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(54) **Title:** BISPECIFIC BINDING MOLECULE BINDING VEGF AND ANG2 AND USE THEREOF

(54) 发明名称: 结合VEGF和Ang2的双特异性结合分子以及其用途

(57) **Abstract:** Provided are an antibody against a vascular endothelial growth factor (VEGF/VEGF-A) and angiopoietin-2 (ANG-2) respectively, a bispecific binding molecule (for example, an antibody) simultaneously against VEGF/VEGF-A and ANG-2, a preparation method therefor, and a pharmaceutical composition containing the antibody or molecule and an application thereof.

(57) 摘要: 提供分别针对血管内皮生长因子(VEGF/VEGF-A)和针对血管生成素-2(ANG-2)的抗体，同时针对血管内皮生长因子(VEGF/VEGF-A)和血管生成素-2(ANG-2)的双特异性结合分子(例如抗体)，以及其制备方法，包含所述抗体或分子的药物组合物，及其应用。

BISPECIFIC BINDING MOLECULE BINDING TO VEGF AND ANG2 AND USE THEREOF

The present invention relates to an antibody directed against a vascular endothelial growth factor (VEGF/VEGF-A) and angiopoietin-2 (ANG-2), respectively, a bispecific binding molecule (e.g., an antibody) directed against the vascular endothelial growth factor (VEGF/VEGF-A) and angiopoietin-2 (ANG-2) simultaneously, a preparation method therefor, a pharmaceutical composition comprising the antibody or molecule, and use thereof.

BACKGROUND

Angiogenesis is involved in the pathogenesis of various diseases including solid tumors, diseases associated with intraocular neovascularization, rheumatoid arthritis, and psoriasis.

VEGF is a potent and ubiquitous angiogenic factor. VEGF family members include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor (PIGF), and endocrine gland-derived VEGF (EG-VEGF). Active forms of VEGF are synthesized with other VEGF family members as homodimers or heterodimers. VEGF-A exists in the following six isotypes that are generated by alternative splicing: VEGF121, VEGF145, VEGF165, VEGF183, VEGF189, and VEGF206. These isotypes differ primarily due to their bioavailability, of which VEGF165 is the major isotype. It is believed that VEGF is an important regulator for normal and disease-related angiogenesis.

In addition to the VEGF family, human angiopoietins are also thought to be involved in vascular development and postnatal angiogenesis. The human angiopoietins include a naturally occurring agonist, angiopoietin-1 (ANG-1), and a naturally occurring antagonist, angiopoietin-2 (ANG-2). The role of ANG-1 is thought to be conserved in adults, where it is widely and constitutively expressed. In contrast, ANG-2 expression is primarily restricted to sites of vascular remodeling where it is thought to block the constitutive stabilizing or maturation functions of ANG-1, allowing vessels to return to and remain in a plastic state that may be more responsive to budding signals.

In recent years, some bispecific antibodies targeting VEGF-A and ANG-2 have been developed (e.g., WO2012131078 and WO2014009465). However, the existing bispecific antibodies have poor blocking ability against VEGF and Ang2, and have too large molecular weights, resulting in lower molar concentrations at a single administration. In particular, for ocular diseases, antibodies with smaller molecular weights applied through vitreous body are typically used and require less frequent administration. Thus, there is still a need for novel bispecific binding molecules targeting VEGF-A and ANG-2, particularly suitable for ocular diseases.

SUMMARY

The present invention develops a novel VHH antibody targeting VEGF-A or ANG-2, and a bispecific binding molecule directed against VEGF-A and ANG2 simultaneously. In particular, compared with known antibodies, the bispecific binding molecule of the present invention has a smaller molecular weight and higher molar concentration at the same mass concentration. Moreover, it has stronger VEGF A and Ang2 blocking activity, and can completely block VEGFA-induced proliferation of primary cells. Therefore, the molecule of the present invention has stronger blocking activity in clinic, can enable the molar concentration of an antibody to be higher at a single administration, maintain the efficacy of the single administration for a longer time, and reduce the frequency of ocular administration (such as intravitreal injection).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the structure of bispecific binding molecules.

FIG. 2 shows that anti-VEGF A VHH antibodies can block the binding of VEGF A to VEGFR2 as determined by ELISA.

FIG. 3 shows the effect of a humanized anti-Ang2 VHH antibody on blocking the binding of Ang2 to Tie2 as determined by ELISA.

FIG. 4 shows the effect of an anti-Ang2 VHH antibody (A) and the humanized anti-Ang2 VHH antibody (B) on inhibiting hAng2-Fc-induced phosphorylation of 293-Tie2 cells as determined by ELISA.

FIG. 5 shows the effect of anti-VEGF A VHH on blocking VEGFA activation of KDR receptors as detected by an HEK293-KDR reporter method.

FIG. 6 shows the effect of the anti-VEGF A VHH antibody on inhibiting VEGF A-induced survival and proliferation of HUVEC cells as determined by CCK-8.

FIG. 7 shows the effect of a VEGF A/Ang2 bispecific binding molecule IEX04-012 on blocking VEGF activation of KDR receptors as detected by the HEK293-KDR reporter method.

FIG. 8 shows the effect of bispecific binding molecules IEX04-008, IEX04-010, and IEX04-012 on inhibiting VEGF-induced survival and proliferation of HUVEC cells.

FIG. 9 shows that the bispecific binding molecules IEX04-008, IEX04-010, and IEX04-012 block the binding of human Ang2 to Tie2 as determined by ELISA.

FIG. 10 shows that the bispecific binding molecules IEX04-008, IEX04-010, and IEX04-012 block the binding of Ang2-Fc to Tie2 as determined by flow cytometry assay.

FIG. 11 shows that the bispecific binding molecule IEX04-012 of the present invention effectively inhibits hAng2-Fc-induced phosphorylation of 293-Tie2 *in vitro* as determined by flow cytometry assay.

