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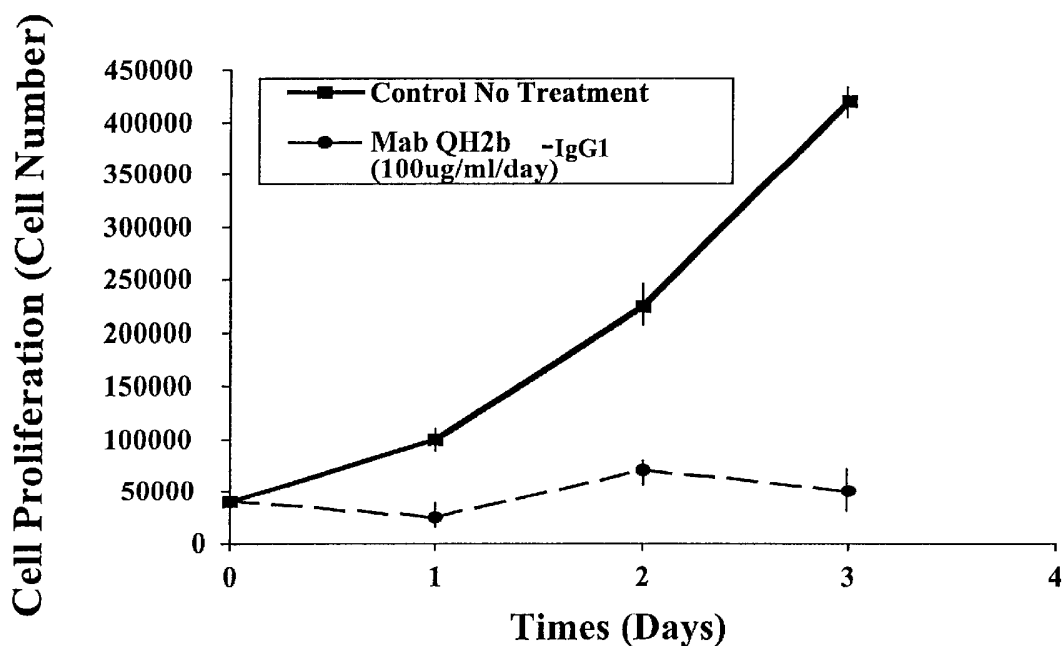
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(54) Title: HUMANIZED COLLAGEN ANTIBODIES AND RELATED METHODS



(57) Abstract: The invention provides a grafted antibody, or functional fragment thereof, comprising one or more complementarily determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR, where the grafted antibody or functional fragment thereof has specific binding activity for a cryptic collagen epitope. The invention also provides methods of using an antibody having specific binding activity for a cryptic collagen epitope, including methods of inhibiting angiogenesis, tumor growth, and metastasis.



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HUMANIZED COLLAGEN ANTIBODIES AND RELATED METHODS

BACKGROUND OF THE INVENTION

The present invention relates generally to immunology and more specifically to humanized
5 antibodies and uses thereof.

The extracellular matrix (ECM) plays a fundamental role in the regulation of normal and pathological processes. The most abundantly expressed component found in the ECM is collagen. Triple helical
10 collagen is known to be highly resistant to proteolytic cleavage except by members of the matrix metalloproteinase (MMP) family of enzymes.

Angiogenesis and tumor growth depend on cellular interactions with the extracellular matrix.
15 During angiogenesis and tumor invasion, both endothelial cells as well as tumor cells proteolytically remodel their extracellular microenvironment. The invasive cells then interact with this newly remodeled extracellular matrix followed
20 by migration and invasion. To this end, a major component of the basement membrane surrounding blood vessels is collagen-IV. Moreover, collagen-I is the major component of the interstitial matrix.

One of the major detrimental consequences of
25 the progression of cancer is metastasis beyond the site of the primary tumor. Such metastasis often requires more aggressive therapies, and once metastasis has occurred, the prognosis for survival of a cancer patient decreases dramatically.

The growth of all solid tumors requires new blood vessel growth for continued expansion of the tumors, particularly beyond a minimal size. Because angiogenesis is required for tumor growth, inhibiting
5 angiogenesis is one approach to inhibiting tumor growth. It is therefore desirable to identify molecules that can target angiogenic vasculature. Particularly attractive molecules for targeting angiogenic vasculature are antibodies that can bind
10 specifically to angiogenic vasculature. However, since most antibodies are developed in non-human animals such as mice, these antibodies often have undesirable immunogenic activity that limits their effectiveness for human therapy.

15 One approach to overcoming the detrimental properties of non-human antibodies is to humanize the antibodies by using human antibody framework region sequences spliced together with the binding domains that confer binding specificity. However, grafting of
20 these binding domains, referred to as complementarity determining regions (CDRs), into human frameworks has often resulted in the loss of binding affinity.

Thus, there exists a need to identify antibodies specific for angiogenic vasculature and to
25 humanize and optimize the antibodies for therapeutic purposes. The following invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The invention provides a grafted antibody, or
30 functional fragment thereof, comprising one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs

of a heavy chain CDR, where the grafted antibody or functional fragment thereof has specific binding activity for a cryptic collagen epitope. The invention also provides methods of using an antibody having
5 specific binding activity for a cryptic collagen epitope, including methods of inhibiting angiogenesis, tumor growth, and metastasis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the sequences of primers used
10 to clone nucleic acids encoding HUIV26 and HUI77 antibodies. Figure 1A shows a set of 5' primers for the signal peptide of mouse antibody light chain (SEQ ID NOS: 184-192). Figure 1B shows a set of 5' primers for the signal peptide of mouse antibody heavy chain
15 (SEQ ID NOS: 193-211). Figure 1C shows a set of primers for the constant region of mouse heavy and light chains. Primer 2650 (SEQ ID NO:212) is the 3' primer for mouse kappa light chain constant region (amino acids 123-115). Primer 2656 (SEQ ID NO:213) is
20 the 3' primer for mouse IgM CH1 region (amino acids 121-114). Primer 2706 (SEQ ID NO:214) is the 3' primer for mouse IgM CH1 region (amino acids 131-124).

Figure 2 shows the sequence of the variable region of anti-cryptic collagen site antibody HUIV26.
25 Figure 2A shows the nucleotide sequence of HUIV26 variable region light chain (SEQ ID NO:1). Figure 2B shows the nucleotide sequence of HUIV26 variable region heavy chain (SEQ ID NO:3). Figure 2C shows an alignment of the amino acid sequence of HUIV26 light
30 chain (V_K) domain of HUIV26 (SEQ ID NO:2) with a human variable region fusion, VKIV/JK2 (SEQ ID NO:6) and an alignment of HUIV26 heavy chain (V_H) domain (SEQ ID NO:4) with a human variable region fusion VHIII/JH6

(SEQ ID NO:8), with CDRs underlined. Amino acids in the framework region that differ between the aligned sequences are indicated by lines.

Figure 3 shows the sequence of the variable
5 region of anti-cryptic collagen site antibody HUI77.
Figure 3A shows the nucleotide sequence of HUI77
variable region light chain (SEQ ID NO:9). Figure 3B
shows the nucleotide sequence of HUI77 variable region
heavy chain (SEQ ID NO:11). Figure 3C shows an
10 alignment of the amino acid sequence of HUI77 light
chain (V_K) domain of HUI77 (SEQ ID NO:10) with a human
variable region fusion, VKII/JK1 (SEQ ID NO:14) and an
alignment of HUI77 heavy chain (V_H) domain (SEQ ID
NO:12) with a human variable region fusion VHIII/JH6
15 (SEQ ID NO:16), with CDRs underlined. Amino acids in
the framework region that differ between the aligned
sequences are indicated by lines. Figure 3D shows an
alignment of the nucleotide sequence of HUI77 variable
region with the sequence of the human framework fusion
20 of DPK13 and JK1 (SEQ ID NO:17).

Figure 4 shows beneficial CDR mutations for
anti-cryptic collagen site antibody HUIV26. Figure 4A
shows a set of primers used to generate random
mutations in LCDR3 and HCDR3 of HUIV26 (HUIV26 LCDR3
25 primers, SEQ ID NOS:224-232; HUIV26 HCDR3 primers, SEQ
ID NOS:233-243). Figure 4B shows a set of primers used
to generate random mutations in LCDR1a (SEQ ID NOS:266-
273), LCDR1b (SEQ ID NOS:274-282), LCDR2 (SEQ ID
NOS:283-289), HCDR1 (SEQ ID NOS:290-294), HCDR2a (SEQ
30 ID NOS:295-303) and HCDR2b (SEQ ID NOS:304-311) of
HUIV26. Figure 4C shows beneficial CDR mutations of
the HUIV26 antibody.

Figure 5 shows beneficial CDR mutations for anti-cryptic collagen site antibody HUI77. Figure 5A shows a set of primers used to generate random mutations in LCDR3 and HCDR3 of HUI77. Figure 5B shows
5 a set of primers used to generate random mutations in LCDR1a (SEQ ID NOS:312-319), LCDR1b (SEQ ID NOS:320-327), LCDR2 (SEQ ID NOS:328-334), HCDR1 (SEQ ID NOS:335-341), HCDR2a (SEQ ID NOS:342-349) and HCDR2b (SEQ ID NOS:350-357) of HUI77. Figure 5C shows
10 beneficial CDR mutations of the HUI77 antibody.

Figure 6 shows mutations in combinatorial variants of the HUIV26 antibody. The position of amino acids are shown, with mutations different than wild type shown in bold. The relative activity of
15 combinatorial variants is shown as "SPEK_{on}" and "SPEK_{off}" (last column). Primers used to create the combinatorial libraries are also shown (SEQ ID NOS:163-173).

Figure 7 shows mutations in combinatorial
20 variants of the HUI77 antibody. The position of amino acids are shown, with mutations different than wild type shown in bold. The relative activity of combinatorial variants is shown as "SPEK_{on}" and "SPEK_{off}" (last column). Primers used to create the
25 combinatorial libraries are also shown (SEQ ID NOS:174-183).

Figure 8 shows the activity and specificity of HUIV26 variants. The binding of purified Fabs of IX-IV26, containing wild type HUIV26 CDRs, and the
30 HUIV26 variants 2D4H1-C3 and DhuG5 is shown for denatured collagen IV (Figure 8A), denatured collagen I (Figure 8B) and native collagen IV (Figure 8C).

Figure 9 shows the activity and specificity of HUI77 variants. The binding of purified Fabs of IX-I77, containing wild type HUI77 CDRs, and HUI77 variants Qh2b-B7 and QhuD9 is shown for denatured collagen I (Figure 9A), denatured collagen IV (Figure 9B) and native collagen I (Figure 9C).

Figure 10 shows the binding activity of the HUIV26 variant DhuH8. The binding activity of the Fab form and the IgG form of the antibody to denatured (d-IV) and native (n-IV) human collagen IV is shown.

Figure 11 shows the effect of the HUI77 variant QH2b on B16 melanoma cell proliferation. B16 melanoma cells grown in culture were not treated (control; squares) or treated with the IgG form of the QH2b variant (circles).

DETAILED DESCRIPTION OF THE INVENTION

The invention provides antibodies specific for a cryptic collagen site, which is exposed during angiogenesis and tumor cell invasion through collagenous tissue and thus serves as an antibody that can target angiogenic vasculature. The antibodies are optimized for binding activity to a cryptic collagen site. The antibodies can be used to target angiogenic vasculature for diagnostic or therapeutic purposes. The antibodies can also be used to inhibit tumor growth.

As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been

described by Kabat et al., J. Biol. Chem. 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); by Chothia et al., J. Mol. Biol. 196:901-917 (1987); and MacCallum et al., J. Mol. Biol. 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison.

Table 1: CDR Definitions

	<u>Kabat</u> ¹	<u>Chothia</u> ²	<u>MacCallum</u> ³
V _H CDR1	31-35	26-32	30-35
V _H CDR2	50-65	53-55	47-58
V _H CDR3	95-102	96-101	93-101
V _L CDR1	24-34	26-32	30-36
V _L CDR2	50-56	50-52	46-55
V _L CDR3	89-97	91-96	89-96

¹ Residue numbering follows the nomenclature of Kabat et al., *supra*

² Residue numbering follows the nomenclature of Chothia et al., *supra*

³ Residue numbering follows the nomenclature of MacCallum et al., *supra*

As used herein, the term "framework" when used in reference to an antibody variable region is intended to mean all amino acid residues outside the

CDR regions within the variable region of an antibody. A variable region framework is generally between about 100-120 amino acids in length but is intended to reference only those amino acids outside of the CDRs.

5 As used herein, the term "framework region" is intended to mean each domain of the framework that is separated by the CDRs.

As used herein, the term "donor" is intended to mean a parent antibody molecule or fragment thereof
10 from which a portion is derived from, given to or contributes to another antibody molecule or fragment thereof so as to confer either a structural or functional characteristic of the parent molecule onto the receiving molecule. For the specific example of
15 CDR grafting, the parent molecule from which the grafted CDRs are derived is a donor molecule. The donor CDRs confer binding affinity of the parent molecule onto the receiving molecule. The donor molecule can be a different species or the same species
20 as the receiving molecule. If the donor and receiving molecules are of the same species, it is understood that it is sufficient that the donor is a separate and distinct molecule from the receiving molecule.

As used herein, the term "acceptor" is
25 intended to mean an antibody molecule or fragment thereof which is to receive the donated portion from the parent or donor antibody molecule or fragment thereof. An acceptor antibody molecule or fragment thereof is therefore imparted with the structural or
30 functional characteristic of the donated portion of the parent molecule. For the specific example of CDR grafting, an acceptor molecule, including framework and/or other antibody fragments, is the receiving molecule into which the CDRs are grafted. The acceptor

antibody molecule or fragment is imparted with the binding affinity of the donor CDRs or parent molecule. As with a donor molecule, it is understood that an acceptor molecule can be the same or a different
5 species as the donor.

A "variable region" when used in reference to an antibody or a heavy or light chain thereof is intended to mean the amino terminal portion of an antibody which confers antigen binding onto the
10 molecule and which is not the constant region. The term is intended to include functional fragments thereof which maintain some of all of the binding function of the whole variable region. Therefore, the term "heteromeric variable region binding fragments" is
15 intended to mean at least one heavy chain variable region and at least one light chain variable regions or functional fragments thereof assembled into a heteromeric complex. Heteromeric variable region binding fragments include, for example, functional
20 fragments such as Fab, F(ab)₂, Fv, single chain Fv (scFv) and the like. Such functional fragments are well known to those skilled in the art. Accordingly, the use of these terms in describing functional fragments of a heteromeric variable region is intended to
25 correspond to the definitions well known to those skilled in the art. Such terms are described in, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1989); Molec. Biology and Biotechnology: A Comprehensive Desk
30 Reference (Myers, R.A. (ed.), New York: VCH Publisher, Inc.); Huston et al., Cell Biophysics, 22:189-224 (1993); Plückthun and Skerra, Meth. Enzymol., 178:497-515 (1989); and in Day, E.D., Advanced Immunochemistry, Second Ed., Wiley-Liss, Inc., New York, NY (1990).

As used herein, the term "population" is intended to refer to a group of two or more different molecules. A population can be as large as the number of individual molecules currently available to the user or able to be made by one skilled in the art. Populations can be as small as 2-4 molecules or as large as 10^{13} molecules. Generally, a population will contain two or more, three or more, five or more, nine or more, ten or more, twelve or more, fifteen or more, or twenty or more different molecules. A population can also contain tens or hundreds of different molecules or even thousands of different molecules. For example, a population can contain about 20 to about 100,000 different molecules or more, for example about 25 or more, 30 or more, 40 or more, 50 or more, 75 or more, 100 or more, 150 or more, 200 or more, 300 or more, 500 or more, or 1000 or more different molecules, and can contain 10,000, 100,000 or even 1×10^6 or more different molecules. Those skilled in the art will know what size and diversity of a population is suitable for a particular application.

As used herein, the term "altered" when used in reference to an antibody variable region is intended to mean a heavy or light chain variable region that contains one or more amino acid changes in a framework region, a CDR or both compared to the parent amino acid sequence at the same position. Where an altered variable region is derived from or composed of donor and acceptor regions, the changed amino acid residues within the altered species are to be compared to their respective amino acid positions within the parent donor and acceptor regions.

As used herein, the term "nucleic acid" or "nucleic acids" is intended to mean a single- or

double-stranded DNA or RNA molecule. A nucleic acid molecule of the invention can be of linear, circular or branched configuration, and can represent either the sense or antisense strand, or both, of a nucleic acid molecule. The term also is intended to include nucleic acid molecules of both synthetic and natural origin. A nucleic acid molecule of natural origin can be derived from any animal, such as a human, non-human primate, mouse, rat, rabbit, bovine, porcine, ovine, canine, feline, or amphibian, or from a lower eukaryote, such as *Drosophila*, *C. elegans*, yeast, and the like. A synthetic nucleic acid includes, for example, chemical and enzymatic synthesis. The term "nucleic acid" or "nucleic acids" is similarly intended to include analogues of natural nucleotides which have similar functional properties as the referenced nucleic acid and which can be utilized in a manner similar to naturally occurring nucleotides and nucleosides.

As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. An antibody useful in the invention, or antigen binding fragment of such an antibody, is characterized by having specific binding activity for a polypeptide or a peptide portion thereof of at least about $1 \times 10^5 \text{ M}^{-1}$. Thus, Fab, F(ab')₂, Fd, Fv, single chain Fv (scFv) fragments of an antibody and the like, which retain specific binding activity for a polypeptide, are included within the definition of an antibody. Specific binding activity of an antibody for a polypeptide can be readily determined by one skilled in the art, for example, by comparing the binding activity of an antibody to a particular polypeptide versus a control polypeptide that is not the particular polypeptide. Methods of preparing polyclonal or

monoclonal antibodies are well known to those skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1988)).

5 In addition, the term "antibody" as used herein includes naturally occurring antibodies as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric, bifunctional and humanized antibodies, as well as
10 antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy
15 chains and variable light chains as described by Huse et al. (Science 246:1275-1281 (1989)). These and other methods of making functional antibodies are well known to those skilled in the art (Winter and Harris, Immunol. Today 14:243-246 (1993); Ward et al., Nature
20 341:544-546 (1989); Harlow and Lane, *supra*, 1988); Hilyard et al., Protein Engineering: A practical approach (IRL Press 1992); Borrabeck, Antibody Engineering, 2d ed. (Oxford University Press 1995)).

 As used herein, specific binding means
25 binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that
30 does not have binding activity, for example, an antibody that binds a distinct epitope or antigen. Specificity of binding also can be determined, for example, by competition with a control molecule, for example, competition with an excess of the same

molecule. In this case, specific binding is indicated if the binding of a molecule is competitively inhibited by itself. Thus, specific binding between an antibody and antigen is measurably different from a non-specific
5 interaction and occurs via the antigen binding site of the antibody.

As used herein, selective binding refers to a binding interaction that is both specific and discriminating between molecules, for example, an
10 antibody that binds to a single molecule or closely related molecules. For example, an antibody can exhibit specificity for an antigen that can be both specific and selective for the antigen if the epitope is unique to a molecule. Thus, a molecule having
15 selective binding can differentiate between molecules, as exemplified by an antibody having specificity for an epitope unique to one molecule or closely related molecules. Alternatively, an antibody can have specificity for an epitope that is common to many
20 molecules, for example, a carbohydrate that is expressed on a number of molecules. Such an antibody has specific binding but is not selective for one molecule or closely related molecules.

As used herein the term "binding affinity" is
25 intended to mean the strength of a binding interaction and includes both the actual binding affinity as well as the apparent binding affinity. The actual binding affinity is a ratio of the association rate over the disassociation rate. Therefore, conferring or
30 optimizing binding affinity includes altering either or both of these components to achieve the desired level of binding affinity. The apparent affinity can include, for example, the avidity of the interaction. For example, a bivalent heteromeric variable region

binding fragment can exhibit altered or optimized binding affinity due to its valency.

As used herein, the term "substantially the same" when used in reference to binding affinity is intended to mean similar or identical binding affinities where one molecule has a binding affinity that is similar to another molecule within the experimental variability of the affinity measurement. The experimental variability of the binding affinity measurement is dependent upon the specific assay used and is known to those skilled in the art.

As used herein, the term "optimizing" when used in reference to a variable region or a functional fragment thereof is intended to mean that the functional activity of the variable region has been modified compared to the activity of a parent variable region or a donor variable region, resulting in a desirable change in activity. A variable region or functional fragment thereof exhibiting optimized activity can exhibit, for example, higher affinity or lower affinity binding, or increased or decreased association or dissociation rates compared to an unaltered variable region. A variable region or functional fragment thereof exhibiting optimized activity also can exhibit increased stability such as increased half-life in a particular organism. For example, an antibody activity can be optimized to increase stability by decreasing susceptibility to proteolysis. An antibody exhibiting optimized activity also can exhibit lower affinity binding, including decreased association rates or increased dissociation rates, if desired. An optimized variable region exhibiting lower affinity binding is useful, for example, for penetrating a solid tumor. In contrast to

a higher affinity variable region, which would bind to the peripheral regions of the tumor but would be unable to penetrate to the inner regions of the tumor due to its high affinity, a lower affinity variable region
5 would be advantageous for penetrating the inner regions of the tumor. As with optimization of binding affinities above, optimization of a catalytic variable region can be, for example, increased or decreased catalytic rates, disassociation constants or
10 association constants.

As used herein, a "cryptic collagen site" or "cryptic collagen epitope" refers to an epitope of a collagen molecule that is less accessible to binding of an antibody, or functional fragment thereof, in native
15 collagen than in denatured collagen. An antibody having binding activity for a cryptic collagen epitope preferentially recognizes denatured collagen over native collagen, that is, has a higher binding affinity for denatured over native collagen. For example, such
20 an antibody can have at least about a 2-fold or greater preference, that is, at least about 2-fold higher binding activity, for denatured collagen over native collagen, and can exhibit about a 3-fold or greater preference, about a 5-fold or greater preference, about
25 a 10-fold or greater preference, about a 15-fold or greater preference, about a 20-fold or greater preference, about a 25-fold or greater preference, about a 50-fold or greater preference, about a 100-fold or greater preference, or even a higher preference for
30 denatured over native collagen.

Native collagen herein refers to a molecule where three alpha-chains are organized in a triple helical molecule. Native collagen can be of different stages of post-translational processing such as

pro-collagen and any intermediates in the generation of a mature tissue form of collagen, or collagen molecules isolated by limited proteolysis of tissues under conditions where the triple-helical structure of collagen is not disrupted. Thus, native collagen can be an intact collagen molecule or can contain non-triple-helical sequences flanking triple-helical regions, so long as the triple-helical is not disrupted. Denatured collagen herein refers to collagen where the triple helix is completely or partially disrupted such that a cryptic epitope is made accessible. Denaturation of collagen can occur *in situ* by the action of proteinases, for example, matrix metalloproteinases, that cleave collagen within triple helical regions, rendering the resulting fragments of the triple helix unstable. Denaturation of collagen can be induced *in vitro* by thermal or chemical denaturation of native collagen. Denatured collagen can also be prepared *in vitro* by treatment of native collagen with proteinases capable of cleaving a triple helical region(s), which are commonly referred to as collagenolytic enzymes, at temperatures where the resulting fragments of the triple helix are thermally unstable. Denatured collagen can be obtained by denaturation of native collagens of different stages of post-translational processing or denaturation of native collagen isolated from tissues by limited proteolysis. One skilled in the art will know a variety of methods for isolation of native collagens and a variety of methods to denature a triple helix that contains a cryptic collagen epitope.

An antibody of the invention can have binding activity for a cryptic collagen epitope that is the same as the respective parental mouse antibody. For example, an antibody of the invention having CDRs

derived from HUIV26 can have essentially the same binding specificity as the mouse HUIV26 antibody described by Xu et al., Hybridoma 19:375-385 (2000); Xu et al., J. Cell Biol. 154:1069-1079 (2001); and WO 00/40597, each of which is incorporated herein by reference. Similarly, an antibody of the invention having CDRs derived from HUI77 can have essentially the same binding specificity as the mouse HUI77 antibody described by Xu et al., *supra*, 2000; Xu et al., *supra*, 2001; and WO 00/40597. Such binding specificity can be tested by the methods disclosed herein, for example, by comparing the activity of an antibody of the invention to the corresponding parental mouse antibody. For example, an antibody of the invention derived from HUIV26 can be compared to a corresponding mouse antibody having the variable region amino acid sequence shown in Figure 2C (SEQ ID NOS:2 and 4). Similarly, an antibody of the invention derived from HUI77 can be compared to a corresponding mouse antibody having the variable region amino acid sequence shown in Figure 3C (SEQ ID NOS:10 and 12). Similar binding specificity can be determined, for example, by competitive binding with the corresponding parental antibody. It is understood that an antibody of the invention can have essentially the same specificity as the corresponding parental antibody or can have altered specificity so long as the antibody has binding activity for a cryptic collagen epitope.

The invention provides antibodies having specific binding activity for a cryptic collagen epitope. The antibodies contain at least one CDR having at least one amino acid substitution in a CDR of the antibodies HUIV26 and HUI77, which are antibodies that bind to a cryptic collagen site. The invention also provides nucleic acids encoding these antibodies.

The invention further provides methods using the antibodies.

Highly specific monoclonal antibodies have been developed that recognize a cryptic domain of human collagen, designated HUIV26 and HUI77 (see Xu et al., Hybridoma 19:375-385 (2000); Xu et al., J. Cell Biol. 154:1069-1079 (2001); WO 00/40597, each of which is incorporated herein by reference). Monoclonal antibody HUIV26 recognizes a cryptic domain of human collagen-IV, and HUI77 recognizes a cryptic domain of human collagen-I and IV that is also common to collagens II, III and V. This cryptic domain(s) is less accessible under most normal physiological conditions but becomes accessible following proteolytic remodeling of the collagen triple helix *in vivo*. Thus, cryptic collagen epitope(s) can become more accessible during invasive cellular processes. Importantly, the cryptic domain(s) defined by these antibodies was shown to be exposed within the basement membrane of tumor associated angiogenic blood vessels from human tumors including, breast, bladder and melanoma tumors. However, this cryptic domain was less exposed within the vessels or normal tissues tested. Therefore, the antibodies HUIV26 and HUI77 represent important and specific markers of angiogenic blood vessels. These cryptic domain(s) plays an important role in regulating angiogenesis and tumor growth since the monoclonal antibodies HUIV26 and HUI77 potentially inhibit angiogenesis and human tumor growth in the chick embryo, rat and mouse models following systemic administration (Xu et al., *supra*, 2001). Thus, these monoclonal antibodies and the antibodies of the invention having specific binding activity for these cryptic collagen site(s) represent a highly potent and effective new

therapeutic reagent for the treatment for diseases characterized by aberrant neovascularization.

A nucleic acid sequence of the invention can include a sequence that is the same or substantially the same as a specifically recited SEQ ID NO. Similarly, an amino acid sequence of the invention can include a sequence that is the same or substantially the same as a specifically recited SEQ ID NO. As used herein, the term "substantially" or "substantially the same" when used in reference to a nucleotide or amino acid sequence is intended to mean that the nucleotide or amino acid sequence shows a considerable degree, amount or extent of sequence identity when compared to a reference sequence, for example, the sequence of a parent antibody. Such a considerable degree, amount or extent of sequence identity is further considered to be significant and meaningful and therefore exhibit characteristics which are definitively recognizable or known. Thus, a nucleotide sequence which is substantially the same nucleotide sequence as a heavy or light chain of an antibody of the invention, including fragments thereof, refers to a sequence which exhibits characteristics that are definitively known or recognizable as encoding or as being the amino acid sequence as the parent antibody sequence. Minor modifications thereof are included so long as they are recognizable as a parent antibody sequence. Similarly, an amino acid sequence which is substantially the same amino acid sequence as a heavy or light chain of an antibody of the invention, or functional fragment thereof, refers to a sequence which exhibits characteristics that are definitively known or recognizable as representing the amino acid sequence of parent antibody and minor modifications thereof. When determining whether a nucleotide or amino acid sequence

is substantially the same as a parent antibody, consideration is given to the number of changes relative to the parent antibody together with whether the function is maintained, for example, whether the function of binding to a cryptic collagen site is maintained for antibodies of the invention.

