader sequence. Therefore, leaders were designed that contain a sequence upstream of the SalI restriction site that together with the SalI restriction site encoded sequence and the sequence downstream of the restriction site after transcription and translation form a functional leader in eukaryotic cells. The VH region is cut from a phage display vector with restriction enzymes and cloned in frame between a 'truncated' eukaryotic leader peptide and the hinge and constant domains of an immunoglobulin heavy chain present in the expression vector. In an identical way the VL region is cloned in frame with a 'truncated' eukaryotic leader peptide and the constant domain of the immunoglobulin light (either lambda or kappa) chain. The newly designed leader peptides have a sequence that is recognized by the cellular secretory apparatus and is proteolitically cleaved during secretion of the immunoglobulin chains from host cells, e.g. eukaryotic host cells, resulting

in correctly processed mature Ig heavy and light chain proteins. The invention also provides an expression vector (pIG-CHG1) for production of a full length IgG1 heavy chain immunoglobulin molecule and two vectors encoding a complete
5 light chain fragment. One vector (pIG-Ckappa) produces the kappa light chain sub-type and the other (pIG-Clambda) the lambda light chain variant. It is essential to determine beforehand what sub-type the VL region belongs to, so that the right constant light chain domain can be fused to it. Of
10 course, the IgG1 constant domain in the vector can be replaced by an IgG2, IgG3 or IgG4 domain to produce heavy chains of the IgG2, IgG3 or IgG4 format. Other proteins that need a leader sequence in both prokaryotes and eukaryotes can also be made by means of the new leaders. For convenient and efficient
15 cloning the pIG vectors were extended with large stuffer fragments such that double cut vectors are easily separated from linear single cut vectors.

In an aspect the invention pertains to an eukaryotic leader peptide comprising an amino acid sequence selected from the
20 group consisting of SEQ ID NO:150, SEQ ID NO:152 and SEQ ID NO:154. In a further aspect the invention provides a recombinant polypeptide (e.g. recombinant protein) comprising an eukaryotic leader peptide of the invention and a polypeptide of interest. The polypeptide of interest can be
25 any recombinant protein, but preferably it is an eukaryotic protein such as a mammalian protein, or more preferably a human protein. In an embodiment the polypeptide of interest is selected from the group consisting of an immunoglobulin heavy chain, an immunoglobulin light chain, an immunoglobulin heavy
30 chain fragment and an immunoglobulin light chain fragment. Preferred fragments are immunoglobulin variable regions (VH or VL regions). Preferably, the immunoglobulin chains and

fragments are human. In one aspect of the invention, the leader peptide is used to direct or even enhance the secretion of the recombinant polypeptide produced in a recombinant (*i.e.* transformed) host organism, *e.g.* a host cell. In an embodiment

5 the carboxy terminus of the eukaryotic leader peptide is joined/fused to the amino terminus of the polypeptide of interest. Preferably, the leader peptide comprising SEQ ID NO:150 is fused to an immunoglobulin heavy chain or fragment thereof. This leader peptide is the result of joining a

10 'truncated' eukaryotic leader peptide encoded by the nucleotide sequence of SEQ ID NO:155 to a nucleotide sequence encoding an immunoglobulin heavy chain or fragment thereof, *e.g.* a heavy chain variable region from plasmid pHEN1 or PDV-C06. The leader peptides comprising SEQ ID NO:152 and SEQ ID

15 NO:154 are joined to an immunoglobulin kappa and lambda light chain or fragment thereof, respectively. These leader peptides are the result of joining a 'truncated' eukaryotic leader peptide encoded by the nucleotide sequence of SEQ ID NO:156 or SEQ ID NO:157 to a nucleotide sequence encoding an

20 immunoglobulin kappa or lambda light chain or fragment thereof, *e.g.* a kappa or lambda light chain variable region from plasmid pHEN1 or PDV-C06. Thus, the invention provides a fusion polypeptide comprising a leader peptide sequence of the invention and a recombinant protein sequence. The fusion

25 polypeptide may be designed such that there are additional amino acids present between the leader peptide and the recombinant protein. In these instances, cleavage of the leader peptide from the fusion polypeptide may produce a modified recombinant protein having additional amino acids at

30 the N-terminus. Alternatively, the fusion polypeptide may be designed such that the site for cleavage of the leader peptide occurs a few amino acids into the sequence of the recombinant

protein. In these instances, a modified recombinant protein may be produced which has an altered N-terminus.

In another aspect the invention provides a nucleic acid sequence encoding an eukaryotic leader peptide of the invention. The eukaryotic leader peptides according to the invention comprise nucleotides GGCCCAGCCGGCC (SEQ ID NO:158), *i.e.* a SfiI-site, or nucleotides TCGAC (SEQ ID NO:159), *i.e.* a combined XhoI/SalI-site, within the nucleic acid sequence encoding the leader peptides. The combined XhoI/SalI-site is made by ligating the XhoI-site in a prokaryotic leader into an eukaryotic vector that has been digested with the restriction enzyme SalI. Preferably, the sites are located within the carboxy terminal part of the leader peptide encoding sequences. In a preferred embodiment the nucleic acid sequence encoding the new leader peptides according to the invention comprise a nucleic acid sequence selected from the group consisting of SEQ ID NO:149, SEQ ID NO:151 and SEQ ID NO:153. The nucleic acid sequence may further comprise a nucleic acid sequence encoding a polypeptide of interest. Suitable polypeptides of interest are mentioned above. The nucleic acid sequence encoding the leader peptides and the nucleic acid sequence encoding the polypeptides of interest can form a so-called fusion construct. By "fusion construct" is intended a nucleic acid comprising the coding sequence for a leader peptide and the coding sequence, with or without introns, for a recombinant protein, in which the coding sequences are adjacent and in the same reading frame such that, when the fusion construct is transcribed and translated in a host cell, a protein is produced in which the C-terminus of the leader peptide is joined to the N-terminus of the recombinant protein. The protein product of the fusion construct may be referred to herein as "fusion polypeptide". Nucleic acid

encoding the leader peptide can be operably joined/linked to nucleic acid containing the coding region of the recombinant protein in such manner that the leader peptide coding region is upstream of (that is, 5' of) and in the same reading frame with the recombinant protein coding region to provide a fusion construct. Typically, the 3'-end of the nucleic acid encoding the leader peptide is joined to the 5'-end of the nucleic acid encoding the recombinant protein. The two coding regions are joined such that they are in the same reading frame. In this way, the fusion construct will encode a single protein, having the leader peptide at the N-terminal end followed by the recombinant protein at the C-terminal end. The leader peptide and the recombinant protein may be joined directly or there may be one or several amino acids connecting them. Certain amino acids are well known to interfere with cleavage by signal peptidases and these residues are avoided in designing the cleavage site for the fusion polypeptide. The fusion construct can be expressed in a host cell to provide a fusion polypeptide comprising the leader peptide joined, at its carboxy terminus, to the recombinant protein at its amino terminus. The fusion polypeptide can be secreted from the host cell. Typically, the leader peptide is cleaved from the fusion polypeptide during the secretion process, resulting in the accumulation of secreted recombinant protein in the external cellular environment.

In a further aspect the invention provides an expression vector comprising a nucleic acid sequence according to the invention. Preferably, the nucleic acid sequence of the invention comprises a nucleic acid sequence encoding a leader peptide according to the invention. They may further comprise a nucleic acid sequence encoding the polypeptide of interest. Expression vectors can be prepared containing the nucleic

acids encoding the leader peptide or the fusion construct by methods that are well known in the art. In general, the expression vectors will contain nucleic acid encoding the leader peptide, or the fusion construct, under the control of a promoter. In some embodiments, more than one leader peptide or fusion construct may be placed under the control of a single promoter. In such embodiments, the additional fusion construct(s) will be placed downstream of the first fusion construct and separated from the upstream fusion construct by nucleotides. The promoter is chosen so that it is capable of directing transcription in a host of interest. Promoters capable of directing transcription in various host cells are well known. In an embodiment the promoter is the CMVlong promoter, but any other suitable promoter may also be chosen.

In general, a "promoter" will include all nucleotide sequences upstream of the translational start necessary for the transcription of the leader peptide and/or fusion polypeptide coding region. The promoter may include or overlap the sequence of the ribosome binding site. Selection of promoter will often influence the selection of ribosome binding site as well. The expression vector may also contain other expression regulating nucleic acid sequences including selectable marker genes, origins of replication, polyadenylation signal sequences, etc. Other expression regulating nucleic acid sequences and further sequences, e.g. sequences of polypeptides useful for isolation, that can be included in the vectors are mentioned above. The expression vectors may contain one or more selectable marker genes, including ampicillin and/or neomycin, for selection in the host of interest and/or one or more origins of replication, including a pUc ori and/or a SV40 ori and/or a f1 ori, to provide autonomous replication of the vector in the host.

Additionally, they may contain one or more polyadenylation signal sequences including a SV40-polyA signal and/or a BGH-polyA signal. Alternatively, or in addition, the expression vector may contain nucleotide sequences to aid in integration of the vector into the host chromosome. An expression vector according to the invention may further comprise a XhoI-site or a NotI-site. An immunoglobulin heavy and/or light chain variable region may for instance be located between a Sfi-site and a XhoI-site (e.g. in an expression vector for the production of heavy chain (IgG1) immunoglobulin chains), and located between a XhoI-site and a NotI-site (e.g. in an expression vector for the production of light chain (kappa and lambda) immunoglobulin chains). The restriction sites may be located downstream of a nucleic acid sequence encoding the promoter, e.g. the CMVlong promoter, and downstream of the eukaryotic leader peptide of the invention. The sites may be located upstream of a nucleic acid sequence encoding an immunoglobulin constant domain.

In a further embodiment the invention provides an expression vector a nucleic acid sequence selected from the group consisting of SEQ ID NO:155, SEQ ID NO:156 and SEQ ID NO:157, i.e. a 'truncated' eukaryotic leader peptide. The expression vector comprising the nucleic acid sequence of SEQ ID NO:155 comprises a SfiI-site at its carboxy terminus. The expression vectors comprising the nucleic acid sequence of SEQ ID NO:156 or SEQ ID NO:157 comprise a XhoI-site at their carboxy termini. The vectors may further comprise downstream of the nucleic acid sequence selected from the group consisting of SEQ ID NO:155, SEQ ID NO:156 and SEQ ID NO:157 a XhoI-site (for SEQ ID NO:155) or a NotI-site (for SEQ ID NO:156 and SEQ ID NO:157). They may further comprise a stuffer sequence between the nucleic acid sequence selected from the

group consisting of SEQ ID NO:155, SEQ ID NO:156 and SEQ ID NO:157 and the XhoI-site or NotI-site. The nucleic acid sequence selected from the group consisting of SEQ ID NO:155, SEQ ID NO:156 and SEQ ID NO:157, the stuffer sequence and the XhoI-site and NotI-site may be located downstream of a promoter sequence such as a CMVlong promoter sequence. They may be located upstream of an immunoglobulin heavy or light chain constant region. The expression vector may further comprise other expression regulating nucleic acid sequences or other sequences. Examples of suitable expression regulating nucleic acid sequences or other sequences are mentioned above. In an embodiment the expression vector according to the invention comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:53, SEQ ID NO:54 and SEQ ID NO:58. These vectors comprise a stuffer sequence.

A host cell comprising at least an expression vector according to the invention is another aspect of the invention. Preferably, the host cell is a human host cell. Other suitable host cells are mentioned above. Furthermore, the invention pertains to the use of an expression vector according to the invention and/or a host cell according to the invention for the production of an immunoglobulin chain and/or molecule.

In yet a further aspect the invention provides a method for producing expression vectors. In one embodiment the expression vector produced is suitable for producing an immunoglobulin heavy chain. The method comprises the steps of: digesting/cutting an expression vector comprising the nucleic acid sequence of SEQ ID NO:155 and downstream thereof an XhoI-site and an immunoglobulin heavy chain constant region with the restriction enzymes SfiI and XhoI, digesting/cutting a phage display vector comprising an immunoglobulin heavy chain variable region with the restriction enzymes SfiI and XhoI,

inserting the immunoglobulin heavy chain variable region into the expression vector, and isolating the expression vector. In a specific embodiment the expression vector comprising the nucleic acid sequence of SEQ ID NO:155 and downstream thereof
5 an XhoI-site and an immunoglobulin heavy chain constant region comprises the nucleic acid sequence of SEQ ID NO:53. In another embodiment the expression vector produced is suitable for producing an immunoglobulin light chain. The method comprises the steps of digesting/cutting an expression vector
10 comprising the nucleic acid sequence of SEQ ID NO:156 or SEQ ID NO:157 and downstream thereof a NotI-site and an immunoglobulin light chain constant region with the restriction enzymes XhoI and NotI, digesting/cutting a phage display vector comprising an immunoglobulin light chain
15 variable region with the restriction enzymes SalI and NotI, inserting the immunoglobulin light chain variable region into the expression vector, and isolating the expression vector. In a specific embodiment the expression vector comprising the nucleic acid sequence of SEQ ID NO:156 or SEQ ID NO:157 and
20 downstream thereof an NotI-site and an immunoglobulin light chain constant region comprises the nucleic acid sequence of SEQ ID NO:54 or SEQ ID NO:58, respectively. The phage display vector can be a phagemid but also any other suitable vehicle. It is clear that the digesting/cutting steps can be performed
25 in any order and even concomitantly. It is further to be understood that the variable heavy chain and variable light chain regions in the phage display vector, such as pHEN1 or PDV, are located between the sites SfiI and XhoI and SalI and NotI, respectively. By digesting the display vectors with the
30 respective restriction enzymes the regions are obtained and can be used for insertion into the eukaryotic expression vectors.

In a further aspect the invention is directed to a method for producing an immunoglobulin heavy or light chain, the method comprising the steps of transforming at least one host cell with an expression vector as produced above, culturing the host cell under conditions conducive to the expression of an immunoglobulin chain, and optionally, purifying the immunoglobulin chain from the medium or cellular extract. In an embodiment the host cell may be transformed with an expression vector suitable for producing a heavy chain and an expression vector suitable for producing a light chain and a complete immunoglobulin may be produced. Many commercially significant proteins are produced by recombinant gene expression in appropriate prokaryotic or eukaryotic host cells. It is frequently desirable to isolate the expressed protein product after secretion into the culture medium. Secreted proteins are typically soluble and can be separated readily from contaminating host proteins and other cellular components. Method for separation and/or purification are well known in the art.

20

EXAMPLES

To illustrate the invention, the following examples are provided. The examples are not intended to limit the scope of the invention in any way.

25

Example 1

Construction of a scFv phage display library using RNA extracted from peripheral blood of WNV convalescent donors.

From three convalescent WNV patients samples of blood were taken 1, 2 and 3 months after infection. Peripheral blood leukocytes were isolated by centrifugation and the blood serum was saved and frozen at -80°C. All donors at all time points

30

had high titers of neutralizing antibodies to WNV as determined using a virus neutralization assay. Total RNA was prepared from the cells using organic phase separation and subsequent ethanol precipitation. The obtained RNA was
5 dissolved in RNase free water and the concentration was determined by OD 260nm measurement. Thereafter, the RNA was diluted to a concentration of 100 ng/ μ l. Next, 1 μ g of RNA was converted into cDNA as follows: To 10 μ l total RNA, 13 μ l DEPC-treated ultrapure water and 1 μ l random hexamers (500
10 ng/ μ l) were added and the obtained mixture was heated at 65°C for 5 minutes and quickly cooled on wet-ice. Then, 8 μ l 5X First-Strand buffer, 2 μ l dNTP (10 mM each), 2 μ l DTT (0.1 M), 2 μ l Rnase-inhibitor (40 U/ μ l) and 2 μ l SuperscriptTMIII MMLV reverse transcriptase (200 U/ μ l) were added to the mixture,
15 incubated at room temperature for 5 minutes and incubated for 1 hour at 50°C. The reaction was terminated by heat inactivation, *i.e.* by incubating the mixture for 15 minutes at 75°C.

The obtained cDNA products were diluted to a final volume
20 of 200 μ l with DEPC-treated ultrapure water. The OD 260nm of a 50 times diluted solution (in 10 mM Tris buffer) of the dilution of the obtained cDNA products gave a value of 0.1.

For each donor 5 to 10 μ l of the diluted cDNA products were used as template for PCR amplification of the
25 immunoglobulin gamma heavy chain family and kappa or lambda light chain sequences using specific oligonucleotide primers (see Tables 1-6). PCR reaction mixtures contained, besides the diluted cDNA products, 25 pmol sense primer and 25 pmol anti-sense primer in a final volume of 50 μ l of 20 mM Tris-HCl (pH
30 8.4), 50 mM KCl, 2.5 mM MgCl₂, 250 μ M dNTPs and 1.25 units Taq polymerase. In a heated-lid thermal cycler having a temperature of 96°C, the mixtures obtained were quickly melted

for 2 minutes, followed by 30 cycles of: 30 seconds at 96°C, 30 seconds at 60°C and 60 seconds at 72°C.

In a first round amplification, each of seventeen light chain variable region sense primers (eleven for the lambda light chain (see Table 1) and six for the kappa light chain (see Table 2) were combined with an anti-sense primer recognizing the C-kappa called HuCk 5'-ACACTCTCCCCTGTTGAAGCTCTT-3' (see SEQ ID NO:37) or C-lambda constant region HuCλ2 5'-TGAACATTCTGTAGGGGCCACTG-3' (see SEQ ID NO:38) and HuCλ7 5'-AGAGCATTCTGCAGGGGCCACTG-3' (see SEQ ID NO:39) (the HuCλ2 and HuCλ7 anti-sense primers were mixed to equimolarity before use), yielding 4 times 17 products of about 600 base pairs. These products were purified on a 2% agarose gel and isolated from the gel using Qiagen gel-extraction columns. 1/10 of each of the isolated products was used in an identical PCR reaction as described above using the same seventeen sense primers, whereby each lambda light chain sense primer was combined with one of the three Jlambda-region specific anti-sense primers and each kappa light chain sense primer was combined with one of the five Jkappa-region specific anti-sense primers. The primers used in the second amplification were extended with restriction sites (see Table 3) to enable directed cloning in the phage display vector PDV-C06 (see SEQ ID NO:40). This resulted in 4 times 63 products of approximately 350 base pairs that were pooled to a total of 10 fractions. This number of fractions was chosen to maintain the natural distribution of the different light chain families within the library and not to over or under represent certain families. The number of alleles within a family was used to determine the percentage of representation within a library (see Table 4). In the next step, 2.5 µg of pooled fraction and 100 µg PDV-C06 vector were digested with SalI and NotI and purified from gel. Thereafter,

a ligation was performed overnight at 16°C as follows. To 500 ng PDV-C06 vector 70 ng pooled fraction was added in a total volume of 50 µl ligation mix containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, 25 µg/ml BSA and 2.5 µl T4 DNA Ligase (400 U/µl). This procedure was followed for each pooled fraction. The ligation mixes were purified by phenol/chloroform, followed by a chloroform extraction and ethanol precipitation, methods well known to the skilled artisan. The DNA obtained was dissolved in 50 µl ultrapure water and per ligation mix two times 2.5 µl aliquots were electroporated into 40 µl of TG1 competent *E. coli* bacteria according to the manufacturer's protocol (Stratagene). Transformants were grown overnight at 37°C in a total of 30 dishes (three dishes per pooled fraction; dimension of dish: 240 mm x 240 mm) containing 2TY agar supplemented with 50 µg/ml ampicillin and 4.5% glucose. A (sub)library of variable light chain regions was obtained by scraping the transformants from the agar plates. This (sub)library was directly used for plasmid DNA preparation using a QiagenTM QIAfilter MAXI prep kit.

For each donor the heavy chain immunoglobulin sequences were amplified from the same cDNA preparations in a similar two round PCR procedure and identical reaction parameters as described above for the light chain regions with the proviso that the primers depicted in Tables 5 and 6 were used. The first amplification was performed using a set of nine sense directed primers (see Table 5; covering all families of heavy chain variable regions) each combined with an IgG specific constant region anti-sense primer called HuCIgG 5'-GTC CAC CTT GGT GTT GCT GGG CTT-3' (SEQ ID NO:41) yielding four times nine products of about 650 base pairs. These products were purified on a 2% agarose gel and isolated from the gel using Qiagen

gel-extraction columns. 1/10 of each of the isolated products was used in an identical PCR reaction as described above using the same nine sense primers, whereby each heavy chain sense primer was combined with one of the four JH-region specific anti-sense primers. The primers used in the second round were extended with restriction sites (see Table 6) to enable directed cloning in the light chain (sub)library vector. This resulted per donor in 36 products of approximately 350 base pairs. These products were pooled for each donor per used (VH) sense primer into nine fractions. The products obtained were purified using Qiagen PCR purification columns. Next, the fractions were digested with SfiI and XhoI and ligated in the light chain (sub)library vector, which was cut with the same restriction enzymes, using the same ligation procedure and volumes as described above for the light chain (sub)library. Alternatively, the fractions were digested with NcoI and XhoI and ligated in the light chain vector, which was cut with the same restriction enzymes, using the same ligation procedure and volumes as described above for the light chain (sub)library. Ligation purification and subsequent transformation of the resulting definitive library was also performed as described above for the light chain (sub)library and at this point the ligation mixes of each donor were combined per VH pool. The transformants were grown in 27 dishes (three dishes per pooled fraction; dimension of dish: 240 mm x 240 mm) containing 2TY agar supplemented with 50 µg/ml ampicillin and 4.5% glucose. All bacteria were harvested in 2TY culture medium containing 50 µg/ml ampicillin and 4.5% glucose, mixed with glycerol to 15% (v/v) and frozen in 1.5 ml aliquots at -80°C. Rescue and selection of each library were performed as described below.

Example 2*Selection of phages carrying single chain Fv fragments specifically recognizing WNV envelope (E) protein*

Antibody fragments were selected using antibody phage display libraries, general phage display technology and MAbstract® technology, essentially as described in US Patent Number 6,265,150 and in WO 98/15833 (both of which are incorporated by reference herein). The antibody phage libraries used were two different semi-synthetic scFv phage libraries (JK1994 and WT2000) and the immune library prepared as described in Example 1. The first semi-synthetic scFv phage library (JK1994) has been described in de Kruif *et al.*, 1995b, the second one (WT2000) was build essentially as described in de Kruif *et al.*, 1995b. Briefly, the library has a semi-synthetic format whereby variation was incorporated in the heavy and light chain V genes using degenerated oligonucleotides that incorporate variation within CDR regions. Only VH3 heavy chain genes were used, in combination with kappa- and lambda light chain genes. CDR1 and CDR3 of the heavy chain and CDR3 of the light chain were recreated synthetically in a PCR-based approach similar as described in de Kruif *et al.*, 1995b. The thus created V region genes were cloned sequentially in scFv format in a phagemid vector and amplified to generate a phage library as described before. Furthermore, the methods and helper phages as described in WO 02/103012 (incorporated by reference herein) were used in the present invention. For identifying phage antibodies recognizing WNV E-protein, phage selection experiments were performed using whole WNV (called strain USA99b or strain 385-99) inactivated by gamma irradiation (50 Gy for 1 hour), recombinantly expressed WNV E-protein (strain 382-99), and/or

WNV-like particles expressing WNV E-protein (strain 382-99) on their surface.

The recombinantly expressed E-protein was produced as follows. The nucleotide sequence coding for the preM/M-protein and the full length E-protein of WNV strain 382-99 (see SEQ ID NO:42 for the amino acid sequence of a fusion protein comprising both WNV polypeptides) was synthesised. Amino acids 1-93 of SEQ ID NO:42 constitute the WNV preM-protein, amino acids 94-168 of SEQ ID NO:42 constitute the WNV M-protein, amino acids 169-669 of SEQ ID NO:42 constitute the WNV E-protein (the soluble WNV E-protein (ectodomain) constitutes amino acids 169-574 of SEQ ID NO:42, while the WNV E-protein stem and transmembrane region constitutes amino acids 575-669 of SEQ ID NO:42) The synthesised nucleotide sequence was cloned into the plasmid pAdapt and the plasmid obtained was called pAdapt.WNV.prM-E (FL).