FIG. 12 shows that the bispecific binding molecule IEX04-012 of the present invention reduces VEGF-induced vascular endothelial cell permeability, that is, inhibits VEGF-induced HUVEC cell leakage.

FIG. 13 shows laser spot ratio statistics at grade 4 (panel A) and grade 3 or higher (panel B) in a laser-induced choroidal neovascularization model.

FIG. 14 shows retinal thickness statistics in the laser-induced choroidal neovascularization model.

FIG. 15 shows leakage area statistics in the laser-induced choroidal neovascularization model.

FIG. 16 shows H&E staining patterns of fundus tissue (A) and lesion area statistics (B) in the laser-induced choroidal neovascularization model.

FIG. 17 shows CD31 staining patterns of fundus tissue (A) and positive cell statistics (B) in the laser-induced choroidal neovascularization model.

Definitions

Before the present invention is described in detail below, it should be understood that the present invention is not limited to the particular methodology, protocols, and reagents described herein, as these may vary. It should also be understood that the terminology used herein is only intended to describe specific embodiments rather than limit the scope of the present invention, which will be limited only by the appended claims. Unless otherwise defined, any technical and scientific term used herein has the same meaning as commonly understood by those of ordinary skill in the art to which the present invention belongs.

For the purpose of explaining this specification, the following definitions will be used, and wherever appropriate, terms used in the singular form may also include the plural form, and vice versa. It should be understood that the terms used herein are for the purpose of describing specific embodiments only, and are not intended to be limiting. The term "about" used in combination with a numerical value is intended to encompass the numerical values in a range from a lower limit less than the specified numerical value by 5% to an upper limit greater than the specified numerical value by 5%.

As used herein, the term "and/or" refers to any one of the options or any two or more or all of the options.

As used herein, the term "comprise" or "include" is intended to mean that the described elements, integers or steps are included, but not to the exclusion of any other elements, integers or steps. The term "comprise" or "include" used herein, unless indicated otherwise, also encompasses the situation where the entirety consists of the described elements, integers, or steps. For example, when referring to an antibody variable region "comprising" a specific sequence, it is also intended to encompass an antibody variable region consisting of the specific sequence.

The term "VEGF" as used herein refers to an angiogenic factor. VEGF family members include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor (PIGF), and endocrine gland-derived VEGF (EG-VEGF). Active forms of VEGF are synthesized with other VEGF family members as homodimers or heterodimers. VEGF-A exists in the following six isotypes that are generated by alternative splicing: VEGF121, VEGF145, VEGF165, VEGF183, VEGF189, and VEGF206. These isotypes differ primarily due to their bioavailability, of which VEGF165 is the major isotype. In some embodiments, VEGF A of the present invention refers to VEGF A from a human, e.g., VEGF 165 from a human. In one embodiment, the amino acid sequence of VEGFA of the present invention is an amino acid sequence under accession No. P15692 (uniprot database).

As used herein, the term "ANG2" refers to human angiopoietin-2 (ANG-2) (alternatively abbreviated as ANGPT2 or ANG2) which is described, for example, in Maisonpierre, P.C. et al., *Science*, 277 (1997) 55-60 and Cheung, A.H. et al., *Genomics*, 48 (1998) 389-91. Ang1 and Ang2 were discovered as ligands for tyrosine kinase family Tie selectively expressed in vascular endothelium. There are currently 4 defined members of the angiopoietin family. Angiopoietins-3 and -4 (ANG3 and ANG4) can represent a wide variety of counterparts of the same locus in mice and humans. Ang1 and Ang2 were originally identified in tissue culture experiments as an agonist and an antagonist, respectively (for ANG1, see Davis, S., et al., *Cell*, 87 (1996) 1161-69; for ANG2, see Maisonpierre, P.C., et al., *Science* 277 (1997) 55-60). All known angiopoietins mainly bind to Tie2. In some embodiments, ANG2 of the present invention refers to Ang2 from a human. In some embodiments, human Ang2 comprises an amino acid sequence under accession No. O15123 (uniprot database).

The term "multispecific binding molecule" refers to a multispecific binding molecule that is at least bispecific, such as a bispecific binding molecule, that is, the molecule comprises at least a first target-binding region and a second target-binding region, wherein the first target-binding region binds to one target or antigen, and the second target-binding region binds to another antigen or target. Accordingly, the molecule according to the present invention comprises specificities for at least two different antigens or targets. The molecule according to the present invention also encompasses a multispecific molecule comprising multiple target-binding regions/sites, such as a trispecific binding molecule. In some embodiments, the bispecific binding molecule of the present invention is a bispecific antibody.

The term "linker" as used herein refers to any molecule that enables direct connection of different portions of a bispecific binding molecule. Examples of linkers to establish covalent linkages between different portions of a molecule include peptide linkers and non-protein polymers including, but not limited to, polyethylene glycol (PEG), polypropylene glycol, polyalkylene oxide, or copolymers of polyethylene glycol and polypropylene glycol. In some embodiments, the linker is a peptide linker, which refers to an amino acid sequence that links the amino acid sequence of a first portion of a binding molecule to a second portion of the binding molecule. For example, the peptide linker may link a first target-binding region of a binding molecule to a second target-binding region. For example, the peptide linker may also link one portion of an antibody to another portion of the antibody, for example, a light chain variable region to a heavy chain variable region. Preferably, the peptide linker has a length sufficient to link two entities in a manner that maintains their conformation relative to each other without interference with the desired activities.

The peptide linker may or may not predominantly comprise the following amino acid residues: Gly, Ser, Ala or Thr. Useful linkers include glycine-serine polymers including, for example, (GS)n, (GSGGS)n, (GGGGS)n, (GGGS)n and (GGGGS)nG, wherein n is an integer of at least 1 (and preferably 2, 3, 4, 5, 6, 7, 8, 9, or 10). Useful linkers also include glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Useful linkers also include the polymerization of glycine monomers, such as (G)n, wherein n is an integer of at least 4 (and for example, from 4 to 20, such as 4, 5, 6, 7, 8, 9, 10 and more). Preferably, the linker is (GGGGS)n, wherein n = 1, 2, 3, or 4.