Minor modification of these nucleotide sequences and/or amino acids are intended to be included as heavy and light chain encoding nucleic acids and their functional fragments. Such minor modifications include, for example, those which do not change the encoded amino acid sequence due to the degeneracy of the genetic code as well as those which result in only a conservative substitution of the encoded amino acid sequence. Conservative substitutions of encoded amino acids include, for example, amino acids which belong within the following groups: (1) non-polar amino acids (Gly, Ala, Val, Leu, and Ile); (2) polar neutral amino acids (Cys, Met, Ser, Thr, Asn, and Gln); (3) polar acidic amino acids (Asp and Glu); (4) polar basic amino acids (Lys, Arg and His); and (5) aromatic amino acids (Phe, Trp, Tyr, and His). Other minor modifications are included within the nucleic acids encoding heavy and light chain polypeptides of the invention so long as the nucleic acid or encoded polypeptides retain some or all of their function as described herein.

To generate antibodies of the invention having specific binding activity for a cryptic collagen epitope, the heavy and light chain variable regions of the antibodies HUIV26 and HUI77 were cloned and sequenced (see Example I and Figures 2 and 3). CDRs of the heavy and light chain variable regions were identified. Exemplary heavy and light chain CDRs, as

determined by the numbering of Kabat, are shown in Figures 2C and 3C (underlined). Exemplary heavy and light chain CDRs of HUIV26 include, for example, V_L CDR1, KSSQSLNLSGNQKNYLA (SEQ ID NO:20); V_L CDR2, 5 GASTRES (SEQ ID NO:22); V_L CDR3, QNDHSYPYT (SEQ ID NO:24); V_H CDR1, GFDFSRYWMS (SEQ ID NO:26); V_H CDR2, EINPDSSTINYTPSLKD (SEQ ID NO:28); and V_H CDR3, PVDGYYDAMDY (SEQ ID NO:30). Exemplary heavy and light chain CDRs of HUI77 include, for example, V_L 10 CDR1, RSSQSIVHSNGNTYLE (SEQ ID NO:32); V_L CDR2, KVSNRFS (SEQ ID NO:34); V_L CDR3, FQGSHVPWT (SEQ ID NO:36); V_H CDR1, GFSLSTSGMGVG (SEQ ID NO:38); V_H CDR2, DIWWDNKKYYNPSLKS (SEQ ID NO:40); and V_H CDR3, RANYGNPYYAMDY (SEQ ID NO:42).

15 Libraries of CDR variants containing single amino acid substitutions were generated (Example II). The libraries were screened for binding to a cryptic collagen site, and single amino acid mutations having beneficial activity were identified. Combinatorial 20 mutants, in which two or more variant CDRs containing at least one amino acid substitution relative to parental HUIV26 or HUI77 CDRs were combined and screened for activity (Example III). A number of combinatorial mutants having optimized activity for 25 binding to a cryptic collagen site were identified.

The antibodies of the invention having binding activity for a cryptic collagen epitope. As disclosed herein, the collagen can be denatured by any of a variety of methods so long as an antigenic 30 determinant is exposed that was less accessible in native collagen. Such methods include, for example, proteolytic digestion, heat or thermal denaturation, chemical denaturation, and the like. One skilled in the art will know a variety of methods suitable for

denaturing a collagen molecule to reveal a cryptic collagen site or epitope. Furthermore, the method of denaturation can be a combination of two or more denaturation methods, for example, proteolytic digestion combined with chemical and/or thermal denaturation. For example, proteolytic digestion can be used to cleave collagen, resulting in a collagen molecule that is more susceptible to thermal or chemical denaturation. An exemplary protease that can be used to denature collagen is matrix metalloproteinase, which can be used *in vitro* and can function *in vivo* to cleave collagen within triple helical regions and at body temperature in a mammal.

The invention provides grafted antibodies of the HUIV26 and HUI77 antibodies. In one embodiment, the invention provides a grafted antibody of HUIV26. The grafted antibody, or functional fragment thereof, comprises one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting of SEQ ID NOS:26, 28 and 30 or a light chain CDR selected from the group consisting of SEQ ID NOS:20, 22 and 24, the grafted antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

In another embodiment, the invention provides a grafted antibody of HUI77. The grafted antibody, or functional fragment thereof, comprises one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting of SEQ ID NOS:38, 40 and 42 or a light chain CDR selected from the group consisting of SEQ ID NOS:32, 34 and 36, the grafted antibody or functional fragment

thereof having specific binding activity for a cryptic collagen epitope.

The invention additionally provides antibodies, or functional fragments thereof, containing
5 specifically recited CDRs, where the antibody or functional fragment thereof has specific binding activity for a cryptic collagen epitope. Such antibodies include those having at least a single amino acid substitution and which retain binding activity for
10 a cryptic collagen epitope. Included among such CDR variants are those described in Figures 4 and 5.

Exemplary CDRs of the invention having a single amino acid substitution in a CDR of HuIV26 include, for example, those described below, in which
15 the position of the amino acid mutation in the numbering of Kabat is indicated along with the amino acid substitution from wild type to mutant (wild type-mutant). Such exemplary CDRs include HuIV26 V_H CDR1 31R-H (SEQ ID NO:43); HuIV26 V_H CDR1 34M-I (SEQ ID
20 NO:44); HuIV26 V_H CDR1 35S-T (SEQ ID NO:45); HuIV26 V_H CDR1 35S-A (SEQ ID NO:46); HuIV26 V_H CDR1 35S-G (SEQ ID NO:47); HuIV26 V_H CDR2 57I-A (SEQ ID NO:48); HuIV26 V_H CDR2 57I-S (SEQ ID NO:49); HuIV26 V_H CDR2 62S-Y (SEQ ID NO:50); HuIV26 V_H CDR2 62S-A (SEQ ID NO:51); HuIV26 V_H
25 CDR2 62S-H (SEQ ID NO:52); HuIV26 V_H CDR2 62S-G (SEQ ID NO:53); HuIV26 V_H CDR2 64K-Q (SEQ ID NO:54); HuIV26 V_H CDR2 65D-S (SEQ ID NO:55); HuIV26 V_H CDR3 97D-P (SEQ ID NO:56); HuIV26 V_H CDR3 97D-G (SEQ ID NO:57); HuIV26 V_H CDR3 97D-T (SEQ ID NO:58); HuIV26 V_H CDR3 97D-A (SEQ ID
30 NO:59); HuIV26 V_H CDR3 98G-P (SEQ ID NO:60); HuIV26 V_H CDR3 98G-A (SEQ ID NO:61); HuIV26 V_H CDR3 98G-H (SEQ ID NO:62); HuIV26 V_H CDR3 102Y-P (SEQ ID NO:63); HuIV26 V_H CDR3 102Y-N (SEQ ID NO:64); HuIV26 V_L CDR1 27Q-R (SEQ ID NO:65); HuIV26 V_L CDR1 27Q-S (SEQ ID NO:66); HuIV26

V_L CDR1 27dN→S (SEQ ID NO:67); HuIV26 V_L CDR1 27eS→Y (SEQ ID NO:68); HuIV26 V_L CDR1 27eS→W (SEQ ID NO:69); HuIV26 V_L CDR1 27eS→H (SEQ ID NO:70); HuIV26 V_L CDR1 27eS→R (SEQ ID NO:71); HuIV26 V_L CDR1 27fG→Y (SEQ ID NO:72); HuIV26 V_L CDR1 27fG→R (SEQ ID NO:73); HuIV26 V_L CDR1 27fG→H (SEQ ID NO:74); HuIV26 V_L CDR1 27fG→I (SEQ ID NO:75); HuIV26 V_L CDR1 29Q→K (SEQ ID NO:76); HuIV26 V_L CDR3 93S→Q (SEQ ID NO:77); HuIV26 V_L CDR3 93S→G (SEQ ID NO:78); HuIV26 V_L CDR3 93S→L (SEQ ID NO:79); HuIV26 V_L CDR3 93S→A (SEQ ID NO:80); HuIV26 V_L CDR3 93S→T (SEQ ID NO:81); HuIV26 V_L CDR3 93S→V (SEQ ID NO:82); HuIV26 V_L CDR3 94Y→N (SEQ ID NO:83); HuIV26 V_L CDR3 94Y→S (SEQ ID NO:84); HuIV26 V_L CDR3 94Y→P (SEQ ID NO:85); HuIV26 V_L CDR3 94Y→M (SEQ ID NO:86); and HuIV26 V_L CDR2 57I→V (SEQ ID NO:162).

Exemplary CDRs of the invention having a single amino acid substitution in a CDR of HUI77 include, for example, those described below, in which the position of the amino acid mutation in the numbering of Kabat is indicated along with the amino acid substitution from wild type to mutant (wild type-mutant). Such exemplary CDRs include HUI77 V_H CDR1 32S→P (SEQ ID NO:87); HUI77 V_H CDR1 32S→W (SEQ ID NO:88); HUI77 V_H CDR1 35bG→W (SEQ ID NO:89); HUI77 V_H CDR1 35bG→L (SEQ ID NO:90); HUI77 V_H CDR1 35bG→A (SEQ ID NO:91); HUI77 V_H CDR2 59Y→S (SEQ ID NO:92); HUI77 V_H CDR2 59Y→A (SEQ ID NO:93); HUI77 V_H CDR2 59Y→P (SEQ ID NO:94); HUI77 V_H CDR2 64K→P (SEQ ID NO:95); HUI77 V_H CDR3 95R→P (SEQ ID NO:96); HUI77 V_H CDR3 95R→Q (SEQ ID NO:97); HUI77 V_H CDR3 95R→L (SEQ ID NO:98); HUI77 V_H CDR3 95R→T (SEQ ID NO:99); HUI77 V_H CDR3 95R→V (SEQ ID NO:100); HUI77 V_H CDR3 100N→V (SEQ ID NO:101); HUI77 V_H CDR3 100N→W (SEQ ID NO:102); HUI77 V_H CDR3 100eM→Q (SEQ ID NO:103); HUI77 V_H CDR3 100eM→N (SEQ ID NO:104); HUI77 V_H CDR3 100eM→T (SEQ ID NO:105); HUI77 V_H CDR3

102Y-K (SEQ ID NO:106); HUI77 V_H CDR3 102Y-T (SEQ ID
NO:107); HUI77 V_H CDR3 102Y-M (SEQ ID NO:108); HUI77 V_H
CDR3 102Y-H (SEQ ID NO:109); HUI77 V_L CDR1 27cV-P (SEQ
ID NO:110); HUI77 V_L CDR1 27cV-W (SEQ ID NO:111); HUI77
5 V_L CDR1 27dH-L (SEQ ID NO:112); HUI77 V_L CDR1 27dH-S
(SEQ ID NO:113); HUI77 V_L CDR1 27eS-W (SEQ ID NO:114);
HUI77 V_L CDR1 28N-Y (SEQ ID NO:115); HUI77 V_L CDR1 28N-W
(SEQ ID NO:116); HUI77 V_L CDR1 30N-Y (SEQ ID NO:117);
HUI77 V_L CDR1 33L-F (SEQ ID NO:118); HUI77 V_L CDR1 33L-V
10 (SEQ ID NO:119); HUI77 V_L CDR2 50K-S (SEQ ID NO:120);
HUI77 V_L CDR2 51V-A (SEQ ID NO:121); HUI77 V_L CDR2 53N-S
(SEQ ID NO:122); HUI77 V_L CDR2 54R-L (SEQ ID NO:123);
HUI77 V_L CDR2 56S-W (SEQ ID NO:124); HUI77 V_L CDR2 56S-F
(SEQ ID NO:125); HUI77 V_L CDR3 89F-V (SEQ ID NO:126);
15 HUI77 V_L CDR3 89F-H (SEQ ID NO:127); HUI77 V_L CDR3 90Q-R
(SEQ ID NO:128); HUI77 V_L CDR3 90Q-W (SEQ ID NO:129);
HUI77 V_L CDR3 91G-S (SEQ ID NO:130); HUI77 V_L CDR3 92S-W
(SEQ ID NO:131); HUI77 V_L CDR3 92S-E (SEQ ID NO:132);
HUI77 V_L CDR3 93H-L (SEQ ID NO:133); HUI77 V_L CDR3 93H-T
20 (SEQ ID NO:134); HUI77 V_L CDR3 93H-S (SEQ ID NO:135);
HUI77 V_L CDR3 93H-A (SEQ ID NO:136); HUI77 V_L CDR3 93H-Q
(SEQ ID NO:137); HUI77 V_L CDR3 94V-T (SEQ ID NO:138);
HUI77 V_L CDR3 97T-A (SEQ ID NO:139); HUI77 V_L CDR3 97T-R
(SEQ ID NO:140); HUI77 V_L CDR3 97T-H (SEQ ID NO:141);
25 HUI77 V_L CDR3 97T-K (SEQ ID NO:142); HUI77 V_L CDR3 97T-I
(SEQ ID NO:143); HUI77 V_H CDR2 59Y-T (SEQ ID NO:144);
HUI77 V_L CDR3 94V-F (SEQ ID NO:145); and HUI77 V_L CDR1
28N-Q (SEQ ID NO:146).

In addition to CDRs having single amino acid
30 substitutions, the invention additionally provides
HUIV26 and HUI77 CDRs having two or more amino acid
substitutions. Exemplary CDRs having two or more amino
acid substitutions in HUIV26 include, for example,
HUIV26 V_H CDR2 57I-A/62S-A (SEQ ID NO:154); HUIV26 V_H
35 CDR2 57I-A/62S-Y (SEQ ID NO:155); HUIV26 V_H CDR2

57I-A/62S-H (SEQ ID NO:156); HUIV26 V_L CDR1
27eS-W/27fG-Y (SEQ ID NO:157); HUIV26 V_L CDR1
27eS-Y/27fG-Y (SEQ ID NO:158); HUIV26 V_L CDR1
27eS-Y/27fG-H (SEQ ID NO:159); HUIV26 V_L CDR1
5 27eS-R/27fG-Y (SEQ ID NO:160); and HUIV26 V_L CDR1
27eS-W/27fG-H (SEQ ID NO:161) (see Figure 6).
Exemplary CDRs having two or more amino acid
substitutions in HUI77 include, for example, HUI77 V_H
CDR1 32S-P/35bG-W (SEQ ID NO:147); HUI77 V_H CDR1
10 32S-P/35bG-A (SEQ ID NO:148); HUI77 V_L CDR1
27dH-S/28N-W (SEQ ID NO:149); HUI77 V_L CDR1
27dH-S/28N-Y (SEQ ID NO:150); HUI77 V_L CDR1
27dH-S/28N-Q (SEQ ID NO:151); HUI77 V_L CDR1 28N-Q/33L-F
(SEQ ID NO:152); and HUI77 V_L CDR1 27H-S/28N-W/33L-F
15 (SEQ ID NO:153) (see Figure 7).

The invention provides an antibody having at
least one of the above variant CDR sequences. It is
understood that any combination of HUIV26 CDRs can be
combined with mutant and/or wild type CDRs to generate
20 an HUIV26 grafted antibody, so long as binding activity
to a cryptic collagen site is maintained. Similarly,
any combination of HUI77 CDRs can be combined with
mutant and/or wild type CDRs to generate a HUI77
grafted antibody so long as binding activity to a
25 cryptic collagen site is maintained. Thus, any
combination of single amino acid substitutions can be
combined with other CDR mutants to generate an antibody
having at least two variant CDRs. Furthermore, any
single mutation at different positions within the same
30 CDR can be combined to generate a CDR having 2 or more
amino acid substitutions at two or more positions. Any
of the single or multiple mutations can be combined so
long as binding activity to a cryptic collagen site is
maintained.

Thus, the invention provides an antibody, or functional fragment thereof, comprising one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78; SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162, the antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

The invention additionally provides an antibody, or functional fragment thereof, comprising one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132,

SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, 5 SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, and SEQ ID NO:153, the antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

10 The invention further provides an antibody, or functional fragment thereof, comprising a heavy chain polypeptide comprising one or more CDRs having at least one amino acid substitution in one or more heavy chain CDRs, the heavy chain CDRs selected from the 15 group consisting of a heavy chain CDR1 selected from the group consisting of CDRs referenced as SEQ ID NOS:26, 43, 44, 45, 46, and 47; a heavy chain CDR2 selected from the group consisting of CDRs referenced as SEQ ID NOS:28, 48, 49, 50, 51, 52, 53, 54, and 55; 20 and a heavy chain CDR3 selected from the group consisting of CDRs referenced as SEQ ID NOS:30, 56, 57, 58, 59, 60, 61, 62, 63, and 64, the antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

25 The invention also provides an antibody, or functional fragment thereof, comprising a light chain polypeptide comprising one or more CDRs having at least one amino acid substitution in one or more light chain CDRs, the light chain CDRs selected from the group 30 consisting of a light chain CDR1 selected from the group consisting of CDRs referenced as SEQ ID NOS:20, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, and 76; a light chain CDR2 referenced as SEQ ID NO:22;; and a light chain CDR3 selected from the group consisting of 35 CDRs referenced as SEQ ID NOS:24, 77, 78, 79, 80, 81,

82, 83, 84, 85, and 86, the antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

The invention further provides an antibody,
5 or functional fragment thereof, comprising a heavy chain polypeptide comprising one or more CDRs having at least one amino acid substitution in one or more heavy chain CDRs, the heavy chain CDRs selected from the group consisting of a heavy chain CDR1 selected from
10 the group consisting of CDRs referenced as SEQ ID NOS:38, 87, 88, 89, 90, 91, 147 and 148; a heavy chain CDR2 selected from the group consisting of CDRs referenced as SEQ ID NOS:40, 92, 93, 94, 95 and 144; and a heavy chain CDR3 selected from the group
15 consisting of CDRs referenced as SEQ ID NOS:42, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108 and 109, the antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

20 Additionally provided is an antibody, or functional fragment thereof, comprising a light chain polypeptide comprising one or more CDRs having at least one amino acid substitution in one or more light chain CDRs, the light chain CDRs selected from the group
25 consisting of a light chain CDR1 selected from the group consisting of CDRs referenced as SEQ ID NOS:32, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 146, 149, 150, 151, 152 and 153; a light chain CDR2 referenced as SEQ ID NOS:34, 120, 121, 122, 123, 124
30 and 125; and a light chain CDR3 selected from the group consisting of CDRs referenced as SEQ ID NOS:36, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, and 145, the antibody or

functional fragment thereof having specific binding activity for a cryptic collagen epitope.

As described above, an antibody of the invention can be generated from any combination of the variant and/or wild type CDRs, so long as binding activity to a cryptic collagen site is maintained. As disclosed herein, a variety of combinatorial antibodies containing multiple CDRs having at least a single amino acid substitution were identified having binding activity for a cryptic collagen site. In addition to antibodies containing any combination of the respective CDRs disclosed herein, the following specific combinations of CDRs are also provided by the invention.

Exemplary HUIV26 variants include, for example, the following antibodies:

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:28; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:20; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (4.1-2D4).

An antibody comprises a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:28; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:72; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (L1b-F11).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:48; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:20; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (H2a-G8).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy chain CDR2 referenced as SEQ ID NO:154; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomA2).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:158; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomA4).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:159; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomB1).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:48; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:160; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomD2).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:72; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomD3).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light

chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomD6).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:160; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomE3).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:160; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomG2).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy chain CDR2 referenced as SEQ ID NO:162; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:158; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomA7).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy chain CDR2 referenced as SEQ ID NO:156; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomB10).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:154; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomC8).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy chain CDR2 referenced as SEQ ID

NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomD7).

5 An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy chain CDR2 referenced as SEQ ID NO:154; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:161; a light chain CDR2 referenced as SEQ ID NO:22; and a light
10 chain CDR3 referenced as SEQ ID NO:77 (DcomD11).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy chain CDR2 referenced as SEQ ID NO:156; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:161; a light
15 chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (DcomE11).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy chain CDR2 referenced as SEQ ID NO:28; a heavy chain CDR3 referenced as SEQ ID NO:63; a
20 light chain CDR1 referenced as SEQ ID NO:20; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77 (2D4H1-C3).

Exemplary HUI77 variants include, for example, the following antibodies:

25 An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:38; a heavy chain CDR2 referenced as SEQ ID NO:40; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:32; a light chain CDR2 referenced as SEQ ID NO:34; and a light
30 chain CDR3 referenced as SEQ ID NO:36 (12F10Q).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:38; a heavy chain CDR2 referenced as SEQ ID NO:92; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:32; a light

chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (QH2b-A3).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:92; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:149; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qcom1B6).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:92; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:150; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qcom1B8).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:149; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qcom1C3).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:144; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:149; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qcom1D3).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:151; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qcom1E3).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID

NO:92; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:151; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qcom1H6).

- 5 An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:152; a light chain CDR2 referenced as SEQ ID NO:34; and a light
10 chain CDR3 referenced as SEQ ID NO:145 (Qcom1H7).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:148; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:150; a light
15 chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qcom2A4).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:115; a light chain CDR2 referenced as SEQ ID NO:34; and a light
20 chain CDR3 referenced as SEQ ID NO:36 (Qcom2B11).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:40; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:153; a light chain CDR2 referenced as SEQ ID NO:34; and a light
25 chain CDR3 referenced as SEQ ID NO:36 (Qcom2C1).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:92; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:116; a light chain CDR2 referenced as SEQ ID NO:34; and a light
30 chain CDR3 referenced as SEQ ID NO:36 (Qcom2D9).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:116; a light
5 chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qcom2E3).

An antibody comprising a heavy chain CDR1 referenced as SEQ ID NO:38; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103;
10 a light chain CDR1 referenced as SEQ ID NO:32; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36 (Qh2b-B7).

The invention also provides grafted antibodies containing CDRs derived from HUIV26 and
15 HUI77, respectively. Such grafted CDRs include humanized antibodies, in which CDRs from HUIV26 or HUI77 have been grafted or in which a CDR containing one or more amino acid substitutions is grafted. The CDRs can be grafted directly into a human framework, as
20 disclosed herein. If desired, framework changes can also be incorporated by generating framework libraries. The optimization of CDRs and/or framework sequences can be performed independently and sequentially combined or can be performed simultaneously, as described in more
25 detail below.

Thus, the invention additionally provides a grafted antibody in which HUIV26 CDRs (SEQ ID NOS:20, 22, 24, 26, 28 and 30) are grafted into a human framework sequence. Also provided is a grafted
30 antibody in which HUI77 CDRs (SEQ ID NOS:32, 34, 36, 38, 40 and 42) are grafted into a human framework.

To generate grafted antibodies, donor CDRs of collagen-specific antibodies are grafted onto an

antibody acceptor variable region framework. Methods for grafting antibodies and generating CDR variants to optimize activity have been described previously (WO 98/33919; WO 00/78815; WO 01/27160). The procedure can
5 be performed to achieve grafting of donor CDRs and affinity reacquisition in a simultaneous process. The methods similarly can be used, either alone or in combination with CDR grafting, to modify or optimize the binding affinity of a variable region. The methods
10 for conferring donor CDR binding affinity onto an acceptor variable region are applicable to both heavy and light chain variable regions and as such can be used to simultaneously graft and optimize the binding affinity of an antibody variable region.

15 The donor CDRs can be altered to contain a plurality of different amino acid residue changes at all or selected positions within the donor CDRs. For example, random or biased incorporation of the twenty naturally occurring amino acid residues, or preselected
20 subsets, can be introduced into the donor CDRs to produce a diverse population of CDR species. Inclusion of CDR variant species into the diverse population of variable regions allows for the generation of variant species that exhibit optimized binding affinity for a
25 predetermined antigen.

A range of possible changes can be made in the donor CDR positions. Some or all of the possible changes that can be selected for change can be introduced into the population of grafted donor CDRs.
30 A single position in a CDR can be selected to introduce changes or a variety of positions having altered amino acids can be combined and screened for activity.

One approach is to change all amino acid positions along a CDR by replacement at each position with, for example, all twenty naturally occurring amino acids. The replacement of each position can occur in the context of other donor CDR amino acid positions so that a significant portion of the CDR maintains the authentic donor CDR sequence, and therefore, the binding affinity of the donor CDR. For example, an acceptor variable region framework, either a native or altered framework, can be grafted with a population of CDRs containing single position replacements at each position within the CDRs. Similarly, an acceptor variable region framework can be targeted for grafting with a population of CDRs containing more than one position changed to incorporate all twenty amino acid residues, or a subset of amino acids. One or more amino acid positions within a CDR, or within a group of CDRs to be grafted, can be altered and grafted into an acceptor variable region framework to generate a population of grafted antibodies. It is understood that a CDR having one or more altered positions can be combined with one or more other CDRs having one or more altered positions, if desired.

A population of CDR variant species having one or more altered positions can be combined with any or all of the CDRs which constitute the binding pocket of a variable region. Therefore, an acceptor variable region framework can be targeted for the simultaneous incorporation of donor CDR variant populations at one, two or all three recipient CDR locations in a heavy or light chain. The choice of which CDR or the number of CDRs to target with amino acid position changes will depend on, for example, if a full CDR grafting into an acceptor is desired or whether the method is being performed for optimization of binding affinity.

Another approach for selecting donor CDR amino acids to change for conferring donor CDR binding affinity onto an antibody acceptor variable region framework is to select known or readily identifiable CDR positions that are highly variable. For example, the variable region CDR3 is generally highly variable. This region therefore can be selectively targeted for amino acid position changes during grafting procedures to ensure binding affinity reacquisition or augmentation, either alone or together with relevant acceptor variable framework changes, as described herein.

If desired, CDR variant populations having one or more altered amino acid positions can be advantageously combined with a framework variant population having one or more altered amino acid positions. Such a combination can result in beneficial combinations of changes, which are identified by screening for an optimized activity.

The resultant population of CDR grafted variable regions therefore contain a species corresponding to the authentic parent amino acid residue at each position as well as a diverse number of different species which correspond to the possible combinations and permutations of the authentic parent amino acid residues together with the variant residues at each of the selected CDR positions. Such a diverse population of CDR grafted variable regions are screened for an altered variable region species which retains donor CDR binding activity, or which has optimized binding activity.

An acceptor can be selected so that it is closely similar to the variable region amino acid

sequence harboring the donor CDRs. In addition, a variety of acceptors less closely related to the donor antibody can be used. Alternatively, a library of all possible or relevant changes in the acceptor framework
5 can be made and then screened for those variable regions, or heteromeric binding fragments thereof, that maintain or exhibit increased binding affinity compared to the donor molecule. The donor CDRs can be grafted into a variety of naturally occurring acceptor
10 frameworks or altered frameworks having one or more changes or even a library containing changes at one or more positions. Therefore, the applicability is not preconditioned on the availability or search for an acceptor framework variable region similar to that of
15 the donor.

The methods for conferring donor CDR binding affinity onto a variable region can involve identifying the relevant amino acid positions in the acceptor framework that are known or predicted to influence a
20 CDR conformation, or that are known or predicted to influence the spacial context of amino acid side chains within the CDR that participate in binding, and then generating a population of altered variable region species that incorporate a plurality of different amino
25 acid residues at those positions. For example, the different amino acid residues at those positions can be incorporated either randomly or with a predetermined bias and can include all of the twenty naturally occurring amino acid residues at each of the relevant
30 positions. Subsets, including less than all of the naturally occurring amino acids can additionally be chosen for incorporation at the relevant framework positions. Including a plurality of different amino acid residues at each of the relevant framework
35 positions ensures that there will be at least one

species within the population that will have framework changes which allows the CDRs to reacquire their donor binding affinity in the context of the acceptor framework variable region.