To produce a soluble secreted form of the E-protein a construct was made lacking the transmembrane spanning regions present in the final 95 amino acids at the carboxyl terminal of the full length E-protein (truncated form). For that purpose the full length construct pAdapt.WNV.prM-E (FL) was PCR amplified with the primers CMV-Spe (SEQ ID NO:43) and WNV-E-95 REV (SEQ ID NO:44) and the fragment obtained was cloned into the plasmid pAdapt.myc.his to create the plasmid called pAdapt.WNV -95. Next, the region coding for the preM-protein, the truncated E-protein, the Myc tag and His tag were PCR amplified with the primers clefsmaquwnv (SEQ ID NO:45) and reverse WNVmychis (SEQ ID NO:46) and cloned into the vector pSyn-C03 containing the HAVT20 leader peptide using the restriction sites EcoRI and SpeI. The expression construct obtained, pSyn-C03-WNV-E -95, was transfected into 90% confluent HEK293T cells using lipofectamine according to the

manufacturers instructions. The cells were cultured for 5 days in serum-free ultra CHO medium, then the medium was harvested and purified by passage over HisTrap chelating columns (Amersham Bioscience) pre-charged with nickel ions. The truncated E protein was eluted with 5 ml of 250 mM imidazole and further purified by passage over a G-75 gel filtration column equilibrated with phosphate buffered saline (PBS). Fractions obtained were analysed by SDS-PAGE analysis and Western blotting using the WNV-E protein specific murine antibody 7H2 (Bioreliance, see Beasley and Barrett 2002). Three 5 ml fractions containing a single band of ~45 kDa that was immunoreactive with antibody 7H2 were aliquoted and stored at -20°C until further use. The protein concentration was determined by OD 280nm.

WNV-like particles were produced as follows. The construct pSyn-C03-WNV-E -95 described above and pcDNA3.1 (Invitrogen) were digested with the restriction endonucleases MunI and XbaI and the construct pAdapt.WNV.prM-E (FL) described above was digested with the restriction endonucleases ClaI and XbaI. The resulting fragments were combined in a three-point ligation to produce the construct pSyn-H-preM/E FL. This construct contained the full length E protein and expressed the two structural WNV proteins, protein M and E, required for assembly of an enveloped virion. The construct was transfected into 70% confluent HEK293T cells using lipofectamine according to the manufacturers instructions. The cells were cultured for 3 days in serum-free ultra CHO medium, then the medium was harvested, layered on to a 30% glycerol solution at a 2:1 ratio and pelleted by centrifugation for 2 hours at 120,000*g at 4°C. The WNV-like particles were resuspended in PBS, aliquoted and stored at -

80°C. Aliquots were analysed by SDS-PAGE analysis and Western blotting using the WNV E-protein specific murine antibody 7H2 (Bioreliance).

Before inactivation, whole WNV was purified by pelleting
5 through a 30% glycerol solution as described above for WNV-like particles. The purified WNV was resuspended in 10 mM Tris/HCl pH 7.4 containing 10 mM EDTA and 200 mM NaCl, the obtained preparation was kept on dry ice during inactivation, tested for infectivity and stored at -80°C in small aliquots.
10 Aliquots were analysed by SDS-PAGE analysis and Western blotting using the WNV E-protein specific murine antibody 7H2 (Bioreliance).

Whole inactivated WNV, WNV-like particles or recombinantly expressed soluble E-protein were diluted in PBS.
15 2-3 ml of the preparation was added to MaxiSorp™ Nunc-Immuno Tubes (Nunc) and incubated overnight at 4°C on a rotating wheel. An aliquot of a phage library (500 µl, approximately 10¹³ cfu, amplified using CT helper phage (see WO 02/103012)) was blocked in blocking buffer (2% Protifar in PBS) for 1-2
20 hours at room temperature. The blocked phage library was added to the immunotubes, incubated for 2 hours at room temperature, and washed with wash buffer (0.1% v/v Tween-20 in PBS) to remove unbound phages. Bound phages were eluted from the antigen by incubation with 1 ml of 50 mM Glycine-HCl pH 2.2
25 for 10 minutes at room temperature. Subsequently, the eluted phages were mixed with 0.5 ml of 1 M Tris-HCl pH 7.5 to neutralize the pH. This mixture was used to infect 5 ml of an XL1-Blue *E.coli* culture that had been grown at 37°C to an OD 600nm of approximately 0.3. The phages were allowed to infect
30 the XL1-Blue bacteria for 30 minutes at 37°C. Then, the mixture was centrifuged for 10 minutes at 3200*g at room

temperature and the bacterial pellet was resuspended in 0.5 ml 2-trypton yeast extract (2TY) medium. The obtained bacterial suspension was divided over two 2TY agar plates supplemented with tetracyclin, ampicillin and glucose. After overnight
5 incubation of the plates at 37°C, the colonies were scraped from the plates and used to prepare an enriched phage library, essentially as described by De Kruif *et al.* (1995a) and WO 02/103012. Briefly, scraped bacteria were used to inoculate 2TY medium containing ampicillin, tetracycline and glucose and
10 grown at a temperature of 37°C to an OD 600nm of ~0.3. CT helper phages were added and allowed to infect the bacteria after which the medium was changed to 2TY containing ampicillin, tetracycline and kanamycin. Incubation was continued overnight at 30°C. The next day, the bacteria were
15 removed from the 2TY medium by centrifugation after which the phages in the medium were precipitated using polyethylene glycol (PEG) 6000/NaCl. Finally, the phages were dissolved in 2 ml of PBS with 1% bovine serum albumin (BSA), filter-sterilized and used for the next round of selection.

20 Typically, two rounds of selections were performed before isolation of individual phage antibodies. After the second round of selection, individual *E.coli* colonies were used to prepare monoclonal phage antibodies. Essentially, individual colonies were grown to log-phase in 96 well plate format and
25 infected with CT helper phages after which phage antibody production was allowed to proceed overnight. The produced phage antibodies were PEG/NaCl-precipitated and filter-sterilized and tested in ELISA for binding to WNV-like particles purified as described *supra*.

Example 3

Validation of the WNV specific single-chain phage antibodies

Selected single-chain phage antibodies that were obtained in the screens described above were validated in ELISA for specificity, *i.e.* binding to WNV E-protein, whole inactivated WNV and WNV-like particles, all purified as described *supra*.

5 For this purpose, whole inactivated WNV, the WNV E-protein, or WNV-like particles were coated to MaxisorpTM ELISA plates. In addition, whole inactivated rabies virus was coated onto the plates as a control. After coating, the plates were blocked in PBS containing 1% Protifar for 1 hour at room temperature. The
10 selected single-chain phage antibodies were incubated for 15 minutes in an equal volume of PBS containing 1% Protifar to obtain blocked phage antibodies. The plates were emptied, and the blocked single-chain phage antibodies were added to the wells. Incubation was allowed to proceed for one hour, the
15 plates were washed in PBS containing 0.1% v/v Tween-20 and bound phage antibodies were detected (using OD 492nm measurement) using an anti-M13 antibody conjugated to peroxidase. As a control, the procedure was performed simultaneously without single-chain phage antibody, with a
20 negative control single-chain phage antibody directed against rabies virus glycoprotein (antibody called SC02-447), with a negative control single-chain phage antibody directed against SARS-CoV (antibody called SC03-014) and a positive control single-chain phage antibody directed against rabies virus. As
25 shown in Table 7, the selected phage antibodies called SC04-348 and SC04-354 displayed significant binding to immobilized whole inactivated WNV and WNV-like particles. Both single-chain phage antibodies were selected when WNV-like particles were used in the first and second round of selection. When the
30 ELISA was performed with recombinantly expressed purified soluble WNV E-protein prepared as described *supra* or rabies virus, the single-chain phage antibodies SC04-348 and SC04-354

did not bind, suggesting they either bind to a region not present in the truncated soluble E-protein, bind to an unrelated protein on the virion surface, do not bind to the monomeric form of the E-protein or do not bind because of the phage antibody format.

Example 4

Characterization of the WNV specific scFvs

From the selected specific single-chain phage antibody (scFv) clones plasmid DNA was obtained and nucleotide sequences were determined according to standard techniques. The nucleotide sequences of the scFvs (including restriction sites for cloning) called SC04-348 and SC04-354 are shown in SEQ ID NO:25 and SEQ ID NO:27, respectively. The amino acid sequences of the scFvs called SC04-348 and SC04-354 are shown in SEQ ID NO:26 and SEQ ID NO:28, respectively.

The VH and VL gene identity (see Tomlinson IM, Williams SC, Ignatovitch O, Corbett SJ, Winter G. V-BASE Sequence Directory. Cambridge United Kingdom: MRC Centre for Protein Engineering (1997)) and heavy chain CDR3 sequences of the scFvs specifically binding WNV are depicted in Table 8. Table 9 shows the other CDR regions of the WNV specific scFvs.

Example 5

Construction of fully human immunoglobulin molecules (human monoclonal anti-WNV antibodies) from the selected anti-WNV single chain Fvs

Heavy and light chain variable regions of the scFv called SC04-354 was PCR-amplified using oligonucleotides to append restriction sites and/or sequences for expression in the IgG expression vectors pSyn-C18-HCγ1 (see SEQ ID No:47) and pSyn-C04-Cλ (see SEQ ID No:48). The heavy chain variable region of

the scFv called SC04-354 was cloned into the vector pSyn-C18-HC γ 1; the light chain variable region of the scFv called SC04-354 was cloned into the vector pSyn-C04-C λ . The VL lambda gene was first amplified using the following oligonucleotides set:

5 SC04-354, 5L-C (SEQ ID NO:49) and sy3L-Cmod (SEQ ID NO:50) and the PCR product cloned into vector pSyn-C04-C λ . Nucleotide sequence for the construct was verified according to standard techniques known to the skilled artisan. VH gene was first amplified using the following oligonucleotide set: SC04-354,
10 5H-A (SEQ ID NO:51) and sy3H-A (SEQ ID NO:52). Thereafter, the PCR product was cloned into vector pSyn-C18-HC γ 1 and nucleotide sequence was verified according to standard techniques known to the skilled person in the art.

Heavy and light chain variable region of the scFv called
15 SC04-348 was cloned directly by restriction digest for expression in the IgG expression vectors pIg-C911-HC γ 1 (see SEQ ID NO:53) and pIg-C909-C κ (see SEQ ID NO:54). The heavy chain variable regions of the scFv called SC04-348 was cloned into the vector pIg-C911-HC γ 1 by restriction digest
20 using the enzymes SfiI and XhoI and the light chain variable region of the scFv called SC04-348 was cloned into the vector pIg-C909-C κ by restriction digest using the enzymes SalI and NotI. Thereafter the nucleotide sequence was verified according to standard techniques known to the person skilled
25 in the art.

The resulting expression constructs pgG104-348C911 and pgG104-354C18 encoding the human IgG1 heavy chains and pgG104-348C909 and pgG104-354C04 encoding the human IgG1 light chains were transiently expressed in combination in 293T cells and
30 supernatants containing human IgG1 antibodies were obtained. The nucleotide sequences of the heavy chains of the antibodies called CR4348 and CR4354 are shown in SEQ ID Nos 29 and 31,

respectively. The amino acid sequences of the heavy chains of the antibodies called CR4348 and CR4354 are shown in SEQ ID NOs 30 and 32, respectively. The nucleotide sequences of the light chain of antibodies CR4348 and CR4354 are shown in SEQ ID NOs 33 and 35, respectively. The amino acid sequences of the light chain of antibodies CR4348 and CR4354 are shown in SEQ ID NOs 34 and 36, respectively. A person skilled in the art can determine the variable regions of the heavy and light chains of the above antibodies by following Kabat *et al.*

(1991) as described in Sequences of Proteins of Immunological Interest. Alternatively, batches of greater than 1 mg of each antibody were produced and purified using standard procedures. The antibodies were then titrated on a fixed concentration of irradiated West Nile virus and tested in ELISA as described above. The results are shown in Table 10. As a negative control an anti-rabies virus antibody was used. Both antibodies showed binding to the virus in a dose dependent manner. The antibodies were also capable of binding WNV-like particles (data not shown).

Additionally, binding of CR4348 and CR4354L4328 (an optimized variant of antibody CR4354; see Example 8 for selection of this variant) to viral material was tested in a capture ELISA. For this purpose, CR4348, CR4283 (an anti-WNV monoclonal antibody; positive control for the inactivated WNV and WNV-like particle and negative control for rabies virus), CR4354L4328 and CR4104 (an anti-rabies virus monoclonal antibody; positive control for rabies virus and negative control for the inactivated WNV and WNV-like particle) were coated in a concentration of 5 µg/ml to Maxisorp™ ELISA plates. After coating, the plates were blocked in PBS containing 1% Protifar for 1 hour at room temperature. Next, a serial dilution of whole inactivated WNV, WNV-like particles,

or rabies virus (BPL-inactivated) was performed in PBS/protifar in a volume of 100 μ l until a dilution of 1/2048 was reached. The viral material was allowed to incubate for 1 hour at room temperature. The plates were emptied, and washed
5 3 times with 100 μ l PBS containing 0.1% v/v Tween-20. Next, the mouse monoclonal antibody 7H2 (Bioreliance) was added at a concentration of 1 μ g/ml (diluted in in PBS/protifar) for detection of the inactivated WNV and WNV-like particles. Moreover, the mouse monoclonal antibody 1112 directed against
10 rabies virus was added in a 1:1000 dilution (diluted in PBS/protifar) for detection of rabies virus. Bound monoclonal antibodies were detected by OD 492nm measurement with an anti-mouse antibody conjugated to peroxidase (Jackson) in a 1:2000 dilution in PBS/protifar. CR4348, CR4283 and CR4354L4328
15 showed a very high binding to WNV-like particles with CR4348 binding twice as efficient compared to CR4354L4328 (data not shown). Moreover, CR4348, CR4283 and CR4354L4328 also bound to WNV (data not shown). When the capture ELISA was performed with rabies virus, binding was observed with the positive
20 control CR4104 and antibody CR4348. No binding of antibody CR4354L4328 was observed (data not shown).

Example 6

In vitro neutralization of WNV by WNV specific scFvs and IgGs
25 *(virus neutralization assay)*

In order to determine whether the selected scFvs are capable of blocking WNV infection, *in vitro* virus neutralization assays (VNA) are performed. The VNA are performed on Vero cells (ATCC CCL 81). The WNV strain 385-99
30 which is used in the assay is diluted to a titer of 4×10^3 TCID₅₀/ml (50% tissue culture infective dose per ml), with the titer calculated according to the method of Spearman and

Kaerber. The scFv preparations are serially 2-fold-diluted in PBS starting from 1:2 (1:2 - 1:1024). 25 µl of the respective scFv dilution is mixed with 25 µl of virus suspension (100 TCID₅₀/25 µl) and incubated for one hour at 37°C. The
5 suspension is then pipetted twice in triplicate into 96-well plates. Next, 50 µl of a freshly trypsinized and homogenous suspension of Vero cells (1:3 split of the confluent cell monolayer of a T75-flask) resuspended in DMEM with 10% v/v fetal calf serum and antibiotics is added. The inoculated
10 cells are cultured for 3-4 days at 37°C and observed daily for the development of cytopathic effect (CPE). CPE is compared to the positive control (WNV inoculated cells) and negative controls (mock-inoculated cells or cells incubated with scFV only). The complete absence of CPE in an individual cell
15 culture is defined as protection (= 100% titer reduction). The serum dilution giving protection in 50% percent of wells (*i.e.* three out of six wells) is defined as the 50% neutralizing antibody titer. The murine neutralising antibody 7H2 (Bioreliance) is used as a positive control in the assay. A
20 50% neutralization titer of $\leq 1:4$ (meaning the antibody is diluted 4 times or more) is regarded as specific evidence of neutralizing activity of the scFv against WNV.

Alternatively, *in vitro* virus neutralization assays (VNA) were performed in order to determine whether the anti-WNV IgGs
25 were capable of blocking WNV infection. The VNA were performed essentially as described for scFvs, with the proviso that the serum dilution giving protection in 66% percent of wells (*i.e.* two out of three wells) was defined as the 66% neutralizing antibody titer and a 66% neutralization titer of $\leq 1:2$ was
30 regarded as specific evidence of neutralizing activity of the IgG against WNV.

Supernatants containing the human anti-WNV antibodies called CR4348 and CR354 were expressed as described in Example 5 and subjected to the above-described VNA. All antibodies had a neutralising titer $\leq 1:2$. The potency of the antibodies (in 5 $\mu\text{g/ml}$) is given in Table 11.

Example 7

In vivo protection by monoclonal antibodies from lethal WNV infection in a murine challenge model

10 A murine challenge model was adapted from the literature (see Ben-Nathan *et al.* 2003; Beasley *et al.* 2002; Wang *et al.* 2001). In Ben-Nathan *et al.* (2003) 4-week old BALB/c mice were used and the animals were inoculated intraperitoneally (i.p.) with 20-times the viral dose resulting in 50% survival (LD_{50}) 15 of WNV strain ISR52 (LD_{50} was equivalent to 5 pfu). Under this dosing mice succumbed to infection 6-7 days after inoculation and reached 100% mortality after 11 days. In another study, the WNV strain USA99 (used in the experiments described here) was shown to have an LD_{50} of 0.5 pfu. This is 10-fold lower 20 than the LD_{50} of ISR52, which may indicate a higher degree of neuroinvasiveness for this viral strain or differences associated with the mouse strain used (see Beasley *et al.* 2002).

To determine the i.p., LD_{50} of USA99 in 4-week BALB/c 25 mice, animals (5 per group) were injected with USA99 at TCID_{50} (tissue culture infectious dose) of 100, 30, 10, 3, 1, 0.3, 0.1, 0.03 the 50% in two separate experiments. The LD_{50} calculated from the first experiment was 5.75 TCID_{50} and from the second experiment 13.25 TCID_{50} . For the calculation of the 30 viral dose in further experiments the average of the two experiments, i.e. 9.5 TCID_{50} , was calculated by probit regression analysis.

The protective capacity of the *in vitro* neutralizing antibodies CR4348 and CR4354 was tested in the *in vivo* model. Purified antibodies were injected i.p. into 4-week BALB/c mice (5 animals per group) at a concentration of 15 mg/kg. After 24 hours the WNV strain USA99 was injected i.p. at a dose of 20-
5 times the LD₅₀ calculated. The animals were observed for signs of disease over 21 days and sacrificed when symptoms of encephalitis were evident. In the model unprotected animals generally succumbed to infection between day 8 and day 10.

10 Table 12 shows that the two antibodies, CR4348 and CR4354, are 100% protective *in vivo* at the dose of 15 mg/kg. The positive control antibody 7H2 (an anti-WNV murine monoclonal) was fully protective and the negative control antibody (binding an irrelevant antigen) showed no protection
15 in the experiment.

To establish a dose protection relationship, the protective antibody CR4348 was titrated in the mouse model using doses of 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003 and 0.001 mg/kg. Using the same doses, an optimized variant of antibody
20 CR4354, *i.e.* CR4354L4328 (see Example 8 for selection of this variant), was titrated in the mouse model. A negative control antibody binding an irrelevant antigen was included as a control at a dose 10 mg/kg.

Figure 1 shows that the antibody CR4354L4328 is 100%
25 protective at a dose of 0.03 mg/kg. The doses 10, 3, 1, 0.3 and 0.1 mg/kg were also 100% protective (data not shown). Figure 1 also shows that there is a direct correlation between dose and protective capacity. The 50% protective dose calculated by probit regression analysis is 0.003 mg/kg.

30 Figure 2 shows that the antibody CR4348 is 100% protective at a dose of 0.1 mg/kg. The doses 10, 3, 1 and 0.3 mg/kg were also 100% protective (data not shown). Figure 2

further shows that there is a direct correlation between dose and protective capacity. The 50% protective dose calculated by probit regression analysis is 0.006 mg/kg.

The titration data of the antibodies were compared by
5 probit regression analysis. Values for the Pearson Goodness-of-Fit test (Chi Square = 10.38, DF = 30, p = 1.00) demonstrated that the model was valid and the results of the Parallelism Test (Chi Square = 3.47, DF = 3, p = 0.324) meant that the curves could be reliably compared. The values for the
10 50% protective dose and 95% protective dose are summarized in Table 13.

Example 8

*Selection of optimized variants of neutralizing monoclonal
15 antibody CR4354*

Monoclonal antibody CR4354 showing to have *in vitro* WNV neutralizing activity and being 100% protective *in vivo* was selected for improving potency and affinity. This was done based on the following hypothesis. The specificity of CR4354
20 (as determined by the CDR3 region on the heavy chain variable chain) is one that targets a potent neutralizing epitope of WNV, but the light chain that is randomly paired with the heavy chain (through the phage-display process) does not optimally recreate the original antigen binding site. Pairing
25 with a more optimally mutated light chain might improve the 'fit' of the antibody-binding pocket for the cognate antigen. Thus, replacement of the light chain might be a way of improving the potency and affinity of the antibody.

Analysis of the heavy and light chain of antibody CR4354
30 showed that they belong to the VH1 1-46 (DP-7) and Vlambd1 (1c-V1-16) gene family, respectively. Further analysis of WNV specific scFvs selected from the WNV immune library as

described in Example 1 revealed 5 scFvs, *i.e.* SC04-261, SC04-267, SC04-328, SC04-335 and SC04-383 (these scFvs were not included in Table 8), that had light chains having the same gene family as the light chain of CR4354. None of the scFvs or
5 their respective IgGs showed WNV neutralizing activity. Each of these light chains contained mutations in the CDR and framework regions away from the germline indicating that they had been modified as part of the natural affinity maturation process.

10 In short, the construction of the antibodies went as follows. CR4354 was prepared as described in Example 5. Heavy chain variable regions of the scFvs called SC04-261, SC04-267, SC04-328 and SC04-335 were PCR-amplified using
15 oligonucleotides to append restriction sites and/or sequences for expression in the IgG expression vector pSyn-C18-HCγ1 and cloned into this vector. Amplification was done using the following oligonucleotide sets: SC04-261, 5H-A (SEQ ID NO:51) and sy3H-A (SEQ ID NO:52); SC04-267, 5H-A (SEQ ID NO:51) and sy3H-C (SEQ ID NO:55); SC04-328, 5H-A (SEQ ID NO:51) and sy3H-
20 A (SEQ ID NO:52); and SC04-335, 5H-C (SEQ ID NO:56) and sy3H-A (SEQ ID NO:52).

The heavy chain variable region of the scFv called SC04-383 was cloned by restriction digest using the enzymes SfiI and XhoI in the IgG expression vector pIg-C911-HCγ1.

25 The light chain variable region of the scFv called SC04-267 was first amplified using the oligonucleotides SC04-267, 5L-C (SEQ ID NO:49) and sy3L-Amod (SEQ ID NO:57) and the PCR product cloned into vector pSyn-C04-Clambda.

Light chain variable regions of the scFvs called SC04-
30 261, SC04-328, SC04-335, and SC04-383 were cloned directly by restriction digest using the enzymes SalI and NotI for

expression in the IgG expression vector pIg-C910-Clambda (SEQ ID NO:58).

Nucleotide sequences for all constructs were verified according to standard techniques known to the skilled artisan.

5 The resulting expression constructs pgG104-261C18, pgG104-267C18, pgG104-328C18, pgG104-335C18 and pgG104-383C911 encoding the anti-WNV human IgG1 heavy chains and pgG104-261C910, pgG104-267C04, pgG104-328C910, pgG104-335C910 and pgG104-383C910 encoding the anti-WNV human IgG1 light chains
10 were transiently expressed in combination in 293T cells and supernatants containing human IgG1 antibodies were obtained.

 The nucleotide sequences of the heavy chains of the antibodies called CR4261, CR4267, CR4328, CR4335, CR4354 and CR4383 are shown in SEQ ID NOs:59, 61, 63, 65, 31 and 67,
15 respectively (the variable regions are from nucleotides 1-348; 1-381; 1-348; 1-351; 1-363; and 1-372, respectively). The amino acid sequences of the heavy chains of the antibodies called CR4261, CR4267, CR4328, CR4335, CR4354 and CR4383 are shown in SEQ ID Nos:60, 62, 64, 66, 32 and 68, respectively
20 (the variable regions are from amino acids 1-116; 1-127; 1-116; 1-117; 1-121; and 1-124, respectively). The nucleotide sequences of the light chain of antibodies CR4261, CR4267, CR4328, CR4335, CR4354 and CR438 are shown in SEQ ID NOs:69, 71, 73, 75, 35 and 77, respectively (the variable regions are
25 from nucleotides 1-342; 1-330; 1-339; 1-339; 1-330; and 1-339, respectively). The amino acid sequences of the light chain of antibodies CR4261, CR4267, CR4328, CR4335, CR4354 and CR4383 are shown in SEQ ID NOs:70, 72, 74, 76, 36 and 78, respectively (the variable regions are from amino acids 1-114;
30 1-110; 1-113; 1-113; 1-110; and 1-113, respectively).