The term "valent" according to the present invention refers to the presence of a specified number of binding sites in a binding molecule, e.g., in an antibody molecule. Thus, the terms divalent, trivalent, and tetravalent indicate the presence of two, three, and four binding sites (target-binding regions), respectively, in a binding molecule. The bispecific binding molecule according to the present invention is at least bivalent and may be multivalent, e.g., bivalent, trivalent, tetravalent, or hexavalent.

The term "target-binding region" as used herein refers to any portion of a multispecific binding molecule, e.g., a bispecific binding molecule, that binds to a particular target or antigen. The target-binding region may be, for example, an antibody or immunoglobulin per se or an antibody fragment. Such target-binding regions may or may not have a tertiary structure independent of the remainder of BsAB, and may or may not bind to their targets as separate entities. The target-binding region may also be a receptor or a ligand, or a domain of a receptor capable of binding to a ligand.

The term "antibody fragment" includes a portion of an intact antibody. In a preferred embodiment, the antibody fragment is an antigen-binding fragment.

"Antigen-binding fragment" refers to a molecule different from an intact antibody, which comprises a portion of the intact antibody and binds to an antigen to which the intact antibody binds. Examples of the antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, $F(ab')_2$, a domain antibody (dAb), a linear antibody, a single-chain antibody (e.g., scFv), a single-domain antibody (e.g., VHH), a bi-valent antibody or a fragment thereof, or a camelid antibody.

"VHH", also referred to as single domain antibody (sdAb), refers to a genetically engineered antibody consisting of only the variable region of a heavy chain antibody, which contains only 3 HCDRs of the heavy chain variable region. VHH has specificity and high affinity for an antigen with only 3 HCDRs, whereas a common antibody requires 6

CDRs. The crystal structure indicates that VHH consists of 2 β-sheets forming a scaffold, which is similar to the traditional antibody VH immunoglobulin folding.

The term "target" refers to the bound substance against which the binding molecule is directed. The target may be an antigen, or may be a ligand or a receptor.

The term "antigen" refers to a molecule that induces an immune response. Such an immune response may involve antibody production or activation of specific immune cells, or both. Those skilled will understand that any macromolecules, including essentially all proteins or peptides, can be used as antigens. In addition, an antigen may be derived from recombinant or genomic DNA. As used herein, the term "epitope" refers to a moiety of an antigen (e.g., VEGF or Ang2) that specifically interacts with an antibody molecule.

"Complementarity determining region" or "CDR region" or "CDR" is a region in an antibody variable domain that is highly variable in sequence and forms a structurally defined loop ("hypervariable loop") and/or comprises antigen-contacting residues ("antigen contact sites"). CDRs are primarily responsible for binding to antigen epitopes. The CDRs of the heavy and light chains are generally referred to as CDR1, CDR2, and CDR3, and are numbered sequentially from the N-terminus. The CDRs located in the heavy chain variable domain of the antibody are referred to as HCDR1, HCDR2, and HCDR3, whereas the CDRs located in the light chain variable domain of the antibody are referred to as LCDR1, LCDR2, and LCDR3. In a given amino acid sequence of a light chain variable region or a heavy chain variable region, the exact amino acid sequence boundary of each CDR can be determined using any one or a combination of many well-known antibody CDR assignment systems including, e.g., Chothia based on the three-dimensional structure of antibodies and the topology of the CDR loops (Chothia *et al.* (1989) *Nature* 342: 877-883; Al-Lazikani *et al.*, *Standard conformations for the canonical structures of immunoglobulins*, *Journal of Molecular Biology*, 273: 927-948 (1997)), Kabat based on antibody sequence variability (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 4th edition, U.S. Department of Health and Human Services, National Institutes of Health (1987)), AbM (University of Bath), Contact (University College London), International ImMunoGeneTics database (IMGT) (imgt.cines.fr/ on the World Wide Web), and North CDR definition based on the affinity propagation clustering using a large number of crystal structures.

For example, according to different CDR determination schemes, the residues of each CDR are described as follows.

CDR	Kabat scheme	AbM scheme	Chothia scheme	Contact scheme
LCDR1	L24-L34	L24-L34	L26-L32	L30-L36
LCDR2	L50-L56	L50-L56	L50-L52	L46-L55
LCDR3	L89-L97	L89-L97	L91-L96	L89-L96
HCDR1	H31-H35B	H26-H35B	H26-H32	H30-H35B
(Kabat numbering system)				
HCDR1	H31-H35	H26-H35	H26-H32	H30-H35
(Chothia numbering system)				
HCDR2	H50-H65	H50-H58	H53-H55	H47-H58
HCDR3	H95-H102	H95-H102	H96-H101	H93-H101
(Kabat numbering system)				

CDRs can also be determined based on having the same Kabat numbering positions as a reference CDR sequence (*e.g.*, any of the exemplary CDRs of the present invention).

Unless otherwise stated, the term "CDR" or "CDR sequence" used herein encompasses CDR sequences determined by any one of the schemes described above.

Unless otherwise stated, residue positions of an antibody variable region (including heavy chain variable region residues and light chain variable region residues) are numbered according to the Kabat numbering system (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)).

In one embodiment, the CDRs in the VHH of the present invention are determined according to the following rules: HCDR1 is determined according to AbM, and HCDR2 and HCDR3 are determined according to Kabat.

It should be noted that boundaries of CDRs in variable regions of the same antibody determined by different assignment systems may differ. That is, CDR sequences of variable regions of the same antibody defined by different assignment systems differ. Therefore, when it comes to defining an antibody with specific CDR sequences defined in the present invention, the scope of antibody also encompasses such antibodies whose variable region sequences comprise the specific CDR sequences, but having claimed CDR boundaries different from the specific CDR boundaries defined by the present invention due to a different protocol (*e.g.*, different assignment system rules or their combinations) applied.