5 For humanizing an antibody, any of a variety of human frameworks can be selected for CDR grafting. For example, CDRs of HUIV26 or HUI77 can be cloned into a variety of human framework sequences. The frameworks can be generated using human germline genes encoding
10 heavy and light chain variable regions as well as J regions to obtain human framework sequences for CDR grafting. Exemplary human framework nucleotide sequences include, for example, the framework sequences of DPK24 (VKIV) (SEQ ID NO:5), DP-54 (VHIII) (SEQ ID
15 NO:7), DPK13 (VKII) (SEQ ID NO:13), DP-28 (VHII) (SEQ ID NO:15), as well as J regions JK1 (SEQ ID NO:217), JK2 (SEQ ID NO:218) and JH6 (SEQ ID NO:219). It is understood that framework regions from any available germline sequence can be combined with any available J
20 sequence, as desired, to generate a human framework for grafting CDRs. For example, an alignment of mouse variable regions of HUIV26 and HUI77 with an exemplary human framework is shown in Figures 2C and 3C, respectively. A fusion of VKIV/JK2 light chain
25 variable region and VHIII/JH6 heavy chain variable region are aligned with HUIV26 (Figure 2C). A fusion of VKII/JK1 light chain variable region and VHIII/JH6 heavy chain variable region are aligned with HUI77 (Figure 3C). An exemplary fusion of a germline and J
30 region is shown in Figure 3D, which is aligned with the HUI77 light chain. It is understood that any available human framework can be selected for CDR grafting and, if desired, optimized by the methods disclosed herein. As disclosed herein, CDRs having beneficial mutations

can be grafted into a variety of frameworks and have retained or improved activity (see Example III).

Selection of the relevant framework amino acid positions to alter depends on a variety of criteria well known to those skilled in the art. One criteria for selecting relevant framework amino acids to change can be the relative differences in amino acid framework residues between the donor and acceptor molecules. Selection of relevant framework positions to alter using this approach is simple and has the advantage of avoiding any subjective bias in residue determination or any bias in CDR binding affinity contribution by the residue.

Another criteria that can be used for determining the relevant amino acid positions to change can be, for example, selection of framework residues that are known to be important or to contribute to CDR conformation. For example, canonical framework residues are important for CDR conformation or structure. Targeting of a canonical framework residue as a relevant position to change can identify a more compatible amino acid residue in context with its associated donor CDR sequence.

The frequency of an amino acid residue at a particular framework position is another criteria which can be used for selecting relevant framework amino acid positions to change. For example, comparison of the selected framework with other framework sequences within its subfamily can reveal residues that occur at minor frequencies at a particular position or positions. Such positions harboring less abundant residues are similarly applicable for selection as a position to alter in the acceptor variable region framework.

The relevant amino acid positions to change also can be selected, for example, based on proximity to a CDR. In certain contexts, such residues can participate in CDR conformation or antigen binding.

5 Moreover, this criteria can similarly be used to prioritize relevant positions selected by other criteria described herein. Therefore, differentiating between residues proximal and distal to one or more CDRs is an efficient way to reduce the number of

10 relevant positions to change.

Other criteria for selecting relevant amino acid framework positions to alter include, for example, residues that are known or predicted to reside in three-dimensional space near the antigen-CDR interface

15 or predicted to modulate CDR activity. Similarly, framework residues that are known or predicted to form contacts between the heavy (V_H) and light (V_L) chain variable region interface can be selected. Such framework positions can affect the conformation or

20 affinity of a CDR by modulating the CDR binding pocket, antigen interaction or the V_H and V_L interaction. Therefore, selection of these amino acid positions for constructing a diverse population for screening of binding activity can be used to identify framework

25 changes which replace residues having detrimental effects on CDR conformation or compensate for detrimental effects of residues occurring elsewhere in the framework.

Other framework residues that can be selected

30 for alteration include amino acid positions that are inaccessible to solvent. Such residues are generally buried in the variable region and are therefore capable of influencing the conformation of the CDR or V_H and V_L interactions. Solvent accessibility can be predicted,

for example, from the relative hydrophobicity of the environment created by the amino acid side chains of the polypeptide or by known three-dimensional structural data.

5 Following selection of relevant amino acid positions in the donor CDRs, as well as any relevant amino acid positions in the framework regions desired to be varied, amino acid changes at some or all of the selected positions can be incorporated into encoding
10 nucleic acids for the acceptor variable region framework and donor CDRs. Altered framework or CDR sequences can be individually made and tested, or can be simultaneously combined and tested, if desired.

 The variability at any or all of the altered
15 positions can range from a few to a plurality of different amino acid residues, including all twenty naturally occurring amino acids or functional equivalents and analogues thereof.

 Selection of the number and location of the
20 amino acid positions to vary is flexible and can depend on the intended use and desired efficiency for identification of the altered variable region having a desirable activity such as substantially the same or greater binding affinity compared to the donor variable
25 region. In this regard, the greater the number of changes that are incorporated into a altered variable region population, the more efficient it is to identify at least one species that exhibits a desirable activity, for example, substantially the same or
30 greater binding affinity as the donor. Alternatively, where the user has empirical or actual data to the affect that certain amino acid residues or positions contribute disproportionately to binding affinity, then

it can be desirable to produce a limited population of altered variable regions which focuses on changes within or around those identified residues or positions.

5 For example, if CDR grafted variable regions are desired, a large, diverse population of altered variable regions can include all the non-identical framework region positions between the donor and acceptor framework and all single CDR amino acid
10 position changes. Alternatively, a population of intermediate diversity can include subsets, for example, of only the proximal non-identical framework positions to be incorporated together with all single CDR amino acid position changes. The diversity of the
15 above populations can be further increased by, for example, additionally including all pairwise CDR amino acid position changes. In contrast, populations focusing on predetermined residues or positions which incorporate variant residues at as few as one framework
20 and/or one CDR amino acid position can similarly be constructed for screening and identification of an altered antibody variable region of the invention. As with the above populations, the diversity of such focused populations can be further increased by
25 additionally expanding the positions selected for change to include other relevant positions in either or both of the framework and CDR regions. There are numerous other combinations ranging from few changes to many changes in either or both of the framework regions
30 and CDRs that can additionally be employed, all of which will result in a population of altered variable regions that can be screened for the identification of at least one CDR grafted altered variable region having desired activity, for example, binding activity to a
35 cryptic collagen site. Those skilled in the art will

know, or can determine, which selected residue positions in the framework or donor CDRs, or subsets thereof, can be varied to produce a population for screening and identification of an altered antibody of the invention given the teachings and guidance provided herein.

Simultaneous incorporation of all of the CDR encoding nucleic acids and all of the selected amino acid position changes can be accomplished by a variety of methods known to those skilled in the art, including for example, recombinant and chemical synthesis. For example, simultaneous incorporation can be accomplished by, for example, chemically synthesizing the nucleotide sequence for the acceptor variable region, fused together with the donor CDR encoding nucleic acids, and incorporating at the positions selected for harboring variable amino acid residues a plurality of corresponding amino acid codons.

One such method well known in the art for rapidly and efficiently producing a large number of alterations in a known amino acid sequence or for generating a diverse population of variable or random sequences is known as codon-based synthesis or mutagenesis. This method is the subject matter of U.S. Patent Nos. 5,264,563 and 5,523,388 and is also described in Glaser et al. J. Immunology 149:3903 (1992). Briefly, coupling reactions for the randomization of, for example, all twenty codons which specify the amino acids of the genetic code are performed in separate reaction vessels and randomization for a particular codon position occurs by mixing the products of each of the reaction vessels. Following mixing, the randomized reaction products corresponding to codons encoding an equal mixture of

all twenty amino acids are then divided into separate reaction vessels for the synthesis of each randomized codon at the next position. For the synthesis of equal frequencies of all twenty amino acids, up to two codons
5 can be synthesized in each reaction vessel.

Variations to these synthesis methods also exist and include for example, the synthesis of predetermined codons at desired positions and the biased synthesis of a predetermined sequence at one or
10 more codon positions. Biased synthesis involves the use of two reaction vessels where the predetermined or parent codon is synthesized in one vessel and the random codon sequence is synthesized in the second vessel. The second vessel can be divided into multiple
15 reaction vessels such as that described above for the synthesis of codons specifying totally random amino acids at a particular position. Alternatively, a population of degenerate codons can be synthesized in the second reaction vessel such as through the coupling
20 of NNG/T nucleotides where N is a mixture of all four nucleotides. Following synthesis of the predetermined and random codons, the reaction products in each of the two reaction vessels are mixed and then redivided into an additional two vessels for synthesis at the next
25 codon position.

A modification to the above-described codon-based synthesis for producing a diverse number of variant sequences can similarly be employed for the production of the variant populations described herein.
30 This modification is based on the two vessel method described above, which biases synthesis toward the parent sequence and allows the user to separate the variants into populations containing a specified number of codon positions that have random codon changes.

Briefly, this synthesis is performed by continuing to divide the reaction vessels after the synthesis of each codon position into two new vessels. After the division, the reaction products from each consecutive pair of reaction vessels, starting with the second vessel, is mixed. This mixing brings together the reaction products having the same number of codon positions with random changes. Synthesis proceeds by then dividing the products of the first and last vessel and the newly mixed products from each consecutive pair of reaction vessels and redividing into two new vessels. In one of the new vessels, the parent codon is synthesized and in the second vessel, the random codon is synthesized. For example, synthesis at the first codon position entails synthesis of the parent codon in one reaction vessel and synthesis of a random codon in the second reaction vessel. For synthesis at the second codon position, each of the first two reaction vessels is divided into two vessels yielding two pairs of vessels. For each pair, a parent codon is synthesized in one of the vessels and a random codon is synthesized in the second vessel. When arranged linearly, the reaction products in the second and third vessels are mixed to bring together those products having random codon sequences at single codon positions. This mixing also reduces the product populations to three, which are the starting populations for the next round of synthesis. Similarly, for the third, fourth and each remaining position, each reaction product population for the preceding position are divided and a parent and random codon synthesized.

Following the above modification of codon-based synthesis, populations containing random codon changes at one, two, three and four positions as well

as others can be conveniently separated out and used based on the need of the individual. Moreover, this synthesis scheme also allows enrichment of the populations for the randomized sequences over the
5 parent sequence since the vessel containing only the parent sequence synthesis is similarly separated out from the random codon synthesis.

Other methods well known in the art for producing a large number of alterations in a known
10 amino acid sequence or for generating a diverse population of variable or random sequences include, for example, degenerate or partially degenerate oligonucleotide synthesis. Codons specifying equal mixtures of all four nucleotide monomers, represented
15 as NNN, results in degenerate synthesis. Whereas partially degenerate synthesis can be accomplished using, for example, the NNG/T codon described previously. Other methods well known in the art can alternatively be used such as the use of statistically
20 predetermined, or varigated, codon synthesis, which is the subject matter of U.S. Patent Nos. 5,223,409 and 5,403,484.

Once the populations of altered variable region encoding nucleic acids have been constructed as
25 described above, they can be expressed to generate a population of altered variable region polypeptides that can be screened for binding affinity. For example, the altered variable region encoding nucleic acids can be cloned into an appropriate vector for propagation,
30 manipulation and expression. Such vectors are known or can be constructed by those skilled in the art and should contain all expression elements sufficient for the transcription, translation, regulation, and if desired, sorting and secretion of the altered variable

region polypeptides. The vectors can be suitable for expression in either procaryotic or eukaryotic host systems so long as the expression and regulatory elements function in the respective host system. The expression vectors can additionally include regulatory elements for inducible or cell type-specific expression. One skilled in the art will know which host systems are compatible with a particular vector and which regulatory or functional elements are sufficient to achieve expression of the polypeptides in soluble, secreted or cell surface forms.

Appropriate host cells, include for example, bacteria and corresponding bacteriophage expression systems, yeast, avian, insect and mammalian cells. Methods for recombinant expression, screening and purification of populations of altered variable regions or altered variable region polypeptides within such populations in various host systems are well known in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1992) and in Ausubel et al., Current Protocols in Molecular Biology, (Supplement 54), John Wiley & Sons, New York (2001). The choice of a particular vector and host system for expression and screening of altered variable regions are known to those skilled in the art and will depend on the preference of the user. Moreover, expression of diverse populations of heteromeric receptors in either soluble or cell surface form using filamentous bacteriophage vector/host systems is well known in the art and is the subject matter of U.S. Patent No. 5,871,974.

The expressed population of altered variable region polypeptides can be screened for the

identification of one or more altered variable region species exhibiting optimized binding activity, for example, binding affinity substantially the same or greater than the donor CDR variable region. Screening
5 can be accomplished using various methods well known in the art for determining the binding affinity of a polypeptide or compound. Additionally, methods based on determining the relative affinity of binding molecules to their partner by comparing the amount of binding
10 between the altered variable region polypeptides and the donor CDR variable region can similarly be used for the identification of species exhibiting binding affinity substantially the same or greater than the donor CDR variable region. All of such methods can be
15 performed, for example, in solution or in solid phase. Moreover, various formats of binding assays are well known in the art and include, for example, immobilization to filters such as nylon or nitrocellulose; two-dimensional arrays, enzyme linked
20 immunosorbant assay (ELISA), radioimmunoassay (RIA), panning and plasmon resonance. Such methods can be found described in, for example, Harlow and Lane, *supra*, 1988.

For the screening of populations of
25 polypeptides such as the altered variable region populations produced by the methods of the invention, immobilization of the populations of altered variable regions to filters or other solid substrate can be advantageous because large numbers of different species
30 can be efficiently screened for antigen binding. Such filter lifts allow for the identification of altered variable regions that exhibit substantially the same or greater binding affinity compared to the donor CDR variable region. Alternatively, if the populations of
35 altered variable regions are expressed on the surface

of a cell or bacteriophage, panning on immobilized antigen can be used to efficiently screen for variants having antigen binding activity or to determine the relative binding affinity of species within the
5 population.

Another affinity method for screening populations of altered variable regions polypeptides is a capture lift assay that is useful for identifying a binding molecule having selective affinity for a ligand
10 (Watkins et. al., (1997); WO 99/06834). This method employs the selective immobilization of altered variable regions to a solid support and then screening of the selectively immobilized altered variable regions for selective binding interactions against the cognate
15 antigen or binding partner. Selective immobilization functions to increase the sensitivity of the binding interaction being measured since initial immobilization of a population of altered variable regions onto a solid support reduces non-specific binding interactions
20 with irrelevant molecules or contaminants which can be present in the reaction.

Another method for screening populations or for measuring the affinity of individual altered variable region polypeptides is through surface plasmon
25 resonance (SPR). This method is based on the phenomenon which occurs when surface plasmon waves are excited at a metal/liquid interface. Light is directed at, and reflected from, the side of the surface not in contact with sample, and SPR causes a reduction in the reflected light intensity at a specific combination of
30 angle and wavelength. Biomolecular binding events cause changes in the refractive index at the surface layer, which are detected as changes in the SPR signal. The binding event can be either binding association or

disassociation between a receptor-ligand pair. The changes in refractive index can be measured essentially instantaneously and therefore allows for determination of the individual components of an affinity constant.

- 5 More specifically, the method enables accurate measurements of association rates (k_{on}) and disassociation rates (k_{off}).

Measurements of k_{on} and k_{off} values can be used identify altered variable regions or optimized variable
10 regions that are therapeutically more efficacious. For example, an altered variable region, or heteromeric binding fragment thereof, can be more efficacious because it has, for example, a higher k_{on} valued compared to variable regions and heteromeric binding
15 fragments that exhibit similar binding affinity. Increased efficacy is conferred because molecules with higher k_{on} values can specifically bind and inhibit their target at a faster rate. Similarly, a molecule of the invention can be more efficacious because it
20 exhibits a lower k_{off} value compared to molecules having similar binding affinity. Increased efficacy observed with molecules having lower k_{off} rates can be observed because, once bound, the molecules are slower to dissociate from their target. Although described with
25 reference to the altered variable regions and optimized variable regions of the invention, the methods described above for measuring association and dissociation rates are applicable to essentially any antibody or fragment thereof for identifying more
30 effective binders for therapeutic or diagnostic purposes.

Methods for measuring the affinity, including association and dissociation rates using surface plasmon resonance are well known in the art and can be
35 found described in, for example, Jönsson and Malmquist,

Advances in Biosensors, 2:291-336 (1992) and Wu et al.
Proc. Natl. Acad. Sci. USA, 95:6037-6042 (1998).

Moreover, one apparatus well known in the art for measuring binding interactions is a BIAcore 2000
5 instrument which is commercially available through Pharmacia Biosensor, (Uppsala, Sweden).

Using any of the above described screening methods, as well as others well known in the art, an altered variable region having optimized binding
10 activity, for example, binding affinity substantially the same or greater than the donor CDR variable region is identified by detecting the binding of at least one altered variable region within the population to its antigen or cognate ligand. In addition to optimizing
15 for antigen binding activity, catalytic activity can also be included in an invention antibody and optimized using the methods disclosed herein for binding affinity optimization. Accordingly, the above methods can be modified to include the addition of substrate and
20 reactants to screen for optimized catalytic activity. Comparison, either independently or simultaneously in the same screen, with the donor variable region will identify those binders that have substantially the same or greater binding affinity as the donor. Those
25 skilled in the art will know, or can determine using the donor variable region, binding conditions which are sufficient to identify selective interactions over non-specific binding.

Detection methods for identification of
30 binding species within the population of altered variable regions can be direct or indirect and can include, for example, the measurement of light emission, radioisotopes, colorimetric dyes and fluorochromes. Direct detection includes methods that

function without intermediates or secondary measuring procedures to assess the amount of bound antigen or ligand. Such methods generally employ ligands that are themselves labeled with a detectable moiety, for
5 example, a radioactive, light emitting, fluorescent, colorimetric or enzyme moiety. In contrast, indirect detection includes methods that function through an intermediate or secondary measuring procedure. These methods generally employ molecules that specifically
10 react with the antigen or ligand and can themselves be directly labeled with a detectable moiety or detected by a secondary reagent. For example, an antibody specific for a ligand can be detected using a secondary antibody capable of interacting with the first antibody
15 specific for the ligand, again using the detection methods described above for direct detection. Moreover, for the specific example of screening for catalytic antibodies, the disappearance of a substrate or the appearance of a product can be used as an
20 indirect measure of binding affinity or catalytic activity.

Isolated variable regions exhibit binding affinity as single chains, in the absence of assembly into a heteromeric structure with their respective V_H or V_L subunits. As such, populations of V_H and V_L
25 altered variable regions polypeptides can be expressed alone and screened for binding activity, for example, optimized activity having substantially the same or greater binding affinity compared to the CDR donor V_H or V_L variable region. Alternatively, populations of
30 V_H and V_L altered variable regions polypeptides can be coexpressed so that they self-assemble into heteromeric altered variable region binding fragments. The heteromeric binding fragment population can then be
35 screened for species exhibiting binding affinity

substantially the same or greater than the CDR donor variable region binding fragment.

Employing the methods for simultaneously grafting and optimizing, or for optimizing, it is possible to generate heteromeric variable region binding fragments having increases in affinities of greater than about 2-fold, 3-fold, 4-fold, 5-fold, 8-fold or 10-fold. In particular, heteromeric variable region binding fragments can be generated having increases in affinities of greater than 12-fold, 15-fold, 20-fold, and 25-fold as well as affinities greater than 50-fold, 100-fold, 200-fold, 500-fold or 1000-fold compared to the donor or parent molecule.

Additionally, the methods described herein for optimizing are also applicable for producing catalytic heteromeric variable region fragments or for optimizing their catalytic activity. Catalytic activity can be optimized by changing, for example, the on or off rate of substrate binding, the substrate binding affinity, the transition state binding affinity, the turnover rate (k_{cat}) or the K_m. Methods for measuring these characteristics are well known in the art (see, for example Segel, Enzyme Kinetics, John Wiley & Sons, New York (1975)). Such methods can be employed in the screening steps of the methods described above when used for optimizing the catalytic activity of a heteromeric variable region binding fragment.

Additionally, the methods for conferring donor CDR binding affinity onto an antibody acceptor variable region framework are applicable for grafting CDRs as described by Kabat et al., *supra*, Chothia et al., *supra* or MacCallum et al., *supra*. The methods

similarly can be used for grafting into an acceptor framework overlapping regions or combinations of CDRs as described in Kabat et al., *supra*, Chothia et al., *supra* or MacCallum et al., *supra*. Generally, variable
5 region CDRs are grafted by identifying the boundaries described by one of the CDR definitions known in the art and set forth herein. However, because the methods are directed to constructing and screening populations of CDR grafted altered variable regions, which can
10 incorporate relevant amino acid position changes in both the framework and CDR regions, and such variations can, for example, compensate or augment amino acid changes elsewhere in the variable region, the exact boundary of a particular CDR or set of variable region
15 CDRs can be varied. Therefore, the exact CDR region to graft, whether it is the region described by Kabat et al., Chothia et al. or MacCallum et al., or any combination thereof, will essentially depend on the preference of the user.

20 Similarly, the methods described previously for optimizing the binding affinity of an antibody also are applicable for use with essentially any variable region for which an encoding nucleic acid is, or can be made, available. As with the methods for conferring
25 donor CDR binding affinity, many applications of the methods for optimizing binding affinity will be for modifying the binding affinity of CDR grafted variable regions having human frameworks. Again, such molecules are significantly less antigenic in human patients and
30 therefore therapeutically valuable in the treatment of human diseases. However, the methods of the invention for optimizing the binding affinity of a variable region are applicable to all species of variable regions. Therefore, the invention includes binding
35 affinity optimization of variable regions derived from

human, mouse, rat, rabbit, goat and chicken, or any other desired species.

The methods of the invention have been described with reference to variable regions and heteromeric variable region binding fragments. Those skilled in the art will understand that all of such methods are applicable to whole antibodies and functional fragments thereof as well as to regions and functional domains other than the antigen binding variable region of antibodies, if desired.

An association rate can be determined in any non-equilibrium mixture including, for example, one formed by rapidly contacting a binding polypeptide and ligand or by rapidly changing temperature. A non-equilibrium mixture can be a pre-equilibrium mixture. A pre-equilibrium mixture can be formed, for example, by contacting a soluble binding polypeptide and soluble ligand in a condition where the amount of total ligand and total binding polypeptide in the detection chamber are constant. Measurements of association rates in pre-equilibrium mixtures can be made in formats providing rapid mixing of binding polypeptide with ligand and rapid detection of changing properties of the binding polypeptide or ligand on a timescale of milliseconds or faster. Stopped flow and rapid quench flow instruments such as those described below provide a convenient means to measure non-equilibrium kinetics. The association rate can also be measured in non-equilibrium mixtures including, for example, solutions containing insoluble species of binding polypeptide, ligand or binding polypeptide bound to ligand, or solutions containing variable concentrations of total ligand or total binding polypeptide. Measurement of an association rate in a non-equilibrium mixture can be

made in formats providing attachment of a ligand to a surface and continuous flow of a solution containing the binding polypeptide over the surface, or vice-versa, combined with rapid detection of changing
5 properties of the binding polypeptide, ligand or surface such that measurements are made on a timescale of milliseconds or faster. Examples of formats providing non-equilibrium measurement of association rates include surface plasmon resonance instruments and
10 evanescent wave instruments.

Association rate measurements can be made by detecting the change in a property of the binding polypeptide or ligand that exists between the bound and unbound state or by detecting a change in the
15 surrounding environment when binding polypeptide and ligand associate. Properties of the binding polypeptide or ligand that can change upon association and that can be used to measure association rates include, for example, absorption and emission of heat,
20 absorption and emission of electromagnetic radiation, affinity for a receptor, molecular weight, density, mass, electric charge, conductivity, magnetic moment of nuclei, spin state of electrons, polarity, molecular shape, or molecular size. Properties of the surrounding
25 environment that can change when binding polypeptide associates with ligand include, for example, temperature and refractive index of surrounding solvent.

Formats for measuring association rates in
30 pre-equilibrium mixtures include, for example, stopped flow kinetic instruments and rapid quench flow instruments. A stopped flow instrument can be used to push solutions containing a binding polypeptide and ligand from separate reservoirs into a mixing chamber

just prior to passage into a detection cell. The instrument can then detect a change in one or more of the above described properties to monitor progress of the binding event. A rapid quench flow instrument can be used to rapidly mix a solution containing a binding polypeptide with a solution containing a ligand followed by quenching the binding reaction after a finite amount of time. A change in one or more of the above described properties can then be detected for quenched mixtures produced by quenching at different times following mixing. Quenching can be performed for example by freezing or addition of a chemical quenching agent so long as the quenching step does not inhibit detection of the property relied upon for measurement of binding rate. Thus, a rapid quench instrument can be useful, for example, in situations where spectroscopic detection is not convenient. A variety of instruments are commercially available from vendors such as KinTek Corp. (State College, PA) and Hi-Tech Scientific (Salisbury, UK).

Formats for measuring association rates in non-equilibrium mixtures include, for example, surface plasmon resonance and evanescent wave instruments. Surface plasmon resonance and evanescent wave technology utilize a ligand or binding polypeptide attached to a biosensor surface and a solution containing either the binding polypeptide or ligand respectively that is passed over the biosensor surface. The change in refractive index of the solution that occurs at the surface of a chip when binding polypeptide associates with ligand can be measured in a time dependent fashion. For example, surface plasmon resonance is based on the phenomenon which occurs when surface plasmon waves are excited at a metal/liquid interface. Light is directed at, and reflected from,

the side of the surface not in contact with sample, and SPR causes a reduction in the reflected light intensity at a specific combination of angle and wavelength. Biomolecular binding events cause changes in the refractive index at the surface layer, which are detected as changes in the SPR signal. The binding event can be either binding association or disassociation between a receptor-ligand pair. The changes in refractive index can be measured essentially instantaneously and therefore allows for determination of the individual components of an affinity constant. More specifically, the method enables accurate measurements of association rates (k_{on}) and disassociation rates (k_{off}). Surface plasmon resonance instruments are available in the art including, for example, the BIAcore instrument, IBIS system, SPR-CELLIA system, Spreeta, and Plasmon SPR and evanescent wave technology is available in the Iasys system as described, for example, in Rich and Myszka, Curr. Opin. Biotech. 11:54-61 (2000).

Another method for measuring binding affinity includes comparative ELISA. As disclosed herein, an approximation of changes in affinity based on shifts in half-maximal binding was used to identify k_{on} and k_{off} values relative to wild type (Example III). Such a method is particularly useful for screening large numbers of variants, whereas the above-described methods can be used for detailed analysis of binding activity.

The association rate can be determined by measuring a change in a property of a ligand or binding polypeptide at one or more discrete time intervals during the binding event using, for example, the methods described above. Measurements determined at

discreet time intervals during the binding event can be used to determine a quantitative measure of association rate or a relative measure of association rate.

Quantitative measures of association rate can include,

5 for example, an association rate value or k_{on} value.

Quantitative values of association rate or k_{on} can be determined from a mathematical or graphical analysis of a time dependent measurement. Such analyses are well known in the art and include algorithms for fitting

10 data to a sum of exponential or linear terms or algorithms for computer simulation to fit data to a binding model as described for example in Johnson, Cur. Opin. Biotech. 9:87-89 (1998), which is incorporated herein by reference.

15 Association rates can be determined from mixtures containing insoluble species or variable concentrations of total ligand or total binding polypeptide using mathematical and graphical analyses such as those described above if effects of mass

20 transport are accounted for in the reaction. One skilled in the art can account for mass transport by comparing association rates under conditions having similar limitations with respect to mass transport or by adjusting the calculated association rate according

25 to models available in the art including, for example those described in Myszkowski et al., Biophys. J. 75:583-594 (1998), which is incorporated herein by reference.