 The expression construct encoding the heavy chain of CR4354 was combined with the constructs expressing the light

chains of the respective antibodies for transfection of HEK293T cells essentially as described in Example 5. The obtained antibodies were designated CR4354L4261, CR4354L4267, CR4354L4328, CR4354L4335 and CR4354L4383. Supernatants were
5 tested for binding by ELISA staining as described in Example 5 and for potency in the *in vitro* neutralization assay as described in Example 6.

The binding data showed that all shuffled variants have specificity for the pre-selected antigen (see Figure 3).

10 In terms of functional activity, it was concluded that two chain shuffled variants CR4354L4328 and CR4354L4335 had a higher affinity for WNV compared to CR4354. CR4354L4261 bound the virus with a similar affinity compared to CR4354, while both CR4354L4383 and CR4354L4267 bound with a lower affinity
15 to the virus compared to CR4354 (see Figure 3).

Furthermore, the antibodies CR4354L4383 and CR4354L4267 did not show any WNV neutralizing activity, which was consistent with their lower binding affinity. CR4354L4261 had a neutralization endpoint concentration similar to the
20 original antibody CR4534, again consistent with the binding data. CR4354L4335 that bound WNV with a higher affinity compared to CR4354 had a lower neutralizing activity compared to the original antibody CR4534. In contrast, the antibody variant CR4354L4328 that had a higher affinity for WNV
25 compared to CR4354 also had a higher neutralizing activity compared to the original antibody CR4534 (see Table 14). In 4 out of 5 cases there was a direct correlation between binding affinity and neutralization potency of the variants. It was demonstrated that substituting similar light chains can
30 improve a functionality of interest of an antibody, e.g. affinity or neutralizing activity.

Moreover, CR4354 was converted into a fully human IgM format by removing the gamma Fc region from construct pgG104-354C18 by restriction digestion with the endonucleases NheI and XbaI. The vector pCR-IgM (SEQ ID NO:146) containing a mu Fc region was digested with the same restriction enzymes and the obtained mu Fc region was ligated into vector pgG104-354C18 and fused in frame with the variable heavy chain gene derived from SC04-354 to make vector pgM104-354C899. This construct was transiently expressed in combination together with the light chain construct pgG104-354C04 (see above) in 293T cells and supernatants containing human IgM antibodies were obtained. The nucleotide sequence of vector pgM104-354C899 is shown in SEQ ID NO:147. The amino acid sequence of the heavy chain the antibody called CRM4354 is shown in SEQ ID NO:148. The IgM antibody was purified from the supernatant by adding ammonium sulphate to a final concentration of 2.4 M and incubating the mixture overnight on ice, while stirring. The precipitated IgM was recovered by centrifugation at 10,395xg for 30 minutes. The pellet was resuspended in PBS and further purified by gel filtration. A HiLoad 26/60 Superdex 200 prep grade column (GE heathcare) equilibrated with PBS was loaded with the resuspended IgM and fractions were collected from the column, while being flushed under a constant flow rate with PBS. The first major elution peak, which contained the purified IgM, was collected. Binding activity of the antibody was confirmed by titration on West Nile virus-like particles (VLPs) (data not shown).

Example 9

In vitro neutralization potency by plaque reduction neutralization test (PRNT)

To further investigate the neutralizing activity of the antibodies of the invention a PRNT was developed. Briefly, Vero-E6 cells were trypsinized and counted. 2.5×10^5 cells were added to each well of a 12-well plate and incubated overnight at 37°C in a humidified CO₂ incubator. Serial dilutions (10-fold) of a titrated stock of West Nile virus USA99b were made in complete medium. Equal volume (250 µl) mixtures of virus (100 pfu) and serial dilutions of purified IgG1 antibodies were incubated in duplicate at 37°C for 1 hour. Dilutions of both virus and antibodies were done in DMEM medium. The mixture was then added (400 µl) to the 12-well plates containing Vero cell monolayers after careful aspiration of the overnight medium. After the plates had been incubated at 37°C for 1 hour, an 1.5 ml overlay of CMC carboxymethyl-cellulose medium with 10% FBS (v/v) (CMC:complete medium) was added per well and the plates placed in a humidified CO₂ incubator for 3 days at 37°C. One day before staining the CMC:complete medium was removed from the wells and replaced with a mixture of CMC:PBS (1:1; v/v) containing 8.25 mg/ml of neutral red (2 ml neutral red at 3.3 g/l in 80 ml CMC:PBS). Plates were incubated 1 day further at 37°C in a humidified CO₂ incubator, after which the number of visible plaques was quantified.

To analyze the antibody potency data from the PRNT a binary regression model known as probit analysis was used. Probit analysis is valid, if it can be assumed that the probability of neutralizing WNV *in vitro* follows a normal distribution with regard to the amount of antibodies used. The assumption of normality most likely holds on a logarithmic scale, hence the neutralization of virus was modeled as a function of the logarithm of the amount of antibodies administered. Antibodies were compared directly in the

regression model, with significance level alpha set at 0.05. Antibody concentrations yielding 50% and 90% neutralization were estimated from the model, together with 95% confidence intervals. A summary of the final analysis of the antibodies is given in Table 15. By converting CR4354 into IgM format (CRM4354) the *in vitro* potency was increased dramatically (see Table 15).

Using the assay described above, the antibodies were tested for their neutralizing potency against other flaviviruses including yellow fever virus, Japanese encephalitis virus, St. Louis encephalitis virus and dengue virus 2. In Figure 4 is shown that CR4348 had significant neutralizing activity against St. Louis encephalitis virus and dengue virus 2.

Example 10

Identification of the target antigens of CR4348 and CR4354L4328

To identify the target antigens of the CR4348 and CR4354L4328 antibodies a human fetal brain protein expression library was screened with the antibodies. The cDNA library was constructed in the bacterial expression vector pQE-30 (Qiagen) for IPTG inducible expression of (His)₆-tagged fusion proteins. The library was composed of 38,016 clones with an average insert of 1500bps and these were printed in duplicate onto the membranes. To identify target antigens CR4348, CR4354L4328 and a negative control antibody (CR4374) were subjected to protein micro-array analysis (RZPD (Heidelberg, Germany)). Two independent experiments were performed with the antibodies. The protein arrays were incubated with the primary antibody and binding was detected with a conjugated anti-human secondary antibody. Clones were considered positive, if

duplicate spots appeared that were not present with the secondary antibody alone. The negative control did not show any reactivity, whereas 5 different clones reacted with CR4348 and 4 different clones with CR4354L4328. The reactive clones
5 were sequenced and used for database search against the NCBI database using the nucleotide-nucleotide BLAST programm. The identity of the clones is depicted in Table 16.

To confirm the protein-array data obtained with CR4348 and CR4354L4328 the different cDNA clones were expressed. The
10 9 different clones and a bacterial clone expressing the E-protein of WNV were streaked on an agar plate and grown overnight at 37°C. Next, 10 ml LB volumes were inoculated with the nine different clones and the control WNV E-protein clone. The bacteria were grown overnight at 37°C. The next day the
15 cultures were diluted 1:50 and grown until an OD600nm of 0.6 was reached, subsequently the expression of the protein was induced by adding IPTG and the cells were harvested after 4 hours of induction in three portions of 1 ml. Next, each portion underwent a different extraction procedure that
20 allowed for the extraction of soluble and non-soluble proteins. The WNV E-protein clone was included as a positive control in the test, since this protein is expressed as a soluble protein and is therefore present in all three extraction procedures. All methods started with a cycle of
25 three freeze-thawings of the pellet. In the first method the pellet was extracted in a volume twice the volume of the cell pellet in a mild extraction buffer (50 mM Na₂HP0₄, 300 mM NaCl, 0.05% Tween-20 buffer with lysozyme 1 mg/ml) by incubation of the cell pellet for 30 minutes on ice, next cells were
30 pelleted and the supernatant was stored for analysis (fraction 1). Subsequently, the pellet was extracted with a more stringent buffer (0.2% DOC - 1% Triton X-100) and resuspended

in a volume twice the volume of the cell pellet. After an incubation period of 30 minutes on ice, cells were pelleted and the supernatant was stored for analysis (fraction 2). In the second method (an adaptation of the colony blot procedure), the pellet was lysed by addition of 10% SDS (twice the volume of the cell pellet). After 10 minutes of incubation at room temperature, the suspension was denatured with an equal volume 0.5 M NaOH containing 1.5 M NaCl for 5 minutes at room temperature. By adding an equal volume of 1.5 M NaCl, 0.5 M Tris pH 7.4 the suspension was neutralized. Next, cell rests were pelleted and the supernatant was stored for analysis (fraction 3). In the third method inclusion bodies were solublized and the pellet was extracted by addition of 8 M ureum in 100 mM Tris/HCl, 100 mM NaH₂PO₄ in a volume twice the volume of the cell pellet. After 30 minutes of incubation at room temperature, cells rests were pelleted and the supernatant was stored for analysis (fraction 4). Proteins extracted with each procedure were spotted on a nitrocellulose filter. Moreover, controls, *i.e.* purified WNV E-protein (negative control) and human IgG (positive control), were spotted. Membranes were blocked overnight with 4% milk powder in TBST at 4°C. Subsequently, the blots were incubated with 5 µg/ml CR4348, CR4354L4328 or the murine anti-WNV E-protein monoclonal antibody 7H2. The antibody used for WNV E-protein detection recognizes a linear epitope and still reacts with recombinant protein after treatment with denaturing reagents like 8 M urea (method 3). After an incubation period of 1 hour at room temperature in a rolling incubator, the membranes were washed three times for five minutes with TBST. Binding of antibodies was detected with HRP conjugated goat anti-human (Pharmingen) or goat anti-mouse (DAKO) antibodies. Finally, the membranes were washed extensively in TBST followed by a

PBS washing step. Reactive proteins were revealed by the ECL chemofluorescence detection system (Amersham).

CR4348 reacted with fractions 1, 2 and 3 of the FAF-1 expression clone (data not shown). CR4354L4328 solely reacted
5 with fraction 3 of the NADH dehydrogenase flavoprotein 1 (NDUFV1) expression clone (data not shown). This indicates that both antibodies react with a single expression clone. It was further concluded that both antibodies recognize a conformational epitope, since they did not react with protein
10 extracted using 8 M ureum, whereas the anti-WNV antibody that recognizes a linear epitope did react with protein extracted using this procedure (data not shown). In conclusion, CR4348 recognizes an epitope present in the protein FAF-1, while CR4354L4328 recognizes an epitope present in the protein
15 NDUFV-1.

To confirm the identification of NDUFV-1 as the target antigen recognised by CR4354L4328, mRNA was extracted from 2×10^7 293T cells using the nucleotrap mRNA mini purification kit (Beckton Dickinson) according to protocols provided by the
20 manufacturer. RT-PCR was performed on the isolated mRNA. For the PCR procedure, the following primers were designed: forward primer 5'-ATGAAGGTGACAGCGTGAGGTGAC-3' (SEQ ID NO:160) and reverse primer 5'-ACATGGATAGACGCAGGACAGCAG-3' (SEQ ID NO:161). PCR was performed with Pfu (Promega) in the presence
25 of 5% DMSO and resulted in a 1500 bp product. The resulting fragment was cloned in the pCR4TOPO vector (Invitrogen) and transformed into DH5 α cells. The resulting clone, TOPONDUFV1 was verified by sequence analysis and aligned with the sequence present in the database. To simplify the detection of
30 the protein in the subsequent transfection experiments, the protein was fused with a myc tag at the 5'prime end by means of PCR (using the construct as a template). For the 5'myc

construct the following primers were designed: forward primer 5'- AAGCTTAGCATGGAACAAAACTTATTTCTGAAGAAGATCTGCTGGCAACACG GCGGCTGCTCGGCTG-3' (SEQ ID NO:162) and reversed primer 5'- GATATCCTTTATTGTCCAGCATTCCAC-3' (SEQ ID NO:163). PCR was

5 performed using Pfu polymerase and the resulting fragment of the 5'myc tag was cloned in PCRblunt4-TOPO, and subsequently digested with HindIII-EcoRV. The resulting fragment was cloned in the corresponding sites of pcDNA3.1/zeo (Invitrogen) resulting in the mycNDUFV-1 construct. The construct was

10 verified by sequencing. All cloning procedures were performed according to standard molecular techniques. 3×10^5 293T cells were seeded in T175 flasks and subjected to a transfection procedure after 72 hours. The expression construct mycNDUFV-1 and a positive control construct ATAD3Amyc were transfected

15 with lipofectamin (Gibco) according to the manufacturers instructions. 72 hours after transfection, cells were lysed in DOC buffer (1% Triton X-100 and 0.5% w/v desoxycholate in 0.2 M phosphate buffer containing 0.12 M NaCl, pH 7.4 and protease inhibitors (Sigma)). The insoluble material was removed by

20 centrifugation for 30 minutes at 4°C at 20,000*g. Next, the lysates of the transfected cells were analyzed for the quantity of myc tagged protein expressed. Hereafter, the lysate was pre-cleared with protein A beads (Amersham) for 2 hours at 4°C. In the mean time, 4 µg of CR4354L4328, control

25 antibody CR4374 (negative control, antibody directed against WNV E-protein), and control antibody CR2361 IgG1 (positive control; antibody directed against ATAD3A) were coupled to protein A beads at room temperature. Next, the pre-cleared samples were incubated with the IgGs coupled to the beads for

30 2 hours at 4°C. The protein A beads were washed three times for 5 minutes with 1 ml of DOC lysis buffer and bound complexes were eluted by the addition of sample loading

buffer. The samples were subjected to SDS-PAGE under non-reducing conditions. After blotting on PVDF membranes, the myc tagged proteins were detected with the HRP conjugated anti-myc monoclonal antibody 9E10 (Amersham). Immunoblot developed with anti-myc antibody demonstrated that mycNDUFV-1 was only immunoprecipitated by CR4354L4328 and not by the any of the other antibodies (see Figure 5). In addition, ATAD3A_{myc} protein was only immunoprecipitated by CR2361 (data not shown).

Example 11

Distribution of the antigen recognized by CR4348 and CR4354L4328 on 293T cells and VLP-transfected HEK293T cells as shown by FACS

The distribution of the target antigens recognized by the antibodies CR4348 and CR4354L4328 was analyzed by flow cytometry in three different ways: a) using HEK293T cells in permeabilized format, b) using HEK293T cells in non-permeabilized format, and c) using non-permeabilized WNV VLP-transfected HEK293T cells. 293T cells were obtained from the ATCC CRL-11268 and were harvested with trypsin/EDTA. One part of the cells was fixed and permeabilized for intracellular staining with the DakoCytomation IntraStain (K2311) according to the manufacturer's instruction, the other part of the cells was stained extracellularly and was incubated directly after harvest with the antibodies. For each sample, 100.000 cells were incubated with 2.5 µg/ml CR4354L4328, CR4348 or the control antibodies CR2300, CR4374 and CR4104 diluted in PBS containing 1% BSA. CR2300 is a positive control antibody (recognizing CD46 which is present on nucleated cells); CR4374 recognizes WNV E-protein and is the positive control antibody for VLP-transfected 293T cells and the negative antibody for

non-transfected 293T cells; and CR4104 recognizes rabies glycoprotein and is included as negative control for all stainings. The antibodies were incubated with the cells for 1 hour on ice and the cells were washed three times with
5 PBS/BSA. Binding of the antibodies to the cells was visualized after incubation for 1 hour on ice with goat anti-human phycoerythrin antibody (Pharmingen). The cells were washed twice in PBS/BSA prior to analysis. In the extracellular staining experiment dead or permeable cells were excluded from
10 analysis by staining with 7-AAD, a dye that stains nuclear DNA. VLP-transfected 293T cells were harvested 24 hour after transfection and stained according to the above-described method for extracellular staining. Cells were analyzed on a FACS calibur (BD) using CellQuest software. For final analysis
15 of the extracellular stained cells, cells were gated based on forward scatter versus low 7-AAD signal. A sample was considered positive if the mean fluorescence intensity was more than two times the signal obtained with the negative control antibody. As is shown in Table 17, CR4348 and
20 CR4354L4328 both recognised an intracellular target antigen that is not expressed at the cell surface under normal culturing conditions. However, after transfection with the DNA construct for WNV-VLP production the target antigens of CR4348 and CR4354L4328 were detected at the cell surface. The
25 antibodies specifically reacted with antigens expressed after WNV-VLP transfection, since they did not bind the cell surface of mock-transfected cells (data not shown). When the antibodies were applied in a serial dilution, they bound the target antigens in a dose dependent way (data not shown).

30 From these combined expression data it was concluded that the antigens recognized by CR4348 and CR4354L4328 are located intracellularly in healthy, normal cells. However, after

mimicking a WNV-infection by introducing a WNV-VLP construct in the cells the antigens became expressed at the cellular surface.

5 Example 12

Intracellular location of the target antigens of CR4354L4328 and CR4348 demonstrated by immunofluorescence

The intracellular location of the CR4354L4328 and CR4348 target antigens was analyzed by immunofluorescent staining of
10 cells. VERO cells and 293T cells were seeded at a confluency of 20% in 4 well LAB-TEK chambers (Nunc). 24 Hours after seeding of the cells one part of the VERO cells was infected with $8.1\text{E-}3$ TCID₅₀/cell of WNV strain 385-99. One part of the 293T cells was transfected with the WNV-VLP construct. The
15 VERO and 293T cells that were not used for infection or transfection were allowed to grow for an additional 24 hours. 24 Hours after infection or transfection all VERO and 293T cells were fixed with 2% formaldehyde in PBS for 15 minutes at room temperature. Thereafter they were kept in PBS until the
20 staining procedure. To allow the antibodies to react with the intracellular antigens in the cell, the cells were permeabilized with 0.2% Triton X-100 in PBS for 5 minutes at room temperature and rinsed for 15 minutes. Next, the cells were blocked with 2% BSA in PBS for 1 hour at room
25 temperature. Subsequently, different stainings were performed. Dual stainings: CR4354L4328, CR4348, CR4104 and CR4283 antibodies were applied at a concentration of 5 µg/ml in 2% BSA for 1 hour at room temperature. After 3 washes of 5 minutes in PBS the antibodies were visualized by adding Alexa
30 fluor 488 goat anti-human antibody at a concentration of 5 µg/ml in 2% BSA containing 5 units of phalloidin bodipy (Molecular probes) to stain the cytoskeletal structure. The

cells were incubated for 1 hour at room temperature in the dark. Next, 3 washes of 5 minutes were performed with PBS and anti fading agent was added (Vectashield) to prevent bleaching of the fluorescent signal. Then, a glass coverslip was glued
5 on top with opaque nailpolish (Miss Helen, HEMA). Triple stainings were performed to analyze co-localization of CR4348 and CR4354L4328 with their target antigens FAF-1 and NDUFV1. Slides were viewed under a Leica TCS-NT confocal laser-scanning microscope. Staining of uninfected VERO cells with
10 CR4348 demonstrated a network like pattern consisting of tiny vescicles. After infection with WNV, bigger vesicles appeared that were scattered throughout the cytoplasm. Staining of uninfected VERO cells with CR4354L4328 demonstrated a bright nuclear staining. After infection of VERO cells with WNV,
15 densely packed vesicles appeared throughout the cytoplasm and staining of the nucleus disappeared. In 293T cells the target for CR4348 was localized in vesicle-like structures close to the cellular membrane. After transfection with the VLP construct a brighter staining pattern appeared and vescicles
20 were also localized throughout the cytoplasm. In 293T cells CR4354L4328 stains small vescicles that were localized throughout the cytoplasm in densely packed structures. After transfection of the VLP construct the appearance of the vesicles the densely packed structures disappeared and the
25 vesicles became scattered throughout the cell and showed a bright staining pattern.

Example 12

Neutralization of rabies virus with CR4354L4328

30 To investigate the neutralizing activity of CR4354L4328 on other viral species, the antibody was tested in a RFITT experiment for the neutralization of rabies virus. The RFFIT

was performed by mixing serial five-fold dilutions of CR4354L4328, CR4374 (negative control; anti-WNV E-protein antibody) and CR57 (positive control; anti-rabies virus antibody) with a constant amount of rabies virus (50 FFD50/0.1 ml) in a multi-chambered slide. One unit of virus is determined as the dilution at which 50% of the observed microscopic fields contain one or more foci of infected cells, *i.e.* the focus forming dose, FFD50. After allowing the mixture to react in a CO₂-incubator at 37°C for 90 minutes, mouse neuroblastoma (MNA) cells in Eagle's minimum essential medium with 10% fetal bovine serum (MEM-10) were added to each monoclonal antibody-virus mixture resulting in a final concentration of 1×10^5 cells/ml. The monoclonal antibody-virus-cell cultures were incubated for 20 hours in a CO₂-incubator at 37°C. The cultures were removed from the incubator, washed, fixed and then stained with an anti-rabies virus antibody conjugate directed against the nucleoprotein and observed under a fluorescence microscope for the presence of fluorescent cells; 20 microscopic fields (160X - 200X) were read for each monoclonal antibody dilution and compared against the virus control (50 FFD50/0.1 ml), which should contain 18 to 20 fields with fluorescent cells. The 50% neutralization endpoint of a particular antibody is defined as the dilution by which 50% or more of the observed microscopic fields contain one or more infected cells and was calculated using the Reed-Muench method. Virus neutralizing antibody titers were normalized to international units (IU) using the NIH US standard human Rabies Immune Globulin reference lot R3 (SRIG). The 50% neutralization point of CR57 was obtained at a concentration of 7.3 ng/ml. The 50% neutralization point of CR4354 was obtained at a concentration of 9.2 µg/ml. The control antibody CR4374 could not neutralize rabies virus at

any concentration tested. The results clearly show that CR4354L4328 has rabies virus neutralizing activity.