Antibodies with different specificities (*i.e.*, different binding sites for different antigens) have different CDRs (under the same assignment system). However, although CDRs vary from antibody to antibody, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. The smallest overlapping region can be determined using at least two of the Kabat, Chothia, AbM, Contact, and North methods, thereby providing a "minimal binding unit" for antigen binding. The minimal binding unit may be a sub-portion of the CDR. As will be clear to those skilled in the art, residues in the remaining portions of the CDR sequences can be determined by the structure and protein folding of the antibody. Therefore, variants of any CDR presented herein are also considered. For example, in a variant of one CDR, the amino acid residue of the smallest binding unit may remain unchanged, while the remaining CDR residues defined according to Kabat or Chothia may be replaced by conservative amino acid residues.

The term "Fc region" is used herein to define the constant regions of CH2 and CH3 of immunoglobulin heavy chains, and includes Fc regions of native sequences and variant Fc regions. The native or wild-type Fc region can bind to different Fc receptors on immune cell surface, thereby causing CDC\ADCC\ADCP effector functions. Such effector functions generally require that the Fc region is associated with a binding domain (e.g., an antibody variable region). In some embodiments, the Fc region is mutated to enhance its CDC\ADCC\ADCP effector functions. In some embodiments, the Fc region is mutated to attenuate or delete its CDC\ADCC\ADCP effector functions.

"Humanized" antibody refers to an antibody comprising amino acid residues from non-human CDRs and amino acid residues from human FRs. In some embodiments, a humanized antibody will comprise at least one, or generally two of substantially all variable domains in which all or substantially all CDRs (*e.g.*, CDRs) correspond to those of a non-human antibody, and all or substantially all FRs correspond to those of a human antibody. A humanized antibody may optionally comprise at least a portion of an antibody constant region derived from a human antibody. The "humanized form" of an antibody (such as a non-human antibody) refers to an antibody that has been

humanized. "Human antibody", "fully human antibody" and "fully humanized antibody" are used interchangeably, and refer to an antibody having an amino acid sequence which corresponds to the amino acid sequence of an antibody generated by a human or human cell or derived from a non-human source that utilizes human antibody libraries or other human antibody encoding sequences. This definition of a human antibody explicitly excludes humanized antibodies comprising non-human antigen-binding residues.

As used herein, the term "anti", "binding", or "specific binding" means that the binding effect is selective for targets or antigens and may be distinguished from unwanted or non-specific interactions. The ability of a binding site to bind to a particular target or antigen may be determined by enzyme-linked immunosorbent assay (ELISA) or conventional binding assays known in the art, such as radioimmunoassay (RIA), bio-layer interferometry, MSD assay, or surface plasmon resonance (SPR).

The term "effective amount" refers to an amount or dosage of the antibody, fragment, composition, or combination of the present invention which generates expected effects in a patient in need of treatment or prevention after administered to the patient in a single or multiple doses.

"Therapeutically effective amount" refers to an amount effective to achieve a desired therapeutic result at a necessary dose for a necessary period of time. The therapeutically effective amount is also such an amount that any toxic or undesired effect of the antibody, fragment thereof, composition, or combination is inferior to the therapeutically beneficial effect. The "therapeutically effective amount" preferably inhibits a measurable parameter or improves a measurable parameter by at least about 40%, and even more preferably by at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or even 100%, relative to untreated subjects.

"Prophylactically effective amount" refers to an amount effective to achieve a desired prophylactic result at a necessary dose for a necessary period of time. Generally, since a prophylactic dose is administered in a subject before or at an earlier stage of a disease, a prophylactically effective amount will be less than a therapeutically effective amount.

The terms "host cell", "host cell line" and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acids are introduced, including progenies of such cells. Host cells include "transformants" and "transformed cells", which include primary transformed cells and progenies derived therefrom, regardless of the number of passages. Progeny may not be exactly the same as parent cells in terms of nucleic acid content, and may comprise mutations. Mutant progenies having the same function or biological activities that are screened or selected from the initially transformed cells are included herein.

The term "label" used herein refers to a compound or composition which is directly or indirectly conjugated or fused to an agent, such as a polynucleotide probe or an antibody, and facilitates the detection of the agent to which it is conjugated or fused. The label itself can be detectable (e.g., a radioisotope label or a fluorescent label) or can catalyze a chemical change to a detectable substrate compound or composition in the case of enzymatic labeling. The term is intended to encompass direct labeling of a probe or an antibody by coupling (i.e., physical linking) a detectable substance to the probe or an antibody and indirect labeling of a probe or antibody by reacting with another reagent which is directly labeled. In some embodiments, the label is hFc or biotin.

"Individual" or "subject" includes mammals. The mammals include, but are not limited to, domestic animals (e.g., cattle, goats, cats, dogs, and horses), primates (e.g., human and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In some embodiments, the individual or subject is a human.

An "isolated" antibody or molecule is an antibody or molecule which has been separated from components of its natural environment. In some embodiments, the antibody or molecule is purified to a purity greater than 95% or 99% as determined by, e.g., electrophoresis (e.g., SDS-PAGE, isoelectric focusing (IEF), and capillary electrophoresis) or chromatography (e.g., ion exchange or reverse-phase HPLC).

The calculation of sequence identity between sequences is performed as follows.

To determine the percent identity of two amino acid sequences or two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., for optimal alignment, gaps can be introduced in one or both of the first and second amino acid sequences or nucleic acid sequences, or non-homologous sequences can be discarded for comparison). In one preferred embodiment, for comparison purposes, the length of the aligned reference sequence is at least 30%, preferably at least 40%, more preferably at least 50% or 60%, and even more preferably at least 70%, 80%, 90%, or 100% of the length of the reference sequence. Amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide at the corresponding position in the second sequence, the molecules are identical at this position.