A higher value of either the association rate or k_{on} is generally indicative of improved therapeutic

30 potency. Thus, quantitative determinations provide an advantage by allowing comparison between an association rate of a binding polypeptide and a therapeutic control determined by different methods so long as the methods

used are understood by one skilled in the art to yield consistent results.

A relative measure of association rate can include, for example, comparison of association rate
5 for two or more binding polypeptides binding to ligand under similar conditions or comparison of association rate for a binding polypeptide binding to ligand with a predefined rate. Comparison of association rate for two or more binding polypeptides can include a standard
10 of known association rate or a molecule of known therapeutic effect. A predefined rate used for comparison can be determined by calibrating the measurement relative to a previously measured rate including, for example, one available in the scientific
15 literature or in a database. An example of a comparison with a predefined rate is selection of the species of binding polypeptide bound to ligand at a discreet time interval defined by the predefined rate by using a time actuated selection device.

20 For purposes of comparison, the association rate of a binding polypeptide and ligand can be determined relative to association rate for a therapeutic control and the same ligand. A comparison can also be made according to a quantitative
25 association rate for binding polypeptide and ligand compared to a quantitative association rate for a therapeutic control and ligand. Relative or quantitative association rates can be determined by the methods described above. Determination of association
30 rates for a binding polypeptide associating with a ligand can be performed simultaneously with a binding polypeptide and therapeutic control or at separate times, provided conditions are sufficiently similar in each assay to allow valid comparison. Thus,

association rate determined for a binding polypeptide can be compared to a previously measured association rate for a therapeutic control.

A binding polypeptide having improved
5 therapeutic potency can be distinguished from a binding polypeptide that has an increased K_a for a ligand but not improved therapeutic potency. Methods for identifying a therapeutic binding polypeptide based on K_a rely on an equilibrium measurement which, absent
10 time dependent measurements made in a non-equilibrium condition, are inaccurate for identifying a binding polypeptide having increased association rate and therefore improved therapeutic potency. According to the relationship $K_a = k_{on}/k_{off}$, an increased K_a for
15 association of a binding polypeptide and ligand can be due to changes in k_{on} or k_{off} . For example, a binding polypeptide having improved therapeutic potency can have a reduced K_a if a reduction in k_{off} occurs that over compensates for an increase in k_{on} . Thus, changes
20 in K_a , being influenced by changes in k_{off} , do not unambiguously correlate with changes in therapeutic potency since binding polypeptides having improved therapeutic potency can display either reduced or increased K_a .

25 For optimization of binding activity of an antibody of the invention, the fold increase in association rate can be indicated by an increase in k_{on} . Therefore, k_{on} can be about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or
30 10-fold or more using methods described herein. The k_{on} can be at least about $1 \times 10^2 \text{ M}^{-1}\text{s}^{-1}$, $2 \times 10^2 \text{ M}^{-1}\text{s}^{-1}$, $5 \times 10^2 \text{ M}^{-1}\text{s}^{-1}$, $1 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$, $2 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$, $5 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$, $1 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, $2 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, $5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, $1 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, $2 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, or $3 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$. The k_{on} can also be

increased to at least about $5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, $7 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, $9 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, $1 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, $3 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, $5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, $7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, $9 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ or $1 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ or more.

Furthermore, the increase in k_{on} resulting in improved
5 therapeutic potency can be independent of an effect of a change in K_{a} for the binding polypeptide. The binding polypeptide having an increase in k_{on} can have a K_{a} value similar to K_{a} for its parent polypeptide or a K_{a} value lower than K_{a} for its parent polypeptide.

10 The invention also provides nucleic acids encoding the antibodies and CDRs of the invention. The invention further provides nucleic acids encoding the mouse antibodies HUIV26 (SEQ ID NOS:1 and 3) and HUI77 (SEQ ID NOS:5 and 7) (see Figures 2 and 3). Further
15 provided are nucleic acids encoding HUIV26 CDRs (SEQ ID NOS:20, 22, 24, 26, 28 and 30) and encoding HUI77 CDRs (SEQ ID NOS:32, 34, 36, 38, 40 and 42). Such nucleic acids include nucleic acids having degenerate codons encoding any or all of the amino acids in the CDRs.
20 For example, the invention provides nucleic acids encoding HUIV26 CDRs: V_{L} CDR1, SEQ ID NOS:19; V_{L} CDR2, SEQ ID NO:21; V_{L} CDR3, SEQ ID NO:23; V_{H} CDR1, SEQ ID NO:25; V_{H} CDR2, SEQ ID NO:27; and V_{H} CDR3, SEQ ID NO:29. The invention also provides nucleic acids
25 encoding HUI77 CDRs: V_{L} CDR1, SEQ ID NOS:31; V_{L} CDR2, SEQ ID NO:33; V_{L} CDR3, SEQ ID NO:35; V_{H} CDR1, SEQ ID NO:37; V_{H} CDR2, SEQ ID NO:39; and V_{H} CDR3, SEQ ID NO:41. Also included are degenerate versions of such nucleic acids such that they encode the amino acid
30 sequences referenced as SEQ ID NOS:20, 22, 24, 26, 28 and 30 for HUIV26 and SEQ ID NOS:32, 34, 36, 38, 40 and 42 for HUI77.

Further provided are nucleic acids encoding a HUIV26 or HUI77 CDR containing one or more amino acid

substitutions. For example, the invention provides nucleic acids encoding the CDRs of HUIV26 and HUI77 having single or multiple amino acid substitutions, as disclosed herein. If a nucleic acid encoding a CDR
5 having one or more amino acid substitution is derived, for example, from one of SEQ ID NOS:19, 21, 23, 25, 27 or 29 for HUIV26 or SEQ ID NOS:31, 33, 35, 37, 39 or 41 for HUI77, the amino acid substitutions can be encoded by any of the corresponding degenerate codons for that
10 amino acid. Nucleic acids encoding such CDR variants can also include degenerate codons at any or all of the wild type amino acid positions.

Throughout the application, various nucleic acids and oligonucleotide primers, in addition to the
15 naturally occurring nucleotides A, C, G, T or U, refer to standard abbreviations: R = G or A; Y = T/U or C; M = A or C; K = G or T/U; S = G or C; W = A or T/U; B = G, C or T/U; D = A, G or T/U; H = A, C or T/U; V = A, G or C; N = any nucleotide.

20 The antibodies of the invention have binding activity for a cryptic collagen epitope. The HUIV26 and HUI77 antibodies have been shown to target to angiogenic vasculature (see Xu et al., *supra*, 2001; WO 00/40597). Accordingly, the grafted HUIV26 and HUI77
25 antibodies of the invention, which specifically bind to a cryptic collagen epitope, similarly can target to angiogenic vasculature. One of the most significant and important aspects of the monoclonal antibodies HUIV26 and HUI77, and the grafted forms thereof
30 disclosed herein, is that of their specificity. It is expected that systemic administration of antibodies of the invention will have minimal if any toxic side effects since the cryptic epitope(s) that is recognized by the HUIV26 and HUI77 antibodies is/are not exposed

in mature native triple helical collagen but is only exposed upon denaturaion, for example, heat denaturation or proteolytic denaturation. Thus, little, if any, binding under normal physiological
5 conditions is expected.

Moreover, the cryptic collagen domain(s) to which HUIV26 and HUI77 bind represents a novel therapeutic target for the treatment of numerous neovascular diseases including tumor growth and
10 metastasis, diabetic retinopathy and other related ocular diseases such as macular degeneration, psoriasis, and rheumatoid arthritis. Other exemplary diseases associated with angiogenesis include, but are not limited to, inflammatory disorders such as immune
15 and non-immune inflammation, chronic articular rheumatism and psoriasis, disorders associated with inappropriate or inopportune invasion of vessels such as diabetic retinopathy, neovascular glaucoma, restenosis, capillary proliferation in atherosclerotic
20 plaques and osteoporosis, and cancer associated disorders, such as solid tumors, solid tumor metastases, angiofibromas, retrolental fibroplasia, hemangiomas, Kaposi's sarcoma and the like cancers which require neovascularization to support tumor
25 growth. Other exemplary tumors include melanoma, carcinoma, sarcoma, fibrosarcoma, glioma and astrocytoma, and the like.

Thus, the methods of the invention can be used to treat an individual having a disease associated
30 with angiogenesis, including those described above. The methods can be used to ameliorate a sign or symptom associated with a disease. For example, in the case of cancer treatment, the methods can be used to inhibit tumor growth. One skilled in the art will know or can

readily determine an appropriate sign or symptom associated with a disease suitable for determining the effectiveness of a therapeutic application using an antibody of the invention.

5 The antibodies of the invention can also be used as an important diagnostic and imaging reagent for the early detection of aberrant neovascularization associated with invasive tumor growth and metastasis. The antibodies of the invention can also be used in
10 staging and grading of tumors since invasive tumor in contrast to benign lesions are likely to be associated with degradation of the surrounding basement membrane.

 Thus, the invention provides a method of targeting angiogenic vasculature, comprising
15 administering an antibody, or functional fragment thereof, the antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope, wherein the antibody or functional fragment is an antibody of the invention. For example, the
20 antibodies can comprise one or more CDRs, including wild type CDRs or variants thereof, of the HUIV26 and HUI77 antibodies, as disclosed herein. The methods of targeting angiogenic vasculature can be used for therapeutic and/or diagnostic purposes.

25 For therapeutic purposes, the antibody, or functional fragment thereof, can be administered as a therapeutic agent itself or can further comprise a therapeutic moiety. In the case of a therapeutic moiety, the moiety can be a drug such as a
30 chemotherapeutic agent, cytotoxic agent, toxin, or anti-angiogenic agent, which refers to a molecule that reduces or inhibits angiogenesis. For example, a cytotoxic agent can be a radionuclide or chemical

compound. Exemplary radionuclides useful as therapeutic agents include, for example, X-ray or γ -ray emitters. In addition, a moiety can be a drug delivery vehicle such as a chambered microdevice, a cell, a
5 liposome or a virus, which can contain an agent such as a drug or a nucleic acid.

Exemplary therapeutic agents include, for example, the anthracyclin, doxorubicin, which has been linked to antibodies and the antibody/doxorubicin
10 conjugates have been therapeutically effective in treating tumors (Sivam et al., Cancer Res. 55:2352-2356 (1995); Lau et al., Bioorg. Med. Chem. 3:1299-1304 (1995); Shih et al., Cancer Immunol. Immunother. 38:92-98 (1994)). Similarly, other anthracyclins, including
15 idarubicin and daunorubicin, have been chemically conjugated to antibodies, which have delivered effective doses of the agents to tumors (Rowland et al., Cancer Immunol. Immunother. 37:195-202 (1993); Aboud-Pirak et al., Biochem. Pharmacol. 38:641-648
20 (1989)).

In addition to the anthracyclins, alkylating agents such as melphalan and chlorambucil have been linked to antibodies to produce therapeutically effective conjugates (Rowland et al., Cancer Immunol.
25 Immunother. 37:195-202 (1993); Smyth et al., Immunol. Cell Biol. 65:315-321 (1987)), as have vinca alkaloids such as vindesine and vinblastine (Aboud-Pirak et al., *supra*, 1989; Starling et al., Bioconj. Chem. 3:315-322 (1992)). Similarly, conjugates of antibodies and
30 antimetabolites such as 5-fluorouracil, 5-fluorouridine and derivatives thereof have been effective in treating tumors (Krauer et al., Cancer Res. 52:132-137 (1992); Henn et al., J. Med. Chem. 36:1570-1579 (1993)). Other chemotherapeutic agents, including cis-platinum

(Schechter et al., Int. J. Cancer 48:167-172 (1991)), methotrexate (Shawler et al., J. Biol. Resp. Mod. 7:608-618 (1988); Fitzpatrick and Garnett, Anticancer Drug Des. 10:11-24 (1995)) and mitomycin-C (Dillman et al., Mol. Biother. 1:250-255 (1989)) also are therapeutically effective when administered as conjugates with various different antibodies. A therapeutic agent can also be a toxin such as ricin.

A therapeutic agent can also be a physical, chemical or biological material such as a liposome, microcapsule, micropump or other chambered microdevice, which can be used, for example, as a drug delivery system. Generally, such microdevices, should be nontoxic and, if desired, biodegradable. Various moieties, including microcapsules, which can contain an agent, and methods for linking a moiety, including a chambered microdevice, to an antibody of the invention are well known in the art and commercially available (see, for example, "Remington's Pharmaceutical Sciences" 18th ed. (Mack Publishing Co. 1990), chapters 89-91; Harlow and Lane, Antibodies: A laboratory manual (Cold Spring Harbor Laboratory Press 1988)).

For diagnostic purposes the antibody, or functional fragment thereof, can further comprise a detectable moiety. A detectable moiety can be, for example, a radionuclide, fluorescent, magnetic, colorimetric moiety, and the like. For *in vivo* diagnostic purposes, a moiety such as a gamma ray emitting radionuclide, for example, indium-111 or technetium-99, can be linked to an antibody of the invention and, following administration to a subject, can be detected using a solid scintillation detector. Similarly, a positron emitting radionuclide such as carbon-11 or a paramagnetic spin label such as carbon-

13 can be linked to the molecule and, following administration to a subject, the localization of the moiety can be detected using positron emission transaxial tomography or magnetic resonance imaging, respectively. Such methods can identify a primary tumor as well as a metastatic lesion.

For diagnostic purposes, the antibodies of the invention can be used to determine the levels of denatured collagen in a tissue or in a bodily fluid. The level of denatured collagen can be determined in a tissue sample obtained from an individual, for example, by tissue biopsy. Exemplary bodily fluids include, but are not limited to, serum, plasma, urine, synovial fluid, and the like.

The invention also provides a method of inhibiting angiogenesis by administering an antibody, or functional fragment thereof, where the antibody or functional fragment thereof has specific binding activity for a cryptic collagen epitope, where the antibody comprises one or more CDRs of the invention. For example, an antibody of the invention can be administered so that angiogenesis is inhibited in a tissue of an individual. The invention further provides a method of targeting a tumor by administering an invention antibody. The invention also provides a method of inhibiting tumor growth by administering an antibody, or functional fragment thereof, of the invention.

The antibodies of the invention can also be used for *in vivo* or *in vitro* diagnostic applications. Thus, the invention provides a method of detecting angiogenic vasculature by contacting angiogenic vasculature with an antibody, or functional fragment

thereof, of the invention. Angiogenic vasculature can be imaged *in vivo* by administering an antibody of the invention, either alone or attached to a detectable moiety, to an individual. The angiogenic vasculature
5 can thus be detected *in vivo*. Alternatively, the antibody can be administered to a tissue obtained from an individual, for example, a tissue biopsy, such that an antibody of the invention can be used *in vitro* for diagnostic purposes to detect angiogenic vasculature.

10

A therapeutic or detectable moiety can be coupled to an antibody of the invention, or functional fragment thereof, by any of a number of well known methods for coupling or conjugating moieties. It is
15 understood that such coupling methods allow the attachment of a therapeutic or detectable moiety without interfering or inhibiting the binding activity of the antibody, that is, the ability to bind a cryptic collagen site. Methods for conjugating moieties to an
20 antibody of the invention, or functional fragment thereof, are well known to those skilled in the art (see, for example, Hermanson, Bioconjugate Techniques, Academic Press, San Diego (1996)).

When administered to a subject, the antibody
25 of the invention is administered as a pharmaceutical composition containing, for example, the antibody and a pharmaceutically acceptable carrier. As disclosed herein, the antibody can be coupled to a therapeutic or detectable moiety. Pharmaceutically acceptable
30 carriers are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters.

A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that act, for example, to stabilize or to increase the absorption of the conjugate. Such physiologically acceptable
5 compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. One skilled in the art will know that the
10 choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the composition. The pharmaceutical composition also can contain an agent such as a cancer therapeutic
15 agent.

One skilled in the art will know that a pharmaceutical composition containing an antibody of the invention can be administered to a subject by various routes including, for example, orally or
20 parenterally, such as intravenously. The composition can be administered by injection or by intubation. The pharmaceutical composition also can be an antibody linked to liposomes or other polymer matrices, which can have incorporated therein, for example, a drug such
25 as a chemotherapeutic agent (Gregoriadis, Liposome Technology, Vols. I to III, 2nd ed. (CRC Press, Boca Raton FL (1993), which is incorporated herein by reference). Liposomes, for example, which consist of phospholipids or other lipids, are nontoxic,
30 physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

For diagnostic or therapeutic methods disclosed herein, an effective amount of the antibody and therapeutic moiety is administered to the subject.

As used herein, the term "effective amount" means the amount of the pharmaceutical composition that produces the desired effect. An effective amount often will depend on whether the antibody itself is administered
5 or whether the antibody is linked to a moiety and the type of moiety. Thus, a lesser amount of a radiolabeled molecule can be required for imaging as compared to the amount of a radioactive drug/antibody conjugate administered for therapeutic purposes. An
10 effective amount of a particular antibody/moiety for a specific purpose can be determined using methods well known to those in the art. One skilled in the art can readily determine an appropriate dose of an antibody of the invention for an effective amount for therapeutic
15 or diagnostic purposes.

For therapeutic or *in vivo* diagnostic purposes, it is understood that any of a variety of methods of administration can be used so long as the administration is effective for a desired purpose.
20 Such methods of administration include, for example, intravenous, transdermal, intrasynovial, intramuscular, intratumoral, intraocular, intranasal, intrathecal, topical, oral, or the like. One skilled in the art can readily determine an appropriate mode of administration
25 depending on the desired therapeutic effect or desired diagnostic purpose.

Furthermore, it is understood that for therapeutic or diagnostic applications, an antibody of the invention in general is administered to a mammal,
30 for example, a human. Applications of an antibody of the invention for domestic animals or agricultural purposes include other mammals, for example, a non-human primate, pig, cow, horse, goat, sheep, mule, donkey, dog, cat, rabbit, mouse, rat, and the like.

It is understood that any of the therapeutic methods disclosed herein using an antibody of the invention can be used in combination with other therapeutic methods. For example, an antibody of the invention, either the antibody itself or an antibody attached to a therapeutic agent, can be administered simultaneously or sequentially with other therapeutic treatment regimens. For example, an antibody of the invention can be administered alone or in combination with another therapeutic treatment, including any of the therapeutic drugs disclosed herein as well as other drugs well known to those skilled in the art for treating a particular disease. For example, in the case of treating a cancer, an antibody of the invention can be administered simultaneously or sequentially with another chemotherapeutic agent such as a drug or radionuclide. Similarly, an antibody of the invention can be combined with other treatment regimens such as surgery by administering the antibody before, during or after surgery. One skilled in the art will know or can readily determine a desirable therapeutic treatment to be used in combination with an antibody of the invention, as desired. Thus, an antibody of the invention can be administered in conjunction with other therapeutic regimens, including but not limited to chemotherapy, radiation therapy, surgery, and the like.

The invention additionally provides a method of inhibiting metastasis using an antibody of the invention. The method can include the step of administering an antibody, or functional fragment thereof, having binding activity for a cryptic collagen epitope. The antibody can be, for example, an antibody comprising one or more CDRs having a least one amino acid substitution in one or more heavy or light chain CDRs of antibodies HUIV26 and HUI77. As used herein,

inhibiting metastasis refers to decreasing the number and/or size of metastatic sites remote from a primary tumor site. The method of inhibiting metastasis can involve using an antibody of the invention that blocks
5 adhesion of tumor cells to a cryptic collagen epitope that is exposed after remodeling of tissues by the action of collagen-degrading enzymes secreted by tumor cells.

As disclosed herein, a variant of HUI77
10 having one or more amino acid substitutions in one or more CDRs inhibited proliferation of melanoma cells *in vitro* (see Example VI). An antibody of the invention can block access to or inhibit binding of a survival or proliferative signal delivered to a tumor cell. Thus,
15 the invention also provides a method of targeting a tumor cell by administration of an antibody of the invention having binding activity for a cryptic collagen epitope that blocks access to a survival or proliferative signal delivered to the tumor cell by a
20 cryptic collagen site.

For methods of inhibiting angiogenesis, the angiogenic vasculature can be associated with a tumor. The methods of the invention can also be used to inhibit tumor growth directly, alone or in combination
25 with inhibiting angiogenic vasculature of the tumor. The methods of the invention can additionally be used to inhibit metastasis, alone or in combination with inhibiting tumor angiogenic vasculature and/or tumor growth. Exemplary tumors include, but are not limited
30 to, those disclosed herein, including melanoma, carcinoma, sarcoma, fibrosacroma, glioma, astrocytoma, and the like. Methods for testing the effect a HUIV26 or HUI77 variant for inhibition of angiogenesis or inhibition of tumor growth can be performed as

described previously using, for example, assays such as the rat corneal micropocket angiogenesis assay, chick embryo tumor growth assay, or SCID mouse tumor growth assay, as described in Xu et al., *supra*, 2001, or any
5 other well known assays for measuring inhibition of angiogenesis, inhibition of tumor growth, or inhibition of metastasis.

The methods of the invention can also be applied to inhibiting non-tumor angiogenic vasculature.
10 Such applications to non-tumor angiogenic vasculature can include tissue that is inflamed and in which angiogenesis is occurring. Exemplary non-tumor diseases associated with angiogenic vasculature suitable for treatment with an antibody of the
15 invention include, but are not limited to, those disclosed herein, including arthritis, ocular disease, retinal disease, hemangioma, and the like. The antibodies of the invention can also be used to inhibit psoriasis, macular degeneration, restenosis, and the
20 like, or any tumor or non-tumor disease associated with increased accessibility of a cryptic collagen epitope for which an antibody of the invention has binding activity.

It is understood that modifications which do
25 not substantially affect the activity of the various embodiments of this invention are also provided within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the present invention.

EXAMPLE I**Cloning of Heavy and Light Chain Variable Regions of
HUIV26 and HUI77 Antibodies**

This example describes the cloning of HUIV26
5 and HUI77 antibody variable regions.

The variable regions of the HUIV26 and HUI77
antibodies were cloned from hybridomas expressing these
mouse monoclonal antibodies and sequenced. Briefly,
total mRNA was isolated from the respective mouse
10 hybridoma cells using Oligotex® Direct mRNA Micro kit
(Qiagen; Valencia CA). First strand cDNA was
synthesized from the mRNA using SuperScript
Preamplification System (GibcoBRL/Invitrogen; Carlsbad
CA). Antibody variable region sequences were amplified
15 by PCR using a set of 5' primers designed for signal
sequences of mouse light chains or heavy chains to pair
with single 3' primer to mouse kappa chain constant
region for V_L or IgM CH1 region for V_H sequences. The
sequences of the 5' primers for the signal peptide of
20 mouse antibody heavy and light chain as well as
constant region primers are shown in Figure 1. The 3'
primer for mouse kappa light chain constant region
(primer 2650; SEQ ID NO:212) corresponds to amino acids
115-123. The 3' primer for mouse IgM CH1 region
25 (primer 2656; SEQ ID NO:213) corresponds to amino acids
121-114. The 3' primer for mouse IgM CH1 region
(primer 2706; SEQ ID NO:214) corresponds to amino acids
131-124.

The DNA fragments were isolated from PCR
30 reactions, with a main product of about 400 bp in
length. The DNA fragments were cloned into the pCR2.1

vector. The inserted DNA fragments were sequenced with both forward and reversed M13 primers. The DNA sequences were compared with an antibody sequence database. The N-terminal amino acid sequence of the HUIV26 and HUI77 antibodies were determined, and the sequences of the DNA fragments were also compared to the N-terminal amino acid sequences of the corresponding antibody.

The HUIV26 V_L encoding nucleic acid was cloned with 5' primer mK2 (primer 2664; SEQ ID NO:185) and 3' primer 2650 (SEQ ID NO:212). A partial sequence of HUIV25 V_L is ATCTTCTTGCTGTTCTGGGTATCTGGAACCTGTGGG (SEQ ID NO:215), with the MK2 primer underlined and the partial sequence coding for mouse signal peptide in italics. The HUIV26 V_H encoding nucleic acid was cloned with 5' primer MH12 (primer 2731; SEQ ID NO:203) and 3' primer 2706 (SEQ ID NO:214).

The HUI77 V_L encoding nucleic acid was cloned with 5' primer mK1 (primer 2663; SEQ ID NO:184) and 3' primer 2650 (SEQ ID NO:212). A partial sequence of HUI77 V_L is TTGGTGCTGATGTTCTGGATTCTGCTTCCAGCAGT (SEQ ID NO:216), with the mK1 primer underlined and the partial sequence coding for mouse signal peptide in italics. The HUI77 encoding nucleic acid was cloned with 5' primers MH15 (primer 2734; SEQ ID NO:206) or MH16 (primer 2735; SEQ ID NO:207) and 3' primer 2656 (SEQ ID NO:213).

The sequences of the heavy and light chain nucleotide and amino acid sequences for HUIV26 and HUI77 are shown in Figures 2 and 3, respectively. Using the numbering system of Kabat, *supra*, the CDRs of the heavy and light chains were identified for each of

the HUIV26 and HUI77 antibodies (underlined in Figures 2C and 3C).

An alignment of the HUI77 V_L nucleotide sequence (SEQ ID NO:9) with the nucleotide sequence of the human framework fusion DPK13/JK1 (SEQ ID NO:17) is shown in Figure 3D. The corresponding light chain amino acid sequences are referenced as SEQ ID NO:10 and SEQ ID NO:18 for HUI77 and DPK13/JK1, respectively.

This example describes the cloning and the sequence of mouse antibodies HUIV26 and HUI77.

EXAMPLE II

Generation of CDR Variant Libraries of HUIV26 and HUI77 Antibodies

This example describes the generation of CDR variant libraries of HUIV26 and HUI77 antibodies for CDR optimization.

The CDR3 regions of antibodies HUIV26 and HUI77 were optimized by generating a library of CDR variants. Primers for light chain CDR3 and heavy chain CDR3 were used to generate a library of CDR3 variants, where the primer was synthesized to encode more than one amino acid one or more positions in CDR3. Following synthesis of primers encoding CDR3 variants, the variant CDR3 regions were assembled into light chain (V_L) and heavy chain (V_H) regions.

Briefly, humanized V_L and V_H genes of HUI77 and HUIV26 antibodies were assembled with the primers shown in Figures 4A and 5A, respectively, using PCR or primer-elongation-ligation. Variable region genes

containing CDR3 mutations were assembled by replacing the wild type CDR3 primer (IV26-17, IV26-h7, I77-17 or I77-h7) with the group of mutant primers corresponding to that CDR. The assembled variable regions were then
5 amplified and asymmetrically biotinylated on plus strand by PCR using primers B-pelB and 224 for V_L and B-phA and 1200a for H_V genes. The primers for amplification of humanized V_L and V_H sequences and the isolation of minus strand DNA were: B-pelB, Biotin-TTA
10 CTC GCT GCC CAA CCA GCC ATG GCC (SEQ ID NO:220); 224, GAC AGA TGG TGC AGC CAC AGT (SEQ ID NO:221); B-phoA, Biotin-TTA CTG TTT ACC CCT GTG ACA AAA GCC (SEQ ID NO:222); and 1200a, GAA GAC CGA TGG GCC CTT GGT (SEQ ID NO:223).

15 The assembled V_L and V_H regions were introduced into a Fab expression vector by mutagenesis. Briefly, the non-biotinylated minus strands were isolated after binding the PCR products to NeutrAvidin-conjugated magnetic beads and introduced into the Fab
20 expression vector IX-104CSA by hybridization mutagenesis (Kristensson et al., Vaccines 95, pp. 39-43, Cold Spring Harbor Laboratory, Cold Spring Harbor (1995); Kunkel, Proc. Natl. Acad. Sci. USA 82:488-492 (1985); Wu et al., J. Mol. Bio. 294:151-162 (1999)).

25 Three humanization-CDR3-mutation libraries were constructed for each the HUI77 and HUIV26 antibodies. The three libraries introduced random mutations but differed in CDR3 mutations. One library had mutations only in LCDR3, the second library had
30 mutations only in HCDR3, and the third library had mutations in both LCDR3 and HCDR3.