5

Table 1: Human lambda chain variable region primers (sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuV λ 1A	5'-CAGTCTGTGCTGACT CAGCCACC-3'	SEQ ID NO:79
HuV λ 1B	5'-CAGTCTGTGYTGACG CAGCCGCC-3'	SEQ ID NO:80
HuV λ 1C	5'-CAGTCTGTCGTGACG CAGCCGCC-3'	SEQ ID NO:81
HuV λ 2	5'-CARTCTGCCCTGACT CAGCCT-3'	SEQ ID NO:82
HuV λ 3A	5'-TCCTATGWGCTGACT CAGCCACC-3'	SEQ ID NO:83
HuV λ 3B	5'-TCTTCTGAGCTGACT CAGGACCC-3'	SEQ ID NO:84
HuV λ 4	5'-CACGTTATACTGACT CAACCGCC-3'	SEQ ID NO:85
HuV λ 5	5'-CAGGCTGTGCTGACT CAGCCGTC-3'	SEQ ID NO:86
HuV λ 6	5'-AATTTTATGCTGACT CAGCCCCA-3'	SEQ ID NO:87
HuV λ 7/8	5'-CAGRCTGTGGTGACY CAGGAGCC-3'	SEQ ID NO:88
HuV λ 9	5'-CWGCCTGTGCTGACT CAGCCMCC-3'	SEQ ID NO:89

Table 2: Human kappa chain variable region primers (sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVk1B	5'-GACATCCAGWTGACCC AGTCTCC-3'	SEQ ID NO:90
HuVk2	5'-GATGTTGTGATGACT CAGTCTCC-3'	SEQ ID NO:91
HuVk3	5'-GAAATTGTGWTGACR CAGTCTCC-3'	SEQ ID NO:92
HuVk4	5'-GATATTGTGATGACC CACACTCC-3'	SEQ ID NO:93
HuVk5	5'-GAAACGACACTCACG CAGTCTCC-3'	SEQ ID NO:94
HuVk6	5'-GAAATTGTGCTGACTC AGTCTCC-3'	SEQ ID NO:95

Table 3: Human kappa chain variable region primers extended with SalI restriction sites (sense), human kappa chain J-region primers extended with NotI restriction sites (anti-sense), human lambda chain variable region primers extended with SalI restriction sites (sense) and human lambda chain J-region primers extended with NotI restriction sites (anti-sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVk1B-SalI	5'-TGAGCACACAGGTCG ACGGACATCCAGWTGACC CAGTCTCC-3'	SEQ ID NO:96
HuVk2-SalI	5'-TGAGCACACAGGTCG ACGGATGTTGTGATGACT CAGTCTCC-3'	SEQ ID NO:97
HuVk3B-SalI	5'-TGAGCACACAGGTCG ACGGAAATTGTGWTGACR CAGTCTCC-3'	SEQ ID NO:98
HuVk4B-SalI	5'-TGAGCACACAGGTCG ACGGATATTGTGATGACC CACACTCC-3'	SEQ ID NO:99
HuVk5-SalI	5'-TGAGCACACAGGTCGACG GAAACGACACTCACGCAGTCT CC-3'	SEQ ID NO:100
HuVk6-SalI	5'-TGAGCACACAGGTCG ACGGAAATTGTGCTGACT CAGTCTCC-3'	SEQ ID NO:101
HuJk1-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACGTTTGATTTCCAC CTTGGTCCC-3'	SEQ ID NO:102
HuJk2-NotI	5'-GAGTCATTCTCGACT	SEQ ID NO:103

	TGCGGCCGCACGTTTGAT CTCCAGCTTGGTCCC-3'	
HuJk3-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACGTTTGATATCCAC TTTGGTCCC-3'	SEQ ID NO:104
HuJk4-NotI	5'-GAGTCATTCTCGACT TGCGGCCGCACGTTTGAT CTCCACCTTGGTCCC-3'	SEQ ID NO:105
HuJk5-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACGTTTAATCTCCAG TCGTGTCCC-3'	SEQ ID NO:106
HuVλ1A-SalI	5'-TGAGCACACAGGTCGACG CAGTCTGTGCTGACTCAGCCA CC-3'	SEQ ID NO:107
HuVλ1B-SalI	5'-TGAGCACACAGGTCGACG CAGTCTGTGYTGACGCAGCCG CC-3'	SEQ ID NO:108
HuVλ1C-SalI	5'-TGAGCACACAGGTCGACG CAGTCTGTCGTGACGCAGCCG CC-3'	SEQ ID NO:109
HuVλ2-SalI	5'-TGAGCACACAGGTCGACG CARTCTGCCCTGACTCAGCCT- 3'	SEQ ID NO:110
HuVλ3A-SalI	5'-TGAGCACACAGGTCGACG TCCTATGWGCTGACTCAGCCA CC-3'	SEQ ID NO:111
HuVλ3B-SalI	5'-TGAGCACACAGGTCGACG TCTTCTGAGCTGACTCAGGAC CC-3'	SEQ ID NO:112
HuVλ4-SalI	5'-TGAGCACACAGGTCGACG CACGTTATACTGACTCAACCG	SEQ ID NO:113

	CC-3'	
HuVλ5-SalI	5'-TGAGCACACAGGTCGACG CAGGCTGTGCTGACTCAGCCG TC-3'	SEQ ID NO:114
HuVλ6-SalI	5'-TGAGCACACAGGTCGACG AATTTTATGCTGACTCAGCCC CA-3'	SEQ ID NO:115
HuVλ7/8-SalI	5'-TGAGCACACAGGTCGACG CAGRCTGTGGTGACYCAGGAG CC-3'	SEQ ID NO:116
HuVλ9-SalI	5'-TGAGCACACAGGTCGACG CWGCCTGTGCTGACTCAGCCM CC-3'	SEQ ID NO:117
HuJλ1-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACCTAGGACGGTGAC CTTGGTCCC-3'	SEQ ID NO:118
HuJλ2/3-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACCTAGGACGGTCAG CTTGGTCCC-3'	SEQ ID NO:119
HuJλ4/5-NotI	5'-GAGTCATTCTCGACTTGC GGCCGCACYTAAACGGTGAG CTGGGTCCC-3'	SEQ ID NO:120

Table 4: Distribution of the different light chain products over the 10 fractions.

Light chain products	Number of alleles	Fraction number	alleles/fraction
Vk1B/Jk1-5	19	1 and 2	9.5
Vk2/Jk1-5	9	3	9
Vk3B/Jk1-5	7	4	7
Vk4B/Jk1-5	1	5	5
Vk5/Jk1-5	1		
Vk6/Jk1-5	3		
Vλ1A/Jl1-3	5	6	5
Vλ1B/Jl1-3			
Vλ1C/Jl1-3			
Vλ2/Jl1-3	5	7	5
Vλ3A/Jl1-3	9	8	9
Vλ3B/Jl1-3			
Vλ4/Jl1-3	3	9	5
Vλ5/Jl1-3	1		
Vλ6/Jl1-3	1		
Vλ7/8/Jl1-3	3	10	6
Vλ9/Jl1-3	3		

Table 5: Human IgG heavy chain variable region primers (sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVH1B/7A	5'-CAGRTGCAGCTGGTG CARTCTGG-3'	SEQ ID NO:121
HuVH1C	5'-SAGGTCCAGCTGGTR CAGTCTGG-3'	SEQ ID NO:122
HuVH2B	5'-SAGGTGCAGCTGGTG GAGTCTGG-3'	SEQ ID NO:123
HuVH3B	5'-SAGGTGCAGCTGGTG GAGTCTGG-3'	SEQ ID NO:124
HuVH3C	5'-GAGGTGCAGCTGGTG GAGWCYGG-3'	SEQ ID NO:125
HuVH4B	5'-CAGGTGCAGCTACAG CAGTGGGG-3'	SEQ ID NO:126
HuVH4C	5'-CAGSTGCAGCTGCAG GAGTCSGG-3'	SEQ ID NO:127
HuVH5B	5'-GARGTGCAGCTGGTG CAGTCTGG-3'	SEQ ID NO:128
HuVH6A	5'-CAGGTACAGCTGCAG CAGTCAGG-3'	SEQ ID NO:129

Table 6: Human IgG heavy chain variable region primers extended with SfiI/NcoI restriction sites (sense) and human IgG heavy chain J-region primers extended with XhoI/BstEII restriction sites (anti-sense).

Primer name	Primer nucleotide sequence	SEQ ID NO
HuVH1B/7A-SfiI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC CAGRTGCAGCTGGTGCAR TCTGG-3'	SEQ ID NO:130
HuVH1C-SfiI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC SAGGTCCAGCTGGTRCAG TCTGG-3'	SEQ ID NO:131
HuVH2B-SfiI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC CAGRTCACCTTGAAGGAG TCTGG-3'	SEQ ID NO:132
HuVH3B-SfiI	5'-GTCCTCGCAACTGCGGCC CAGCCGGCCATGGCCSAGGTG CAGCTGGTGGAGTCTGG-3'	SEQ ID NO:133
HuVH3C-SfiI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC GAGGTGCAGCTGGTGGAG WCYGG-3'	SEQ ID NO:134
HuVH4B-SfiI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC CAGGTGCAGCTACAGCAG TGGGG-3'	SEQ ID NO:135
HuVH4C-SfiI	5'-GTCCTCGCAACTGCGGCC CAGCCGGCCATGGCCCAGSTG	SEQ ID NO:136

	CAGCTGCAGGAGTCSGG-3'	
HuVH5B-SfiI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC GARGTGCAGCTGGTGCAG TCTGG-3'	SEQ ID NO:137
HuVH6A-SfiI	5'-GTCCTCGCAACTGCG GCCCAGCCGGCCATGGCC CAGGTACAGCTGCAGCAG TCAGG-3'	SEQ ID NO:138
HuJH1/2-XhoI	5'-GAGTCATTCTCGACTCGA GACGGTGACCAGGGTGCC-3'	SEQ ID NO:139
HuJH3-XhoI	5'-GAGTCATTCTCGACT CGAGACGGTGACCATTTGT CCC-3'	SEQ ID NO:140
HuJH4/5-XhoI	5'-GAGTCATTCTCGACT CGAGACGGTGACCAGGGT TCC-3'	SEQ ID NO:141
HuJH6-XhoI	5'-GAGTCATTCTCGACTCGA GACGGTGACCGTGGTCCC-3'	SEQ ID NO:142

Table 7: Binding of single-chain (scFv) phage antibodies to
 5 West Nile virus (WNV), recombinant WNV E protein, FBS, and
 rabies virus as measured by ELISA at 492nm).

Name phage antibody	WN virus	WNV E protein	WNV-like particle	FBS (5%)	Rabies virus
SC04-348	1.026	0.093	0.771	ND	0.063
SC04-354	1.041	0.069	0.811	ND	0.063
SC02-447	0.094	0.057	ND	0.041	ND
SC03-014	0.061	0.060	ND	ND	0.062

Pos. control	0.067	0.056	0.062	ND	0.991
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ND means not determined

Table 8: Data of the WNV specific single-chain Fvs.

Name scFv	SEQ ID NO of nucl. sequence	SEQ ID NO of amino acid sequence*	HCDR3 (SEQ ID NO:)	VH- locus	VL-locus
SC04-348	25	26 (Vh 1-124; Vl 143-250)	DKSYYYGSGTSGGWF DP (SEQ ID NO:5)	3-09 (DP-31)	Vk I (O12/O2- DPK9)
SC04-354	27	28 (Vh 1-121; Vl 140-250)	DWGSNYVWGSYPKY (SEQ ID NO:6)	1-46 (DP-7)	Vl 1 (1c - Vl- 16)

* between brackets the amino acids making up the heavy chain variable region (VH) and the light chain variable region (VL) is shown

5

Table 9: Data of the CDR regions of the WNV specific single-chain Fvs.

Name scFv	HCDR1 (SEQ ID NO:)	HCDR2 (SEQ ID NO:)	LCDR1 (SEQ ID NO:)	LCDR2 (SEQ ID NO:)	LCDR3 (SEQ ID NO:)
SC04-348	7	8	9	10	11
SC04-354	12	13	14	15	16

10

Table 10: Binding of IgG1 antibodies to WNV as measured by ELISA (OD 492nm).

Ab	Antibody Concentration (µg/ml)										
	20.000	10.000	5.000	2.500	1.250	0.630	0.310	0.160	0.078	0.039	0.000
CR4348	ND	0.370	0.343	0.300	0.251	0.192	0.143	0.122	0.104	0.085	0.003
CR4354	0.291	0.262	0.217	0.167	0.151	0.106	0.067	0.034	0.014	0.010	0.003
Neg. control	0.051	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND means not determined

Table 11: Potency of the anti-WNV antibodies in the 66% neutralizing antibody titer assay.

Antibody name	$\mu\text{g/ml}$
CR4348	1.97
CR4354	0.48

5

Table 12: Protection from lethal WNV challenge in mice by monoclonal antibodies.

Antibody (15 mg/kg)	Surviving animals
CR4348	5/5
CR4354	5/5
7H2	5/5
Negative Control IgG1	0/5

10 Table 13: Probit analysis of the protective activity of human IgG1 in a murine lethal WNV challenge model

Antibody	50% protection ($\mu\text{g/kg}$)	95% protection ($\mu\text{g/kg}$)
CR4354L4328	2.74	57.4
CR4348	6.26	131

15 Table 14: Percentage difference in 66% neutralization concentration of IgG1 variants of CR4354 against WNV as measured by VNA.

Antibody	Potency (%) *
CR4354	100
CR4354L4261	106

CR4354L4267	Below detection
CR4354L4328	286
CR4354L4335	60
CR4354L4383	Below detection

* Potency is represented in comparison to original antibody CR4354 (the 66% neutralising concentration of which was set at 100%) and was calculated by dividing the 66% neutralising concentration (in µg/ml) of CR4354 by the 66% neutralising concentration (in µg/ml) of the chain shuffled variants and multiplying the resulting number by 100%.

Table 15: Neutralizing potency against West Nile virus strain USA99b as measured by PRNT.

Antibody	PRNT50 (95% CI) (µg/ml)	PRNT90 (95% CI) (µg/ml)
CR4348	3.72 (3.21 - 4.27)	65.2 (52.3 - 84.1)
CR4354L4328	21.2 (10.1 - 53.4)	1602 (397 - 20000)
CRM4354	0.17 (0.11 - 0.25)	4.3 (2.85 - 7.29)

Table 16. Clones reactive with CR4348 and CR4354L4328 as recognised by protein micro-array analysis.

Antibody	Reactive clone	Blast	Target (Homo sapiens)
CR4348	MPMGp800B14568	gi 19528653 ref NM_007051.2	Fas (TNFRSF6) associated factor 1 (FAF1)
CR4348	MPMGp800G07549Q170	gi 27924136 gb BC044933.1	clone IMAGE:4540326, mRNA, partial cds
CR4348	MPMGp800O05569Q170	gi 54607125 ref NM_013364.2	paraneoplastic antigen MA3 (PNMA3), mRNA
CR4348	MPMGp800E02577Q	gi 34364672 emb BX640642.1	mRNA; cDNA DKFZp686K01114

121

CR4348	MPMGp800M04560Q183	gi 39761682 gb AY405708.1	CYLN2 gene, VIRTUAL TRANSCRIPT
CR4354L4328	MPMGp800J14549Q	gi 14198175 gb BC008146.1	NADH dehydrogenase (ubiquinone) flavoprotein 1
CR4354L4328	MPMGp800K13552Q	gi 62087473 dbj AB208947.1	mRNA for chordin isoform a variant protein
CR4354L4328	MPMGp800A02549Q	gi 17149812 ref NM_057161.2	kelch domain containing 3 (KLHDC3)
CR4354L4328	MPMGp800I05513Q	gi 4753262 gb AC006360.2	PAC clone RP5- 1140N14 from 14q24.3

Table 17. Mean fluorescence measured after incubation with 2.5 µg/ml antibody.

Antibody	293T extracellular	293T intracellular	293T-VLP extracellular
CR4348	16	225	15
CR4354L4328	16	840	11
CR4104	15	55	4
CR4374	15	57	19
CR2300	484	236	10

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	200 205 210 215	
70	att gag ggc aag cag ggc aag ccc cgc ctg aag ccc ccc ttc ccc gca	726
	Ile Glu Gly Lys Gln Gly Lys Pro Arg Leu Lys Pro Pro Phe Pro Ala	
	220 225 230	

133

	gac	gtg	gga	gtg	ttt	ggc	tgc	ccc	aca	act	gtg	gcc	aac	gtg	gag	aca	774
	Asp	Val	Gly	Val	Phe	Gly	Cys	Pro	Thr	Thr	Val	Ala	Asn	Val	Glu	Thr	
				235					240					245			
5	gtg	gca	gtg	tcc	ccc	aca	atc	tgc	cgc	cgt	gga	ggg	acc	tgg	ttt	gct	822
	Val	Ala	Val	Ser	Pro	Thr	Ile	Cys	Arg	Arg	Gly	Gly	Thr	Trp	Phe	Ala	
			250					255					260				
10	ggc	ttt	ggc	aga	gaa	cgc	aac	tca	ggc	acc	aaa	cta	ttc	aac	atc	tct	870
	Gly	Phe	Gly	Arg	Glu	Arg	Asn	Ser	Gly	Thr	Lys	Leu	Phe	Asn	Ile	Ser	
		265					270					275					
15	ggc	cat	gtc	aac	cac	cct	tgc	act	gtg	gag	gag	gag	atg	tct	gtg	ccc	918
	Gly	His	Val	Asn	His	Pro	Cys	Thr	Val	Glu	Glu	Glu	Met	Ser	Val	Pro	
	280					285					290					295	
20	ttg	aaa	gaa	ctg	att	gag	aag	cat	gct	ggg	ggg	gtc	acg	ggc	ggc	tgg	966
	Leu	Lys	Glu	Leu	Ile	Glu	Lys	His	Ala	Gly	Gly	Val	Thr	Gly	Gly	Trp	
				300						305					310		
25	gac	aac	ctc	ctt	gct	gtg	atc	cct	ggc	ggc	tcg	tct	acc	cca	ctg	atc	1014
	Asp	Asn	Leu	Leu	Ala	Val	Ile	Pro	Gly	Gly	Ser	Ser	Thr	Pro	Leu	Ile	
				315					320					325			
30	ccc	aag	tct	gtg	tgt	gag	acg	gtg	ctg	atg	gac	ttc	gat	gcg	ctg	gtg	1062
	Pro	Lys	Ser	Val	Cys	Glu	Thr	Val	Leu	Met	Asp	Phe	Asp	Ala	Leu	Val	
			330					335					340				
35	cag	gca	cag	aca	ggc	ctg	ggc	aca	gct	gcg	gtg	atc	gtc	atg	gac	cgc	1110
	Gln	Ala	Gln	Thr	Gly	Leu	Gly	Thr	Ala	Ala	Val	Ile	Val	Met	Asp	Arg	
		345				350						355					
40	tcg	acg	gac	atc	gtg	aaa	gcc	atc	gcc	cgc	ctc	att	gag	ttc	tat	aag	1158
	Ser	Thr	Asp	Ile	Val	Lys	Ala	Ile	Ala	Arg	Leu	Ile	Glu	Phe	Tyr	Lys	
	360					365				370						375	
45	cac	gag	agc	tgt	ggc	cag	tgt	acc	cca	tgc	cgt	gag	ggg	gtg	gac	tgg	1206
	His	Glu	Ser	Cys	Gly	Gln	Cys	Thr	Pro	Cys	Arg	Glu	Gly	Val	Asp	Trp	
				380						385					390		
50	atg	aac	aag	gtg	atg	gca	cgt	ttc	gtg	agg	ggg	gat	gcc	cgg	ccg	gcc	1254
	Met	Asn	Lys	Val	Met	Ala	Arg	Phe	Val	Arg	Gly	Asp	Ala	Arg	Pro	Ala	
				395					400					405			
55	gag	atc	gac	tcc	ctg	tgg	gag	atc	agc	aag	cag	ata	gaa	ggc	cat	acg	1302
	Glu	Ile	Asp	Ser	Leu	Trp	Glu	Ile	Ser	Lys	Gln	Ile	Glu	Gly	His	Thr	
			410				415						420				
60	att	tgt	gct	ctg	ggg	gac	ggg	gcc	gcc	tgg	cct	gtg	cag	ggg	ctg	atc	1350
	Ile	Cys	Ala	Leu	Gly	Asp	Gly	Ala	Ala	Trp	Pro	Val	Gln	Gly	Leu	Ile	
		425				430						435					
65	cgc	cac	ttt	cgg	ccg	gag	ctc	gag	gag	cgg	atg	cag	cgg	ttt	gcc	cag	1398
	Arg	His	Phe	Arg	Pro	Glu	Leu	Glu	Glu	Arg	Met	Gln	Arg	Phe	Ala	Gln	
	440					445				450					455		

134

cag cat cag gcc cgg cag gct gcc tct tag cccaccaccc tggcctgctg 1448
 Gln His Gln Ala Arg Gln Ala Ala Ser
 460

5 tcctgcgtct atccatgtgg aatgctggac aataaagcga gtgctgcca ccctccaaaa 1508
 aaaaaaaaaa aaaaaaaaaa a 1529

10 <210> 4

<211> 464

<212> PRT

15

<213> Homo sapiens

20 <400> 4

Met Leu Ala Thr Arg Arg Leu Leu Gly Trp Ser Leu Pro Ala Arg Val
 1 5 10 15

25

Ser Val Arg Phe Ser Gly Asp Thr Thr Ala Pro Lys Lys Thr Ser Phe
 20 25 30

30

Gly Ser Leu Lys Asp Glu Asp Arg Ile Phe Thr Asn Leu Tyr Gly Arg
 35 40 45

35

His Asp Trp Arg Leu Lys Gly Ser Leu Ser Arg Gly Asp Trp Tyr Lys
 50 55 60

40

Thr Lys Glu Ile Leu Leu Lys Gly Pro Asp Trp Ile Leu Gly Glu Ile
 65 70 75 80

Lys Thr Ser Gly Leu Arg Gly Arg Gly Gly Ala Gly Phe Pro Thr Gly
 85 90 95

45

Leu Lys Trp Ser Phe Met Asn Lys Pro Ser Asp Gly Arg Pro Lys Tyr
 100 105 110

50

Leu Val Val Asn Ala Asp Glu Gly Glu Pro Gly Thr Cys Lys Asp Arg
 115 120 125

55

Glu Ile Leu Arg His Asp Pro His Lys Leu Leu Glu Gly Cys Leu Val
 130 135 140

135

	Gly	Gly	Arg	Ala	Met	Gly	Ala	Arg	Ala	Ala	Tyr	Ile	Tyr	Ile	Arg	Gly	145	150	155	160
5	Glu	Phe	Tyr	Asn	Glu	Ala	Ser	Asn	Leu	Gln	Val	Ala	Ile	Arg	Glu	Ala	165	170	175	
10	Tyr	Glu	Ala	Gly	Leu	Ile	Gly	Lys	Asn	Ala	Cys	Gly	Ser	Gly	Tyr	Asp	180	185	190	
15	Phe	Asp	Val	Phe	Val	Val	Arg	Gly	Ala	Gly	Ala	Tyr	Ile	Cys	Gly	Glu	195	200	205	
20	Glu	Thr	Ala	Leu	Ile	Glu	Ser	Ile	Glu	Gly	Lys	Gln	Gly	Lys	Pro	Arg	210	215	220	
25	Leu	Lys	Pro	Pro	Phe	Pro	Ala	Asp	Val	Gly	Val	Phe	Gly	Cys	Pro	Thr	225	230	235	240
30	Thr	Val	Ala	Asn	Val	Glu	Thr	Val	Ala	Val	Ser	Pro	Thr	Ile	Cys	Arg	245	250	255	
35	Arg	Gly	Gly	Thr	Trp	Phe	Ala	Gly	Phe	Gly	Arg	Glu	Arg	Asn	Ser	Gly	260	265	270	
40	Thr	Lys	Leu	Phe	Asn	Ile	Ser	Gly	His	Val	Asn	His	Pro	Cys	Thr	Val	275	280	285	
45	Glu	Glu	Glu	Met	Ser	Val	Pro	Leu	Lys	Glu	Leu	Ile	Glu	Lys	His	Ala	290	295	300	
50	Gly	Gly	Val	Thr	Gly	Gly	Trp	Asp	Asn	Leu	Leu	Ala	Val	Ile	Pro	Gly	305	310	315	320
55	Gly	Ser	Ser	Thr	Pro	Leu	Ile	Pro	Lys	Ser	Val	Cys	Glu	Thr	Val	Leu	325	330	335	
	Met	Asp	Phe	Asp	Ala	Leu	Val	Gln	Ala	Gln	Thr	Gly	Leu	Gly	Thr	Ala	340	345	350	
	Ala	Val	Ile	Val	Met	Asp	Arg	Ser	Thr	Asp	Ile	Val	Lys	Ala	Ile	Ala	355	360	365	
	Arg	Leu	Ile	Glu	Phe	Tyr	Lys	His	Glu	Ser	Cys	Gly	Gln	Cys	Thr	Pro				

136

370

375

380

5 Cys Arg Glu Gly Val Asp Trp Met Asn Lys Val Met Ala Arg Phe Val
385 390 395 400

10 Arg Gly Asp Ala Arg Pro Ala Glu Ile Asp Ser Leu Trp Glu Ile Ser
405 410 415

15 Lys Gln Ile Glu Gly His Thr Ile Cys Ala Leu Gly Asp Gly Ala Ala
420 425 430

Trp Pro Val Gln Gly Leu Ile Arg His Phe Arg Pro Glu Leu Glu Glu
435 440 445

20 Arg Met Gln Arg Phe Ala Gln Gln His Gln Ala Arg Gln Ala Ala Ser
450 455 460

25 <210> 5

<211> 17

<212> PRT

30 <213> Artificial sequence

35 <220>

<223> HCDR3

<400> 5

40 Asp Lys Ser Tyr Tyr Tyr Gly Ser Gly Thr Ser Gly Gly Trp Phe Asp
1 5 10 15

45 Pro

<210> 6

50 <211> 14

<212> PRT

55 <213> Artificial sequence

137

<220>

<223> HCDR3

5 <400> 6

Asp Trp Gly Ser Asn Tyr Val Trp Gly Ser Tyr Pro Lys Tyr
1 5 10

10

<210> 7

<211> 5

15 <212> PRT

<213> Artificial sequence

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<220>

<223> HCDR1

25 <400> 7

Asp Tyr Ala Met His
1 5

30

<210> 8

<211> 17

35 <212> PRT

<213> Artificial sequence

40

<220>

<223> HCDR2

45 <400> 8

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

50

Gly

55 <210> 9

138

<211> 11

<212> PRT

5 <213> Artificial sequence

<220>

10

<223> LCDR1

<400> 9

15 Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Asn
1 5 10

<210> 10

20

<211> 7

<212> PRT

25 <213> Artificial sequence

<220>

30

<223> LCDR2

<400> 10

35 Ala Ala Ser Ser Leu Gln Ser
1 5

<210> 11

40

<211> 9

<212> PRT

45 <213> Artificial sequence

<220>

50

<223> LCDR3

<400> 11

55 Gln Gln Ser Tyr Ser Thr Pro Arg Thr
1 5

139

<210> 12
<211> 5
5 <212> PRT
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15 <400> 12
His Tyr Tyr Met His
1 5
20
<210> 13
<211> 17
25 <212> PRT
<213> Artificial sequence
30
<220>
<223> HCDR2
35 <400> 13
Ile Ile Asn Pro Ser Gly Gly Ser Thr Thr Tyr Ala Gln Lys Leu Gln
1 5 10 15
40
Gly
45
<210> 14
<211> 13
50 <212> PRT
<213> Artificial sequence
55
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140