A mathematical algorithm can be used to compare two sequences and calculate percent identity between the sequences. In one preferred embodiment, the percent identity between two amino acid sequences is determined with the Needlema and Wunsch algorithm ((1970) *J. Mol. Biol.*, 48: 444-453; available at http://www.gcg.com) which has been integrated into the GAP program of the GCG software package, using the Blossum 62 matrix or PAM250 matrix and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In another preferred embodiment, the percent identity between two nucleotide acid sequences is determined with the GAP program of the GCG software package (available at http://www.gcg.com), using the NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred parameter set (and one that should be used unless otherwise stated) is a Blossum 62 scoring matrix with a gap penalty of 12, a gap extension penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid sequences or nucleotide sequences can also be determined with a PAM120 weighted remainder table, a gap length penalty of 12, and a gap penalty of 4, using the E. Meyers and W. Miller algorithm ((1989) CABIOS, 4: 11-17) which has been incorporated into the ALIGN program (version 2.0). Additionally or alternatively, the nucleic acid sequences and protein sequences described herein can be further used as "query sequences" to perform searches against public databases to, e.g., identify other family member sequences or related sequences.

The "ocular disease" described herein encompasses ocular diseases (e.g., diseases occurring in the eye) involving angiogenesis, such as ocular diseases associated with corneal neovascularization.

The term "pharmaceutical supplementary material" refers to diluents, adjuvants (e.g., Freund's adjuvants (complete and incomplete)), excipients, carriers, stabilizers, or the like, which are administered with the active substance.

The term "pharmaceutical composition" refers to a composition that exists in a form allowing effective biological activity of the active ingredient contained therein, and does not contain additional ingredients having unacceptable toxicity to a subject to which the composition is administered.

As used herein, "treatment" (or "treat" or "treating") refers to slowing, interrupting, arresting, alleviating, stopping, lowering, or reversing the progression or severity of an existing symptom, disorder, condition, or disease.

As used herein, "prevention" (or "prevent" or "preventing") includes the inhibition of the development or progression of symptoms of a disease or disorder, or a specific disease or disorder.

The term "vector" used herein refers to a nucleic acid molecule capable of proliferating another nucleic acid to which it is linked. The term includes vectors that serve as self-replicating nucleic acid structures as well as vectors binding to the genome of a host cell into which they have been introduced. Some vectors are capable of directing the expression of a nucleic acid to which they are operably linked. Such vectors are referred to as "expression vectors" herein.

"Subject/patient/individual sample" refers to a collection of cells or fluids obtained from a patient or a subject. The source of tissue or cell samples can be solid tissues, e.g., from fresh, frozen and/or preserved organ or tissue samples or biopsy samples or puncture samples; blood or any blood component; body fluids such as tears, vitreous humors, cerebrospinal fluids, amniotic fluids, peritoneal fluids, or interstitial fluids; and cells from a subject at any time during pregnancy or development. In some embodiments, the tissue sample is an ocular tissue, such as vitreous body. In some embodiments, the sample is tear or vitreous humor. Tissue samples may comprise compounds which are naturally not mixed with tissues, such as preservatives, anticoagulants, buffers, fixatives, nutrients, and antibiotics.

DETAILED DESCRIPTION

I. Anti-VEGF A VHH antibody

The present invention relates to a VHH antibody directed against VEGF A. In some embodiments, the anti-VEGF VHH of the present invention comprises 3 CDRs, HCDR1, HCDR2, and HCDR3, as follows, wherein

the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 1;

the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 2; and

the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 3;

or

the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 6;

the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 7 or 10; and

the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 8.

In some embodiments, the VHH comprises or consists of an amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11.

In some embodiments, the VHH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.

In some preferred embodiments, the mutations are not present in the CDRs, such as HCDR1, HCDR2, or HCDR3.

II. Anti-Ang2 VHH antibody

The present invention relates to a VHH antibody directed against Ang2. In some embodiments, the anti-Ang2 VHH of the present invention comprises 3 CDRs, HCDR1, HCDR2, and HCDR3, as follows, wherein

the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 16;

the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 17 or 20; and
the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 18.
In some embodiments, the VHH comprises or consists of an amino acid sequence set forth in SEQ ID NO: 19 or 21, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 19 or 21.
In some embodiments, the VHH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 19 or 21, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.
In some preferred embodiments, the mutations are not present in the CDRs, such as HCDR1, HCDR2, or HCDR3.

III. Anti-VEGF A × ANG2 bispecific binding molecule

In one aspect of the present invention, the present invention relates to a bispecific binding molecule binding to VEGF A and Ang2, comprising a first target-binding region specifically binding to VEGF A and a second target-binding region specifically binding to Ang2, wherein the second target-binding region is anti-Ang2 VHH, e.g., the anti-Ang2 VHH of the present invention as described above.
In some embodiments, the first target-binding region is selected from:
a VHH specifically binding to VEGF A;
an antigen-binding fragment of an antibody, e.g., an scFv, specifically binding to VEGF A, for example, the antibody is a fully human or humanized antibody; or
a VEGF receptor (VEGF R) specifically binding to VEGF A or an extracellular domain thereof or a fusion protein comprising the extracellular domain thereof, e.g., a fusion protein of the extracellular domain with Fc.

In some embodiments, the bispecific binding molecule of the present invention comprises 1, 2, 3, or 4 first or second target-binding regions. In some embodiments, the bispecific binding molecule of the present invention comprises 2, 3, or 4 target-binding regions. In some embodiments, the bispecific binding molecule of the present invention is bivalent, trivalent, or tetravalent. In some embodiments, the bispecific binding molecule of the present invention is a bispecific antibody.
In one aspect of the present invention, the bispecific binding molecule of the present invention, such as a bispecific antibody, has the following structure:
light chain variable region VL of the anti-VEGF antibody-linker-heavy chain variable region VH of the anti-VEGF antibody-linker-anti-Ang2 VHH or
heavy chain variable region VH of the anti-VEGF antibody-linker-light chain variable region VL of the anti-VEGF antibody-linker-anti-Ang2 VHH;
wherein the anti-Ang2 VHH comprises HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 16; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 17 or 20; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 18.
In some embodiments, the structure of the bispecific binding molecule described above is shown in FIG. 1A or FIG. 1B. In some embodiments, the bispecific binding molecule described above consists of one chain. In some embodiments, the bispecific binding molecule described above is bivalent.