Methods essentially the same as those described above for CDR3 mutagenesis were also

performed on CDR1 and CDR2 of the HUIV26 and HUI77 antibodies. After assembling into a Fab expression vector, the Fabs containing HUIV26 and HUI77 variant CDRs were expressed in bacteria and tested for binding
5 to denatured collagen. The mutant libraries were screened with filter lift screening and ELISA. The assays were performed essentially as described previously (Huse et al., J. Immunol. 149:3914-3920 (1992); Watkins et al., Anal. Biochem. 253:37-45
10 (1997)). Briefly, nitrocellulose membranes were pre-coated with heat-denatured human collagen I or IV and used to lift *E. coli*-expressed variant FABs from phage plates. The membranes were then incubated with
15 antibodies, either anti-human kappa chain or anti-hemagglutinin (HA) tag conjugated to alkaline phosphatase to detect bound variant Fabs. Positive clones were screened again by single point ELISA (Watkins et al., *supra*, 1997) for binding to denatured-biotinylated human collagen I and IV, correspondingly.
20 Beneficial variants were characterized for binding to both collagens in native and heat-denatured forms by ELISA. Beneficial mutations were determined as those having higher affinity binding to denatured collagen relative to the corresponding wild type Fab, as
25 demonstrated by ELISA.

Shown in Figures 4B and 5B is a summary of beneficial CDR mutations in the HUIV26 and HUI77 antibodies, respectively. Figure 4B summarizes beneficial single amino acid mutations in heavy chain
30 CDR1, CDR2, and CDR3 and light chain CDR1 and CDR3 of HUIV26. An exemplary HUIV26 variant having a single amino acid substitution is the 12F10Q variant, which exhibited k_{on} of 0.055 and k_{off} of 0.049 as estimated by the fold improvement based on shifts in half-maximal
35 binding obtained from ELISA titrations.

Figure 5B summarizes beneficial single amino acid mutations in heavy chain CDR1, CDR2 and CDR3 and light chain CDR1, CDR2 and CDR3 of HUI77. As can be seen, numerous single amino acid mutations in various CDRs were found to maintain or enhance binding to a cryptic collagen site.

This example describes CDR variants of HUIV26 and HUI77 having beneficial mutations.

EXAMPLE III

10 Identification of Combinatorial Variants of HUIV26 and HUI77 Antibodies Having Enhanced Activity

This example describes the generation and identification of combinatorial variants incorporating various beneficial CDR mutations in HUIV26 and HUI77.

15 To further optimize HUIV26 and HUI77 antibody CDR variants, combinatorial variants, which incorporate at least two CDRs containing one or more mutations, were generated and tested for binding to a cryptic collagen site. Combinatorial variants were synthesized using primers with one or more positions encoding variant amino acids as described in Example II. The primers used are shown in Figures 6 and 7.

Shown in Figures 6 and 7 is a summary of the beneficial combinatorial variants of HUIV26 and HUI77 antibodies, respectively. The k_{on} and k_{off} values shown in Figures 6 and 7 ("SPEKon" and "SPEkoff") were estimated as the fold improvement of variants based on shifts in half-maximal binding obtained from ELISA titrations. Also shown are several variants having the same beneficial CDR mutations but having different

framework sequences. These results show that beneficial CDR mutations can be grafted into a variety of frameworks and can retain or have improved binding activity.

5 This example shows the generation of combinatorial CDR variants of HUIV26 and HUI77. A number of variants were identified having increased affinity relative to wild type forms of the respective antibodies.

10 **EXAMPLE IV**
Binding Activity and Specificity of HUIV26 and HUI77
Variants

 This example describes the binding activity and specificity of HUIV26 and HUI77 antibodies on
15 native and denatured collagen.

 The activity and specificity of wild type and selected exemplary HUIV26 and HUI77 variants were determined. As shown in Figure 8, the activity and specificity of IX-IV26, a Fab containing wild type
20 HUIV26 CDRs, and the HUIV26 variants 2D4H1-C3 and DhuG5 were determined. The antibodies were tested for binding to denatured collagen IV (Figure 8A), denatured collagen I (Figure 8B), and native collagen IV (Figure 8C). None of the antibodies had significant binding
25 activity for native collagen IV (Figure 8C). All three antibodies exhibited binding activity for denatured collagen IV (Figure 8A). However, the 2D4H1-C3 and DhuG5 variants exhibited significantly increased binding activity relative to IX-IV26 (Figure 8A). IX-
30 IV26 did not exhibit significant binding activity to denatured collagen I, and 2D4H1-C3 and DhuG5 exhibited

low binding activity at the highest measured concentration of antibody (Figure 8B). These results indicate that the HUIV26 variants have similar binding activity and specificity as that of wild type HUIV26 and maintain activity and specificity for a cryptic collagen epitope. These results further show that variants having mutated CDRs can have maintained or increased binding affinity relative to wild type.

As shown in Figure 9, the activity and specificity of IX-I77, a Fab containing wild type HUI77 CDRs, and the HUI77 variants Qh2b-B7 and QhuD9 were determined. The antibodies were tested for binding to denatured collagen I (Figure 9A), denatured collagen IV (Figure 9B) and native collagen I (Figure 9C), and the results indicate that these variants exhibited similar binding specificities as wild type. Neither IX-I77 nor Qhu2b-B7 exhibited significant binding activity for native collagen I, although the variant QhuD9 exhibited modest binding activity to native collagen at higher concentrations of antibody. The antibodies all exhibited binding activity for denatured collagen I (Figure 9A) and denatured collagen IV (Figure 9B). However, the Qhu2b-B7 and QhuD9 variants exhibited significantly increased binding activity relative to IX-I77 on both denatured collagen I and IV. These results indicate that variants having mutated CDRs can have maintained or increased binding affinity relative to wild type.

To further examine the effect of CDR mutations on binding activity, the HUIV26 variant DhuH8 was selected and expressed in two forms, as a Fab and immunoglobulin (IgG). The binding activity of these two forms was determined for native (n-IV) and denatured (d-IV) human collagen IV. As shown in Figure

10, neither the Fab nor IgG form of the Dhu8 variant exhibited significant binding to native collagen IV. The Fab form exhibited binding activity for denatured collagen IV, and the binding affinity was significantly increased for the IgG form. These results indicate that a HUIV26 variant having one or more CDR amino acid substitutions relative to wild type can exhibit binding to a cryptic collagen epitope and that the binding affinity can be significantly increased in the IgG form relative to the Fab form of the antibody variant.

These results indicate that HUIV26 and HUI77 variants having one or more CDR amino acid substitutions can exhibit similar binding specificity and increased binding affinity relative to wild type.

15

EXAMPLE V**Generation of Grafted HUIV26 and HUI77 Antibodies
Having Optimized CDRs**

This example describes the generation of humanized HUIV26 and HUI77 antibodies incorporating beneficial CDR mutations.

A CDR variant have a beneficial mutation is identified as described in Examples II and III. Once a beneficial CDR variant is identified, the CDR variant is grafted into a human framework sequence. In addition to the CDR variant having a beneficial mutation, other CDRs can be a wild type sequence of the respective antibody or one or more variant CDRs. At least one of the CDRs will be a variant containing a beneficial mutation. For example, if the grafted antibody contains a heavy and light chain, at least one of the heavy or light chain CDRs will have at least one

amino acid mutation relative to the corresponding wild type CDR.

A human framework sequence is selected as the recipient for grafting. The human framework can be
5 closely related to the donor antibody framework sequence or can be relatively divergent from the parental donor antibody. Once a human framework is selected for grafting, overlapping oligonucleotides are synthesized encoding the selected human framework and
10 the appropriate donor CDRs, including at least one variant CDR containing at least one beneficial mutation. The overlapping oligonucleotides are used to assemble a nucleic acid encoding a variable region including the selected human framework, the CDR
15 variant, and appropriate other CDRs to generate an antibody or fragment having binding activity for a cryptic collagen site.

The assembled variable region is cloned into an expression vector, for example, a Fab expression
20 vector such as described in Example II, and binding activity to denatured collagen is tested, as described in Examples II and III.

This example describes the generation of humanized antibodies containing beneficial CDR
25 mutations of HUIV26 and HUI77 antibodies.

EXAMPLE VI

Inhibition of B16 Melanoma Cell Proliferation by a Variant HUI77 Antibody

This example describes the effect of the
30 HUI77 variant QH2b on B16 melanoma cell proliferation.

The humanized Fab designated QH2b, which is the QH2b-B7 variant of the HUI77 antibody, was engineered into a full length IgG1 antibody (QH2b-IgG1). The QH2b-IgG1 antibody was expressed in mammalian cell culture in NSO cells and purified.

The purified QH2b-IgG1 antibody was used in a cell proliferation assay *in vitro*. B16 melanoma cells were plated on denatured human Type I collagen. QH2b-IgG1 (100 µg/ml/day) was added to one set of culture dishes and cell numbers were determined at the indicated times (Figure 11). As a control, the cells were not treated with antibody.

As shown in Figure 11, B16 melanoma cells proliferated on denatured collagen type-I, as indicated by the increase in cell numbers over 3 days. The B16 melanoma cell cultures treated with QH2b-IgG1 exhibited essentially no cell growth over a period of 3 days, indicating that the melanoma cells did not proliferate in the presence of the HUI77 variant QH2b-IgG1.

These results indicate that a HUI77 variant having one or more CDR amino acid substitutions can inhibit cell proliferation of B16 melanoma cells.

Throughout this application various publications have been referenced. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains. Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

1. A grafted antibody, or functional fragment thereof, comprising one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting of SEQ ID NOS:26, 28 and 30 or a light chain CDR selected from the group consisting of SEQ ID NOS:20, 22 and 24, said grafted antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

2. An antibody, or functional fragment thereof, comprising one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162, said antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

3. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy

chain CDR2 referenced as SEQ ID NO:28; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:20; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3
5 referenced as SEQ ID NO:77.

4. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:28; a heavy chain
10 CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:72; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

5. The antibody of claim 2, wherein said
15 antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:48; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:20; a light chain CDR2
20 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

6. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy
25 chain CDR2 referenced as SEQ ID NO:154; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

30 7. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy

chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:158; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3
5 referenced as SEQ ID NO:77.

8. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy chain CDR2 referenced as SEQ ID NO:155; a heavy chain
10 CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:159; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

9. The antibody of claim 2, wherein said
15 antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:48; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:160; a light chain CDR2
20 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

10. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy
25 chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:72; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

30 11. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy

chain CDR2 referenced as SEQ ID NO:155; a heavy chain
CDR3 referenced as SEQ ID NO:63; a light chain CDR1
referenced as SEQ ID NO:157; a light chain CDR2
referenced as SEQ ID NO:22; and a light chain CDR3
5 referenced as SEQ ID NO:77.

12. The antibody of claim 2, wherein said
antibody, or functional fragment thereof, comprises a
heavy chain CDR1 referenced as SEQ ID NO:45; a heavy
chain CDR2 referenced as SEQ ID NO:155; a heavy chain
10 CDR3 referenced as SEQ ID NO:63; a light chain CDR1
referenced as SEQ ID NO:160; a light chain CDR2
referenced as SEQ ID NO:22; and a light chain CDR3
referenced as SEQ ID NO:77.

13. The antibody of claim 2, wherein said
15 antibody, or functional fragment thereof, comprises a
heavy chain CDR1 referenced as SEQ ID NO:46; a heavy
chain CDR2 referenced as SEQ ID NO:155; a heavy chain
CDR3 referenced as SEQ ID NO:63; a light chain CDR1
referenced as SEQ ID NO:160; a light chain CDR2
20 referenced as SEQ ID NO:22; and a light chain CDR3
referenced as SEQ ID NO:77.

14. The antibody of claim 2, wherein said
antibody, or functional fragment thereof, comprises a
heavy chain CDR1 referenced as SEQ ID NO:45; a heavy
25 chain CDR2 referenced as SEQ ID NO:162; a heavy chain
CDR3 referenced as SEQ ID NO:63; a light chain CDR1
referenced as SEQ ID NO:158; a light chain CDR2
referenced as SEQ ID NO:22; and a light chain CDR3
referenced as SEQ ID NO:77.

30 15. The antibody of claim 2, wherein said
antibody, or functional fragment thereof, comprises a
heavy chain CDR1 referenced as SEQ ID NO:45; a heavy

chain CDR2 referenced as SEQ ID NO:156; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3
5 referenced as SEQ ID NO:77.

16. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:26; a heavy chain CDR2 referenced as SEQ ID NO:154; a heavy chain
10 CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

17. The antibody of claim 2, wherein said
15 antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:45; a heavy chain CDR2 referenced as SEQ ID NO:155; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:157; a light chain CDR2
20 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

18. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy
25 chain CDR2 referenced as SEQ ID NO:154; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:161; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

30 19. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy

chain CDR2 referenced as SEQ ID NO:156; a heavy chain CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:161; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3
5 referenced as SEQ ID NO:77.

20. The antibody of claim 2, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:46; a heavy chain CDR2 referenced as SEQ ID NO:28; a heavy chain
10 CDR3 referenced as SEQ ID NO:63; a light chain CDR1 referenced as SEQ ID NO:20; a light chain CDR2 referenced as SEQ ID NO:22; and a light chain CDR3 referenced as SEQ ID NO:77.

21. An antibody, or functional fragment
15 thereof, comprising a heavy chain polypeptide comprising one or more CDRs having at least one amino acid substitution in one or more heavy chain CDRs, said heavy chain CDRs selected from the group consisting of a heavy chain CDR1 selected from the group consisting
20 of CDRs referenced as SEQ ID NOS:26, 43, 44, 45, 46, and 47; a heavy chain CDR2 selected from the group consisting of CDRs referenced as SEQ ID NOS:28, 48, 49, 50, 51, 52, 53, 54, and 55; and a heavy chain CDR3 selected from the group consisting of CDRs referenced
25 as SEQ ID NOS:30, 56, 57, 58, 59, 60, 61, 62, 63, and 64, said antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

22. An antibody, or functional fragment
30 thereof, comprising a light chain polypeptide comprising one or more CDRs having at least one amino acid substitution in one or more light chain CDRs, said light chain CDRs selected from the group consisting of

a light chain CDR1 selected from the group consisting of CDRs referenced as SEQ ID NOS:20, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, and 76; a light chain CDR2 referenced as SEQ ID NO:22;; and a light chain CDR3
5 selected from the group consisting of CDRs referenced as SEQ ID NOS:24, 77, 78, 79, 80, 81, 82, 83, 84, 85, and 86, said antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

10 23. A grafted antibody, or functional fragment thereof, comprising one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting
15 of SEQ ID NOS:38, 40 and 42 or a light chain CDR selected from the group consisting of SEQ ID NOS:32, 34 and 36, said grafted antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

20 24. An antibody, or functional fragment thereof, comprising one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95,
25 SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113,
30 SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID

NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134,
SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID
NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141,
SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID
5 NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148,
SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID
NO:152, and SEQ ID NO:153, said antibody or functional
fragment thereof having specific binding activity for a
cryptic collagen epitope.

10

25. The antibody of claim 24, wherein said
antibody, or functional fragment thereof, comprises a
heavy chain CDR1 referenced as SEQ ID NO:38; a heavy
chain CDR2 referenced as SEQ ID NO:40; a heavy chain
15 CDR3 referenced as SEQ ID NO:103; a light chain CDR1
referenced as SEQ ID NO:32; a light chain CDR2
referenced as SEQ ID NO:34; and a light chain CDR3
referenced as SEQ ID NO:36.

26. The antibody of claim 24, wherein said
20 antibody, or functional fragment thereof, comprises a
heavy chain CDR1 referenced as SEQ ID NO:38; a heavy
chain CDR2 referenced as SEQ ID NO:92; a heavy chain
CDR3 referenced as SEQ ID NO:103; a light chain CDR1
referenced as SEQ ID NO:32; a light chain CDR2
25 referenced as SEQ ID NO:34; and a light chain CDR3
referenced as SEQ ID NO:36.

27. The antibody of claim 24, wherein said
antibody, or functional fragment thereof, comprises a
heavy chain CDR1 referenced as SEQ ID NO:147; a heavy
30 chain CDR2 referenced as SEQ ID NO:92; a heavy chain
CDR3 referenced as SEQ ID NO:103; a light chain CDR1
referenced as SEQ ID NO:149; a light chain CDR2
referenced as SEQ ID NO:34; and a light chain CDR3
referenced as SEQ ID NO:36.

28. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:92; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:150; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

29. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:149; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

30. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:144; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:149; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

31. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:151; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

32. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:92; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:151; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

33. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:152; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:145.

34. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:148; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:150; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

35. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:115; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

36. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:40; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:153; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

37. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:92; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:116; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

38. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:147; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:116; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

39. The antibody of claim 24, wherein said antibody, or functional fragment thereof, comprises a heavy chain CDR1 referenced as SEQ ID NO:38; a heavy chain CDR2 referenced as SEQ ID NO:93; a heavy chain CDR3 referenced as SEQ ID NO:103; a light chain CDR1 referenced as SEQ ID NO:32; a light chain CDR2 referenced as SEQ ID NO:34; and a light chain CDR3 referenced as SEQ ID NO:36.

40. An antibody, or functional fragment thereof, comprising a heavy chain polypeptide comprising one or more CDRs having at least one amino acid substitution in one or more heavy chain CDRs, said
5 heavy chain CDRs selected from the group consisting of a heavy chain CDR1 selected from the group consisting of CDRs referenced as SEQ ID NOS:38, 87, 88, 89, 90, 91, 147 and 148; a heavy chain CDR2 selected from the group consisting of CDRs referenced as SEQ ID NOS:40,
10 92, 93, 94, 95 and 144; and a heavy chain CDR3 selected from the group consisting of CDRs referenced as SEQ ID NOS:42, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108 and 109, said antibody or functional fragment thereof having specific binding activity for a
15 cryptic collagen epitope.

41. An antibody, or functional fragment thereof, comprising a light chain polypeptide comprising one or more CDRs having at least one amino acid substitution in one or more light chain CDRs, said
20 light chain CDRs selected from the group consisting of a light chain CDR1 selected from the group consisting of CDRs referenced as SEQ ID NOS:32, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 146, 149, 150, 151, 152 and 153; a light chain CDR2 referenced as SEQ ID
25 NOS:34, 120, 121, 122, 123, 124 and 125; and a light chain CDR3 selected from the group consisting of CDRs referenced as SEQ ID NOS:36, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, and 145, said antibody or functional fragment
30 thereof having specific binding activity for a cryptic collagen epitope.

42. The grafted antibody of any of claims 1-41, wherein said functional fragment is selected from the group consisting of Fv, Fab, F(ab)₂ and scFV.

43. A nucleic acid encoding the antibody of any of claims 1-41.

44. A method of targeting angiogenic vasculature, comprising administering an antibody, or
5 functional fragment thereof, said antibody or functional fragment thereof comprising one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting
10 of SEQ ID NOS:26, 28 and 30 or a light chain CDR selected from the group consisting of SEQ ID NOS:20, 22 and 24, and said antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

15 45. The method of claim 44, wherein said antibody or functional fragment comprises one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49,
20 SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69,
25 SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:154, SEQ ID NO:155, SEQ ID
30 NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162.

46. The method of claim 44, wherein said antibody, or functional fragment thereof, further comprises a therapeutic moiety.

47. The method of claim 44, wherein said
5 antibody, or functional fragment thereof, further comprises a detectable moiety.

48. A method of inhibiting angiogenesis, comprising administering an antibody, or functional fragment thereof, said antibody or functional fragment
10 thereof comprising one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting of SEQ ID NOS:26, 28 and 30 or a light chain CDR selected from
15 the group consisting of SEQ ID NOS:20, 22 and 24, and said antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

49. The method of claim 48, wherein said
20 antibody or functional fragment comprises one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53,
25 SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73,
30 SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:154, SEQ ID NO:155, SEQ ID

NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159,
SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162.

50. The method of claim 48, wherein said
antibody, or functional fragment thereof, further
5 comprises a therapeutic moiety.

51. A method of targeting a tumor,
comprising administering an antibody, or functional
fragment thereof, said antibody or functional fragment
thereof comprising one or more complementarity
10 determining regions (CDRs) having at least one amino
acid substitution in one or more CDRs of a heavy chain
CDR selected from the group consisting of SEQ ID
NOS:26, 28 and 30 or a light chain CDR selected from
the group consisting of SEQ ID NOS:20, 22 and 24, and
15 said antibody or functional fragment thereof having
specific binding activity for a cryptic collagen
epitope.

52. The method of claim 51, wherein said
antibody or functional fragment comprises one or more
20 CDRs selected from the group consisting of CDRs
referenced as SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45,
SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49,
SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53,
SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57,
25 SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61,
SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65,
SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69,
SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73,
SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77,
30 SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81,
SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85,
SEQ ID NO:86, SEQ ID NO:154, SEQ ID NO:155, SEQ ID

NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159,
SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162.

53. The method of claim 51, wherein said
antibody, or functional fragment thereof, further
5 comprises a therapeutic moiety.

54. The method of claim 51, wherein said
antibody, or functional fragment thereof, further
comprises a detectable moiety.

55. A method of inhibiting tumor growth,
10 comprising administering an antibody, or functional
fragment thereof, said antibody or functional fragment
thereof comprising one or more complementarity
determining regions (CDRs) having at least one amino
acid substitution in one or more CDRs of a heavy chain
15 CDR selected from the group consisting of SEQ ID
NOS:26, 28 and 30 or a light chain CDR selected from
the group consisting of SEQ ID NOS:20, 22 and 24, and
said antibody or functional fragment thereof having
specific binding activity for a cryptic collagen
20 epitope.

56. The method of claim 55, wherein said
antibody or functional fragment comprises one or more
CDRs selected from the group consisting of CDRs
referenced as SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45,
25 SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49,
SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53,
SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57,
SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61,
SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65,
30 SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69,
SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73,
SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77,

SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81,
SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85,
SEQ ID NO:86, SEQ ID NO:154, SEQ ID NO:155, SEQ ID
NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159,
5 SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162.

57. The method of claim 55, wherein said
antibody, or functional fragment thereof, further
comprises a therapeutic moiety.

58. A method of detecting angiogenic
10 vasculature, comprising contacting angiogenic
vasculature with an antibody, or functional fragment
thereof, said antibody or functional fragment thereof
comprising one or more complementarity determining
regions (CDRs) having at least one amino acid
15 substitution in one or more CDRs of a heavy chain CDR
selected from the group consisting of SEQ ID NOS:26, 28
and 30 or a light chain CDR selected from the group
consisting of SEQ ID NOS:20, 22 and 24, and said
antibody or functional fragment thereof having specific
20 binding activity for a cryptic collagen epitope.

59. The method of claim 58, wherein said
antibody or functional fragment comprises one or more
CDRs selected from the group consisting of CDRs
referenced as SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45,
25 SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49,
SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53,
SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57,
SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61,
SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65,
30 SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69,
SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73,
SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77,
SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81,

SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85,
SEQ ID NO:86, SEQ ID NO:154, SEQ ID NO:155, SEQ ID
NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159,
SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162.

5 60. The method of claim 58, wherein said
antibody, or functional fragment thereof, further
comprises a detectable moiety.

 61. A method of inhibiting metastasis,
comprising administering an antibody, or functional
10 fragment thereof, said antibody or functional fragment
thereof comprising one or more complementarity
determining regions (CDRs) having at least one amino
acid substitution in one or more CDRs of a heavy chain
CDR selected from the group consisting of SEQ ID
15 NOS:26, 28 and 30 or a light chain CDR selected from
the group consisting of SEQ ID NOS:20, 22 and 24, and
said antibody or functional fragment thereof having
specific binding activity for a cryptic collagen
epitope.

20 62. The method of claim 61, wherein said
antibody or functional fragment comprises one or more
CDRs selected from the group consisting of CDRs
referenced as SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45,
SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49,
25 SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53,
SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57,
SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61,
SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65,
SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69,
30 SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73,
SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77,
SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81,
SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85,

SEQ ID NO:86, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162.

63. The method of claim 61, wherein said
5 antibody, or functional fragment thereof, further comprises a therapeutic moiety.

64. A method of targeting angiogenic vasculature, comprising administering an antibody, or functional fragment thereof, said antibody or
10 functional fragment thereof comprising one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting of SEQ ID NOS:38, 40 and 42 or a light chain CDR
15 selected from the group consisting of SEQ ID NOS:32, 34 and 36, said grafted antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

65. The method of claim 64, wherein said
20 antibody or functional fragment comprises one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97,
25 SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID
30 NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID

NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132,
SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID
NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139,
SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID
5 NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146,
SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID
NO:150, SEQ ID NO:151, SEQ ID NO:152, and SEQ ID
NO:153.

66. The method of claim 64, wherein said
10 antibody, or functional fragment thereof, further
comprises a therapeutic moiety.

67. The method of claim 64, wherein said
antibody, or functional fragment thereof, further
comprises a detectable moiety.

15 68. A method of inhibiting angiogenesis,
comprising administering an antibody, or functional
fragment thereof, said antibody or functional fragment
thereof comprising one or more complementarity
determining regions (CDRs) having at least one amino
20 acid substitution in one or more CDRs of a heavy chain
CDR selected from the group consisting of SEQ ID
NOS:38, 40 and 42 or a light chain CDR selected from
the group consisting of SEQ ID NOS:32, 34 and 36, said
grafted antibody or functional fragment thereof having
25 specific binding activity for a cryptic collagen
epitope.

69. The method of claim 68, wherein said
antibody or functional fragment comprises one or more
CDRs selected from the group consisting of CDRs
30 referenced as SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89,
SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93,
SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97,

SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID
NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104,
SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID
NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111,
5 SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID
NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118,
SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID
NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125,
SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID
10 NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132,
SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID
NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139,
SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID
NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146,
15 SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID
NO:150, SEQ ID NO:151, SEQ ID NO:152, and SEQ ID
NO:153.

70. The method of claim 68, wherein said
antibody, or functional fragment thereof, further
20 comprises a therapeutic moiety.

71. A method of targeting a tumor,
comprising administering an antibody, or functional
fragment thereof, said antibody or functional fragment
thereof comprising one or more complementarity
25 determining regions (CDRs) having at least one amino
acid substitution in one or more CDRs of a heavy chain
CDR selected from the group consisting of SEQ ID
NOS:38, 40 and 42 or a light chain CDR selected from
the group consisting of SEQ ID NOS:32, 34 and 36, said
30 grafted antibody or functional fragment thereof having
specific binding activity for a cryptic collagen
epitope.

72. The method of claim 71, wherein said antibody or functional fragment comprises one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, and SEQ ID NO:153.

73. The method of claim 71, wherein said antibody, or functional fragment thereof, further comprises a therapeutic moiety.

74. The method of claim 71, wherein said antibody, or functional fragment thereof, further comprises a detectable moiety.

75. A method of inhibiting tumor growth, comprising administering an antibody, or functional fragment thereof, said antibody or functional fragment thereof comprising one or more complementarity

determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting of SEQ ID NOS:38, 40 and 42 or a light chain CDR selected from the group consisting of SEQ ID NOS:32, 34 and 36, said grafted antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

76. The method of claim 75, wherein said antibody or functional fragment comprises one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, and SEQ ID NO:153.

77. The method of claim 75, wherein said antibody, or functional fragment thereof, further comprises a therapeutic moiety.

78. A method of detecting angiogenic vasculature, comprising contacting angiogenic vasculature with an antibody, or functional fragment thereof, said antibody or functional fragment thereof
5 comprising one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain CDR selected from the group consisting of SEQ ID NOS:38, 40 and 42 or a light chain CDR selected from the group
10 consisting of SEQ ID NOS:32, 34 and 36, said grafted antibody or functional fragment thereof having specific binding activity for a cryptic collagen epitope.