<223> LCDR1

<400> 14

5 Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Thr Val Asn
1 5 10

<210> 15

10

<211> 7

<212> PRT

15 <213> Artificial sequence

<220>

20

<223> LCDR2

<400> 15

25 Ser Asn Asn Gln Arg Pro Ser
1 5

<210> 16

30

<211> 11

<212> PRT

35 <213> Artificial sequence

<220>

40

<223> LCDR3

<400> 16

45 Ala Ala Trp Asp Asp Ser Leu Asn Gly Pro Val
1 5 10

<210> 17

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<211> 372

<212> DNA

55 <213> Artificial sequence

141

<220>

5 <223> Heavy chain variable region 348

<220>

<221> CDS

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<223>

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<400> 17

	gag	gtg	cag	ctg	gtg	gag	tct	ggg	gga	ggc	ttg	gta	cag	cct	ggc	agg	48
	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Arg	
20	1				5					10					15		

	tcc	ctg	aga	ctc	tcc	tgt	gca	gcc	tct	gga	ttc	acc	ttt	gat	gat	tat	96
	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asp	Asp	Tyr	
				20					25					30			

25	gcc	atg	cac	tgg	gtc	cgg	caa	gct	cca	ggg	aag	ggc	ctg	gag	tgg	gtc	144
	Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
			35					40					45				

30	tca	ggt	att	agt	tgg	aat	agt	ggt	aac	ata	ggc	tat	gcg	gac	tct	gtg	192
	Ser	Gly	Ile	Ser	Trp	Asn	Ser	Gly	Asn	Ile	Gly	Tyr	Ala	Asp	Ser	Val	
		50					55					60					

35	aag	ggc	cga	ttc	acc	atc	tcc	aga	gac	aac	gcc	aag	aac	tcc	ctg	tat	240
	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr	
	65					70				75					80		

	ctg	caa	atg	aac	agt	ctg	aga	gct	gag	gac	acg	gcc	ttg	tat	tac	tgt	288
	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Leu	Tyr	Tyr	Cys	
40				85						90					95		

	gca	aaa	gat	aaa	tcg	tat	tat	tat	ggt	tcg	ggg	acc	tcg	gga	ggc	tgg	336
	Ala	Lys	Asp	Lys	Ser	Tyr	Tyr	Tyr	Gly	Ser	Gly	Thr	Ser	Gly	Gly	Trp	
				100					105					110			

45	ttc	gac	ccc	tgg	ggc	cag	ggc	acc	ctg	gtg	acc	gtc					372
	Phe	Asp	Pro	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val					
				115				120									

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<210> 18

<211> 124

55 <212> PRT

142

<213> Artificial sequence

5 <220>

<223> Heavy chain variable region 348

<400> 18

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

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

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

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

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

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

35 Ala Lys Asp Lys Ser Tyr Tyr Tyr Gly Ser Gly Thr Ser Gly Gly Trp
 100 105 110

40 Phe Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val
 115 120

<210> 19

45 <211> 363

<212> DNA

50 <213> Artificial sequence

<220>

55 <223> Heavy chain variable region 354

143

<220>

<221> CDS

5 <222> (1) .. (363)

<223>

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<400> 19

cag	gtg	cag	ctg	gtg	cag	tct	ggg	gct	gag	gtg	agg	aag	cct	ggg	gcc	48
Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Arg	Lys	Pro	Gly	Ala	
1				5					10					15		

15

tca	gtg	aag	gtt	tcc	tgc	aag	gca	tct	gga	tac	acc	ttc	acc	cac	tat	96
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	His	Tyr	
			20					25					30			

20

tat	atg	cac	tgg	gtg	cga	cag	gcc	cct	gga	caa	ggg	ctt	gag	tgg	atg	144
Tyr	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	
		35					40					45				

25

gga	ata	atc	aac	cct	agt	ggg	ggg	agc	aca	acc	tac	gca	cag	aag	ctc	192
Gly	Ile	Ile	Asn	Pro	Ser	Gly	Gly	Ser	Thr	Thr	Tyr	Ala	Gln	Lys	Leu	
	50					55					60					

30

cag	ggc	aga	gtc	acc	atg	acc	agg	gac	acg	tcc	acg	agc	aca	gtc	tac	240
Gln	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Thr	Ser	Thr	Val	Tyr	
	65				70					75				80		

35

atg	gag	ctg	agc	agc	ctg	aga	tct	gag	gac	acg	gcc	gtg	tat	tac	tgt	288
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	
				85				90					95			

40

gcg	aga	gat	tgg	ggc	tcc	aat	tac	gtt	tgg	ggg	agt	tat	ccc	aag	tac	336
Ala	Arg	Asp	Trp	Gly	Ser	Asn	Tyr	Val	Trp	Gly	Ser	Tyr	Pro	Lys	Tyr	
			100					105					110			

45

tgg	ggc	cag	ggc	acc	ctg	gtc	acc	gtc								363
Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val								
		115					120									

45

<210> 20

<211> 121

<212> PRT

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<213> Artificial sequence

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<220>

<223> Heavy chain variable region 354

144

<400> 20

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

10 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr
20 25 30

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

Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Thr Tyr Ala Gln Lys Leu
50 55 60

20 Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
65 70 75 80

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

30 Ala Arg Asp Trp Gly Ser Asn Tyr Val Trp Gly Ser Tyr Pro Lys Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val
115 120

35

<210> 21

<211> 330

40

<212> DNA

<213> Artificial sequence

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<220>

<223> Light chain variable region 348

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<220>

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<222> (1) .. (330)

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<223>

145

<400> 21
 5 tcg acg gac atc cag atg acc cag tct cca tcc tcc ctg tct gca tct 48
 Ser Thr Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
 1 5 10 15
 gta gga gac aga gtc acc atc act tgc cgg gca agt cag agc att agc 96
 10 Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
 20 25 30
 agc tat tta aat tgg tat cag cag aaa cca ggg aaa gcc cct aag ctc 144
 Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
 35 40 45
 15 ctg atc tat gct gca tcc agt ttg caa agt ggg gtc cca tca agg ttc 192
 Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe
 50 55 60
 20 agt ggc agt gga tct ggg aca gat ttc act ctc acc atc agc agt ctg 240
 Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu
 65 70 75 80
 25 caa cct gaa gat ttt gca act tac tac tgt caa cag agt tac agt acc 288
 Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr
 85 90 95
 cct cgg acg ttc ggc caa ggg acc aag ctg gag atc aaa cgt 330
 30 Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110

<210> 22
 35 <211> 110
 <212> PRT
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 45 <223> Light chain variable region 348

<400> 22
 50 Ser Thr Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
 1 5 10 15
 Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
 20 25 30
 55 Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu

45

atc tat agt aat aat caq cgg ccc tca ggg atc cct qac cga ttc tct 192

147

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

5 ggc tcc aag tct ggc acc tca gcc tcc ctg gcc atc agt ggg ctc caa 240
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
 65 70 75 80

10 tct gaa gat gag gct gat tat tac tgt gca gca tgg gat gac agc ctg 288
 Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95

15 aat ggt ccg gtg ttc ggc gga ggg acc aag ctg acc gtc cta ggt 333
 Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

<210> 24

<211> 111

<212> PRT

<213> Artificial sequence

<220>

<223> Light chain variable region 354

<400> 24

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

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30

Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

45 Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
 50 55 60

50 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95

Asn Gly Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly

148

100

105

110

<210> 25

5

<211> 750

<212> DNA

10 <213> Artificial sequence

<220>

15

<223> SC04-348

<220>

20 <221> CDS

<222> (1) .. (750)

<223>

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<400> 25

30 gag gtg cag ctg gtg cag tct ggg gga ggc ttg gta cag cct ggc agg 48
 Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1 5 10 15

tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttt gat gat tat 96
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 35 20 25 30

gcc atg cac tgg gtc cgg caa gct cca ggg aag ggc ctg gag tgg gtc 144
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

40 tca ggt att agt tgg aat agt ggt aac ata ggc tat gcg gac tct gtg 192
 Ser Gly Ile Ser Trp Asn Ser Gly Asn Ile Gly Tyr Ala Asp Ser Val
 50 55 60

45 aag ggc cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat 240
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

50 ctg caa atg aac agt ctg aga gct gag gac acg gcc ttg tat tac tgt 288
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

gca aaa gat aaa tcg tat tat tat ggt tcg ggg acc tcg gga ggc tgg 336
 Ala Lys Asp Lys Ser Tyr Tyr Tyr Gly Ser Gly Thr Ser Gly Gly Trp
 55 100 105 110

ttc gac ccc tgg ggc cag ggc acc ctg gtc acc gtc tcg agc ggt acg 384

149

	Phe	Asp	Pro	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Gly	Thr	
	115						120						125				
5	ggc	ggt	tca	ggc	gga	acc	ggc	agc	ggc	act	ggc	ggg	tcg	acg	gac	atc	432
	Gly	Gly	Ser	Gly	Gly	Thr	Gly	Ser	Gly	Thr	Gly	Gly	Ser	Thr	Asp	Ile	
	130						135						140				
10	cag	atg	acc	cag	tct	cca	tcc	tcc	ctg	tct	gca	tct	gta	gga	gac	aga	480
	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	
	145						150						155			160	
15	gtc	acc	atc	act	tgc	cgg	gca	agt	cag	agc	att	agc	agc	tat	tta	aat	528
	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Ser	Tyr	Leu	Asn	
				165						170						175	
	tgg	tat	cag	cag	aaa	cca	ggg	aaa	gcc	cct	aag	ctc	ctg	atc	tat	gct	576
	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Ala	
				180						185						190	
20	gca	tcc	agt	ttg	caa	agt	ggg	gtc	cca	tca	agg	ttc	agt	ggc	agt	gga	624
	Ala	Ser	Ser	Leu	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	
	195						200						205				
25	tct	ggg	aca	gat	ttc	act	ctc	acc	atc	agc	agt	ctg	caa	cct	gaa	gat	672
	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	
	210						215						220				
30	ttt	gca	act	tac	tac	tgt	caa	cag	agt	tac	agt	acc	cct	cgg	acg	ttc	720
	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Tyr	Ser	Thr	Pro	Arg	Thr	Phe	
	225						230						235			240	
35	ggc	caa	ggg	acc	aag	ctg	gag	atc	aaa	cgt							750
	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg							
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5	Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
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10	Ser	Gly	Ile	Ser	Trp	Asn	Ser	Gly	Asn	Ile	Gly	Tyr	Ala	Asp	Ser	Val
		50					55					60				
15	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
	65					70					75					80
20	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Leu	Tyr	Tyr	Cys
					85					90					95	
25	Ala	Lys	Asp	Lys	Ser	Tyr	Tyr	Tyr	Gly	Ser	Gly	Thr	Ser	Gly	Gly	Trp
				100					105					110		
30	Phe	Asp	Pro	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Gly	Thr
			115					120					125			
35	Gly	Gly	Ser	Gly	Gly	Thr	Gly	Ser	Gly	Thr	Gly	Gly	Ser	Thr	Asp	Ile
		130					135					140				
40	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg
	145					150					155					160
45	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Ser	Tyr	Leu	Asn
					165					170					175	
50	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Ala
				180					185					190		
55	Ala	Ser	Ser	Leu	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly
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60	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp
		210					215					220				
65	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Tyr	Ser	Thr	Pro	Arg	Thr	Phe
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 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr His Tyr
 20 25 30
 35 tat atg cac tgg gtg cga cag gcc cct gga caa ggg ctt gag tgg atg 144
 Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45
 40 gga ata atc aac cct agt ggt ggt agc aca acc tac gca cag aag ctc 192
 Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Thr Tyr Ala Gln Lys Leu
 50 55 60
 cag ggc aga gtc acc atg acc agg gac acg tcc acg agc aca gtc tac 240
 Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
 45 65 70 75 80
 atg gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt 288
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 50 85 90 95
 gcg aga gat tgg ggc tcc aat tac gtt tgg ggg agt tat ccc aag tac 336
 Ala Arg Asp Trp Gly Ser Asn Tyr Val Trp Gly Ser Tyr Pro Lys Tyr
 100 105 110
 55 tgg ggc cag ggc acc ctg gtc acc gtc tcg agc ggt acg ggc ggt tca 384
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Thr Gly Gly Ser

152

	115		120		125	
5	ggc gga acc ggc agc ggc act ggc ggg tcg acg cag tct gtg ttg acg Gly Gly Thr Gly Ser Gly Thr Gly Gly Ser Thr Gln Ser Val Leu Thr 130 135 140	432				
10	cag ccg ccc tca gtg tct gcg gcc ccc gga cag aag gtc acc atc tcc Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln Lys Val Thr Ile Ser 145 150 155 160	480				
	tgt tct gga agc agc tcc aac atc gga agt aat act gta aac tgg tac Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Thr Val Asn Trp Tyr 165 170 175	528				
15	cag cag ctc cca gga acg gcc ccc aaa ctc ctc atc tat agt aat aat Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Ser Asn Asn 180 185 190	576				
20	cag cgg ccc tca ggg atc cct gac cga ttc tct ggc tcc aag tct ggc Gln Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly 195 200 205	624				
25	acc tca gcc tcc ctg gcc atc agt ggg ctc caa tct gaa gat gag gct Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala 210 215 220	672				
30	gat tat tac tgt gca gca tgg gat gac agc ctg aat ggt ccg gtg ttc Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu Asn Gly Pro Val Phe 225 230 235 240	720				
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153

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45
 5

Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Thr Tyr Ala Gln Lys Leu
 50 55 60
 10

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
 65 70 75 80
 15

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 20

Ala Arg Asp Trp Gly Ser Asn Tyr Val Trp Gly Ser Tyr Pro Lys Tyr
 100 105 110
 25

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Thr Gly Gly Ser
 115 120 125
 30

Gly Gly Thr Gly Ser Gly Thr Gly Gly Ser Thr Gln Ser Val Leu Thr
 130 135 140
 35

Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln Lys Val Thr Ile Ser
 145 150 155 160
 40

Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Thr Val Asn Trp Tyr
 165 170 175
 45

Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Ser Asn Asn
 180 185 190
 50

Gln Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly
 195 200 205
 55

Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala
 210 215 220
 60

Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu Asn Gly Pro Val Phe
 225 230 235 240
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Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 245 250

154

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 30 tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttt gat gat tat 96
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30
 35 gcc atg cac tgg gtc cgg caa gct cca ggg aag ggc ctg gag tgg gtc 144
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 40 tca ggt att agt tgg aat agt ggt aac ata ggc tat gcg gac tct gtg 192
 Ser Gly Ile Ser Trp Asn Ser Gly Asn Ile Gly Tyr Ala Asp Ser Val
 50 55 60
 aag ggc cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat 240
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 45 65 70 75 80
 ctg caa atg aac agt ctg aga gct gag gac acg gcc ttg tat tac tgt 288
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95
 50 gca aaa gat aaa tcg tat tat tat ggt tcg ggg acc tcg gga ggc tgg 336
 Ala Lys Asp Lys Ser Tyr Tyr Tyr Gly Ser Gly Thr Ser Gly Gly Trp
 100 105 110
 55 ttc gac ccc tgg ggc cag ggc acc ctg gtg acc gtc tcc agc gct agc 384
 Phe Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser
 115 120 125

155

	acc aag ggc ccc agc gtg ttc ccc ctg gcc ccc agc agc aag agc acc	432
	Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr	
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5	agc ggc ggc aca gcc gcc ctg ggc tgc ctg gtg aag gac tac ttc ccc	480
	Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro	
	145 150 155 160	
10	gag ccc gtg acc gtg agc tgg aac agc ggc gcc ttg acc agc ggc gtg	528
	Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val	
	165 170 175	
15	cac acc ttc ccc gcc gtg ctg cag agc agc ggc ctg tac agc ctg agc	576
	His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser	
	180 185 190	
20	agc gtg gtg acc gtg ccc agc agc agc ctg ggc acc cag acc tac atc	624
	Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile	
	195 200 205	
25	tgc aac gtg aac cac aag ccc agc aac acc aag gtg gac aaa cgc gtg	672
	Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val	
	210 215 220	
30	gag ccc aag agc tgc gac aag acc cac acc tgc ccc ccc tgc cct gcc	720
	Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala	
	225 230 235 240	
35	ccc gag ctg ctg ggc gga ccc tcc gtg ttc ctg ttc ccc ccc aag ccc	768
	Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro	
	245 250 255	
40	aag gac acc ctc atg atc agc cgg acc ccc gag gtg acc tgc gtg gtg	816
	Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val	
	260 265 270	
45	gtg gac gtg agc cac gag gac ccc gag gtg aag ttc aac tgg tac gtg	864
	Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val	
	275 280 285	
50	gac ggc gtg gag gtg cac aac gcc aag acc aag ccc cgg gag gag cag	912
	Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln	
	290 295 300	
55	tac aac agc acc tac cgg gtg gtg agc gtg ctc acc gtg ctg cac cag	960
	Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln	
	305 310 315 320	
50	gac tgg ctg aac ggc aag gag tac aag tgc aag gtg agc aac aag gcc	1008
	Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala	
	325 330 335	
55	ctg cct gcc ccc atc gag aag acc atc agc aag gcc aag ggc cag ccc	1056
	Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro	
	340 345 350	

156

	cgg gag ccc cag gtg tac acc ctg ccc ccc agc cgg gag gag atg acc Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr 355 360 365	1104
5	aag aac cag gtg tcc ctc acc tgt ctg gtg aag ggc ttc tac ccc agc Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser 370 375 380	1152
10	gac atc gcc gtg gag tgg gag agc aac ggc cag ccc gag aac aac tac Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr 385 390 395 400	1200
15	aag acc acc ccc cct gtg ctg gac agc gac ggc agc ttc ttc ctg tac Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr 405 410 415	1248
20	agc aag ctc acc gtg gac aag agc cgg tgg cag cag ggc aac gtg ttc Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe 420 425 430	1296
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55	Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45	

157

	Ser	Gly	Ile	Ser	Trp	Asn	Ser	Gly	Asn	Ile	Gly	Tyr	Ala	Asp	Ser	Val	
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5	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr	
	65					70					75					80	
10	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Leu	Tyr	Tyr	Cys	
					85					90					95		
15	Ala	Lys	Asp	Lys	Ser	Tyr	Tyr	Tyr	Gly	Ser	Gly	Thr	Ser	Gly	Gly	Trp	
				100					105					110			
20	Phe	Asp	Pro	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	
			115					120					125				
25	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	
		130					135					140					
30	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	
	145					150					155					160	
35	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	
					165					170					175		
40	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	
				180					185					190			
45	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	
			195					200					205				
50	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	
	210						215					220					
55	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	
	225					230					235					240	
60	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	
					245					250					255		
65	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	
				260					265					270			
70	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	

285

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55

159

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<223> Heavy chain CR4354

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

20	tca	gtg	aag	gtt	tcc	tgc	aag	gca	tct	gga	tac	acc	ttc	acc	cac	tat	96
	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	His	Tyr	
				20					25					30			

25	tat	atg	cac	tgg	gtg	cga	cag	gcc	cct	gga	caa	ggg	ctt	gag	tgg	atg	144
	Tyr	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	
			35					40					45				

30	gga	ata	atc	aac	cct	agt	ggg	ggg	agc	aca	acc	tac	gca	cag	aag	ctc	192
	Gly	Ile	Ile	Asn	Pro	Ser	Gly	Gly	Ser	Thr	Thr	Tyr	Ala	Gln	Lys	Leu	
		50					55					60					

35	cag	ggc	aga	gtc	acc	atg	acc	agg	gac	acg	tcc	acg	agc	aca	gtc	tac	240
	Gln	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Thr	Ser	Thr	Val	Tyr	
	65					70				75					80		

40	atg	gag	ctg	agc	agc	ctg	aga	tct	gag	gac	acg	gcc	gtg	tat	tac	tgt	288
	Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	
				85					90					95			

45	gcg	aga	gat	tgg	ggc	tcc	aat	tac	gtt	tgg	ggg	agt	tat	ccc	aag	tac	336
	Ala	Arg	Asp	Trp	Gly	Ser	Asn	Tyr	Val	Trp	Gly	Ser	Tyr	Pro	Lys	Tyr	
				100					105					110			

50	tgg	ggc	cag	ggc	acc	ctg	gtg	acc	gtc	tcc	agc	gct	agc	acc	aag	ggc	384
	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	
			115					120					125				

55	ccc	agc	gtg	ttc	ccc	ctg	gcc	ccc	agc	agc	aag	agc	acc	agc	ggc	ggc	432
	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	
			130				135					140					

60	aca	gcc	gcc	ctg	ggc	tgc	ctg	gtg	aag	gac	tac	ttc	ccc	gag	ccc	gtg	480
	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	
	145					150					155				160		

65	acc	gtg	agc	tgg	aac	agc	ggc	gcc	ttg	acc	agc	ggc	gtg	cac	acc	ttc	528
	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	

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[illegible]

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	<212>	PRT
25	<213>	Artificial sequence

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      <400>  32

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

162

	Ala	Arg	Asp	Trp	Gly	Ser	Asn	Tyr	Val	Trp	Gly	Ser	Tyr	Pro	Lys	Tyr	
				100					105					110			
5	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	
			115					120					125				
10	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	
		130					135					140					
15	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	
	145					150					155					160	
20	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	
					165					170					175		
25	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	
				180					185					190			
30	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	
			195					200					205				
35	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	
		210					215					220					
40	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	
	225					230					235					240	
45	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	
					245					250					255		
50	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	
				260					265					270			
55	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	
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		290					295					300					
65	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	
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Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
325 330 335

5 Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 340 345 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
10 355 360 365

15 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
370 375 380

Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr
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20 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 405 410 415

25 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
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30 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
435 440 445

Leu Ser Pro Gly Lys
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<211> 645

<212> DNA

<213> Artificial sequence

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<220>

<223> Light chain CR4348

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<221> CDS

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$\langle 223 \rangle$

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	1				5					10					15		
10	gta	gga	gac	aga	gtc	acc	atc	act	tgc	cgg	gca	agt	cag	agc	att	agc	96
	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	
				20					25					30			
15	agc	tat	tta	aat	tgg	tat	cag	cag	aaa	cca	ggg	aaa	gcc	cct	aag	ctc	144
	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	
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	Leu	Ile	Tyr	Ala	Ala	Ser	Ser	Leu	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	
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	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	
	65					70					75					80	
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	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Tyr	Ser	Thr	
					85					90					95		
35	cct	cgg	acg	ttc	ggc	caa	ggg	acc	aag	ctg	gag	atc	aaa	cgt	gcg	gcc	336
	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Ala	Ala	
				100					105					110			
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	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	
			115					120					125				
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	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	
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	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	
	145					150					155					160	
55	cag	gag	agc	gtg	acc	gag	cag	gac	agc	aag	gac	tcc	acc	tac	agc	ctg	528
	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	
				165						170					175		
60	agc	agc	acc	ctc	acc	ctg	agc	aag	gcc	gac	tac	gag	aag	cac	aag	gtg	576
	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	
				180					185					190			
65	tac	gcc	tgc	gag	gtg	acc	cac	cag	ggc	ctg	agc	agc	ccc	gtg	acc	aag	624
	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val			

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Ser Phe Asn Arg Gly Glu Cys
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<223> Light chain CR4348

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Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
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30

Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
 35 40 45

35

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

40

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

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

45

Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala
 100 105 110

50

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
 115 120 125

55

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

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser

[illegible]

167

[illegible]