In some embodiments, the light chain variable region VL of the anti-VEGF antibody comprises LCDR1, LCDR2, and LCDR3, wherein the LCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 31; the LCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 32; and the LCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 33.

In some embodiments, the heavy chain variable region VH of the anti-VEGF antibody comprises HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 35; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 36; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 37.

In some embodiments, the heavy chain variable region VH of the anti-VEGF antibody of the present invention comprises or consists of an amino acid sequence set forth in SEQ ID NO: 34, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 34. In some embodiments, the heavy chain variable region VH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 34, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs, such as HCDR1, HCDR2, or HCDR3.

In some embodiments, the light chain variable region VL of the anti-VEGF antibody of the present invention comprises or consists of an amino acid sequence set forth in SEQ ID NO: 30, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 30. In some embodiments, the light chain variable region VL comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 30, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs, such as LCDR1, LCDR2, or LCDR3.

In some embodiments, the anti-Ang2 VHH of the present invention comprises or consists of an amino acid sequence set forth in SEQ ID NO: 19 or 21, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 19 or 21. In some embodiments, the VHH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 19 or 21, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs, such as HCDR1, HCDR2, or HCDR3.

In some embodiments, the linker comprises or consists of an amino acid sequence set forth in SEQ ID NO: 23. In some embodiments, the linker between the light chain variable region and the heavy chain variable region of the anti-VEGF antibody comprises or consists of an amino acid sequence set forth in SEQ ID NO: 23, wherein, for example, n = 4. In some embodiments, the linker between the anti-VEGF variable region and the anti-Ang2 VHH comprises or consists of an amino acid sequence set forth in SEQ ID NO: 23, wherein, for example, n = 2 or 3, such as 3.

In some embodiments, the anti-VEGF A × ANG2 bispecific binding molecule of the present invention comprises or consists of an amino acid sequence set forth in SEQ ID NO: 28, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 28. In some embodiments, the bispecific binding molecule comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 28, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the variable regions of the anti-VEGF antibody and the CDRs of the anti-Ang2 VHH.

In another aspect of the present invention, the bispecific binding molecule of the present invention, such as a bispecific antibody, has the following structure:

first anti-VEGF VHH-linker-second anti-VEGF VHH-linker-anti-Ang2 VHH,

wherein the anti-Ang2 VHH comprises HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 16; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 17 or 20; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 18.

In some embodiments, the structure of the bispecific binding molecule described above is shown in FIG. 1C. In some embodiments, the bispecific binding molecule described above consists of one chain. In some embodiments, the bispecific binding molecule described above is trivalent. In some embodiments, the first anti-VEGF VHH is the same as or different from the second anti-VEGF VHH.

In some embodiments, the anti-VEGF VHH comprises HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 1; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 2; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 3;

or

the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 6; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 7 or 10; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 8.

In some embodiments, the anti-VEGF VHH comprises or consists of an amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11. In some embodiments, the VHH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs, such as HCDR1, HCDR2, or HCDR3.

In some embodiments, the anti-VEGF VHH comprises HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 6; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 7 or 10; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 8.

In some embodiments, the anti-VEGF VHH comprises or consists of an amino acid sequence set forth in SEQ ID NO: 9 or 11, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 9 or 11. In some embodiments, the VHH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 9 or 11, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs, such as HCDR1, HCDR2, or HCDR3.

In some embodiments, the anti-Ang2 VHH of the present invention comprises or consists of an amino acid sequence set forth in SEQ ID NO: 19 or 21, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 19 or 21. In some embodiments, the VHH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 19 or 21, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs, such as HCDR1, HCDR2, or HCDR3.

In some embodiments, the linker comprises or consists of an amino acid sequence set forth in SEQ ID NO: 23. In some embodiments, the linker between the first anti-VEGF VHH and the second anti-VEGF VHH or the linker between the second anti-VEGF VHH and the anti-Ang2 VHH comprises or consists of an amino acid sequence set forth in SEQ ID NO: 23, for example, n = 2.

In some embodiments, the anti-VEGF A × ANG2 bispecific binding molecule of the present invention comprises or consists of an amino acid sequence set forth in SEQ ID NO: 22, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 22. In some embodiments, the bispecific binding molecule comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 22, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs of the anti-VEGF VHH and the anti-Ang2 VHH.

In another aspect of the present invention, the bispecific binding molecule of the present invention comprises one or two of the following chains:

VEGF R extracellular domain-Fc-linker-anti-Ang2 VHH,

wherein the anti-Ang2 VHH comprises HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 16; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 17 or 20; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 18.

In some embodiments, the structure of the bispecific binding molecule described above is shown in FIG. 1D. In some embodiments, the bispecific binding molecule described above consists of two chains. In some embodiments, the bispecific binding molecule described above is tetravalent.

In some embodiments, the anti-Ang2 VHH comprises or consists of an amino acid sequence set forth in SEQ ID NO: 19 or 21, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 19 or 21. In some embodiments, the VHH

comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 19 or 21, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs, such as HCDR1, HCDR2, or HCDR3.

In some embodiments, the VEGFR extracellular domain is an extracellular domain of VEGFR from a human. In some embodiments, the VEGFR extracellular domain comprises a second antibody-like domain of VEGFR1 (e.g., FLT1 domain 2) and a third antibody-like domain of VEGFR2 (e.g., KDR domain 3). In some embodiments, the VEGFR extracellular domain comprises a second antibody-like domain of human VEGFR1 and a third antibody-like domain of human VEGFR2. In some embodiments, the VEGFR extracellular domain comprises or consists of an amino acid sequence set forth in SEQ ID NO: 26, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 26. In some embodiments, the VEGFR extracellular domain comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 26, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements; preferably, the VEGFR extracellular domain retains a binding affinity for VEGF similar to (e.g., having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% similarity to) that of a domain set forth in SEQ ID NO: 26.