79. The method of claim 78, wherein said antibody or functional fragment comprises one or more
15 CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID
20 NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118,
25 SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID
30 NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, and SEQ ID
35 NO:153.

80. The method of claim 78, wherein said antibody, or functional fragment thereof, further comprises a detectable moiety.

81. A method of inhibiting tumor growth,
5 comprising administering an antibody, or functional fragment thereof, said antibody or functional fragment thereof comprising one or more complementarity determining regions (CDRs) having at least one amino acid substitution in one or more CDRs of a heavy chain
10 CDR selected from the group consisting of SEQ ID NOS:38, 40 and 42 or a light chain CDR selected from the group consisting of SEQ ID NOS:32, 34 and 36, said grafted antibody or functional fragment thereof having specific binding activity for a cryptic collagen
15 epitope.

82. The method of claim 81, wherein said antibody or functional fragment comprises one or more CDRs selected from the group consisting of CDRs referenced as SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89,
20 SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID
25 NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125,
30 SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID

NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146,
SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID
NO:150, SEQ ID NO:151, SEQ ID NO:152, and SEQ ID
NO:153.

- 5 83. The method of claim 81, wherein said
antibody, or functional fragment thereof, further
comprises a therapeutic moiety.

Oilgo #	Name	Primer Sequences (5'-3')
2663	mK1	TTG GTG CTG ATG TTC TGG
2664	mK2	ATC TTC TTG CTG TTC TGG
2665	mK3	TGG GTG CTG CTG CTC TGG
2666	mK4	GGG CTG CTT GTG CTC TGG
2667	mK5	GGA ATC TTG TTG CTC TGG
2668	mK6	RTR TTS CTG CTG CTR TGG
2669	mK7	GGT CTC CTG TTG CTC TGT
2670	mK8	ATA TTT CTA CTG CTC TGT
2671	mK9	GTC ATA ATR TCC AGA GGA

Oilgo #	Name	Primer Sequences (5'-3')
2672	mH1	CTG AGC TGT GTA TTC CT
2673	mH2	CTC ARM TTG ATT TTC CT
2674	mH3	TGG RTC ATS TTC TTC CT
2675	mH4	TKS RTC TTT CTC TTC CT
2676	mH5	TGT ATC ATS CTC TTC TT
2677	mH6	TGG RTC TTT CTC TTT TT
2678	mH7	TTA AAC TGG GTT TTT CT
2679	mH8	GKG CTG YTC YTC TGC CT
2680	mH9	TTA AGT CTT CTG TAC CTG
2730	MH11	TCAGTAACTGCAGGTGTCCA
2731	MH12	TTTTAAAAGGTGTCCAGTGT
2732	MH13	GCAACAGCTACAGGTGTCCA
2733	MH14	CAGCTACAGRTGTCCACTCC
2734	MH15	ATTTCCAAGCTGTGTCTGTCC
2735	MH16	CTCCTGTCAGGAACTGCAGGTGT
2736	MH17	CAGTGGTTACAGGGGTCAATTCA
2737	MH18	CTGTTSACAGCCHTTCCCKGGT
2738	MH19	CTGATGGCAGCTGCCCAAAGT
2739	MH20	TTTATCAAGGTGTGCATTGT

2650 5' TCACTGGATGGTGGGAAGATGGATACA 3'
2656 5' GACATTTGGGAAGGACTGACTCTC 3'
2706 5' CAG GGG GCT CTC GCA GGA GAC GAG 3'

FIGURE 1

HuIV-26 VL

GACATTGTGATGACACAGTCTCCATCTTTGTTGAGTGTGTCAGCAGGAGAGAAGGTCACT
ATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAACTACTTGGCC
TGGTACCAGCAGAAACCAGGGCAGCCTCCTAAACTGTTGATCTATGGGGCATCCACTAGG
GAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACCGATTTCACTCTTATC
ATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAATGATCATAAGTTAT
CCGTACACGTTCCGAGGGGGGACCAAGCTGGAAATAAAA

FIGURE 2A

HuIV-26 VH

GAGGTGAAGCTTCTCGAGTCTGGAGGTGGCCTGGTGCAGCCTGGAGGATCCCTGAAACTC
TCCTGTGCAGCCTCAGGATTCGATTTTAGTAGATACTGGATGAGTTGGGTCCGGCAGGCT
CCAGGGAAAGGGCTAGAATGGATTGGAGAAATTAATCCAGATAGCAGTACGATAAACTAT
ACGCCATCTCTAAAGGATAAATTCATCATCTCCAGAGACAACGCCAAAAATACGCTGTAC
CTGCAAATGAGCAAAGTGAGATCTGAGGACACAGCCCTTTATTACTGTGCAAGACCGGTT
GATGGTTACTACGATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA

FIGURE 2B

VH Domain

1	10	20	a	b	c	d	e	f	30	40
HUIV26	DIVMTQSP	SLSVSAGEKVT	MSC	KSSQ	SL	SL	SL	SG	NQ	KN
VKIV	DIVMTQSP	DLAVSLGERAT	IN	KSSQ	SV	LY	SS	NN	KN	YL
HUIV26	50	60	70	80	90	100				
	GASTRES	GV	PDR	FT	GS	GS				
VKIV/JK2	WASTRES	GV	PDR	FS	GS	GS				

[illegible]

FIGURE 2C

HUI77 VL sequence

GATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCAT
CTCTTGCAGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAATGGTACC
TGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGT
GTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACTCAAGATCAGCAGAGT
GGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCAAGGTTACATGTTCCGTGGACGTTTCG
GTGGAGGCACCAAGCTGGAAATCAAA

FIGURE 3A

HUI77 VH SEQUENCE

CAGGTTACTCTGAAAGAGACTGGCCCTGGGATATTGCAGCCCTCCCAGACCCTCAGTCTGA
CTTGTTCTTTCTCTGGGTTTTCACTGAGCACTTCTGGTATGGGTGTAGGCTGGATTTCGTCA
GCCTTCAGGAGAGGGTCTAGAGTGGCTGGCAGACATTTGGTGGGATGACAATAAGTACTAT
AACCCATCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAGCAACCAGGTATTCC
TCAAGATCACCAGTGTGGACACTGCAGATACTGCCACTTACTACTGTGCTCGAAGAGCTAA
CTATGGTAACCCCTACTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCC
TCA

FIGURE 3B

HUI77 VL sequence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HUI77 VL	GAT	GTT	TTG	ATG	ACC	CAA	ACT	CCA	CTC	TCC	CTG	CCT	GTC	AGT	CTT
DPK13	---	A--	G--	---	---	--G	---	---	---	---	---	--C	---	--CC	--C-
	CDR1														
	16	17	18	19	20	21	22	23	24	25	26	27	27a	27b	27c
HUI77 VL	GGA	GAT	CAA	GCC	TCC	ATC	TCT	TGC	AGA	TCT	AGT	CAG	AGC	ATT	GTA
DPK13	---	--G	-CG	---	---	---	--C	---	--G	---	---	---	---	C-C	T-G
	27d	27e	27f	28	29	30	31	32	33	34	35	36	37	38	39
HUI77 VL	CAT	AGT	...	AAT	GGA	AAC	ACC	TAT	TTA	GAA	TGG	TAC	CTG	CAG	AAA
DPK13	G--	---	GAT	G--	---	---	---	---	--G	--C	---	---	---	---	--G
	CDR2														
	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
HUI77 VL	CCA	GGC	CAG	TCT	CCA	AAG	CTC	CTG	ATC	TAC	AAA	GTT	TCC	AAC	CGA
DPK13	---	--G	---	---	---	C--	---	---	---	--T	-CG	C--	---	T-T	--G
	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
HUI77 VL	TTT	TCT	GGT	GTC	CCA	GAC	AGG	TTC	AGT	GGC	AGT	GGA	TCA	GGG	ACA
DPK13	GCC	---	--A	---	---	---	---	---	---	---	---	--G	---	--C	--T
	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
HUI77 VL	GAT	TTC	ACA	CTC	AAG	ATC	AGC	AGA	GTG	GAG	GCT	GAG	GAT	CTG	GGA
DPK13	---	---	---	--G	--A	---	---	--G	---	---	---	---	---	G-T	---
	CDR3														
	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
HUI77 VL	GTT	TAT	TAC	TGC	TTT	CAA	GGT	TCA	CAT	GTT	CCG	TGG	ACG	TTC	GGT
DPK13	---	---	---	---	A-G	---	---	---	---	---	---	---	---	---	--C
	100	101	102	103	104	105	106	107							
HUI77 VL	GGA	GGC	ACC	AAG	CTG	GAA	ATC	AAA							
JK1	CA-	--G	---	---	G--	---	---	---							

FIGURE 3D

HUIV26 LCDR3

IV26-17.1 CTT GGT CCC CTG GCC AAA AGT GTA CGG ATA ACT ATG ATC ATT MNN ACA GTA ATA AAC TGC CAC ATC
IV26-17.2 CTT GGT CCC CTG GCC AAA AGT GTA CGG ATA ACT ATG ATC MNN CTG ACA GTA ATA AAC TGC CAC ATC
IV26-17.3 CTT GGT CCC CTG GCC AAA AGT GTA CGG ATA ACT ATG MNN ATT CTG ACA GTA ATA AAC TGC CAC ATC
IV26-17.4 CTT GGT CCC CTG GCC AAA AGT GTA CGG ATA ACT MNN ATC ATT CTG ACA GTA ATA AAC TGC CAC ATC
IV26-17.5 CTT GGT CCC CTG GCC AAA AGT GTA CGG ATA MNN ATG ATC ATT CTG ACA GTA ATA AAC TGC CAC ATC
IV26-17.6 CTT GGT CCC CTG GCC AAA AGT GTA CGG MNN ACT ATG ATC ATT CTG ACA GTA ATA AAC TGC CAC ATC
IV26-17.7 CTT GGT CCC CTG GCC AAA AGT GTA MNN ATA ACT ATG ATC ATT CTG ACA GTA ATA AAC TGC CAC ATC
IV26-17.8 CTT GGT CCC CTG GCC AAA AGT MNN CGG ATA ACT ATG ATC ATT CTG ACA GTA ATA AAC TGC CAC ATC
IV26-17.9 CTT GGT CCC CTG GCC AAA MNN GTA CGG ATA ACT ATG ATC ATT CTG ACA GTA ATA AAC TGC CAC ATC

HUIV26 HCDR3

IV26-h7.1 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATC GTA GTA ACC ATC AAC MNN TCT CGC ACA GTA ATA CAC
IV26-h7.2 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATC GTA GTA ACC ATC MNN CGG TCT CGC ACA GTA ATA CAC
IV26-h7.3 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATC GTA GTA ACC MNN AAC CGG TCT CGC ACA GTA ATA CAC
IV26-h7.4 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATC GTA GTA MNN ATC AAC CGG TCT CGC ACA GTA ATA CAC
IV26-h7.5 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATC GTA MNN ACC ATC AAC CGG TCT CGC ACA GTA ATA CAC
IV26-h7.6 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATC MNN GTA ACC ATC AAC CGG TCT CGC ACA GTA ATA CAC
IV26-h7.7 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC MNN GTA GTA ACC ATC AAC CGG TCT CGC ACA GTA ATA CAC
IV26-h7.8 CGT GGT TCC TTG CCC CCA GTA GTC CAT MNN ATC GTA GTA ACC ATC AAC CGG TCT CGC ACA GTA ATA CAC
IV26-h7.9 CGT GGT TCC TTG CCC CCA GTA GTC MNN AGC ATC GTA GTA ACC ATC AAC CGG TCT CGC ACA GTA ATA CAC
IV26-h7.10 CGT GGT TCC TTG CCC CCA GTA MNN CAT AGC ATC GTA GTA ACC ATC AAC CGG TCT CGC ACA GTA ATA CAC
IV26-h7.11 CGT GGT TCC TTG CCC CCA MNN GTC CAT AGC ATC GTA GTA ACC ATC AAC CGG TCT CGC ACA GTA ATA CAC

FIGURE 4A

HUIV26 LCDR1a

IV26L1-1 GTTCTTTTGGTTTCCGCWGTTTAACAGACTCTGGCTGGAMNNGCAGTTGATGGTGGCCCT
IV26L1-2 GTTCTTTTGGTTTCCGCWGTTTAACAGACTCTGGCTMNNTTGCAGTTGATGGTGGCCCT
IV26L1-3 GTTCTTTTGGTTTCCGCWGTTTAACAGACTCTGMNNGGACTTGCAGTTGATGGTGGCCCT
IV26L1-4 GTTCTTTTGGTTTCCGCWGTTTAACAGACTMNNGCTGGACTTGCAGTTGATGGTGGCCCT
IV26L1-5 GTTCTTTTGGTTTCCGCWGTTTAACAGMNNTTGGCTGGACTTGCAGTTGATGGTGGCCCT
IV26L1-6 GTTCTTTTGGTTTCCGCWGTTTAAMNNACTCTGGCTGGACTTGCAGTTGATGGTGGCCCT
IV26L1-7 GTTCTTTTGGTTTCCGCWGTTMNNAAGACTCTGGCTGGACTTGCAGTTGATGGTGGCCCT
IV26L1-8 GTTCTTTTGGTTTCCGCWMTTAAACAGACTCTGGCTGGACTTGCAGTTGATGGTGGCCCT

HUIV26 LCDR1b

IV26L1-9 TGGTTTCTGCTGGTACCAAGCTAAGTAGTTCTTTTGGTTTCCMNNGTTTAAACAGACTCTGGCT
IV26L1-10 TGGTTTCTGCTGGTACCAAGCTAAGTAGTTCTTTTGGTTMNNCGWTTTAAACAGACTCTGGCT
IV26L1-11 TGGTTTCTGCTGGTACCAAGCTAAGTAGTTCTTTTGMNNTCCGCWGTTTAAACAGACTCTGGCT
IV26L1-12 TGGTTTCTGCTGGTACCAAGCTAAGTAGTTCTTMNNGTTTCCGCWGTTTAAACAGACTCTGGCT
IV26L1-13 TGGTTTCTGCTGGTACCAAGCTAAGTAGTTMNNNTGGTTTCCGCWGTTTAAACAGACTCTGGCT
IV26L1-14 TGGTTTCTGCTGGTACCAAGCTAAGTAMNNTCTTTTGGTTTCCGCWGTTTAAACAGACTCTGGCT
IV26L1-15 TGGTTTCTGCTGGTACCAAGCTAAMNNGTTCTTTTGGTTTCCGCWGTTTAAACAGACTCTGGCT
IV26L1-16 TGGTTTCTGCTGGTACCAAGCMNNGTAGTTCTTTTGGTTTCCGCWGTTTAAACAGACTCTGGCT
IV26L1-17 TGGTTTCTGCTGGTACCAMNNTAAGTAGTTCTTTTGGTTTCCGCWGTTTAAACAGACTCTGGCT

HUIV26 LCDR2

IV26L2-1 GAATCGGTGAGGACCCCGGATTCCCTGGTAGATGCMNNGTAAATGAGCAGCTTAGG
IV26L2-2 GAATCGGTGAGGACCCCGGATTCCCTGGTAGAMNNTCCGTAATGAGCAGCTTAGG
IV26L2-3 GAATCGGTGAGGACCCCGGATTCCCTGGTMNNTGCCCGTAAATGAGCAGCTTAGG
IV26L2-4 GAATCGGTGAGGACCCCGGATTCCCTMNNAGATGCCCGTAAATGAGCAGCTTAGG
IV26L2-5 GAATCGGTGAGGACCCCGGATTMNNNGTAGATGCCCGTAAATGAGCAGCTTAGG
IV26L2-6 GAATCGGTGAGGACCCCGGAMNNTTGGTAGATGCCCGTAAATGAGCAGCTTAGG
IV26L2-7 GAATCGGTGAGGACCCCMNNTTCCCTGGTAGATGCCCGTAAATGAGCAGCTTAGG

HUIV26 HCDR1

IV26H1-1 TGGAGCCTGGCGGACCCAGCTCATCCAATAMNNACTAAAGTGAATCCAGA
IV26H1-2 TGGAGCCTGGCGGACCCAGCTCATCCAMNNTCTACTAAAGTGAATCCAGA
IV26H1-3 TGGAGCCTGGCGGACCCAGCTCATMNNATATCTACTAAAGTGAATCCAGA
IV26H1-4 TGGAGCCTGGCGGACCCAGCTMNNCAATATCTACTAAAGTGAATCCAGA
IV26H1-5 TGGAGCCTGGCGGACCCAMNNTATCAATATCTACTAAAGTGAATCCAGA

HUIV26 HCDR2a

IV26H2-1 TAGAGATGGCGTATAGTTTATCGTACTGCTATCTGGATTTATMNNGCCAAYCCACTCCAGCCCTTTC
IV26H2-2 TAGAGATGGCGTATAGTTTATCGTACTGCTATCTGGATTTMNNNTTCGCCAAYCCACTCCAGCCCTTTC
IV26H2-3 TAGAGATGGCGTATAGTTTATCGTACTGCTATCTGGMNTATTTTCGCCAAYCCACTCCAGCCCTTTC
IV26H2-4 TAGAGATGGCGTATAGTTTATCGTACTGCTATCMNNATTTATTTTCGCCAAYCCACTCCAGCCCTTTC
IV26H2-5 TAGAGATGGCGTATAGTTTATCGTACTGCTMNNNTGGATTTATTTTCGCCAAYCCACTCCAGCCCTTTC
IV26H2-6 TAGAGATGGCGTATAGTTTATCGTACTMNNATCTGGATTTATTTTCGCCAAYCCACTCCAGCCCTTTC
IV26H2-7 TAGAGATGGCGTATAGTTTATCGTMNNGCTATCTGGATTTATTTTCGCCAAYCCACTCCAGCCCTTTC
IV26H2-8 TAGAGATGGCGTATAGTTTATMNNACTGCTATCTGGATTTATTTTCGCCAAYCCACTCCAGCCCTTTC
IV26H2-9 TAGAGATGGCGTATAGTTMNNCTGCTATCTGGATTTATTTTCGCCAAYCCACTCCAGCCCTTTC

HUIV26 HCDR2b

IV26H2-10 CGTTGTCTCTGGAGATGRTGAATYATCCTTTAGAGATGGCGTATAMNNTATCGTACTGCTATCTGG
IV26H2-11 CGTTGTCTCTGGAGATGRTGAATYATCCTTTAGAGATGGCGTMNNGTTTATCGTACTGCTATCTGG
IV26H2-12 CGTTGTCTCTGGAGATGRTGAATYATCCTTTAGAGATGGMNNTAGTTTATCGTACTGCTATCTGG
IV26H2-13 CGTTGTCTCTGGAGATGRTGAATYATCCTTTAGAGAMNNGTATAGTTTATCGTACTGCTATCTGG
IV26H2-14 CGTTGTCTCTGGAGATGRTGAATYATCCTTTAGMNNNTGGCGTATAGTTTATCGTACTGCTATCTGG
IV26H2-15 CGTTGTCTCTGGAGATGRTGAATYATCCTTMNNAGATGGCGTATAGTTTATCGTACTGCTATCTGG
IV26H2-16 CGTTGTCTCTGGAGATGRTGAATYATCMNNNTAGAGATGGCGTATAGTTTATCGTACTGCTATCTGG
IV26H2-17 CGTTGTCTCTGGAGATGRTGAATYTMNNTTTAGAGATGGCGTATAGTTTATCGTACTGCTATCTGG

FIGURE 4B

Beneficial Mutations for HuIV-26 Antibody

CORs	H1			H2				H3			L1				L2		L3	
Kabat Number	31	34	35	57	62	64	65	97	98	102	27	27d	27e	27f	29		93	94
HuIV-26	R	M	S	I	S	K	D	D	G	Y	Q	N	S	G	Q		S	Y
Mutations	R	I	T	A	Y	Q	S	P	P	P	R	S	Y	Y	K		Q	N
			A	S	A			G	A	N	S		W	R		G	S	
			G		H			T	H				H	H		L	P	
					G			A					R	I		A	M	
																T		
																V		

FIGURE 4C

HUI77 LCDR3
 I77-h7.1 CTT GGT GCC CTG GCC GAA CGT CCA CGG AAC ATG TGA ACC TTG MNN GCA GTA ATA AAC TCC AAC ATC
 I77-h7.2 CTT GGT GCC CTG GCC GAA CGT CCA CGG AAC ATG TGA ACC MNN AAA GCA GTA ATA AAC TCC AAC ATC
 I77-h7.3 CTT GGT GCC CTG GCC GAA CGT CCA CGG AAC ATG TGA MNN TTG AAA GCA GTA ATA AAC TCC AAC ATC
 I77-h7.4 CTT GGT GCC CTG GCC GAA CGT CCA CGG AAC ATG MNN ACC TTG AAA GCA GTA ATA AAC TCC AAC ATC
 I77-h7.5 CTT GGT GCC CTG GCC GAA CGT CCA CGG AAC MNN TGA ACC TTG AAA GCA GTA ATA AAC TCC AAC ATC
 I77-h7.6 CTT GGT GCC CTG GCC GAA CGT CCA CGG MNN ATG TGA ACC TTG AAA GCA GTA ATA AAC TCC AAC ATC
 I77-h7.7 CTT GGT GCC CTG GCC GAA CGT CCA MNN AAC ATG TGA ACC TTG AAA GCA GTA ATA AAC TCC AAC ATC
 I77-h7.8 CTT GGT GCC CTG GCC GAA CGT MNN CGG AAC ATG TGA ACC TTG AAA GCA GTA ATA AAC TCC AAC ATC
 I77-h7.9 CTT GGT GCC CTG GCC GAA MNN CCA CGG AAC ATG TGA ACC TTG AAA GCA GTA ATA AAC TCC AAC ATC
 HUI77 HCDR3
 I77-h7.1 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATA GTA GGG GTT ACC ATA GTT AGC MNN TCG AGC ACA GTA ATA CGT
 I77-h7.2 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATA GTA GGG GTT ACC ATA GTT MNN TCT TCG AGC ACA GTA ATA CGT
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 I77-h7.8 CGT GGT TCC TTG CCC CCA GTA GTC CAT AGC ATA MNN GGG GTT ACC ATA GTT AGC TCT TCG AGC ACA GTA ATA CGT
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 I77-h7.10 CGT GGT TCC TTG CCC CCA GTA GTC MNN ATA GTA GGG GTT ACC ATA GTT AGC TCT TCG AGC ACA GTA ATA CGT
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 I77-h7.12 CGT GGT TCC TTG CCC CCA GTA MNN CAT AGC ATA GTA GGG GTT ACC ATA GTT AGC TCT TCG AGC ACA GTA ATA CGT
 I77-h7.13 CGT GGT TCC TTG CCC CCA MNN GTC CAT AGC ATA GTA GGG GTT ACC ATA GTT AGC TCT TCG AGC ACA GTA ATA CGT

FIGURE 5A

HUI77 LCDR1a

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3001 ATA GGT GTT TCC ATT ACT ATG TAC AAT GCT CTG ACT MNN CCT GCA GGA GAT GGA GGC C
3002 ATA GGT GTT TCC ATT ACT ATG TAC AAT GCT MNN ACT AGA CCT GCA GGA GAT GGA GGC C
3003 ATA GGT GTT TCC ATT ACT ATG TAC AAT GCT MNN ACT AGA CCT GCA GGA GAT GGA GGC C
3004 ATA GGT GTT TCC ATT ACT ATG TAC AAT MNN CTG ACT AGA CCT GCA GGA GAT GGA GGC C
3005 ATA GGT GTT TCC ATT ACT ATG TAC MNN GCT CTG ACT AGA CCT GCA GGA GAT GGA GGC C
3006 ATA GGT GTT TCC ATT ACT ATG MNN AAT GCT CTG ACT AGA CCT GCA GGA GAT GGA GGC C
3007 ATA GGT GTT TCC ATT ACT MNN TAC AAT GCT CTG ACT AGA CCT GCA GGA GAT GGA GGC C

HUI77 LCDR1b

3008 TGG CTT CTG CAG GTA CCA TTC CAA ATA GGT GTT TCC ATT MNN ATG TAC AAT GCT CTG ACT
3009 TGG CTT CTG CAG GTA CCA TTC CAA ATA GGT GTT TCC MNN ACT ATG TAC AAT GCT CTG ACT
3010 TGG CTT CTG CAG GTA CCA TTC CAA ATA GGT GTT MNN ATT ACT ATG TAC AAT GCT CTG ACT
3011 TGG CTT CTG CAG GTA CCA TTC CAA ATA GGT MNN TCC ATT ACT ATG TAC AAT GCT CTG ACT
3012 TGG CTT CTG CAG GTA CCA TTC CAA ATA MNN GTT TCC ATT ACT ATG TAC AAT GCT CTG ACT
3013 TGG CTT CTG CAG GTA CCA TTC CAA MNN GGT GTT TCC ATT ACT ATG TAC AAT GCT CTG ACT
3014 TGG CTT CTG CAG GTA CCA TTC MNN GGT GTT TCC ATT ACT ATG TAC AAT GCT CTG ACT
3015 TGG CTT CTG CAG GTA CCA MNN CAA ATA GGT GTT TCC ATT ACT ATG TAC AAT GCT CTG ACT

HUI77 LCDR2

3016 GAA CCT GTC TGG GAC TCC AGA AAA CCG GTT GGA AAC MNN ATA GAT CAG GAG CTG TGG
3017 GAA CCT GTC TGG GAC TCC AGA AAA CCG GTT GGA MNN TTT ATA GAT CAG GAG CTG TGG
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HUI77 HCDR1

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3026 TGG GGG CTG ACG GAT CCA GCC CAC ACC MNN TCC AGA AGT GCT GAG TGA GAA CCC AGA
3027 TGG GGG CTG ACG GAT CCA GCC CAC MNN CAT TCC AGA AGT GCT GAG TGA GAA CCC AGA
3028 TGG GGG CTG ACG GAT CCA GCC MNN ACC CAT TCC AGA AGT GCT GAG TGA GAA CCC AGA
3029 TGG GGG CTG ACG GAT CCA MNN CAC ACC CAT TCC AGA AGT GCT GAG TGA GAA CCC AGA

HUI77 HCDR2a

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3045 CAG AGA TGG GTT GTA GTA MNN ATT GTC ATC CCA CCA AAT GTC TGC AAG CCA CTC CAG GGC

HUI77 HCDR2b

3030 CTT GGA GAT GGT GAG CCT GCT CTT CAG AGA TGG GTT GTA MNN TTT ATT GTC ATC CCA CCA
3031 CTT GGA GAT GGT GAG CCT GCT CTT CAG AGA TGG GTT MNN GTA TTT ATT GTC ATC CCA CCA
3032 CTT GGA GAT GGT GAG CCT GCT CTT CAG AGA TGG MNN GTA GTA TTT ATT GTC ATC CCA CCA
3033 CTT GGA GAT GGT GAG CCT GCT CTT CAG AGA MNN GTT GTA GTA TTT ATT GTC ATC CCA CCA
3034 CTT GGA GAT GGT GAG CCT GCT CTT CAG MNN TGG GTT GTA GTA TTT ATT GTC ATC CCA CCA
3035 CTT GGA GAT GGT GAG CCT GCT CTT MNN AGA TGG GTT GTA GTA TTT ATT GTC ATC CCA CCA
3036 CTT GGA GAT GGT GAG CCT GCT MNN CAG AGA TGG GTT GTA GTA TTT ATT GTC ATC CCA CCA
3037 CTT GGA GAT GGT GAG CCT MNN CTT CAG AGA TGG GTT GTA GTA TTT ATT GTC ATC CCA CCA