168

<400> 36

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15	Thr	Val	Asn 35	Trp	Tyr	Gln	Gln	Leu 40	Pro	Gly	Thr	Ala	Pro 45	Lys	Leu	Leu
20	Ile	Tyr 50	Ser	Asn	Asn	Gln	Arg 55	Pro	Ser	Gly	Ile	Pro 60	Asp	Arg	Phe	Ser
25	Gly 65	Ser	Lys	Ser	Gly	Thr 70	Ser	Ala	Ser	Leu	Ala 75	Ile	Ser	Gly	Leu	Gln 80
30	Ser	Glu	Asp	Glu	Ala 85	Asp	Tyr	Tyr	Cys	Ala 90	Ala	Trp	Asp	Asp	Ser	Leu
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40	Pro	Lys	Ala 115	Ala	Pro	Ser	Val	Thr 120	Leu	Phe	Pro	Pro	Ser 125	Ser	Glu	Glu
45	Leu	Gln 130	Ala	Asn	Lys	Ala	Thr 135	Leu	Val	Cys	Leu	Ile 140	Ser	Asp	Phe	Tyr
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55	Ala	Gly	Val	Glu	Thr 165	Thr	Thr	Pro	Ser	Lys 170	Gln	Ser	Asn	Asn	Lys 175	Tyr
60	Ala	Ala	Ser	Ser	Tyr 180	Leu	Ser	Leu	Thr 185	Pro	Glu	Gln	Trp	Lys 190	Ser	His
65	Arg	Ser	Tyr 195	Ser	Cys	Gln	Val	Thr 200	His	Glu	Gly	Ser	Thr 205	Val	Glu	Lys
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tctttgtctc caggggaaag agccaccctc tctcgaggg ccagtcagag tgtagcagc 300

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171

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173

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<212> DNA

<213> Artificial sequence

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<220>

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<400> 41

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<211> 669

<212> PRT

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<213> West Nile virus

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<400> 42

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 20 25 30

50

Asn Leu Cys Ile Val Arg Ala Met Asp Val Gly Tyr Met Cys Asp Asp
 35 40 45

55

Thr Ile Thr Tyr Glu Cys Pro Val Leu Ser Ala Gly Asn Asp Pro Glu
 50 55 60

174

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10	Val	Gln	Thr	His	Gly	Glu	Ser	Thr	Leu	Ala	Asn	Lys	Lys	Gly	Ala	Trp	
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40	Leu	Glu	Gly	Asp	Ser	Cys	Val	Thr	Ile	Met	Ser	Lys	Asp	Lys	Pro	Thr	
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50	Arg	Ser	Tyr	Cys	Tyr	Leu	Ala	Thr	Val	Ser	Asp	Leu	Ser	Thr	Lys	Ala	
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55	Ala	Cys	Pro	Thr	Met	Gly	Glu	Ala	His	Asn	Asp	Lys	Arg	Ala	Asp	Pro	
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60	Ala	Phe	Val	Cys	Arg	Gln	Gly	Val	Val	Asp	Arg	Gly	Trp	Gly	Asn	Gly	
				260					265					270			
65	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser	Ile	Asp	Thr	Cys	Ala	Lys	Phe	Ala	
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175

290

295

300

5 Tyr Glu Val Ala Ile Phe Val His Gly Pro Thr Thr Val Glu Ser His
 305 310 315 320

10 Gly Asn Tyr Ser Thr Gln Val Gly Ala Thr Gln Ala Gly Arg Phe Ser
 325 330 335

15 Ile Thr Pro Ala Ala Pro Ser Tyr Thr Leu Lys Leu Gly Glu Tyr Gly
 340 345 350

20 Glu Val Thr Val Asp Cys Glu Pro Arg Ser Gly Ile Asp Thr Asn Ala
 355 360 365

25 Tyr Tyr Val Met Thr Val Gly Thr Lys Thr Phe Leu Val His Arg Glu
 370 375 380

30 Trp Phe Met Asp Leu Asn Leu Pro Trp Ser Ser Ala Gly Ser Thr Val
 385 390 395 400

35 Trp Arg Asn Arg Glu Thr Leu Met Glu Phe Glu Glu Pro His Ala Thr
 405 410 415

40 Lys Gln Ser Val Ile Ala Leu Gly Ser Gln Glu Gly Ala Leu His Gln
 420 425 430

45 Ala Leu Ala Gly Ala Ile Pro Val Glu Phe Ser Ser Asn Thr Val Lys
 435 440 445

50 Leu Thr Ser Gly His Leu Lys Cys Arg Val Lys Met Glu Lys Leu Gln
 450 455 460

55 Leu Lys Gly Thr Thr Tyr Gly Val Cys Ser Lys Ala Phe Lys Phe Leu
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60 Gly Thr Pro Ala Asp Thr Gly His Gly Thr Val Val Leu Glu Leu Gln
 485 490 495

65 Tyr Thr Gly Thr Asp Gly Pro Cys Lys Val Pro Ile Ser Ser Val Ala
 500 505 510

70 Ser Leu Asn Asp Leu Thr Pro Val Gly Arg Leu Val Thr Val Asn Pro
 515 520 525

176

5 Phe Val Ser Val Ala Thr Ala Asn Ala Lys Val Leu Ile Glu Leu Glu
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10 Pro Pro Phe Gly Asp Ser Tyr Ile Val Val Gly Arg Gly Glu Gln Gln
 545 550 555 560

15 Ile Asn His His Trp His Lys Ser Gly Ser Ser Ile Gly Lys Ala Phe
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20 Thr Thr Thr Leu Lys Gly Ala Gln Arg Leu Ala Ala Leu Gly Asp Thr
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25 Ala Trp Asp Phe Gly Ser Val Gly Gly Val Phe Thr Ser Val Gly Lys
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30 Ala Val His Gln Val Phe Gly Gly Ala Phe Arg Ser Leu Phe Gly Gly
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181

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185

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204

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207

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208

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210

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					405					410					415	
60	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
				420					425					430		
65	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys
			435					440					445			
70	<210> 61															

211

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5 <213> Artificial sequence

<220>

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<220>

15 <221> CDS

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<400> 61

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

30	tcg	gtg	aag	gtc	tcc	tgc	aag	gct	tct	gga	ggc	acc	ttc	agc	aac	tat	96
	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Gly	Thr	Phe	Ser	Asn	Tyr	
				20					25					30			

35	gct	atc	agc	tgg	gtg	cga	cag	gcc	cct	gga	caa	ggg	ctt	gag	tgg	atg	144
	Ala	Ile	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	
			35					40					45				

40	gga	ggt	atc	gtc	cct	aag	ttt	gct	aca	aca	agc	tac	gca	cag	agg	ttc	192
	Gly	Gly	Ile	Val	Pro	Lys	Phe	Ala	Thr	Thr	Ser	Tyr	Ala	Gln	Arg	Phe	
			50				55					60					

45	cag	ggc	aga	gtc	acg	att	acc	gcg	gac	gaa	tcc	acg	agc	aca	gtc	tac	240
	Gln	Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Glu	Ser	Thr	Ser	Thr	Val	Tyr	
	65					70				75					80		

50	atg	gag	ctg	agc	agc	ctg	aga	tct	gag	gac	acg	gcc	gtg	tat	ttt	tgt	288
	Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Phe	Cys	
				85					90						95		

55	gcg	aga	ttt	gga	gtg	gtg	gta	gct	gcc	gac	cgt	caa	gga	gcc	tac	tac	336
	Ala	Arg	Phe	Gly	Val	Val	Val	Ala	Ala	Asp	Arg	Gln	Gly	Ala	Tyr	Tyr	
				100				105						110			

55	tac	tac	ggt	atg	gac	gtc	tgg	ggc	cag	ggc	acc	acc	gtg	acc	gtc	tcc	384
	Tyr	Tyr	Gly	Met	Asp	Val	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	
			115					120					125				

55	agc	gct	agc	acc	aag	ggc	ccc	agc	gtg	ttc	ccc	ctg	gcc	ccc	agc	agc	432
	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	

212

	130				135				140								
5	aag Lys 145	agc Ser	acc Thr	agc Ser	ggc Gly	ggc Gly 150	aca Thr	gcc Ala	gcc Ala	ctg Leu	ggc Gly 155	tgc Cys	ctg Leu	gtg Val	aag Lys	gac Asp 160	480
10	tac Tyr	ttc Phe	ccc Pro	gag Glu	ccc Pro 165	gtg Val	acc Thr	gtg Val	agc Ser	tgg Trp 170	aac Asn	agc Ser	ggc Gly	gcc Ala	ttg Leu 175	acc Thr	528
	agc Ser	ggc Gly	gtg Val	cac His 180	acc Thr	ttc Phe	ccc Pro	gcc Ala	gtg Val 185	ctg Leu	cag Gln	agc Ser	agc Ser	ggc Gly 190	ctg Leu	tac Tyr	576
15	agc Ser	ctg Leu	agc Ser 195	agc Ser	gtg Val	gtg Val	acc Thr	gtg Val 200	ccc Pro	agc Ser	agc Ser	agc Ser	ctg Leu 205	ggc Gly	acc Thr	cag Gln	624
20	acc Thr 210	tac Tyr	atc Ile	tgc Cys	aac Asn	gtg Val 215	aac Asn	cac His	aag Lys	ccc Pro	agc Ser	aac Asn 220	acc Thr	aag Lys	gtg Val	gac Asp	672
25	aaa Lys 225	cgc Arg	gtg Val	gag Glu	ccc Pro 230	aag Lys	agc Ser	tgc Cys	gac Asp	aag Lys	acc Thr 235	cac His	acc Thr	tgc Cys	ccc Pro 240	ccc Pro	720
30	tgc Cys	cct Pro	gcc Ala	ccc Pro	gag Glu 245	ctg Leu	ctg Leu	ggc Gly	gga Gly	ccc Pro 250	tcc Ser	gtg Val	ttc Phe	ctg Leu	ttc Phe 255	ccc Pro	768
	ccc Pro	aag Lys	ccc Pro	aag Lys 260	gac Asp	acc Thr	ctc Leu	atg Met	atc Ile 265	agc Ser	cgg Arg	acc Thr	ccc Pro	gag Glu 270	gtg Val	acc Thr	816
35	tgc Cys	gtg Val	gtg Val 275	gtg Val	gac Asp	gtg Val	agc Ser	cac His 280	gag Glu	gac Asp	ccc Pro	gag Glu	gtg Val 285	aag Lys	ttc Phe	aac Asn	864
40	tgg Trp 290	tac Tyr	gtg Val	gac Asp	ggc Gly	gtg Val	gag Glu 295	gtg Val	cac His	aac Asn	gcc Ala	aag Lys 300	acc Thr	aag Lys	ccc Pro	cgg Arg	912
45	gag Glu 305	gag Glu	cag Gln	tac Tyr	aac Asn	agc Ser 310	acc Thr	tac Tyr	cgg Arg	gtg Val	gtg Val 315	agc Ser	gtg Val	ctc Leu	acc Thr	gtg Val 320	960
50	ctg Leu	cac His	cag Gln	gac Asp	tgg Trp 325	ctg Leu	aac Asn	ggc Gly	aag Lys	gag Glu 330	tac Tyr	aag Lys	tgc Cys	aag Lys	gtg Val 335	agc Ser	1008
	aac Asn	aag Lys	gcc Ala	ctg Leu 340	cct Pro	gcc Ala	ccc Pro	atc Ile	gag Glu 345	aag Lys	acc Thr	atc Ile	agc Ser	aag Lys 350	gcc Ala	aag Lys	1056
55	ggc Gly	cag Gln	ccc Pro 355	cgg Arg	gag Glu	ccc Pro	cag Gln	gtg Val 360	tac Tyr	acc Thr	ctg Leu	ccc Pro 365	ccc Pro	agc Ser	cgg Arg	gag Glu	1104

213

gag atg acc aag aac cag gtg tcc ctc acc tgt ctg gtg aag ggc ttc 1152
 Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 370 375 380

5

tac ccc agc gac atc gcc gtg gag tgg gag agc aac ggc cag ccc gag 1200
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 385 390 395 400

10

aac aac tac aag acc acc ccc cct gtg ctg gac agc gac ggc agc ttc 1248
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 405 410 415

15

ttc ctg tac agc aag ctc acc gtg gac aag agc cgg tgg cag cag ggc 1296
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 420 425 430

20

aac gtg ttc agc tgc agc gtg atg cac gag gcc ctg cac aac cac tac 1344
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 435 440 445

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acc cag aag agc ctg agc ctg agc ccc ggc aag 1377
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 450 455

<210> 62
 <211> 459
 <212> PRT
 <213> Artificial sequence

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<220>
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<400> 62
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Asn Tyr
 20 25 30

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

55

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

214

	Gln	Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Glu	Ser	Thr	Ser	Thr	Val	Tyr	
	65					70					75					80	
5	Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Phe	Cys	
					85					90					95		
10	Ala	Arg	Phe	Gly	Val	Val	Val	Ala	Ala	Asp	Arg	Gln	Gly	Ala	Tyr	Tyr	
				100					105					110			
15	Tyr	Tyr	Gly	Met	Asp	Val	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	
			115					120					125				
20	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	
		130					135					140					
25	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	
	145					150					155					160	
30	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	
					165					170					175		
35	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	
				180					185					190			
40	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	
			195					200					205				
45	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	
		210					215					220					
50	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	
	225					230					235					240	
55	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	
					245					250					255		
60	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	
				260					265					270			
65	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	
			275					280					285				

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
290 295 300

5 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 305 310 315 320

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
10 325 330 335

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
340 345 350

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
355 360 365

20 Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 370 375 380

25 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 385 390 395 400

30 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
405 410 415

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
420 425 430

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
435 440 445

40 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455

45 <210> 63

<211> 1344

<212> DNA

50 <213> Artificial sequence

55 <220>

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216

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<222> (1) .. (1344)

<223>

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<400> 63

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

	tca	gtg	aag	gtc	tcc	tgc	aag	gct	tct	gga	tac	acc	ttc	act	agc	tat	96
	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	
				20					25					30			

20		gct	atg	cat	tgg	gtg	cgc	cag	gcc	ccc	gga	caa	agg	ctt	gag	tgg	atg	144
		Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Arg	Leu	Glu	Trp	Met	
				35					40					45				

25		gga	tgg	atc	aac	gct	ggc	aat	ggt	aac	aca	aaa	tat	tca	cag	aag	ttc	192
		Gly	Trp	Ile	Asn	Ala	Gly	Asn	Gly	Asn	Thr	Lys	Tyr	Ser	Gln	Lys	Phe	
			50					55					60					

	cag	ggc	aga	gtc	acc	att	acc	agg	gac	aca	tcc	gcg	agc	aca	gcc	tac	240	
30		Gln	Gly	Arg	Val	Thr	Ile	Thr	Arg	Asp	Thr	Ser	Ala	Ser	Thr	Ala	Tyr	
		65					70					75					80	

	atg	gag	ctg	agc	agc	ctg	aga	tct	gaa	gac	acg	gct	gtg	tat	tac	tgt	288	
	Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys		
35					85					90					95			

	gcg	agg	ggg	tac	gac	agc	tgg	tct	ttt	gac	tac	tgg	ggc	cag	ggc	acc	336
	Ala	Arg	Gly	Tyr	Asp	Ser	Trp	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	
				100					105					110			

40		ctg	gtg	acc	gtc	tcc	agc	gct	agc	acc	aag	ggc	ccc	agc	gtg	ttc	ccc	384
		Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	
				115					120					125				

45		ctg	gcc	ccc	agc	agc	aag	agc	acc	agc	ggc	ggc	aca	gcc	gcc	ctg	ggc	432
		Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	
			130					135					140					

	tgc	ctg	gtg	aag	gac	tac	ttc	ccc	gag	ccc	gtg	acc	gtg	agc	tgg	aac	480	
50		Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	
		145				150					155						160	

	agc	ggc	gcc	ttg	acc	agc	ggc	gtg	cac	acc	ttc	ccc	gcc	gtg	ctg	cag	528	
	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln		
55					165					170					175			

	agc	agc	ggc	ctg	tac	agc	ctg	agc	agc	gtg	gtg	acc	gtg	ccc	agc	agc	576
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217

	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	
				180					185					190			
5	agc	ctg	ggc	acc	cag	acc	tac	atc	tgc	aac	gtg	aac	cac	aag	ccc	agc	624
	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	
			195					200					205				
10	aac	acc	aag	gtg	gac	aaa	cgc	gtg	gag	ccc	aag	agc	tgc	gac	aag	acc	672
	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	
			210				215					220					
15	cac	acc	tgc	ccc	ccc	tgc	cct	gcc	ccc	gag	ctg	ctg	ggc	gga	ccc	tcc	720
	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	
						230					235					240	
20	gtg	ttc	ctg	ttc	ccc	ccc	aag	ccc	aag	gac	acc	ctc	atg	atc	agc	cgg	768
	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	
					245					250					255		
25	acc	ccc	gag	gtg	acc	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gag	gac	ccc	816
	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	
				260				265						270			
30	gag	gtg	aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cac	aac	gcc	864
	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	
			275					280					285				
35	aag	acc	aag	ccc	cgg	gag	gag	cag	tac	aac	agc	acc	tac	cgg	gtg	gtg	912
	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	
			290				295					300					
40	agc	gtg	ctc	acc	gtg	ctg	cac	cag	gac	tgg	ctg	aac	ggc	aag	gag	tac	960
	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	
						310					315					320	
45	aag	tgc	aag	gtg	agc	aac	aag	gcc	ctg	cct	gcc	ccc	atc	gag	aag	acc	1008
	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	
					325					330					335		
50	atc	agc	aag	gcc	aag	ggc	cag	ccc	cgg	gag	ccc	cag	gtg	tac	acc	ctg	1056
	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	
				340				345						350			
55	ccc	ccc	agc	cgg	gag	gag	atg	acc	aag	aac	cag	gtg	tcc	ctc	acc	tgt	1104
	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	
				355				360					365				
60	ctg	gtg	aag	ggc	ttc	tac	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	1152
	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	
			370				375					380					
65	aac	ggc	cag	ccc	gag	aac	aac	tac	aag	acc	acc	ccc	cct	gtg	ctg	gac	1200
	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	
						390					395				400		
70	agc	gac	ggc	agc	ttc	ttc	ctg	tac	agc	aag	ctc	acc	gtg	gac	aag	agc	1248
	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	

				405					410					415						
cgg	tgg	cag	cag	ggc	aac	gtg	ttc	agc	tgc	agc	gtg	atg	cac	gag	gcc					1296
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala					
			420					425						430						
ctg	cac	aac	cac	tac	acc	cag	aag	agc	ctg	agc	ctg	agc	ccc	ggc	aag					1344
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys					
		435					440					445								
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Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Met	Lys	Lys	Pro	Gly	Ala					
1				5					10					15						
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr					
			20					25					30							
Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Arg	Leu	Glu	Trp	Met					
		35					40					45								
Gly	Trp	Ile	Asn	Ala	Gly	Asn	Gly	Asn	Thr	Lys	Tyr	Ser	Gln	Lys	Phe					
	50					55					60									
Gln	Gly	Arg	Val	Thr	Ile	Thr	Arg	Asp	Thr	Ser	Ala	Ser	Thr	Ala	Tyr					
65					70					75					80					
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys					
				85					90					95						
Ala	Arg	Gly	Tyr	Asp	Ser	Trp	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr					
			100					105					110							
Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro					
		115					120					125								

219

5	Leu 130	Ala	Pro	Ser	Ser	Lys	Ser 135	Thr	Ser	Gly	Gly	Thr 140	Ala	Ala	Leu	Gly
10	Cys 145	Leu	Val	Lys	Asp	Tyr 150	Phe	Pro	Glu	Pro	Val 155	Thr	Val	Ser	Trp	Asn 160
15	Ser	Ser	Gly	Leu 180	Tyr	Ser	Leu	Ser	Ser 185	Val	Val	Thr	Val	Pro 190	Ser	Ser
20	Ser	Leu	Gly 195	Thr	Gln	Thr	Tyr 200	Ile	Cys	Asn	Val	Asn 205	His	Lys	Pro	Ser
25	Asn 210	Thr	Lys	Val	Asp	Lys	Arg 215	Val	Glu	Pro	Lys	Ser 220	Cys	Asp	Lys	Thr
30	His 225	Thr	Cys	Pro	Pro	Cys 230	Pro	Ala	Pro	Glu	Leu 235	Leu	Gly	Gly	Pro	Ser 240
35	Val	Phe	Leu	Phe 245	Pro	Pro	Lys	Pro	Lys	Asp 250	Thr	Leu	Met	Ile	Ser 255	Arg
40	Thr	Pro	Glu	Val 260	Thr	Cys	Val	Val	Val 265	Asp	Val	Ser	His	Glu 270	Asp	Pro
45	Glu	Val	Lys 275	Phe	Asn	Trp	Tyr 280	Val	Asp	Gly	Val	Glu 285	Val	His	Asn	Ala
50	Lys 290	Thr	Lys	Pro	Arg	Glu 295	Glu	Gln	Tyr	Asn	Ser 300	Thr	Tyr	Arg	Val	Val
55	Ser 305	Val	Leu	Thr	Val	Leu 310	His	Gln	Asp	Trp	Leu 315	Asn	Gly	Lys	Glu	Tyr 320
60	Lys	Cys	Lys	Val	Ser 325	Asn	Lys	Ala	Leu	Pro 330	Ala	Pro	Ile	Glu	Lys 335	Thr
65	Ile	Ser	Lys	Ala 340	Lys	Gly	Gln	Pro	Arg 345	Glu	Pro	Gln	Val	Tyr 350	Thr	Leu

220

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
 355 360 365

5 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
 370 375 380

10 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
 385 390 395 400

15 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
 405 410 415

20 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
 420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

25 <210> 65
 <211> 1347

30 <212> DNA
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35 <220>
 <223> Heavy chain CR4335

40 <220>
 <221> CDS
 <222> (1)..(1347)

45 <223>

50 <400> 65
 cag gtg cag ctg gtg gag tct ggg gga ggc gtg gtc cag cct ggc agg 48
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

55 tcc ctg aga ctc tcc tgt gta gcc tct gga ttc acc ttc agt aag gac 96
 Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Lys Asp
 20 25 30

221

		gct atg cac tgg gtc cgc cag gct cca ggc aag ggg cta gag tgg gtg	144
		Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
		35 40 45	
5		gca gtt ata tca tat gat gga agt gat aaa cac tac gca gac tcc gtg	192
		Ala Val Ile Ser Tyr Asp Gly Ser Asp Lys His Tyr Ala Asp Ser Val	
		50 55 60	
10		aag ggc cga gtc acc atc tcc aga gac aat tcc agg aaa acg ctg cat	240
		Lys Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Arg Lys Thr Leu His	
		65 70 75 80	
15		ctg cga atg gac agc ctg aga gct gag gac acg gct cta tat tac tgt	288
		Leu Arg Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys	
		85 90 95	
20		gcg aga gga tac aac tct ggt cat tac ttt gac tac tgg ggc cag ggc	336
		Ala Arg Gly Tyr Asn Ser Gly His Tyr Phe Asp Tyr Trp Gly Gln Gly	
		100 105 110	
		acc ctg gtg acc gtc tcc agc gct agc acc aag ggc ccc agc gtg ttc	384
		Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe	
		115 120 125	
25		ccc ctg gcc ccc agc agc aag agc acc agc ggc ggc aca gcc gcc ctg	432
		Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu	
		130 135 140	
30		ggc tgc ctg gtg aag gac tac ttc ccc gag ccc gtg acc gtg agc tgg	480
		Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp	
		145 150 155 160	
35		aac agc ggc gcc ttg acc agc ggc gtg cac acc ttc ccc gcc gtg ctg	528
		Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu	
		165 170 175	
40		cag agc agc ggc ctg tac agc ctg agc agc gtg gtg acc gtg ccc agc	576
		Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser	
		180 185 190	
		agc agc ctg ggc acc cag acc tac atc tgc aac gtg aac cac aag ccc	624
		Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro	
		195 200 205	
45		agc aac acc aag gtg gac aaa cgc gtg gag ccc aag agc tgc gac aag	672
		Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys	
		210 215 220	
50		acc cac acc tgc ccc ccc tgc cct gcc ccc gag ctg ctg ggc gga ccc	720
		Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro	
		225 230 235 240	
55		tcc gtg ttc ctg ttc ccc ccc aag ccc aag gac acc ctc atg atc agc	768
		Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser	
		245 250 255	