In some embodiments, the Fc is an Fc derived from human IgG1, IgG2, IgG3, or IgG4, such as a wild-type Fc, or an Fc variant known in the art. In some embodiments, the Fc comprises or consists of an amino acid sequence set forth in SEQ ID NO: 27, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 27.

In some embodiments, the VEGF R extracellular domain-Fc is a fusion protein of the VEGFR extracellular domain and the Fc, such as aflibercept or a derivative thereof.

In some embodiments, the VEGF R extracellular domain-Fc comprises or consists of an amino acid sequence set forth in SEQ ID NO: 25, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 25. In some embodiments, the VEGF R extracellular domain-Fc comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 25, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements; preferably, the VEGF R extracellular domain-Fc retains a binding affinity for VEGF similar to (e.g., having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% similarity to) that of a domain set forth in SEQ ID NO: 25.

In some embodiments, the linker comprises or consists of an amino acid sequence set forth in SEQ ID NO: 23, for example, n = 3.

In some embodiments, one chain of the anti-VEGF A × ANG2 bispecific binding molecule of the present invention comprises or consists of an amino acid sequence set forth in SEQ ID NO: 24, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 24. In some embodiments, the bispecific binding molecule comprises an amino acid sequence

having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 24, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements. In some preferred embodiments, the mutations are not present in the CDRs of the anti-Ang2 VHH. In some embodiments, the VEGF R extracellular domain-Fc having a mutation retains a binding affinity for VEGF similar to (e.g., having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% similarity to) that of a domain set forth in SEQ ID NO: 25.

In one embodiment of the present invention, the antibody or binding molecule described herein comprises one or more amino acid mutations. In some embodiments, the amino acid mutations include amino acid replacements, insertions, or deletions. Preferably, the amino acid change described herein is an amino acid replacement, preferably a conservative replacement.

In a preferred embodiment, the amino acid mutations described herein occur in a region outside the CDRs (e.g., in FRs). In some embodiments, the amino acid mutations described herein occur in the antibody heavy chain constant region, such as an Fc region. In a preferred embodiment, the amino acid mutations in the Fc region attenuate or delete the ADCC and/or CDC effect of the antibody.

In some embodiments, the replacement is a conservative replacement. A conservative replacement refers to the replacement of an amino acid by another amino acid of the same class, e.g., the replacement of an acidic amino acid by another acidic amino acid, the replacement of a basic amino acid by another basic amino acid, or the replacement of a neutral amino acid by another neutral amino acid.

In certain embodiments, one or more amino acid mutations may be introduced into the Fc region of the antibody provided herein, thereby producing an Fc region variant to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, complement-dependent cytotoxicity, Fc receptor binding, and/or antibody-dependent cell-mediated cytotoxicity. The Fc region variant may comprise a human Fc region sequence (such as human IgG1, IgG2, IgG3, or IgG4 Fc region) comprising an amino acid mutation (such as replacement) at one or more amino acid positions.

In certain embodiments, the variable region of the antibody may need to be mutated to produce a disulfide bond, for example, to produce an scFv comprising a disulfide bond mutation.

In certain embodiments, the antibody or binding molecule provided herein can be further modified to comprise other non-proteinaceous portions known in the art and readily available. Suitable portions for derivatization include, but are not limited to, water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited to, polyethylene glycol (PEG), ethylene glycol/propylene glycol copolymer, carboxymethyl cellulose, glucan, polyvinyl alcohol, polyvinylpyrrolidone, poly-1,3-dioxane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyamino acid (homopolymer or random copolymer), and glucan or poly(n-vinylpyrrolidone), polyethylene glycol, propylene glycol homopolymer, polypropylene oxide/ethylene oxide copolymer, polyoxyethylated polyol (such as glycerol), polyvinyl alcohol, and mixtures thereof.

IV. Properties of VHH antibody or bispecific binding molecule of the present invention

In some embodiments, the anti-Ang2 VHH antibody of the present invention can specifically bind to Ang2, such as human Ang2, e.g., with high affinity.

In some embodiments, the anti-VEGFA VHH antibody of the present invention can specifically bind to VEGF A, such as human VEGF A, e.g., with high affinity.

In some embodiments, the bispecific binding molecule of the present invention can specifically bind to Ang2 and VEGFA, such as human Ang2 and human VEGFA, e.g., with high affinity.

In some embodiments, the anti-Ang2 antibody or the anti-VEGFA antibody or the bispecific binding molecule of the present invention has one or more of the following properties:

(i) the anti-Ang2 antibody or bispecific binding molecule has an inhibitory effect on Ang2-induced Tie2 phosphorylation;

(ii) the anti-Ang2 antibody or bispecific binding molecule has a blocking effect on the binding between Ang2 and Tie2;

(iii) the anti-VEGFA antibody or bispecific binding molecule has a blocking effect on VEGFA activation-related receptor signaling pathways, e.g., as detected by KDR reporters;

(iv) the anti-VEGFA antibody or bispecific binding molecule has a blocking effect on the binding between VEGFA and VEGFR;

(v) the anti-VEGFA antibody or bispecific binding molecule has an inhibitory effect on VEGF A-induced survival and proliferation of cells (e.g., primary cells, e.g., vascular endothelial cells, e.g., human umbilical vein endothelial cells, e.g., HUVECs);

(vi) the bispecific binding molecule has an inhibitory effect on the leakage of vascular endothelial cells (e.g., human umbilical vein endothelial cells, e.g., HUVECs); and

(vii) the bispecific binding molecule has an inhibitory effect on neovascularization *in vivo* or *in vitro*, e.g., inhibiting fundus or retinochoroidal neovascularization, e.g., inhibiting the leakage caused by the neovascularization, e.g., protecting vascular integrity.