FIGURE 5B

Beneficial Mutations for H1L-77 Antibody

CDRs	H1	H2	H3	L1	L2	L3
Kabat Number	32 35b	59 64	95 100 100e 102	27c 27d 27e 28 30 33	50 51 53 54 56	89 90 91 92 93 94 97
H1L-77	S G	Y K	R N M Y	V H S N N L	K V N R S	F Q G S H V T
Mutations	P W W L A	S P A P	P V Q K Q W N T L T M H T V	P L W Y Y F W S W V	S A S L W F	V R S W L T A H W E T S H K I

FIGURE 5C

Beneficial mutations chosen for combinatorial library

CDRs	H1	H2	H3	L1	L3	SPKkon	SPKoff
Kabat Number	35	57 62	102	27d 27e 27f	93	O.D.560	O.D.560
wild type	S	I S	Y	N S G	S		
HuIV-26	S	I S	Y	N S G	S		
Mutations	T A	A Y A H	P	S Y Y W R H H R	Q		

Beneficial mutants

4.1-2D4	S	I S	P	N S G	Q		
L1b-F11	S	I S	P	N S Y	Q	0.745	0.483
H2a-G8	S	A S	P	N S G	Q	0.397	0.159
2D4H1-C3	A	I S	P	N S G	Q		
DcomA4	S	A Y	P	N Y Y	Q	0.981	0.769
DcomB1	A	A Y	P	N Y H	Q	1.018	0.714
DcomE1						1.031	0.758
DcomH2						1.07	0.705
DcomD2	S	A S	P	N R Y	Q	1.104	0.729
DcomD3	T	A Y	P	N S Y	Q	1.035	0.736
DcomD6	S	A Y	P	N W Y	Q	1.102	0.753
DcomA11						1.003	0.786
DcomE3	T	A Y	P	N R Y	Q	0.991	0.754
DcomG2	A	A Y	P	N R Y	Q	1.161	0.856
DcomA7	T	V S	P	N Y Y	Q	0.986	0.77
DcomB8	T	A A	P	N W Y	Q	0.998	0.807
DcomA2						1.026	0.74
DcomB10	T	A H	P	N W Y	Q	0.87	0.735
DcomC8	S	A A	P	N W Y	Q	0.978	0.763
DcomD7	T	A Y	P	N W Y	Q	1.083	0.794
DhuG5							
DhuH8							
DcomD11	A	A A	P	N W H	Q	1.125	0.752
DcomE11	A	A H	P	N W H	Q	0.973	0.732

Primers for combinatorial mutation

dH5762 TCTCTGGAGATGGTGAATTTACGTACTGCTATCTGGATT
 dL27def CTAAGTAGTTCTTTTGGTTGTTATAACAGACTCTGGCTGGA
 H1-35 TGGAGCCTGGCGGACCCAGGHCATCCAATATCTACTAAAGGTGAATCCAGA
 H2-5762a TCTCTGGAGATGGTGAATCTATCCTTTAGGGM TGGCGTATAGTTGGCCGTACTGCTATCTGGATT
 H2-5762b TCTCTGGAGATGGTGAATCTATCCTTTAGGTRTGGCGTATAGTTGGCCGTACTGCTATCTGGATT
 L1-27def1 CTAAGTAGTTCTTTTGGTTGTRGTRGYTTAACAGACTCTGGCTGGA
 L1-27def2 CTAAGTAGTTCTTTTGGTTGCSGTRGYTTAACAGACTCTGGCTGGA
 L1-27def3 CTAAGTAGTTCTTTTGGTTGTRGCKGYTTAACAGACTCTGGCTGGA
 L1-27def4 CTAAGTAGTTCTTTTGGTTGCSGCKGYTTAACAGACTCTGGCTGGA
 L1-27def5 CTAAGTAGTTCTTTTGGTTGTRCCAGYTTAACAGACTCTGGCTGGA
 L1-27def6 CTAAGTAGTTCTTTTGGTTGSCCAGYTTAACAGACTCTGGCTGGA

FIGURE 6

Beneficial mutations chosen for combinatorial library

CDRs	H1	H2	H3	L1	L3	Screen	Screen
Kabat Number	32 35b	59	100e	27d 28 33	94	kon	koff
wild type	S G	Y	M	H N L	V		
HuI-77	S G	Y	M	H N L	V		
Mutations	P W L A	S A P	Q	L Y F S W			

Beneficial combinatorial mutants

Qh2b-B7	S G	A	Q	H N L	V		
Qh2b-A3	S G	S	Q	H N L	V		
Qcom1B6	P W	S	Q	S W L	V	1.319	0.534
Qcom1B8	P W	S	Q	S Y L	V	1.266	0.497
Qcom1E7						1.247	0.46
Qcom1G10						1.282	0.452
Qcom2G2						1.304	0.402
Qcom1C3	P W	A	Q	S W L	V	1.342	0.535
Qcom2A2						1.605	0.647
Qcom1G3						1.274	0.464
Qcom1D3	P W	T	Q	S W L	V	1.647	0.984
QhuD9							
QhuD93							
Qcom1E3	P W	A	Q	S Q L	V	1.225	0.347
Qcom2F6						1.311	0.321
Qcom1H6	P W	S	Q	S Q L	V	1.295	0.323
Qcom1H7	P W	A	Q	H Q F	F	1.634	1.08
Qcom2A4	P A	A	Q	S Y L	V	1.304	0.265
Qcom2B11	P W	A	Q	H Y L	V	1.11	0.221
Qcom2C1	P W	Y	Q	S W F	V	1.189	0.213
Qcom2D9	P W	S	Q	H W L	V	1.101	0.209
Qcom2E3	P W	A	Q	H W L	V	1.117	0.248

Primers for combinatorial mutation

dL27d-33 CTT CTG CAG GTA CCA TTC GTTA TAC AAT GCT CTG ACT AGA

H1-35b1 TGG GGG CTG ACG GAT CCA CMA CAC ACC CAT TCC AGR AGT GCT GAG TGA GAA CCC AGA

H1-35b2 TGG GGG CTG ACG GAT CCA GSC CAC ACC CAT TCC AGR AGT GCT GAG TGA GAA CCC AGA

H2-59 GCT CTT CAG AGA TGG GTT AGV GTA TTT ATT GTC ATC CCA C

L27d1 CTT CTG CAG GTA CCA TTC MAA ATA GGT GTT TCC CCA ACT CRA TAC AAT GCT CTG ACT AGA

L27d2 CTT CTG CAG GTA CCA TTC MAA ATA GGT GTT TCC GTA ACT CRA TAC AAT GCT CTG ACT AGA

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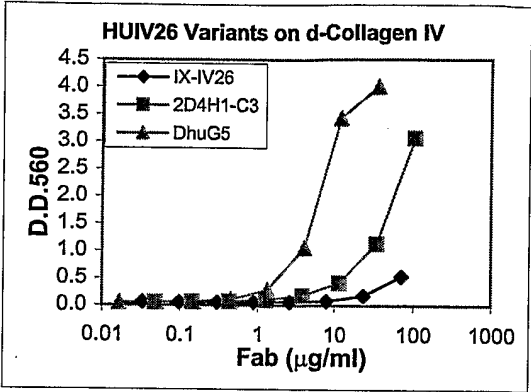
L27d4 CTT CTG CAG GTA CCA TTC MAA ATA GGT GTT TCC CCA ACT GTG TAC AAT GCT CTG ACT AGA

L27d5 CTT CTG CAG GTA CCA TTC MAA ATA GGT GTT TCC GTA ACT GTG TAC AAT GCT CTG ACT AGA

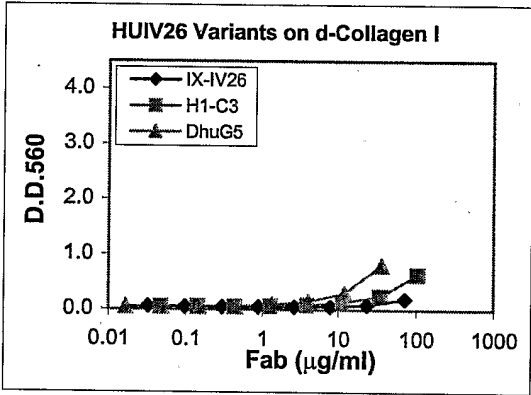
L27d6 CTT CTG CAG GTA CCA TTC MAA ATA GGT GTT TCC CTG ACT GTG TAC AAT GCT CTG ACT AGA

FIGURE 7

A



B



C

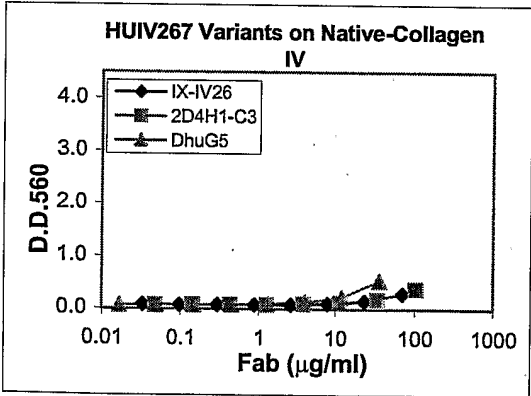


FIGURE 8

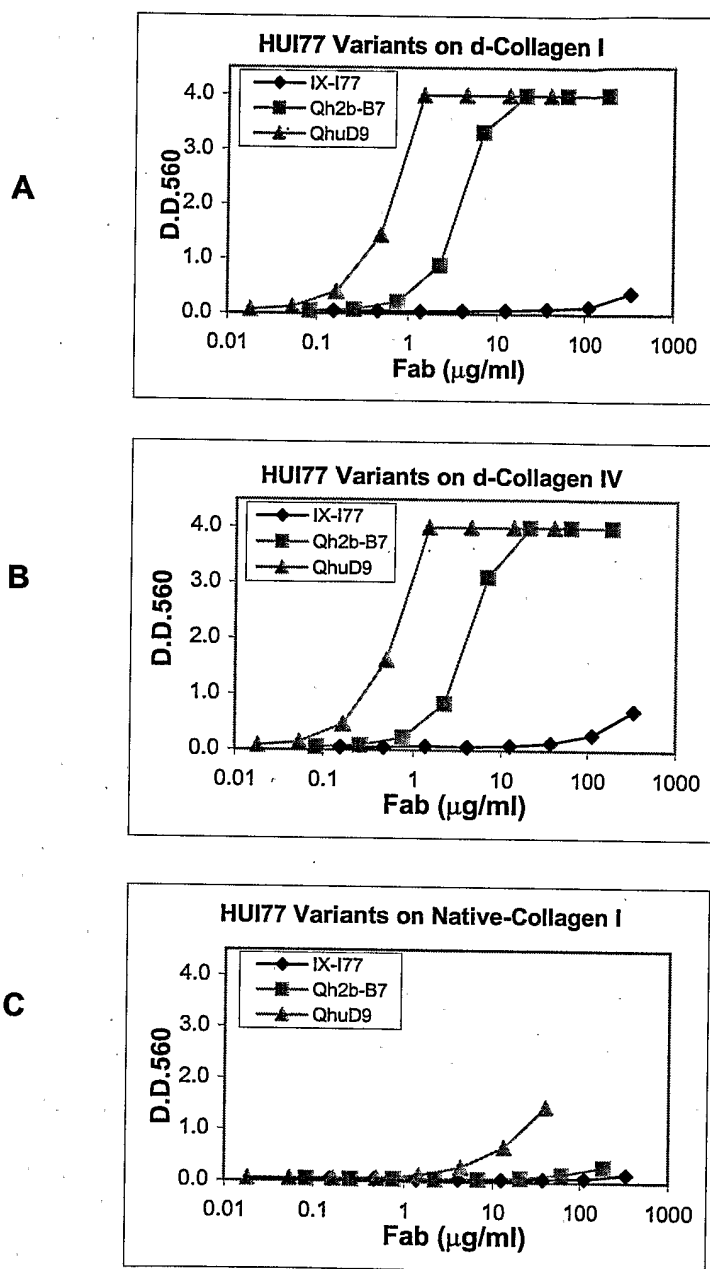


FIGURE 9

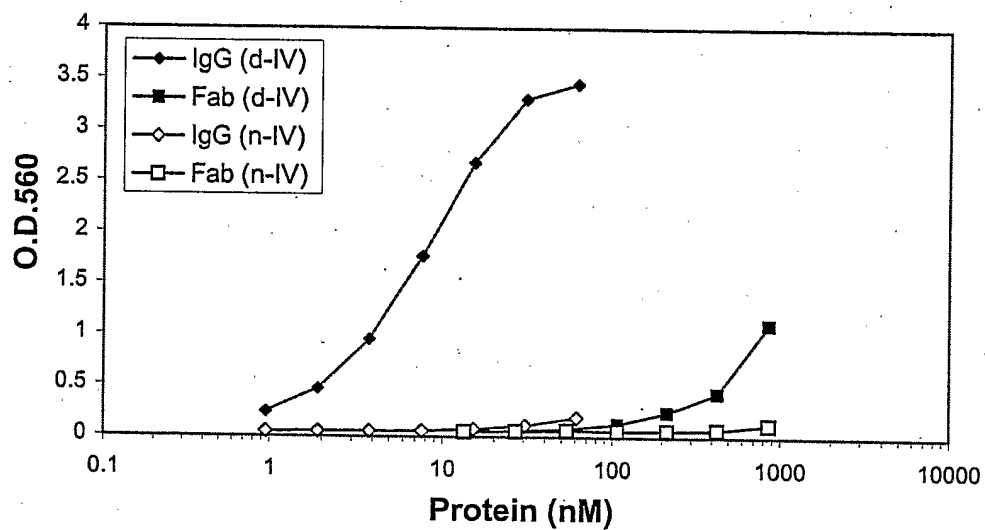


FIGURE 10

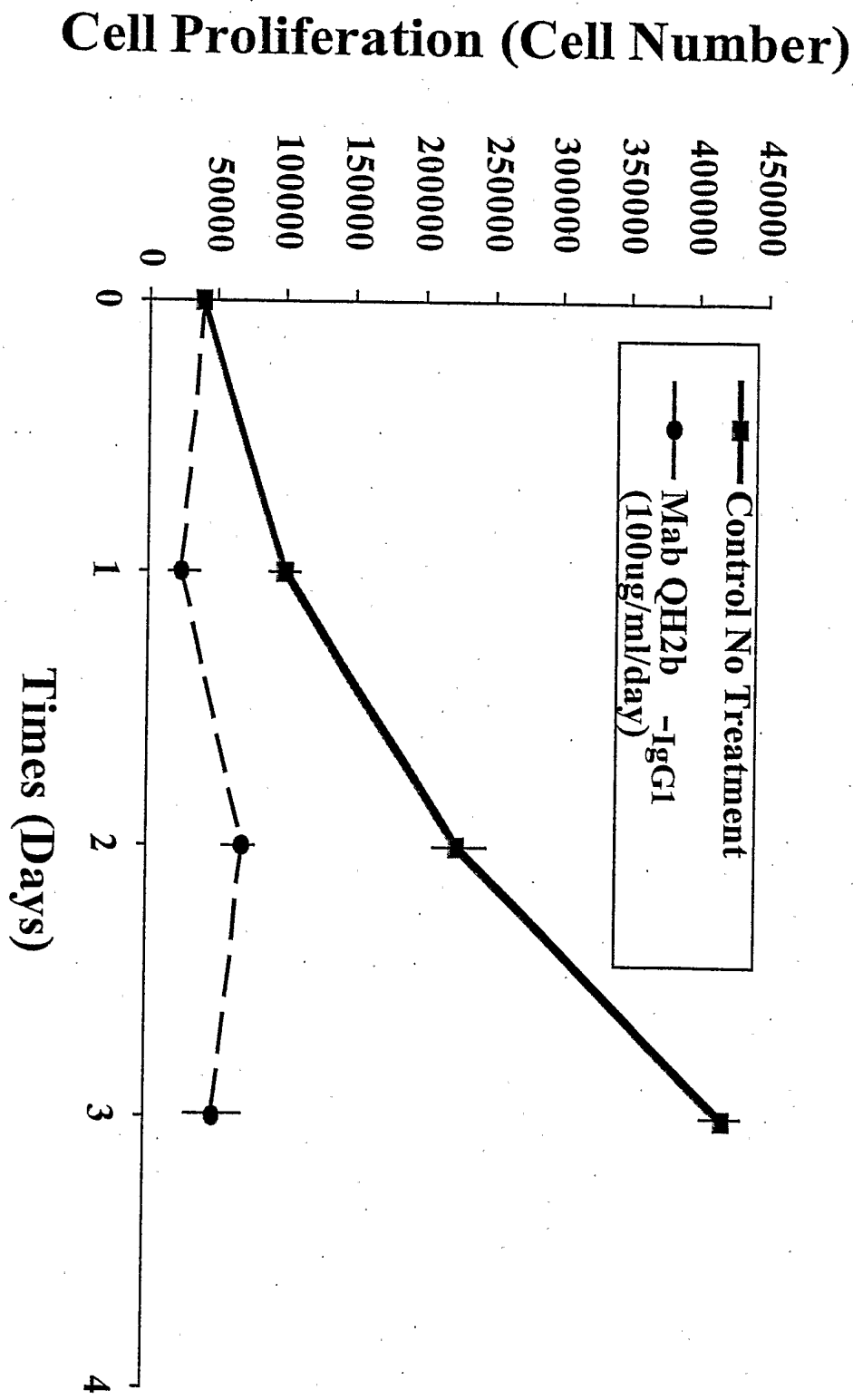


FIGURE 11

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<110> Cell-Matrix, Inc.
 Watkins, Jeffry D.
 Huse, William D.
 Tang, Ying
 Broek, Daniel
 Brooks, Peter C.

<120> Humanized Collagen Antibodies and
 Related Methods

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<150> US 09/995,529

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cct cct aaa ctg ttg atc tat ggg gca tcc act agg gaa tct ggg gtc	192
Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val	
50 55 60	

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Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ile	
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atc agc agt gtg cag gct gaa gac ctg gca gtt tat tac tgt cag aat	288
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Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
20 25 30
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aag gat aaa ttc atc atc tcc aga gac aac gcc aaa aat acg ctg tac			240
Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr			
65	70	75	80
ctg caa atg agc aaa gtg aga tct gag gac aca gcc ctt tat tac tgt			288
Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys			
	85	90	95
gca aga ccg gtt gat ggt tac tac gat gct atg gac tac tgg ggt caa			336
Ala Arg Pro Val Asp Gly Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln			
	100	105	110
gga acc tca gtc acc gtc tcc tca			360
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	115	120	

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Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile			
	35	40	45
Gly Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu			
	50	55	60
Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr			
	65	70	75
Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys			
	85	90	95
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	100	105	110
Gly Thr Ser Val Thr Val Ser Ser			
	115	120	

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cct gac cga ttc agt ggc agc ggg tct ggg aca gat ttc act ctc acc 240
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Ser	Asn	Asn	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
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Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr
65					70					75				80	
Ile	Ser	Ser	Leu	Gln	Ala	Glu	Asp	Val	Ala	Val	Tyr	Tyr	Cys	Gln	Gln
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Lys

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gcc	aac	ata	aag	caa	gat	gga	agt	gag	aaa	tac	tat	gtg	gac	tct	gtg
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Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr
			20					25					30		
Trp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val

35			40			45									
Ala	Asn	Ile	Lys	Gln	Asp	Gly	Ser	Glu	Lys	Tyr	Tyr	Val	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						
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
85			90			95									
Ala	Arg	Pro	Asp	Tyr	Tyr	Tyr	Tyr	Tyr	Gly	Met	Asp	Val	Trp	Gly	Gln
100			105			110									
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser								
115			120												

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<210> 9
<211> 336
<212> DNA
<213> Mus musculus
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<220>
<221> CDS
<222> (1) ... (336)
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<400> 9																	
gat	gtt	ttg	atg	acc	caa	act	cca	ctc	tcc	ctg	cct	gtc	agt	ctt	gga	48	
Asp	Val	Leu	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu	Gly		
1				5					10					15			
gat	caa	gcc	tcc	atc	tct	tgc	aga	tct	agt	cag	agc	att	gta	cat	agt	96	
Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Ile	Val	His	Ser		
			20					25					30				
aat	gga	aac	acc	tat	tta	gaa	tgg	tac	ctg	cag	aaa	cca	ggc	cag	tct	144	
Asn	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser		
		35					40					45					
cca	aag	ctc	ctg	atc	tac	aaa	gtt	tcc	aac	cga	ttt	tct	ggg	gtc	cca	192	
Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro		
	50					55					60						
gac	agg	ttc	agt	ggc	agt	gga	tca	ggg	aca	gat	ttc	aca	ctc	aag	atc	240	
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile		
65					70					75					80		
agc	aga	gtg	gag	gct	gag	gat	ctg	gga	gtt	tat	tac	tgc	ttt	caa	ggg	288	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly		
				85					90					95			
tca	cat	gtt	ccg	tgg	acg	ttc	ggg	gga	ggc	acc	aag	ctg	gaa	atc	aaa	336	
Ser	His	Val	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys		
			100					105					110				

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<210> 10
<211> 112
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<212> PRT

<213> Mus musculus

<400> 10

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Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1           5           10           15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
          20           25           30
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
          35           40           45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
          50           55           60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65           70           75           80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
          85           90           95
Ser His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
          100          105          110

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<210> 11

<211> 369

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1) ... (369)

<400> 11

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cag gtt act ctg aaa gag act ggc cct ggg ata ttg cag ccc tcc cag      48
Gln Val Thr Leu Lys Glu Thr Gly Pro Gly Ile Leu Gln Pro Ser Gln
 1           5           10           15

acc ctc agt ctg act tgt tct ttc tct ggg ttt tca ctg agc act tct      96
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
          20           25           30

ggt atg ggt gta ggc tgg att cgt cag cct tca gga gag ggt cta gag      144
Gly Met Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Glu Gly Leu Glu
          35           40           45

tgg ctg gca gac att tgg tgg gat gac aat aag tac tat aac cca tcc      192
Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser
          50           55           60

ctg aag agc cgg ctc aca atc tcc aag gat acc tcc agc aac cag gta      240
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Ser Asn Gln Val
          65           70           75           80

ttc ctc aag atc acc agt gtg gac act gca gat act gcc act tac tac      288
Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
          85           90           95

tgt gct cga aga gct aac tat ggt aac ccc tac tat gct atg gac tac      336

```

Cys Ala Arg Arg Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Tyr
 100 105 110

tgg ggt caa gga acc tca gtc acc gtc tcc tca 369
 Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
 115 120

<210> 12
 <211> 123
 <212> PRT
 <213> Mus musculus

<400> 12
 Gln Val Thr Leu Lys Glu Thr Gly Pro Gly Ile Leu Gln Pro Ser Gln
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30
 Gly Met Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Glu Gly Leu Glu
 35 40 45
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Ser Asn Gln Val
 65 70 75 80
 Phe Leu Lys Ile Thr Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Tyr
 100 105 110
 Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
 115 120

<210> 13
 <211> 305
 <212> DNA
 <213> Homo sapiens

<400> 13
 gat att gtg atg acc cag act cca ctc tcc ctg ccc gtc acc cct gga 48
 gag ccg gcc tcc atc tcc tgc agg tct agt cag agc ctc ttg gat agt 96
 gat gat gga aac acc tat ttg gac tgg tac ctg cag aag cca ggg cag 144
 tct cca cag ctc ctg atc tat acg ctt tcc tat cgg gcc tct gga gtc 192
 cca gac agg ttc agt ggc agt ggg tca ggc act gat ttc aca ctg aaa 240
 atc agc agg gtg gag gct gag gat gtt gga gtt tat tac tgc atg caa 288
 cgt ata gag ttt cct tc 305

<210> 14
 <211> 111
 <212> PRT
 <213> Homo sapiens

<400> 14
 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15
 Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp Ser

	20		25		30
Asp Gly Asn Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser					
35		40		45	
Pro Gln Leu Leu Ile Tyr Thr Leu Ser Tyr Arg Ala Ser Gly Val Pro					
50		55		60	
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile					
65		70		75	
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser					
	85		90		95
His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys					
100		105		110	

<210> 15
 <211> 288
 <212> DNA
 <213> Homo sapiens

<400> 15
 cagggtcacct tgaaggagtc tggctctgcg ctggtgaaac ccacacagac cctcacactg 60
 acctgcacct tctctgggtt ctactcagc actagtggaa tgcgtgtgag ctggatccgt 120
 cagccccag ggaaggccct ggagtggctt gcacgcattg attggg atg atg ata 175
 aat tct aca gca cat ctc tga agaccaggct caccatctcc aaggacacct 226
 ccaaaaacca ggtggctctt acaatgacca acatggaccc tgtggacaca gccacgtatt 286
 ac 288

<210> 16
 <211> 123
 <212> PRT
 <213> Homo sapiens

<400> 16
 Gln Val Thr Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1 5 10 15
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30
 Gly Met Arg Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45
 Trp Leu Ala Arg Ile Asp Trp Asp Asp Asp Lys Phe Tyr Ser Thr Ser
 50 55 60
 Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80
 Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Arg Arg Ala Asn Tyr Tyr Tyr Tyr Tyr Ala Met Asp Val
 100 105 110
 Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 17
 <211> 340
 <212> DNA
 <213> Homo sapiens

<220>

<221> CDS

<222> (1)...(339)

<400> 17

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gat att gtg atg acc cag act cca ctc tcc ctg ccc gtc acc cct gga . 48
Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
  1             5             10             15

gag ccg gcc tcc atc tcc tgc agg tct agt cag agc ctc ttg gat agt   96
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp Ser
      20             25             30

gat gat gga aac acc tat ttg gac tgg tac ctg cag aag cca ggg cag   144
Asp Asp Gly Asn Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln
      35             40             45

tct cca cag ctc ctg atc tat acg ctt tcc tat cgg gcc tct gga gtc   192
Ser Pro Gln Leu Leu Ile Tyr Thr Leu Ser Tyr Arg Ala Ser Gly Val
      50             55             60

cca gac agg ttc agt ggc agt ggg tca ggc act gat ttc aca ctg aaa   240
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
      65             70             75             80

atc agc agg gtg gag gct gag gat gtt gga gtt tat tac tgc atg caa   288
Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln
      85             90             95

cgg ttc aca tgt tcc gtg gac gtt cgg cca agg gac caa ggt gga aat   336
Arg Phe Thr Cys Ser Val Asp Val Arg Pro Arg Asp Gln Gly Gly Asn
      100            105            110

caa a
Gln
                                     340

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<210> 18

<211> 113

<212> PRT

<213> Homo sapiens

<400> 18

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

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp Ser
      20             25             30

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

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

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
      65             70             75             80

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln

```

				85					90					95	
Arg	Phe	Thr	Cys	Ser	Val	Asp	Val	Arg	Pro	Arg	Asp	Gln	Gly	Gly	Asn
			100					105					110		
Gln															

<210> 19
 <211> 51
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(51)

<400> 19																
aag	tcc	agt	cag	agt	ctg	tta	aac	agt	gga	aat	caa	aag	aac	tac	ttg	48
Lys	Ser	Ser	Gln	Ser	Leu	Leu	Asn	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu	
1				5					10				15			
gcc															51	
Ala																

<210> 20
 <211> 17
 <212> PRT
 <213> Mus musculus

<400> 20															
Lys	Ser	Ser	Gln	Ser	Leu	Leu	Asn	Ser	Gly	Asn	Gln	Lys	Asn	Tyr	Leu
1				5					10				15		
Ala															

<210> 21
 <211> 21
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(21)

<400> 21															
ggg	gca	tcc	act	agg	gaa	tct									21
Gly	Ala	Ser	Thr	Arg	Glu	Ser									
1				5											

<210> 22
 <211> 7

<212> PRT
<213> Mus musculus

<400> 22
Gly Ala Ser Thr Arg Glu Ser
1 5

<210> 23
<211> 27
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(27)

<400> 23
cag aat gat cat agt tat ccg tac acg
Gln Asn Asp His Ser Tyr Pro Tyr Thr
1 5