222

[illegible]

223

<212> PRT

<213> Artificial sequence

5

<220>

<223> Heavy chain CR4335

10

<400> 66

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

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

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

Ala Val Ile Ser Tyr Asp Gly Ser Asp Lys His Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Arg Lys Thr Leu His
 65 70 75 80

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

35

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

40

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
 130 135 140

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

50

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

55

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser

224

180

185

190

5	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	
			195					200					205				
10	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	
		210					215					220					
15	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	
	225					230					235					240	
20	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	
					245					250					255		
25	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	
				260					265					270			
30	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	
			275					280					285				
35	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	
		290					295					300					
40	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	
	305					310					315					320	
45	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	
					325					330					335		
50	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	
				340					345					350			
55	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	
			355					360					365				
60	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	
		370					375					380					
65	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	
	385					390					395					400	
70	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	
					405					410					415		

225

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 420 425 430
 5
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 435 440 445
 10
 Lys
 15 <210> 67
 <211> 1368
 <212> DNA
 20 <213> Artificial sequence
 25 <220>
 <223> Heavy chain CR4383
 <220>
 30 <221> CDS
 <222> (1) .. (1368)
 35 <223>
 <400> 67
 40 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc 48
 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 tca gtg aag gtc tcc tgc aag act tct gga tac acc ttc acc gac tac 96
 45 Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30
 tat ctg cac tgg gtg cga cac gcc cct gga caa ggg ctt gag tgg atg 144
 Tyr Leu His Trp Val Arg His Ala Pro Gly Gln Gly Leu Glu Trp Met
 50 35 40 45
 gga tgg atc aac cct aac agt ggt gac aca aac tat gca cag aag ttt 192
 Gly Trp Ile Asn Pro Asn Ser Gly Asp Thr Asn Tyr Ala Gln Lys Phe
 50 55 60
 55 cag ggc agg gtc acc atg acc agg gac acg tcc atc agc aca gcc tac 240
 Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr

226

	65				70					75					80	
5	atg gac ctg agc agg ctg aga tct gac gac gcg gcc gtg tat tac tgt															288
	Met Asp Leu Ser Arg Leu Arg Ser Asp Asp Ala Ala Val Tyr Tyr Cys															
					85				90					95		
10	gcg aga gat tgg aat acg gtg act acg tac tac tat tac tac tac ggt															336
	Ala Arg Asp Trp Asn Thr Val Thr Thr Tyr Tyr Tyr Tyr Tyr Tyr Gly															
				100				105						110		
15	atg gac gtc tgg ggc caa ggg acc acg gtc acc gtc tcg agt gct agc															384
	Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser															
				115				120					125			
20	acc aag ggc ccc agc gtg ttc ccc ctg gcc ccc agc agc aag agc acc															432
	Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr															
				130				135					140			
25	agc ggc ggc aca gcc gcc ctg ggc tgc ctg gtg aag gac tac ttc ccc															480
	Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro															
																160
				145				150					155			
30	gag ccc gtg acc gtg agc tgg aac agc ggc gcc ttg acc agc ggc gtg															528
	Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val															
					165				170						175	
35	cac acc ttc ccc gcc gtg ctg cag agc agc ggc ctg tac agc ctg agc															576
	His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser															
					180				185						190	
40	agc gtg gtg acc gtg ccc agc agc agc ctg ggc acc cag acc tac atc															624
	Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile															
					195				200				205			
45	tgc aac gtg aac cac aag ccc agc aac acc aag gtg gac aaa cgc gtg															672
	Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val															
					210				215				220			
50	gag ccc aag agc tgc gac aag acc cac acc tgc ccc ccc tgc cct gcc															720
	Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala															
																240
				225				230				235				
55	ccc gag ctg ctg ggc gga ccc tcc gtg ttc ctg ttc ccc ccc aag ccc															768
	Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro															
					245										255	
60	aag gac acc ctc atg atc agc cgg acc ccc gag gtg acc tgc gtg gtg															816
	Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val															
					260				265						270	
65	gtg gac gtg agc cac gag gac ccc gag gtg aag ttc aac tgg tac gtg															864
	Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val															
					275				280					285		
70	gac ggc gtg gag gtg cac aac gcc aag acc aag ccc cgg gag gag cag															912
	Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln															
				290				295					300			

227

	tac aac agc acc tac cgg gtg gtg agc gtg ctc acc gtg ctg cac cag	960
	Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln	
	305 310 315 320	
5	gac tgg ctg aac ggc aag gag tac aag tgc aag gtg agc aac aag gcc	1008
	Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala	
	325 330 335	
10	ctg cct gcc ccc atc gag aag acc atc agc aag gcc aag ggc cag ccc	1056
	Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro	
	340 345 350	
15	cgg gag ccc cag gtg tac acc ctg ccc ccc agc cgg gag gag atg acc	1104
	Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr	
	355 360 365	
20	aag aac cag gtg tcc ctc acc tgt ctg gtg aag ggc ttc tac ccc agc	1152
	Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser	
	370 375 380	
25	gac atc gcc gtg gag tgg gag agc aac ggc cag ccc gag aac aac tac	1200
	Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr	
	385 390 395 400	
30	aag acc acc ccc cct gtg ctg gac agc gac ggc agc ttc ttc ctg tac	1248
	Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr	
	405 410 415	
35	agc aag ctc acc gtg gac aag agc cgg tgg cag cag ggc aac gtg ttc	1296
	Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe	
	420 425 430	
40	agc tgc agc gtg atg cac gag gcc ctg cac aac cac tac acc cag aag	1344
	Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys	
	435 440 445	
45	agc ctg agc ctg agc ccc ggc aag	1368
	Ser Leu Ser Leu Ser Pro Gly Lys	
	450 455	
	<210> 68	
50	<211> 456	
	<212> PRT	
	<213> Artificial sequence	
55	<223> Heavy chain CR4383	
	<400> 68	

228

	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ala	
	1				5					10					15		
5	Ser	Val	Lys	Val	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Asp	Tyr	
				20					25					30			
10	Tyr	Leu	His	Trp	Val	Arg	His	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	
			35					40					45				
15	Gly	Trp	Ile	Asn	Pro	Asn	Ser	Gly	Asp	Thr	Asn	Tyr	Ala	Gln	Lys	Phe	
	50						55					60					
20	Gln	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Ile	Ser	Thr	Ala	Tyr	
	65					70					75					80	
25	Met	Asp	Leu	Ser	Arg	Leu	Arg	Ser	Asp	Asp	Ala	Ala	Val	Tyr	Tyr	Cys	
					85				90					95			
30	Ala	Arg	Asp	Trp	Asn	Thr	Val	Thr	Thr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Gly	
				100				105					110				
35	Met	Asp	Val	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	
			115					120					125				
40	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	
		130					135					140					
45	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	
	145					150					155					160	
50	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	
					165				170						175		
55	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	
				180					185					190			
60	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	
			195					200					205				
65	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	
	210						215					220					

	Glu 225	Pro	Lys	Ser	Cys	Asp 230	Lys	Thr	His	Thr	Cys 235	Pro	Pro	Cys	Pro	Ala 240
5	Pro	Glu	Leu	Leu	Gly 245	Gly	Pro	Ser	Val	Phe 250	Leu	Phe	Pro	Pro	Lys 255	Pro
10	Lys	Asp	Thr	Leu	Met 260	Ile	Ser	Arg	Thr 265	Pro	Glu	Val	Thr	Cys 270	Val	Val
15	Val	Asp	Val 275	Ser	His	Glu	Asp 280	Pro	Glu	Val	Lys	Phe	Asn 285	Trp	Tyr	Val
20	Asp 290	Gly	Val	Glu	Val	His	Asn 295	Ala	Lys	Thr	Lys	Pro 300	Arg	Glu	Glu	Gln
	Tyr 305	Asn	Ser	Thr	Tyr	Arg 310	Val	Val	Ser	Val	Leu 315	Thr	Val	Leu	His	Gln 320
25	Asp	Trp	Leu	Asn	Gly 325	Lys	Glu	Tyr	Lys	Cys 330	Lys	Val	Ser	Asn	Lys 335	Ala
30	Leu	Pro	Ala	Pro 340	Ile	Glu	Lys	Thr	Ile 345	Ser	Lys	Ala	Lys	Gly 350	Gln	Pro
35	Arg	Glu	Pro 355	Gln	Val	Tyr	Thr	Leu 360	Pro	Pro	Ser	Arg	Glu 365	Glu	Met	Thr
40	Lys	Asn 370	Gln	Val	Ser	Leu	Thr 375	Cys	Leu	Val	Lys	Gly 380	Phe	Tyr	Pro	Ser
	Asp 385	Ile	Ala	Val	Glu	Trp 390	Glu	Ser	Asn	Gly	Gln 395	Pro	Glu	Asn	Asn	Tyr 400
45	Lys	Thr	Thr	Pro 405	Pro	Val	Leu	Asp	Ser	Asp 410	Gly	Ser	Phe	Phe	Leu 415	Tyr
50	Ser	Lys	Leu	Thr 420	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn 430	Val	Phe
55	Ser	Cys 435	Ser	Val	Met	His	Glu	Ala 440	Leu	His	Asn	His	Tyr 445	Thr	Gln	Lys
	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys								

230

450

455

<210> 69

5

<211> 669

<212> DNA

10 <213> Artificial sequence

<220>

15

<223> Light chain CR4261

<220>

20 <221> CDS

<222> (1)..(669)

<223>

25

<400> 69

30 tcg acg cag gct gtg ctg act cag ccg tcc tca gcg tct ggg acc ccc 48
 Ser Thr Gln Ala Val Leu Thr Gln Pro Ser Ser Ala Ser Gly Thr Pro
 1 5 10 15

35 ggg cag agg gtc acc atc tct tgt tct gga agc agc ccc aac atc gga 96
 Gly Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Pro Asn Ile Gly
 20 25 30

40 agt aat aat gta aac tgg tac cag cag ctc cca gga acg gcc ccc aaa 144
 Ser Asn Asn Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys
 35 40 45

ctc ctc att tat agt aat aat cag cgg ccc tca ggg gtc cct ggc cga 192
 Leu Leu Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Gly Arg
 50 55 60

45 ttc tct ggc tcc aag tct ggc acc tca gcc tcc ctg gcc atc agt ggg 240
 Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly
 65 70 75 80

50 ctc cag tct gag gat gag gct gat tat tac tgt gca gca tgg gat gac 288
 Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp
 85 90 95

55 ggc cta agt ggt aat tat gtc ttc gga gct ggg acc cag ctc acc gtt 336
 Gly Leu Ser Gly Asn Tyr Val Phe Gly Ala Gly Thr Gln Leu Thr Val
 100 105 110

tta agt gcg gcc gca ggc cag ccc aag gcc gct ccc agc gtg acc ctg 384

231

Leu Ser Ala Ala Ala Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu
 115 120 125

5 ttc ccc ccc tcc tcc gag gag ctg cag gcc aac aag gcc acc ctg gtg 432
 Phe Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val
 130 135 140

10 tgc ctc atc agc gac ttc tac cct ggc gcc gtg acc gtg gcc tgg aag 480
 Cys Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys
 145 150 155 160

15 gcc gac agc agc ccc gtg aag gcc ggc gtg gag acc acc acc ccc agc 528
 Ala Asp Ser Ser Pro Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser
 165 170 175

aag cag agc aac aac aag tac gcc gcc agc agc tac ctg agc ctc acc 576
 Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr
 180 185 190

20 ccc gag cag tgg aag agc cac cgg agc tac agc tgc cag gtg acc cac 624
 Pro Glu Gln Trp Lys Ser His Arg Ser Tyr Ser Cys Gln Val Thr His
 195 200 205

25 gag ggc agc acc gtg gag aag acc gtg gcc ccc acc gag tgc agc 669
 Glu Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
 210 215 220

30 <210> 70
 <211> 223
 <212> PRT

35 <213> Artificial sequence

40 <220>
 <223> Light chain CR4261
 <400> 70

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

50 Gly Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Pro Asn Ile Gly
 20 25 30

55 Ser Asn Asn Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys
 35 40 45

Leu Leu Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Gly Arg

232

50

55

60

5 Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly
 65 70 75 80

10 Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp
 85 90 95

15 Gly Leu Ser Gly Asn Tyr Val Phe Gly Ala Gly Thr Gln Leu Thr Val
 100 105 110

20 Leu Ser Ala Ala Ala Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu
 115 120 125

25 Phe Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val
 130 135 140

30 Cys Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys
 145 150 155 160

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

40 Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr
 180 185 190

45 Pro Glu Gln Trp Lys Ser His Arg Ser Tyr Ser Cys Gln Val Thr His
 195 200 205

50 Glu Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
 210 215 220

<210> 71

<211> 648

<212> DNA

<213> Artificial sequence

<220>

<223> Light chain CR4267

233

<220>

<221> CDS

5 <222> (1) .. (648)

<223>

10

<400> 71

cag	tcc	gtg	ctg	acc	cag	cct	ccc	tca	gcg	tct	ggg	acc	ccc	ggg	cag	48
Gln	Ser	Val	Leu	Thr	Gln	Pro	Pro	Ser	Ala	Ser	Gly	Thr	Pro	Gly	Gln	
1				5					10					15		

15

agg	gtc	acc	atc	tct	tgt	tct	gga	agc	agc	tcc	aac	atc	gga	agt	aat	96
Arg	Val	Thr	Ile	Ser	Cys	Ser	Gly	Ser	Ser	Ser	Asn	Ile	Gly	Ser	Asn	
			20					25					30			

20

act	gta	aac	tgg	tac	cag	cag	ctc	cca	gga	acg	gcc	ccc	aaa	ctc	ctc	144
Thr	Val	Asn	Trp	Tyr	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu	
		35					40					45				

25

atc	tat	agt	aat	aat	cag	cgg	ccc	tca	ggg	gtc	cct	gac	cga	ttc	tct	192
Ile	Tyr	Ser	Asn	Asn	Gln	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	
	50					55					60					

30

ggc	tcc	aag	tct	ggc	acc	tca	gcc	tcc	ctg	gcc	atc	agt	ggg	ctc	cag	240
Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Ser	Leu	Ala	Ile	Ser	Gly	Leu	Gln	
65					70					75					80	

35

tct	gag	gat	gag	gct	gat	tat	tat	tgt	gca	gct	tgg	gat	gac	acc	ctg	288
Ser	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Ala	Ala	Trp	Asp	Asp	Thr	Leu	
				85					90					95		

aat	ggt	tat	gtc	ttc	gga	act	ggc	acc	aag	ctt	acc	gtg	ctg	ggc	cag	336
Asn	Gly	Tyr	Val	Phe	Gly	Thr	Gly	Thr	Lys	Leu	Thr	Val	Leu	Gly	Gln	
			100					105					110			

40

ccc	aag	gcc	gct	ccc	agc	gtg	acc	ctg	ttc	ccc	ccc	tcc	tcc	gag	gag	384
Pro	Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	Pro	Pro	Ser	Ser	Glu	Glu	
		115					120					125				

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ctg	cag	gcc	aac	aag	gcc	acc	ctg	gtg	tgc	ctc	atc	agc	gac	ttc	tac	432
Leu	Gln	Ala	Asn	Lys	Ala	Thr	Leu	Val	Cys	Leu	Ile	Ser	Asp	Phe	Tyr	
	130					135					140					

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cct	ggc	gcc	gtg	acc	gtg	gcc	tgg	aag	gcc	gac	agc	agc	ccc	gtg	aag	480
Pro	Gly	Ala	Val	Thr	Val	Ala	Trp	Lys	Ala	Asp	Ser	Ser	Pro	Val	Lys	
145					150					155					160	

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gcc	ggc	gtg	gag	acc	acc	acc	ccc	agc	aag	cag	agc	aac	aac	aag	tac	528
Ala	Gly	Val	Glu	Thr	Thr	Thr	Pro	Ser	Lys	Gln	Ser	Asn	Asn	Lys	Tyr	
				165					170					175		

gcc	gcc	agc	agc	tac	ctg	agc	ctc	acc	ccc	gag	cag	tgg	aag	agc	cac	576
Ala	Ala	Ser	Ser	Tyr	Leu	Ser	Leu	Thr	Pro	Glu	Gln	Trp	Lys	Ser	His	

234

		180		185		190		
	cgg agc tac agc tgc cag gtg acc cac gag ggc agc acc gtg gag aag							624
5	Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys	195		200		205		
	acc gtg gcc ccc acc gag tgc agc							648
	Thr Val Ala Pro Thr Glu Cys Ser	210		215				
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	Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn							
	20 25 30							
35	Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu							
	35 40 45							
40	Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser							
	50 55 60							
	Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln							
45	65 70 75 80							
	Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Thr Leu							
	85 90 95							
50	Asn Gly Tyr Val Phe Gly Thr Gly Thr Lys Leu Thr Val Leu Gly Gln							
	100 105 110							
55	Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu							
	115 120 125							

235

5 Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
 130 135 140

10 Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
 145 150 155 160

15 Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
 165 170 175

20 Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
 195 200 205

25 Thr Val Ala Pro Thr Glu Cys Ser
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 1 5 10 15

55 ggg cag agg gtc acc atc tct tgt tct gga agc agc tcc aac atc gga 96
 Gly Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly

236

[illegible]

237

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20 25 30

20 Ser Asn Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys
35 40 45

25 Leu Leu Ile Tyr Gly Asn Asn Asn Arg Pro Ser Gly Val Pro Ala Arg
50 55 60

30 Phe Ser Gly Ser Arg Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly
65 70 75 80

35 Leu Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp
85 90 95

40 Gly Ala Ala Ala Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe
115 120 125

45 Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys
130 135 140

50 Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala
145 150 155 160

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

60 Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro
180 185 190

238

Glu Gln Trp Lys Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu
 195 200 205

5 Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
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40 ggg cag agg gtc acc atc tct tgt tct gga agc gac tcc aac atc ggc 96
 Gly Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Asp Ser Asn Ile Gly
 20 25 30

45 agt aat act gta aac tgg tac cag cag ctc cca gga atg gcc ccc aaa 144
 Ser Asn Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Met Ala Pro Lys
 35 40 45

50 ctc ctc atc tat agg aat aat cag cgg ccc tca ggg gtc cct gac cga 192
 Leu Leu Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg
 50 55 60

55 ttc tct ggc tcc aag tct ggc acc tca gcc tcc ctg gcc atc agt ggt 240
 Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly
 65 70 75 80

55 ctc cag tct gaa gat gag gct gac tat ttc tgt gca tca tgg gat gcc 288
 Leu Gln Ser Glu Asp Glu Ala Asp Tyr Phe Cys Ala Ser Trp Asp Ala
 85 90 95

239

	aat ctg ggt ggt ccg ctg ttc ggt ggg ggg acc aag gtc acc gtc cta	336
	Asn Leu Gly Gly Pro Leu Phe Gly Gly Gly Thr Lys Val Thr Val Leu	
	100 105 110	
5	ggt gcg gcc gca ggc cag ccc aag gcc gct ccc agc gtg acc ctg ttc	384
	Gly Ala Ala Ala Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe	
	115 120 125	
10	ccc ccc tcc tcc gag gag ctg cag gcc aac aag gcc acc ctg gtg tgc	432
	Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys	
	130 135 140	
15	ctc atc agc gac ttc tac cct ggc gcc gtg acc gtg gcc tgg aag gcc	480
	Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala	
	145 150 155 160	
20	gac agc agc ccc gtg aag gcc ggc gtg gag acc acc acc ccc agc aag	528
	Asp Ser Ser Pro Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys	
	165 170 175	
25	cag agc aac aac aag tac gcc gcc agc agc tac ctg agc ctc acc ccc	576
	Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro	
	180 185 190	
30	gag cag tgg aag agc cac cgg agc tac agc tgc cag gtg acc cac gag	624
	Glu Gln Trp Lys Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu	
	195 200 205	
35	ggc agc acc gtg gag aag acc gtg gcc ccc acc gag tgc agc	666
	Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr Glu Cys Ser	
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	20 25 30	

240

Ser Asn Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Met Ala Pro Lys
 35 40 45

5 Leu Leu Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg
 50 55 60

10 Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly
 65 70 75 80

15 Leu Gln Ser Glu Asp Glu Ala Asp Tyr Phe Cys Ala Ser Trp Asp Ala
 85 90 95

20 Asn Leu Gly Gly Pro Leu Phe Gly Gly Gly Thr Lys Val Thr Val Leu
 100 105 110

Gly Ala Ala Ala Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe
 115 120 125

25 Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys
 130 135 140

30 Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala
 145 150 155 160

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

40 Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro
 180 185 190

Glu Gln Trp Lys Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu
 195 200 205

45 Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
 210 215 220

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20	1				5					10					15		

	ggg	cag	agg	gtc	acc	atc	tct	tgt	tct	gga	ggc	atc	tcc	aac	atc	gga	96
	Gly	Gln	Arg	Val	Thr	Ile	Ser	Cys	Ser	Gly	Gly	Ile	Ser	Asn	Ile	Gly	
				20					25					30			

25	agt	aat	agt	gta	aat	tgg	ttc	cag	caa	ctc	cca	gga	acg	gcc	ccc	aaa	144
	Ser	Asn	Ser	Val	Asn	Trp	Phe	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	
			35				40						45				

30	ctc	ctc	ctc	tac	agt	aat	aat	cag	cgg	gcc	tca	ggg	gtc	cct	gac	cga	192
	Leu	Leu	Leu	Tyr	Ser	Asn	Asn	Gln	Arg	Ala	Ser	Gly	Val	Pro	Asp	Arg	
			50				55					60					

35	ttc	tct	ggc	tcc	aag	tct	ggc	acc	tca	gcc	tcc	ctg	gcc	atc	agt	ggg	240
	Phe	Ser	Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Ser	Leu	Ala	Ile	Ser	Gly	
	65				70					75				80			

40	ctc	cag	tct	gag	gat	gag	gtt	gat	tat	tac	tgt	gca	gca	tgg	gat	gac	288
	Leu	Gln	Ser	Glu	Asp	Glu	Val	Asp	Tyr	Tyr	Cys	Ala	Ala	Trp	Asp	Asp	
				85					90						95		

	cgc	ctg	att	ggg	tat	gtc	ttc	gga	acg	ggg	acc	aag	gtc	acc	gtc	cta	336
	Arg	Leu	Ile	Gly	Tyr	Val	Phe	Gly	Thr	Gly	Thr	Lys	Val	Thr	Val	Leu	
				100				105						110			

45	ggg	gcg	gcc	gca	ggc	cag	ccc	aag	gcc	gct	ccc	agc	gtg	acc	ctg	ttc	384
	Gly	Ala	Ala	Ala	Gly	Gln	Pro	Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	
			115				120						125				

50	ccc	ccc	tcc	tcc	gag	gag	ctg	cag	gcc	aac	aag	gcc	acc	ctg	gtg	tgc	432
	Pro	Pro	Ser	Ser	Glu	Glu	Leu	Gln	Ala	Asn	Lys	Ala	Thr	Leu	Val	Cys	
			130				135					140					

55	ctc	atc	agc	gac	ttc	tac	cct	ggc	gcc	gtg	acc	gtg	gcc	tgg	aag	gcc	480
	Leu	Ile	Ser	Asp	Phe	Tyr	Pro	Gly	Ala	Val	Thr	Val	Ala	Trp	Lys	Ala	
	145				150					155						160	

242

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

5 gag agc aac aac aag tac gcc gcc agc agc tac ctg agc ctc acc ccc 576
 Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro
 180 185 190

10 gag cag tgg aag agc cac cgg agc tac agc tgc cag gtg acc cac gag 624
 Glu Gln Trp Lys Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu
 195 200 205

15 ggc agc acc gtg gag aag acc gtg gcc ccc acc gag tgc agc 666
 Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
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 20 25 30

Ser Asn Ser Val Asn Trp Phe Gln Gln Leu Pro Gly Thr Ala Pro Lys
 35 40 45

45 Leu Leu Leu Tyr Ser Asn Asn Gln Arg Ala Ser Gly Val Pro Asp Arg
 50 55 60

50 Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly
 65 70 75 80

55 Leu Gln Ser Glu Asp Glu Val Asp Tyr Tyr Cys Ala Ala Trp Asp Asp
 85 90 95

243

Arg Leu Ile Gly Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu
 100 105 110

5 Gly Ala Ala Ala Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe
 115 120 125

10 Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys
 130 135 140

15 Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala
 145 150 155 160

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

20 Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro
 180 185 190

25 Glu Gln Trp Lys Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu
 195 200 205

30 Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
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41

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257

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272

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45	Cys	Val	Pro	Asp	Gln	Asp	Thr	Ala	Ile	Arg	Val	Phe	Ala	Ile	Pro	Pro	
				340					345					350			
50	Val	Thr	Asp	Leu	Thr	Thr	Tyr	Asp	Ser	Val	Thr	Ile	Ser	Trp	Thr	Arg	
		370					375					380					
55	Gln	Asn	Gly	Glu	Ala	Val	Lys	Thr	His	Thr	Asn	Ile	Ser	Glu	Ser	His	
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273

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 405 410 415

5 Asp Trp Asn Ser Gly Glu Arg Phe Thr Cys Thr Val Thr His Thr Asp
 420 425 430

10 Leu Pro Ser Pro Leu Lys Gln Thr Ile Ser Arg Pro Lys Gly Val Ala
 435 440 445

15 Leu His Arg Pro Asp Val Tyr Leu Leu Pro Pro Ala Arg Glu Gln Leu
 450 455 460

Asn Leu Arg Glu Ser Ala Thr Ile Thr Cys Leu Val Thr Gly Phe Ser
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20 Pro Ala Asp Val Phe Val Gln Trp Met Gln Arg Gly Gln Pro Leu Ser
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25 Pro Glu Lys Tyr Val Thr Ser Ala Pro Met Pro Glu Pro Gln Ala Pro
 500 505 510

30 Gly Arg Tyr Phe Ala His Ser Ile Leu Thr Val Ser Glu Glu Glu Trp
 515 520 525

35 Asn Thr Gly Glu Thr Tyr Thr Cys Val Val Ala His Glu Ala Leu Pro
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45

CLAIMS

1. A binding molecule capable of specifically binding to an intracellular host cell protein, which can be incorporated
5 into a viral membrane, characterized in that the binding molecule has virus-neutralizing activity.
2. A binding molecule according to claim 1, characterized in that the viruses are enveloped viruses.
- 10 3. A binding molecule according to claim 1 or 2, characterized in that the binding molecule has virus neutralizing activity against viruses of at least two different genera.
- 15 4. A binding molecule according to any of the claims 1-3, characterized in that the intracellular host cell protein is displayed on the surface of the host cell after infection of the host cell with a virus.
- 20 5. A binding molecule according to any of the claims 1-4, characterized in that the binding molecule has flavivirus neutralizing activity.
- 25 6. A binding molecule according to any of the claims 1-5, characterized in that the binding molecule has WNV neutralizing activity.
- 30 7. A binding molecule according to any of the claims 1-6, characterized in that the intracellular host cell protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.

8. A binding molecule capable of specifically binding to a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.

5

9. A binding molecule according to claim 8, characterized in that the binding molecule has virus neutralizing activity.

10. A binding molecule according to claim 9, characterized in that the binding molecule has virus neutralizing activity against viruses of at least two different genera.

11. A binding molecule according to any of the claims 1-10, characterized in that the binding molecule is a human monoclonal antibody.

12. A binding molecule according to any of the claims 1-11, characterized in that the binding molecule comprises at least a heavy chain CDR3 region comprising the amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:6.

13. A binding molecule according to claim 12, characterized in that the binding molecule further comprises at least a heavy chain CDR1 region comprising the amino acid sequence selected from the group consisting of SEQ ID NO:7 and SEQ ID NO:12, and/or a heavy chain CDR2 region comprising the amino acid sequence selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:13.

30

14. A functional variant of a binding molecule according to any claim 12 or 13, characterized in that the functional variant

is capable of competing for specifically binding to the host cell protein and has virus neutralizing activity against viruses of at least two different genera.

5 15. An immunoconjugate comprising a binding molecule according to any of the claims 1-13 or a functional variant according to claim 14, the immunoconjugate further comprising at least one tag.

10 16. A nucleic acid molecule encoding a binding molecule according to any one of the claims 1-13 or a functional variant according to claim 14.

17. A vector comprising at least one nucleic acid molecule
15 according to claim 16.

18. A host comprising at least one vector according to claim 17.

20 19. A host according to claim 18, wherein the host is a cell derived from a human cell.

20. A method of producing a binding molecule according to any one of the claims 1-13 or a functional variant according to
25 claim 14, wherein the method comprises the steps of:

a) culturing a host according to claim 18 or 19 under conditions conducive to the expression of the binding molecule or functional variant, and optionally,

30 b) recovering the expressed binding molecule or functional variant.

21. A binding molecule or functional variant thereof as obtainable by the method according to claim 20.

22. A composition comprising a binding molecule according to
5 any of the claims 1-13, a functional variant according to claim 14, an immunoconjugate according to claim 15, or a binding molecule or functional variant thereof according to claim 21.

10 23. A composition comprising a nucleic acid molecule according to claim 16.

24. A pharmaceutical composition comprising a binding molecule according to any one of the claims 1-13, a functional
15 variant according to claim 14, an immunoconjugate according to claim 15, a binding molecule or functional variant thereof according to claim 21, or a composition according to claim 22 or 23, the pharmaceutical composition further comprising at least one pharmaceutically acceptable
20 excipient.

25. A pharmaceutical composition according to claim 24 further comprising at least one other therapeutic agent.

25 26. A binding molecule according to any one of the claims 1-13, a functional variant according to claim 14, an immunoconjugate according to claim 15, a binding molecule or functional variant thereof according to claim 21, a composition according to claim 22 or 23, or a pharmaceutical
30 composition according to claim 24 or 25 for use as a medicament.

27. A binding molecule according to any one of the claims 1-13, a functional variant according to claim 14, an immunoconjugate according to claim 15, a binding molecule or functional variant thereof according to claim 21, a composition according to claim 22 or 23, or a pharmaceutical composition according to claim 24 or 25 for use in the diagnosis, prophylaxis, treatment, or combination thereof, of a viral infection.

10 28. Use of a binding molecule according to any one of the claims 1-13, a functional variant according to claim 14, an immunoconjugate according to claim 15, a binding molecule or functional variant thereof according to claim 21, a composition according to claim 22 or 23, or a pharmaceutical composition according to claim 24 or 25 in the preparation of a medicament for the diagnosis, prophylaxis, treatment, or combination thereof, of a viral infection.

29. Use according to claim 28, characterized in that the viral infection is WNV.

30. A kit comprising a binding molecule according to any one of the claims 1-13, a functional variant according to claim 14, an immunoconjugate according to claim 15, a binding molecule or functional variant thereof according to claim 21, a composition according to claim 22 or 23, or a pharmaceutical composition according to claim 24 or 25, or a combination thereof.

31. A method of identifying a binding molecule specifically binding to a host cell protein or a nucleic acid molecule

encoding a binding molecule specifically binding to a host cell protein, wherein the method comprises the steps of:

- a) contacting a collection of binding molecules on the surface of replicable genetic packages with a virus, virus-like particle or fragment thereof under conditions conducive to binding,
- b) selecting at least once for a replicable genetic package binding to the virus, virus-like particle or fragment thereof, and
- c) separating and recovering the replicable genetic package binding to the virus, virus-like particle or fragment thereof from replicable genetic packages that do not bind.

32. A method of obtaining a binding molecule specifically binding to a host cell protein or a nucleic acid molecule encoding a binding molecule specifically binding to a host cell protein, wherein the method comprises the steps of:

- a) performing the method according to claim 31, and
- b) isolating from the recovered replicable genetic package the binding molecule and/or the nucleic acid molecule encoding the binding molecule.

33. A method of obtaining a binding molecule potentially having virus neutralizing activity and being capable of specifically binding to a host cell protein, wherein the method comprises the steps of:

- a) performing the method according to claim 32, and
- b) verifying if the binding molecule isolated has virus neutralizing activity.

34. A method according to any one of claims 31-33,
characterized in that the replicable genetic package is
selected from the group consisting of a phage particle, a
bacterium, a yeast, a fungus, a spore of a microorganism and
5 a ribosome.

35. A method according to any one of claims 31-34,
characterized in that the virus is a flavivirus.

10 36. A method according to claim 35, characterized in that the
virus is WNV.

37. A method according to any one of claims 31-36,
characterized in that the virus, virus-like particle or
15 fragment thereof comprises the host cell protein.

38. A method according to any one of claims 31-37,
characterized in that the collection of binding molecules on
the surface of replicable genetic packages is a scFv phage
20 display library.

39. An eukaryotic leader peptide, characterized in that the
peptide comprises an amino acid sequence selected from the
group consisting of SEQ ID NO:150, SEQ ID NO:152 and SEQ ID
25 NO:154.

40. A recombinant polypeptide comprising a eukaryotic leader
peptide according to claim 39 and a polypeptide of interest.

30 41. A recombinant polypeptide according to claim 40,
characterized in that the polypeptide of interest is
selected from the group consisting of an immunoglobulin

heavy chain, an immunoglobulin light chain, an immunoglobulin heavy chain fragment and an immunoglobulin light chain fragment.

5 42. A recombinant polypeptide according to claim 40 or 41, characterized in that the carboxy terminus of the eukaryotic leader peptide is joined to the amino terminus of the polypeptide of interest.

10 43. A nucleic acid sequence encoding an eukaryotic leader peptide according to claim 39, characterized in that the nucleic acid sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:149, SEQ ID NO:151 and SEQ ID NO:153.

15 44. A nucleic acid sequence according to claim 43, characterized in that the nucleic acid sequence further comprising a nucleic acid sequence encoding a polypeptide of interest.

20 45. A nucleic acid sequence according to claim 44, characterized in that the polypeptide of interest is selected from the group consisting of an immunoglobulin heavy chain, an immunoglobulin light chain, an immunoglobulin heavy chain fragment and an immunoglobulin light chain fragment.

25

46. An expression vector comprising a nucleic acid sequence according to any one of the claims 43-45.

30 47. An expression vector according to claim 46, characterized in that the vector further comprises downstream of the

nucleic acid sequence encoding the eukaryotic leader peptide
a XhoI-site or a NotI-site.

48. An expression vector according to claim 46 or 47,
5 characterized in that the vector further comprises a
promoter sequence and a polyadenylation signal sequence.

49. A host cell comprising at least an expression vector
according to any one of the claims 46-48.

10

50. A host cell according to claim 49, characterized in that
the host cell is a human host cell.

15

51. Use of an expression vector according to any one of the
claims 46-48 and/or a host cell according to claim 49 or 50
for the production of an immunoglobulin molecule.

20

52. An expression vector comprising a nucleic acid sequence
selected from the group consisting of SEQ ID NO:155, SEQ ID
NO:156 and SEQ ID NO:157.

25

53. An expression vector according to claim 52, characterized
in that the vector further comprises downstream of the
nucleic acid sequence selected from the group consisting of
SEQ ID NO:155, SEQ ID NO:156 and SEQ ID NO:157 a XhoI-site
or a NotI-site.

30

54. An expression vector according to claim 52 or 53,
characterized in that the vector further comprises a stuffer
sequence between the nucleic acid sequence selected from the
group consisting of SEQ ID NO:155, SEQ ID NO:156 and SEQ ID
NO:157 and the XhoI-site or NotI-site.

55. An expression vector according to any one of claims 52-54, characterized in that the vector comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:53,
5 SEQ ID NO:54 and SEQ ID NO:58.

56. A method for producing an expression vector for producing an immunoglobulin heavy chain, the method comprising the steps of:

- 10 a. digesting an expression vector comprising the nucleic acid sequence of SEQ ID NO:155 and downstream thereof an XhoI-site and an immunoglobulin heavy chain constant region with the restriction enzymes SfiI and XhoI,
b. digesting a phage display vector comprising an
15 immunoglobulin heavy chain variable region with the restriction enzymes SfiI and XhoI,
c. inserting the immunoglobulin heavy chain variable region into the expression vector, and
d. isolating the expression vector.

20

57. A method for producing an expression vector for producing an immunoglobulin light chain, the method comprising the steps of:

- 25 a. digesting an expression vector comprising the nucleic acid sequence of SEQ ID NO:156 or SEQ ID NO:157 and downstream thereof a NotI-site and an immunoglobulin light chain constant region with the restriction enzymes XhoI and NotI,
b. digesting a phage display vector comprising an
30 immunoglobulin light chain variable region with the restriction enzymes SalI and NotI, and

- c. inserting the immunoglobulin light chain variable region into the expression vector, and
- d. isolating the expression vector.

5 58. A method for producing an immunoglobulin heavy or light chain, the method comprising the steps of:

a. transforming at least one host cell with an expression vector according to claim 56 or 57,

10 b. culturing the host cell under conditions conducive to the expression of an immunoglobulin chain, and

c. optionally, purifying the immunoglobulin chain from the medium or cellular extract.

15

20

1/5

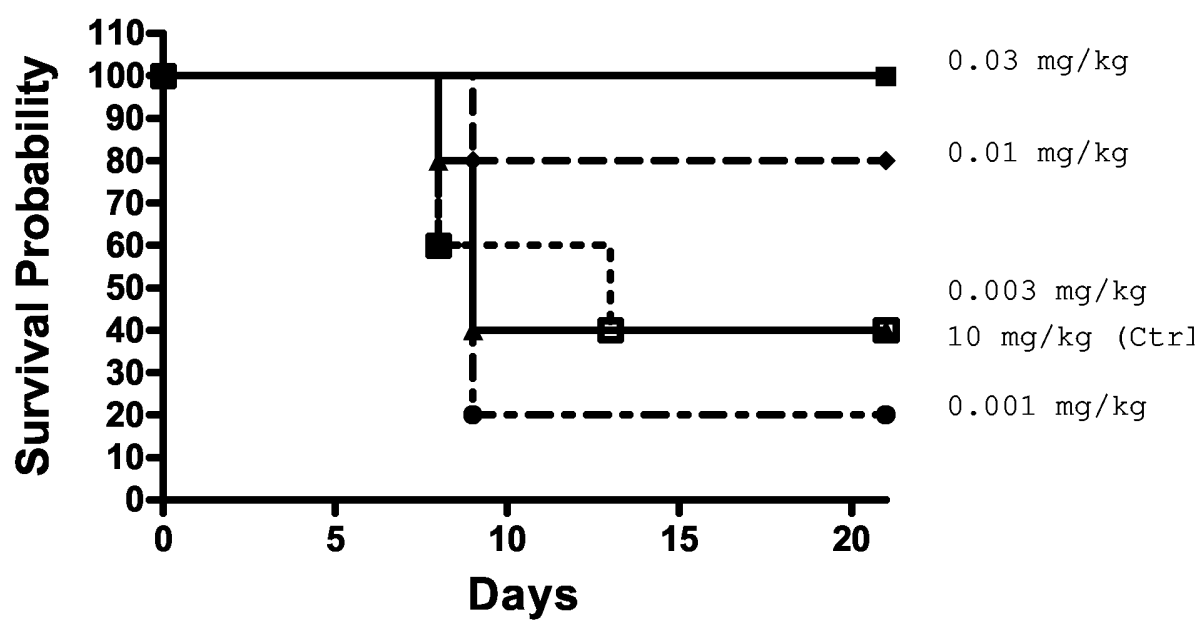


FIGURE 1

2/5

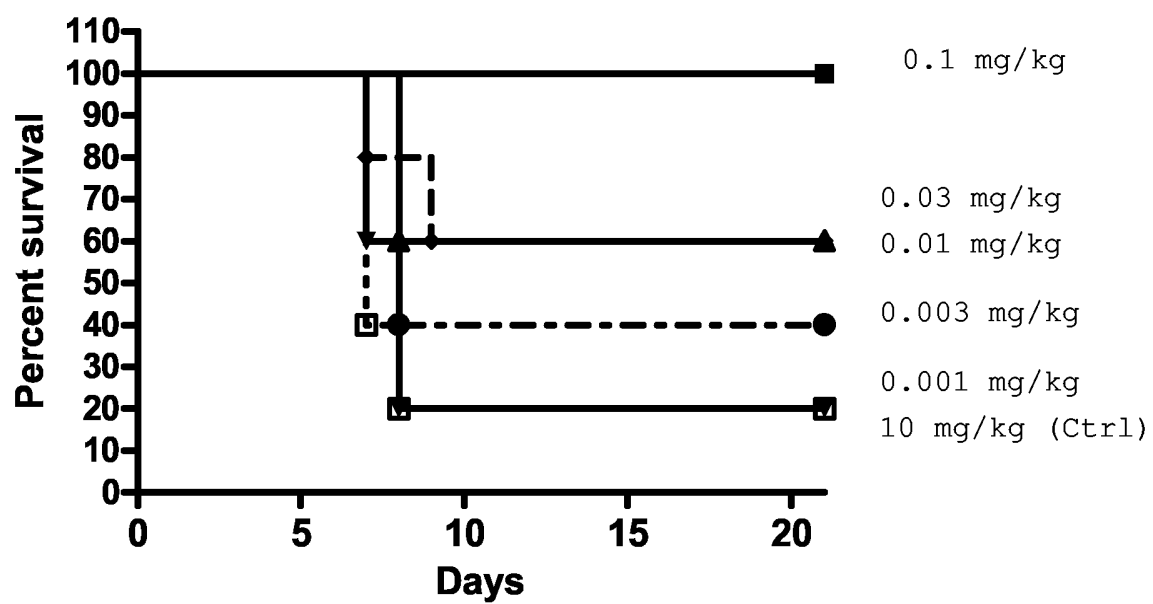


FIGURE 2

3/5

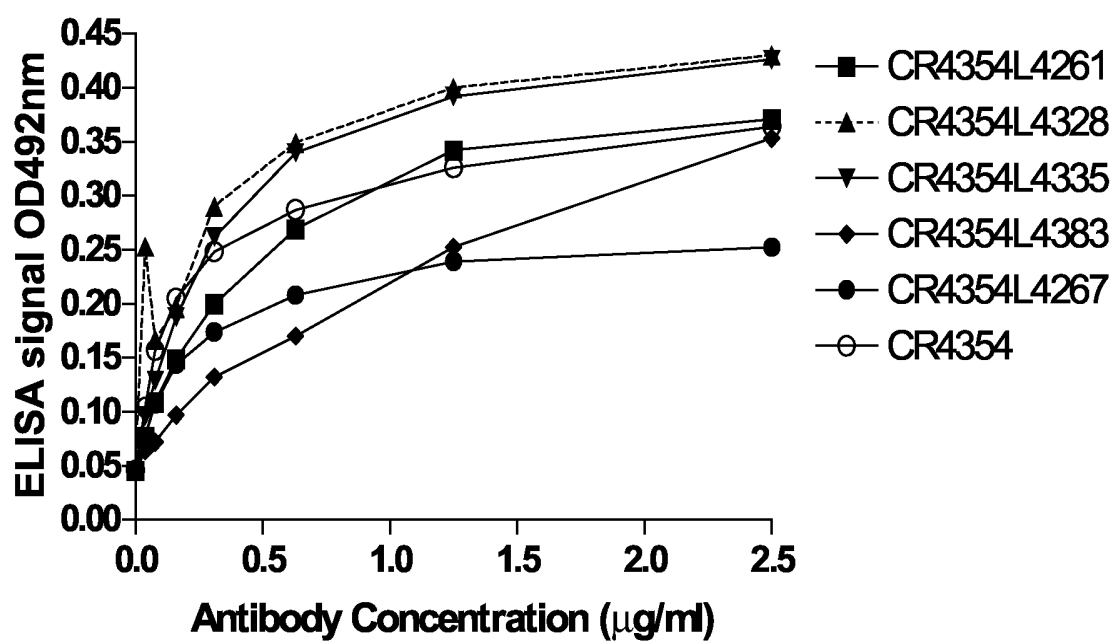


FIGURE 3

4/5

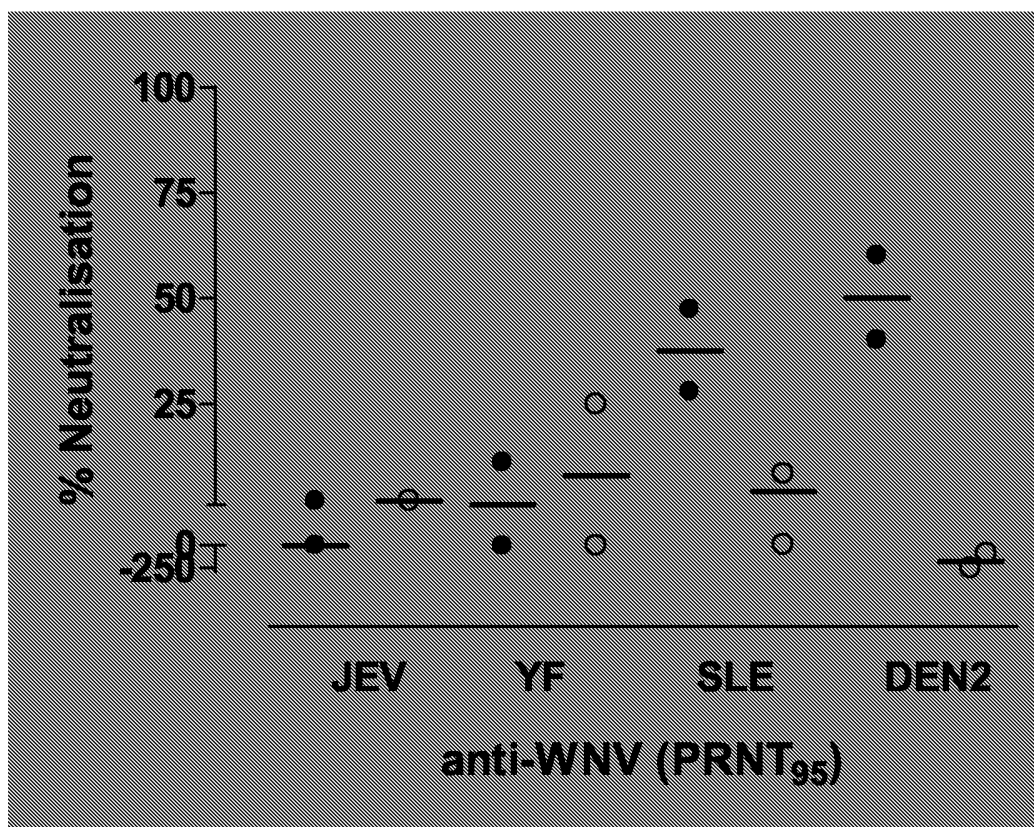
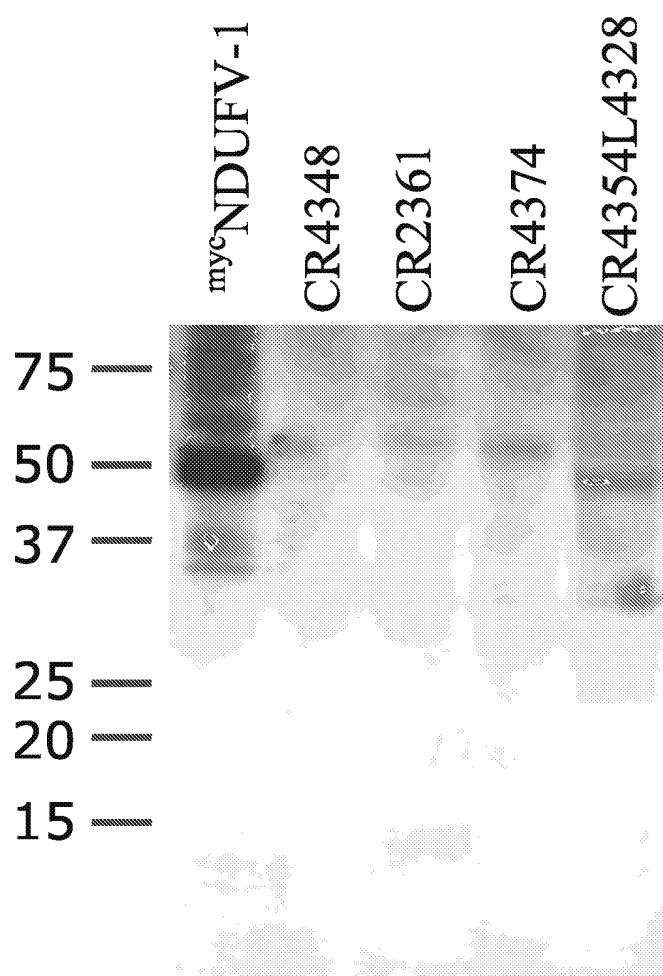


FIGURE 4

5/5

**FIGURE 5**