27

<210> 24
<211> 9
<212> PRT
<213> Mus musculus

<400> 24
Gln Asn Asp His Ser Tyr Pro Tyr Thr
1 5

<210> 25
<211> 30
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(30)

<400> 25
gga ttc gat ttt agt aga tac tgg atg agt
Gly Phe Asp Phe Ser Arg Tyr Trp Met Ser
1 5 10

30

<210> 26
<211> 10
<212> PRT
<213> Mus musculus

<400> 26
Gly Phe Asp Phe Ser Arg Tyr Trp Met Ser

1 5 10

<210> 27
<211> 51
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(51)

<400> 27
gaa att aat cca gat agc agt acg ata aac tat acg cca tct cta aag 48
Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu Lys
1 5 10 15

gat 51
Asp

<210> 28
<211> 17
<212> PRT
<213> Mus musculus

<400> 28
Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu Lys
1 5 10 15
Asp

<210> 29
<211> 33
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(33)

<400> 29
ccg gtt gat ggt tac tac gat gct atg gac tac 33
Pro Val Asp Gly Tyr Tyr Asp Ala Met Asp Tyr
1 5 10

<210> 30
<211> 11
<212> PRT
<213> Mus musculus

<400> 30

Pro Val Asp Gly Tyr Tyr Asp Ala Met Asp Tyr
 1 5 10

<210> 31
 <211> 48
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(48)

<400> 31
 aga tct agt cag agc att gta cat agt aat gga aac acc tat tta gaa 48
 Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
 1 5 10 15

<210> 32
 <211> 16
 <212> PRT
 <213> Mus musculus

<400> 32
 Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
 1 5 10 15

<210> 33
 <211> 21
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(21)

<400> 33
 aaa gtt tcc aac cga ttt tct 21
 Lys Val Ser Asn Arg Phe Ser
 1 5

<210> 34
 <211> 7
 <212> PRT
 <213> Mus musculus

<400> 34
 Lys Val Ser Asn Arg Phe Ser
 1 5

<210> 35
<211> 27
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(27)

<400> 35
ttt caa ggt tca cat gtt ccg tgg acg
Phe Gln Gly Ser His Val Pro Trp Thr
1 5

27

<210> 36
<211> 9
<212> PRT
<213> Mus musculus

<400> 36
Phe Gln Gly Ser His Val Pro Trp Thr
1 5

<210> 37
<211> 36
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(36)

<400> 37
ggg ttt tca ctg agc act tct ggt atg ggt gta ggc
Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Gly
1 5 10

36

<210> 38
<211> 12
<212> PRT
<213> Mus musculus

<400> 38
Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Gly
1 5 10

<210> 39
<211> 48
<212> DNA
<213> Mus musculus

<220>

<221> CDS

<222> (1)...(48)

<400> 39

gac att tgg tgg gat gac aat aag tac tat aac cca tcc ctg aag agc 48
 Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser Leu Lys Ser
 1 5 10 15

<210> 40

<211> 16

<212> PRT

<213> Mus musculus

<400> 40

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

<210> 41

<211> 39

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(39)

<400> 41

aga gct aac tat ggt aac ccc tac tat gct atg gac tac 39
 Arg Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Tyr
 1 5 10

<210> 42

<211> 13

<212> PRT

<213> Mus musculus

<400> 42

Arg Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Tyr
 1 5 10

<210> 43

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 43

Gly Phe Asp Phe Ser His Tyr Trp Met Ser
1 5 10

<210> 44

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 44

Gly Phe Asp Phe Ser Arg Tyr Trp Ile Ser
1 5 10

<210> 45

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 45

Gly Phe Asp Phe Ser Arg Tyr Trp Met Thr
1 5 10

<210> 46

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 46

Gly Phe Asp Phe Ser Arg Tyr Trp Met Ala
1 5 10

<210> 47

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Artificial sequence

<400> 47

Gly Phe Asp Phe Ser Arg Tyr Trp Met Gly
1 5 10

<210> 48
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial sequence

<400> 48
Glu Ile Asn Pro Asp Ser Ser Thr Ala Asn Tyr Thr Pro Ser Leu Lys
1 5 10 15
Asp

<210> 49
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial sequence

<400> 49
Glu Ile Asn Pro Asp Ser Ser Thr Ser Asn Tyr Thr Pro Ser Leu Asp
1 5 10 15
Lys

<210> 50
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial sequence

<400> 50
Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Tyr Leu Lys
1 5 10 15
Asp

<210> 51
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic antibody mutation

<400> 51

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

<210> 52
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Artificial sequence

<400> 52
Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro His Leu Lys
1 5 10 15
Asp

<210> 53
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic antibody mutation

<400> 53
Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Gly Leu Lys
1 5 10 15
Asp

<210> 54
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic antibody mutation

<400> 54
Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu Gln
1 5 10 15
Asp

<210> 55
<211> 17
<212> PRT
<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 55

Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu Lys

1 5 10 15

Ser

<210> 56

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 56

Pro Val Pro Gly Tyr Tyr Asp Ala Met Asp Tyr

1 5 10

<210> 57

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 57

Pro Val Gly Gly Tyr Tyr Asp Ala Met Asp Tyr

1 5 10

<210> 58

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 58

Pro Val Thr Gly Tyr Tyr Asp Ala Met Asp Tyr

1 5 10

<210> 59

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 59

Pro Val Ala Gly Tyr Tyr Asp Ala Met Asp Tyr
1 5 10

<210> 60

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 60

Pro Val Asp Pro Tyr Tyr Asp Ala Met Asp Tyr
1 5 10

<210> 61

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 61

Pro Val Asp Ala Tyr Tyr Asp Ala Met Asp Tyr
1 5 10

<210> 62

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 62

Pro Val Asp His Tyr Tyr Asp Ala Met Asp Tyr
1 5 10

<210> 63

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 63

Pro Val Asp Gly Tyr Tyr Asp Ala Met Asp Pro
1 5 10

<210> 64

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Artificial sequence

<400> 64

Pro Val Asp Gly Tyr Tyr Asp Ala Met Asp Asn
1 5 10

<210> 65

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 65

Lys Ser Ser Arg Ser Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 66

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 66

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

<210> 67

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 67

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

<210> 68

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 68

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

<210> 69

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 69

Lys Ser Ser Gln Ser Leu Leu Asn Trp Gly Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 70

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 70

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

<210> 71

<211> 17

<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic antibody mutation

<400> 71
Lys Ser Ser Gln Ser Leu Leu Asn Arg Gly Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 72
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic antibody mutation

<400> 72
Lys Ser Ser Gln Ser Leu Leu Asn Ser Tyr Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 73
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic antibody mutation

<400> 73
Lys Ser Ser Gln Ser Leu Leu Asn Ser Arg Asn Gln Lys Asn Tyr Leu
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Ala

<210> 74
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<213> Artificial Sequence

<220>
<223> synthetic antibody mutation

<400> 74
Lys Ser Ser Gln Ser Leu Leu Asn Ser His Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 75
<211> 17
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<220>
<223> synthetic antibody mutation

<400> 75
Lys Ser Ser Gln Ser Leu Leu Asn Ser Ile Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 76
<211> 17
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<220>
<223> synthetic antibody mutation

<400> 76
Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Lys Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 77
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<220>
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<400> 77
Gln Asn Asp His Gln Tyr Pro Tyr Thr
1 5

<210> 78
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<220>
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<400> 78

Gln Asn Asp His Gly Tyr Pro Tyr Thr
1 5

<210> 79

<211> 9

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<220>

<223> synthetic antibody mutation

<400> 79

Gln Asn Asp His Leu Tyr Pro Tyr Thr
1 5

<210> 80

<211> 9

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<213> Artificial Sequence

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<223> synthetic antibody mutation

<400> 80

Gln Asn Asp His Ala Tyr Pro Tyr Thr
1 5

<210> 81

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

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<400> 81

Gln Asn Asp His Thr Tyr Pro Tyr Thr
1 5

<210> 82

<211> 9

<212> PRT

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<220>

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<400> 82

Gln Asn Asp His Val Tyr Pro Tyr Thr
1 5

<210> 83
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<220>
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<400> 83
Gln Asn Asp His Ser Asn Pro Tyr Thr
1 5

<210> 84
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<220>
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<400> 84
Gln Asn Asp His Ser Ser Pro Tyr Thr
1 5

<210> 85
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<220>
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<400> 85
Gln Asn Asp His Ser Pro Pro Tyr Thr
1 5

<210> 86
<211> 9
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<220>
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<400> 86
Gln Asn Asp His Ser Met Pro Tyr Thr
1 5

<210> 87
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<220>

<223> synthetic antibody mutation

<400> 87

Gly Phe Ser Leu Ser Thr Pro Gly Met Gly Val Gly
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<210> 88

<211> 12

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<223> synthetic antibody mutation

<400> 88

Gly Phe Ser Leu Ser Thr Trp Gly Met Gly Val Gly
1 5 10

<210> 89

<211> 12

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<223> synthetic antibody mutation

<400> 89

Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Trp
1 5 10

<210> 90

<211> 12

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<223> synthetic antibody mutation

<400> 90

Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Leu
1 5 10

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<211> 12

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<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 91

Gly	Phe	Ser	Leu	Ser	Thr	Ser	Gly	Met	Gly	Val	Ala
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<400> 92

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<211> 16

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<223> synthetic antibody mutation

<400> 93

Asp	Ile	Trp	Trp	Asp	Asp	Asn	Lys	Tyr	Ala	Asn	Pro	Ser	Leu	Lys	Ser
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<211> 16

<212> PRT

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<223> synthetic antibody mutation

<400> 94

Asp	Ile	Trp	Trp	Asp	Asp	Asn	Lys	Tyr	Pro	Asn	Pro	Ser	Leu	Lys	Ser
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<210> 95

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 95

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

<210> 96

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 96

Pro Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Tyr
1 5 10

<210> 97

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 97

Gln Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Tyr
1 5 10

<210> 98

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 98

Leu Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Tyr
1 5 10

<210> 99

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 99

Thr Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Tyr
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<210> 100
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<210> 101
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<400> 101
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1 5 10

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<400> 102
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<211> 13
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<400> 104
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Arg Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Thr Asp Tyr
1 5 10

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<400> 106
Arg Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Lys
1 5 10

<210> 107
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1 5 10

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<211> 13
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<220>

<223> synthetic antibody mutation

<400> 108

Arg Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp Met
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<210> 109

<211> 13

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<223> synthetic antibody mutation

<400> 109

Arg Ala Asn Tyr Gly Asn Pro Tyr Tyr Ala Met Asp His
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<211> 16

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<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 110

Arg Ser Ser Gln Ser Ile Pro His Ser Asn Gly Asn Thr Tyr Leu Glu
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<210> 111

<211> 16

<212> PRT

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<220>

<223> synthetic antibody mutation

<400> 111

Arg Ser Ser Gln Ser Ile Trp His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 112

<211> 16

<212> PRT

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<220>

<223> synthetic antibody mutation

<400> 112

Arg Ser Ser Gln Ser Ile Val Leu Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 113

<211> 16

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<223> synthetic antibody mutation

<400> 113

Arg Ser Ser Gln Ser Ile Val Ser Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 114

<211> 16

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<223> synthetic antibody mutation

<400> 114

Arg Ser Ser Gln Ser Ile Val His Trp Asn Gly Asn Thr Tyr Leu Glu
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<210> 115

<211> 16

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<223> synthetic antibody mutation

<400> 115

Arg Ser Ser Gln Ser Ile Val His Ser Tyr Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 116

<211> 16

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<223> synthetic antibody mutation

<400> 116

Arg Ser Ser Gln Ser Ile Val His Ser Trp Gly Asn Thr Tyr Leu Glu

1 5 10 15

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<400> 117
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Tyr Thr Tyr Leu Glu
1 5 10 15

<210> 118
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<212> PRT
<213> Artificial Sequence

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<400> 118
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Phe Glu
1 5 10 15

<210> 119
<211> 16
<212> PRT
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<400> 119
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Val Glu
1 5 10 15

<210> 120
<211> 7
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<400> 120
Ser Val Ser Asn Arg Phe Ser
1 5

<210> 121
<211> 7
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<220>
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<400> 121
Lys Ala Ser Asn Arg Phe Ser
1 5

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<400> 122
Lys Val Ser Ser Arg Phe Ser
1 5

<210> 123
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<400> 123
Lys Val Ser Asn Leu Phe Ser
1 5

<210> 124
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<400> 124
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1 5

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Lys Val Ser Asn Arg Phe Phe

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<400> 126

Val Gln Gly Ser His Val Pro Trp Thr

1

5

<210> 127

<211> 9

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<400> 127

His Gln Gly Ser His Val Pro Trp Thr

1

5

<210> 128

<211> 9

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<223> synthetic antibody mutation

<400> 128

Phe Arg Gly Ser His Val Pro Trp Thr

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5

<210> 129

<211> 9

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<223> synthetic antibody mutation

<400> 129

Phe Trp Gly Ser His Val Pro Trp Thr
1 5

<210> 130

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<220>

<223> synthetic antibody mutation

<400> 130

Phe Gln Ser Ser His Val Pro Trp Thr
1 5

<210> 131

<211> 9

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<223> synthetic antibody mutation

<400> 131

Phe Gln Gly Trp His Val Pro Trp Thr
1 5

<210> 132

<211> 9

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<220>

<223> synthetic antibody mutation

<400> 132

Phe Gln Gly Glu His Val Pro Trp Thr
1 5

<210> 133

<211> 9

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<223> synthetic antibody mutation

<400> 133

Phe Gln Gly Ser Leu Val Pro Trp Thr
1 5

<210> 134

<211> 9

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<220>

<223> synthetic antibody mutation

<400> 134

Phe Gln Gly Ser Thr Val Pro Trp Thr
1 5

<210> 135

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<223> synthetic antibody mutation

<400> 135

Phe Gln Gly Ser Ser Val Pro Trp Thr
1 5

<210> 136

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<223> synthetic antibody mutation

<400> 136

Phe Gln Gly Ser Ala Val Pro Trp Thr
1 5

<210> 137

<211> 9

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<400> 137

Phe Gln Gly Ser Gln Val Pro Trp Thr
1 5

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<400> 138
Phe Gln Gly Ser His Thr Pro Trp Thr
1 5

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<400> 139
Phe Gln Gly Ser His Val Pro Trp Ala
1 5

<210> 140
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Phe Gln Gly Ser His Val Pro Trp Arg
1 5

<210> 141
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Phe Gln Gly Ser His Val Pro Trp His
1 5

<210> 142
<211> 9

<212> PRT
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<400> 142
Phe Gln Gly Ser His Val Pro Trp Lys
1 5

<210> 143
<211> 9
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<400> 143
Phe Gln Gly Ser His Val Pro Trp Ile
1 5

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

<210> 145
<211> 9
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<213> Artificial Sequence

<220>
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<400> 145
Phe Gln Gly Ser His Phe Pro Trp Thr
1 5

<210> 146
<211> 16
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<220>

<223> synthetic antibody mutation

<400> 146

Arg	Ser	Ser	Gln	Ser	Ile	Val	His	Ser	Gln	Gly	Asn	Thr	Tyr	Leu	Glu
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<210> 147

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<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 147

Gly	Phe	Ser	Leu	Ser	Thr	Pro	Gly	Met	Gly	Val	Trp
1				5				10			

<210> 148

<211> 12

<212> PRT

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<220>

<223> synthetic antibody mutation

<400> 148

Gly	Phe	Ser	Leu	Ser	Thr	Pro	Gly	Met	Gly	Val	Ala
1				5				10			

<210> 149

<211> 15

<212> PRT

<213> Artificial Sequence

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<223> synthetic antibody mutation

<400> 149

Arg	Ser	Ser	Gln	Ser	Ile	Val	Ser	Ser	Trp	Gly	Asn	Thr	Tyr	Leu
1				5					10					15

<210> 150

<211> 15

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<223> synthetic antibody mutation

<400> 150

Arg Ser Ser Gln Ser Ile Val Ser Ser Tyr Gly Asn Thr Tyr Leu
1 5 10 15

<210> 151

<211> 15

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<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 151

Arg Ser Ser Gln Ser Ile Val Ser Ser Gln Gly Asn Thr Tyr Leu
1 5 10 15

<210> 152

<211> 15

<212> PRT

<213> Artificial Sequence

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<223> synthetic antibody mutation

<400> 152

Arg Ser Ser Gln Ser Ile Val His Ser Gln Gly Asn Thr Tyr Phe
1 5 10 15

<210> 153

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 153

Arg Ser Ser Gln Ser Ile Val Ser Ser Trp Gly Asn Thr Tyr Phe
1 5 10 15

<210> 154

<211> 17

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<213> Artificial Sequence

<220>

<223> synthetic antibody mutation

<400> 154

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

Asp

<210> 155
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
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<400> 155
Glu Ile Asn Pro Asp Ser Ser Thr Ala Asn Tyr Thr Pro Tyr Leu Lys
1 5 10 15
Asp

<210> 156
<211> 17
<212> PRT
<213> Artificial Sequence

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<400> 156
Glu Ile Asn Pro Asp Ser Ser Thr Ala Asn Tyr Thr Pro His Leu Lys
1 5 10 15
Asp

<210> 157
<211> 17
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<400> 157
Lys Ser Ser Gln Ser Leu Leu Asn Trp Tyr Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 158
<211> 17
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<400> 158

Lys Ser Ser Gln Ser Leu Leu Asn Tyr Tyr Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 159

<211> 17

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<400> 159

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

<210> 160

<211> 17

<212> PRT

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<400> 160

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

<210> 161

<211> 17

<212> PRT

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<400> 161

Lys Ser Ser Gln Ser Leu Leu Asn Trp His Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 162

<211> 17
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<400> 162
Glu Ile Asn Pro Asp Ser Ser Thr Val Asn Tyr Thr Pro Ser Leu Lys
1 5 10 15
Asp

<210> 163
<211> 39
<212> DNA
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<220>
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<210> 164
<211> 41
<212> DNA
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<223> Primer

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<210> 165
<211> 51
<212> DNA
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<220>
<223> Primer

<400> 165
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<210> 166
<211> 65
<212> DNA
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<220>
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<400> 166
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<210> 167
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<212> DNA
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ggatt 65

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<210> 169
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<400> 171

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46

<210> 172

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46

<210> 173

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<400> 173

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46

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<400> 174

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40

<210> 175

<211> 57

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<400> 175

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57

<210> 176

<211> 57

<212> DNA

<213> Artificial Sequence

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<223> Primer

<400> 176

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<210> 177

<211> 40

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<400> 177

gctcttcaga gatggggttag vgtattttatt gtcatcccac 40

<210> 178

<211> 60

<212> DNA

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<400> 178

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<210> 179

<211> 60

<212> DNA

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<400> 179

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<211> 60

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<400> 180

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<400> 181

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<211> 18

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<400> 184

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atcttcttgc tggttctgg

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cag 63

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<400> 223
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<210> 225
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<221> misc_feature

<222> 41, 42

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<210> 226

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<222> 38, 39

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<210> 227

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<221> misc_feature

<222> 35, 36

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<210> 228

<211> 66

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<221> misc_feature

<222> 32, 33

<223> n = A,T,C or G

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cacatc 66

<210> 229
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<221> misc_feature
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cacatc 66

<210> 230
<211> 66
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<222> 26, 27
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cacatc 66

<210> 231
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<222> 23, 24
<223> n = A,T,C or G

<400> 231
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cacatc 66

<210> 232
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<222> 20, 21

<223> n = A,T,C or G

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<210> 233

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<222> 50, 51

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<400> 233

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gtaatacac 69

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gtaatacac 69

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<222> 44, 45

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gtaatacac 69

<210> 236

<211> 69

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<222> 41, 42

<223> n = A,T,C or G

<400> 236

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gtaatacac 69

<210> 237

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<222> 38, 39

<223> n = A,T,C or G

<400> 237

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gtaatacac 69

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<222> 35, 36

<223> n = A,T,C or G

<400> 238

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gtaatacac 69

<210> 239

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<400> 239
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gtaatacac 69

<210> 240
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<223> n = A,T,C or G

<400> 240
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gtaatacac 69

<210> 241
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<400> 241
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gtaatacac 69

<210> 242
<211> 69
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<222> 23, 24

<223> n = A,T,C or G

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gtaatacac 69

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gtaatacac 69

<210> 244

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<222> 44, 45

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aacatc 66

<210> 245

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<222> 41, 42

<223> n = A,T,C or G

<400> 245

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aacatc 66

<210> 246
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<222> 38, 39
<223> n = A,T,C or G

<400> 246
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aacatc 66

<210> 247
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aacatc 66

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aacatc 66

<210> 249
<211> 66
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<222> 29, 30

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aacatc 66

<210> 250

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<210> 251

<211> 66

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<222> 23, 24

<223> n = A,T,C or G

<400> 251

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aacatc 66

<210> 252

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<223> n = A,T,C or G

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aacatc 66

<210> 253
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agcacagtaa tacgt 75

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<221> misc_feature
<222> 53, 54
<223> n = A,T,C or G

<400> 254
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agcacagtaa tacgt 75

<210> 255
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agcacagtaa tacgt 75

<210> 256
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<222> 47, 48

<223> n = A,T,C or G

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<222> 41, 42

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<222> 38, 39

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agcacagtaa tacgt 75

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agcacagtaa tacgt 75

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<222> 41, 42

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gct 63

<210> 275

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<212> DNA

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<222> 41, 42

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<210> 276

<211> 63

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<222> 38, 39

<223> n = A,T,C or G

<400> 276

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gct

<210> 277
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gct 63

<210> 278
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<222> 32, 33
<223> n = A,T,C or G

<400> 278
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gct 63

<210> 279
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<221> misc_feature
<222> 29, 30
<223> n = A,T,C or G

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gct 63

<210> 280
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<222> 26, 27

<223> n = A,T,C or G

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gct 63

<210> 281

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<223> n = A,T,C or G

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<210> 282

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<223> n = A,T,C or G

<400> 282

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gct 63

<210> 283

<211> 57

<212> DNA

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<221> misc_feature

<222> 38, 39

<223> n = A,T,C or G

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<210> 284
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<221> misc_feature
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<210> 285
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<221> misc_feature
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<221> misc_feature
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<223> n = A,T,C or G

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<221> misc_feature
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<400> 287
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<210> 288
<211> 57
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<221> misc_feature
<222> 23, 24
<223> n = A,T,C or G

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<210> 289
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<222> 20, 21
<223> n = A,T,C or G

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<210> 290
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<220>
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<221> misc_feature
<222> 32, 33
<223> n = A,T,C or G

<400> 290
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<210> 291
<211> 51

<212> DNA
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<221> misc_feature
<222> 29, 30
<223> n = A,T,C or G

<400> 291
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<210> 292
<211> 51
<212> DNA
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<221> misc_feature
<222> 26, 27
<223> n = A,T,C or G

<400> 292
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<210> 293
<211> 51
<212> DNA
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<222> 23, 24
<223> n = A,T,C or G

<400> 293
tggagcctgg cggacccagc tmnnccaata tctactaaag gtgaatccag a 51

<210> 294
<211> 51
<212> DNA
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<221> misc_feature
<222> 20, 21
<223> n = A,T,C or G

<400> 294
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<210> 295
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<221> misc_feature
<222> 44, 45
<223> n = A,T,C or G

<400> 295
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ccctttc 67

<210> 296
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
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<221> misc_feature
<222> 41, 42
<223> n = A,T,C or G

<400> 296
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ccctttc 67

<210> 297
<211> 67
<212> DNA
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<221> misc_feature
<222> 38, 39
<223> n = A,T,C or G

<400> 297
tagagatggc gtatagttta tcgtactgct atctggmnnt atttcgccaa yccactccag 60
ccctttc 67

<210> 298
<211> 67
<212> DNA
<213> Artificial Sequence

<220>

<223> primer

<221> misc_feature

<222> 35, 36

<223> n = A,T,C or G

<400> 298

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ccctttc 67

<210> 299

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<221> misc_feature

<222> 32, 33

<223> n = A,T,C or G

<400> 299

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ccctttc 67

<210> 300

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<221> misc_feature

<222> 29, 30

<223> n = A,T,C or G

<400> 300

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ccctttc 67

<210> 301

<211> 67

<212> DNA

<213> Artificial Sequence

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<223> primer

<221> misc_feature

<222> 26, 27

<223> n = A,T,C or G

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ccctttc 67

<210> 302
<211> 67
<212> DNA
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<221> misc_feature
<222> 23, 24
<223> n = A,T,C or G

<400> 302
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ccctttc 67

<210> 303
<211> 67
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<221> misc_feature
<222> 20, 21
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<400> 303
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ccctttc 67

<210> 304
<211> 67
<212> DNA
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<220>
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<221> misc_feature
<222> 48, 49
<223> n = A,T,C or G

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tatctgg 67

<210> 305
<211> 67

<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<221> misc_feature
<222> 45, 46
<223> n = A,T,C or G

<400> 305
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tatctgg 67

<210> 306
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<221> misc_feature
<222> 42, 43
<223> n = A,T,C or G

<400> 306
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tatctgg 67

<210> 307
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
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<221> misc_feature
<222> 39, 40
<223> n = A,T,C or G

<400> 307
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tatctgg 67

<210> 308
<211> 67
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<221> misc_feature

<222> 36, 37

<223> n = A,T,C or G

<400> 308

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tatctgg 67

<210> 309

<211> 67

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<221> misc_feature

<222> 33, 34

<223> n = A,T,C or G

<400> 309

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tatctgg 67

<210> 310

<211> 67

<212> DNA

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<221> misc_feature

<222> 30, 31

<223> n = A,T,C or G

<400> 310

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tatctgg 67

<210> 311

<211> 67

<212> DNA

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<223> primer

<221> misc_feature

<222> 27, 28

<223> n = A,T,C or G

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tatctgg 67

<210> 312
<211> 58
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<221> misc_feature
<222> 41, 42
<223> n = A,T,C or G

<400> 312
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<210> 313
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<222> 38, 39
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<400> 313
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<210> 314
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<221> misc_feature
<222> 35, 36
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<400> 314
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<210> 315
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<222> 32, 33

<223> n = A,T,C or G

<400> 315
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<222> 29, 30
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<400> 317
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<210> 318
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<223> n = A,T,C or G

<400> 318
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<210> 319
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<222> 20, 21

<223> n = A,T,C or G

<400> 319

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<210> 320

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<222> 41, 42

<223> n = A,T,C or G

<400> 320

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<210> 321

<211> 60

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<223> n = A,T,C or G

<400> 321

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<210> 322

<211> 60

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<222> 35, 36

<223> n = A,T,C or G

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<210> 323
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<400> 325
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<210> 326
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<221> misc_feature

<222> 23, 24

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<400> 326

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<210> 327

<211> 60

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<210> 328

<211> 57

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<222> 38, 39

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<400> 328

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<210> 329

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<222> 35, 36

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<210> 330
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<210> 335
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<210> 336
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<210> 337
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<210> 339
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<210> 340
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<221> misc_feature
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<223> n = A,T,C or G

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<210> 341
<211> 57
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<221> misc_feature
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<400> 341
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<210> 342
<211> 60
<212> DNA
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<221> misc_feature
<222> 41, 42
<223> n = A,T,C or G

<400> 342
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<210> 343
<211> 60
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<221> misc_feature
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<210> 344
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<222> 35, 36

<223> n = A,T,C or G

<400> 344

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<210> 345

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<212> DNA

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<222> 32, 33

<223> n = A,T,C or G

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<210> 346

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<212> DNA

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<223> n = A,T,C or G

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<210> 347

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<212> DNA

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<223> n = A,T,C or G

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<210> 348

<211> 60

<212> DNA

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<221> misc_feature

<222> 23, 24

<223> n = A,T,C or G

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<210> 349

<211> 60

<212> DNA

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<222> 20, 21

<223> n = A,T,C or G

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<210> 350

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<222> 41, 42

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Phe Gln Ser Ser His Phe Pro Trp Thr

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