V. Nucleic Acid of the present invention and host cell comprising same

In one aspect, the present invention provides a nucleic acid encoding any of the above VHHs or bispecific binding molecules or any one of the chains thereof. In one embodiment, provided is a vector comprising the nucleic acid. In one embodiment, the vector is an expression vector, e.g., a eukaryotic expression vector. The vector includes, but is not limited to, a virus, a plasmid, a cosmid, a λ phage, or a yeast artificial chromosome (YAC). In one embodiment, the vector is, for example, pcDNA3.1. In one embodiment, provided is a host cell comprising the nucleic acid or the vector. In one embodiment, the host cell is eukaryotic. In another embodiment, the host cell is selected from yeast cells, mammalian cells (e.g., CHO cells (e.g., CHO-S) or 293 cells (e.g., 293F or HEK293 cells)), or other cells suitable for preparing an antibody or a fragment thereof. In another embodiment, the host cell is prokaryotic, e.g., *E. coli*, e.g., TG1.

For example, the nucleic acid of the present invention includes a nucleic acid encoding an amino acid sequence selected from any one of SEQ ID NOs: 4, 5, 9, 11, 19, 21, 22, 24, and 28, or a nucleic acid encoding an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence selected from any one of SEQ ID NOs: 4, 5, 9, 11, 19, 21, 22, 24, and 28.

In one embodiment, provided is a host cell comprising the vector. The suitable host cell for cloning or expressing the vector encoding the VHH or bispecific binding molecule includes prokaryotic cells or eukaryotic cells described

herein. For example, the VHH may be produced in bacteria. After expression, the VHH is present in the culture supernatant and may be further purified.

In one embodiment, the host cell is prokaryotic, e.g., a bacterium, e.g., *E.coli*, e.g., TG1.

In one embodiment, the host cell is eukaryotic. In another embodiment, the host cell is selected from yeast cells, mammalian cells, and additional cells suitable for producing an antibody or a fragment thereof. For example, eukaryotic microorganisms, such as filamentous fungi or yeast, are suitable cloning or expression hosts for the vector encoding the antibody. For example, fungus and yeast strains in which a glycosylation pathway has been "humanized" produce antibodies having a partial or full human glycosylation pattern. Host cells suitable for expressing a glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Vertebrate cells may also be used as hosts. For example, a mammalian cell line engineered to be suitable for suspension growth may be used. Other examples of useful mammalian host cell lines are monkey kidney CV1 line (COS-7) transformed with SV40, human embryonic kidney line (HEK293, 293F, or 293T cells), and the like. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells, CHO-S cells, ExpiCHO, and the like; and myeloma cell lines such as Y0, NS0, and Sp2/0. Mammalian host cell lines suitable for producing antibodies are known in the art.

VI. Production and purification of VHH or bispecific binding molecule of the present invention

In one embodiment, provided is a method for preparing the VHH or bispecific binding molecule of the present invention, wherein the method comprises culturing the host cell comprising a nucleic acid encoding the VHH or bispecific binding molecule (e.g., any one and/or more polypeptide chains) or an expression vector comprising the nucleic acid, as provided above, under conditions suitable for expressing the VHH or bispecific binding molecule or the chain thereof, and optionally recovering the VHH or bispecific binding molecule from the host cell (or the host cell medium).

For recombinant production of the molecule of the present invention, a nucleic acid encoding the VHH or bispecific binding molecule (e.g., the molecule described above, e.g., any one and/or more polypeptide chains) is isolated and inserted into one or more vectors for further cloning and/or expressing in the host cells. Such nucleic acids are readily isolated and sequenced using conventional procedures.

The VHH or bispecific binding molecule prepared as described herein can be purified by known prior art such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, and size exclusion chromatography. The actual conditions used to purify a particular protein also depend on factors such as net charge, hydrophobicity and hydrophilicity, and these will be apparent to those skilled in the art. The purity of the antibody molecule of the present invention can be determined by any one of a variety of well-known analytical methods including size exclusion chromatography, gel electrophoresis, high performance liquid chromatography, and the like.

VII. Assays

The VHH or bispecific binding molecule provided herein can be identified, screened, or characterized for physical/chemical properties and/or biological activities through a variety of assays known in the art. In one aspect, the VHH or bispecific binding molecule of the present invention is tested for the target- (e.g., antigen-) binding activity, for example, by known methods such as bio-layer interferometry, ELISA, and the like. The binding to VEGF A and/or Ang2 can be determined by methods known in the art, and exemplary methods are disclosed herein.

In some embodiments, the binding is determined by radioimmunoassay (RIA), bio-layer interferometry, MSD assay, surface plasmon resonance (SPR), or flow cytometry.

The present invention further provides an assay for identifying a VHH or a bispecific binding molecule having biological activity. The biological activity is selected from, for example

(i) an inhibitory effect of the anti-Ang2 antibody or bispecific binding molecule on Ang2-induced Tie2 phosphorylation;

(ii) a blocking effect of the anti-Ang2 antibody or bispecific binding molecule on the binding between Ang2 and Tie2;

(iii) a blocking effect of the anti-VEGFA antibody or bispecific binding molecule on VEGFA activation-related receptor signaling pathways, e.g., as detected by KDR reporters;

(v) a blocking effect of the anti-VEGFA antibody or bispecific binding molecule on the binding between VEGFA and VEGFR;

(iv) an inhibitory effect of the anti-VEGFA antibody or bispecific binding molecule on VEGF A-induced survival and proliferation of cells (e.g., primary cells, e.g., vascular endothelial cells, e.g., human umbilical vein endothelial cells, e.g., HUVECs);

(vi) an inhibitory effect of the bispecific binding molecule on the leakage of vascular endothelial cells (e.g., human umbilical vein endothelial cells, e.g., HUVECs); and

(vii) an inhibitory effect of the bispecific binding molecule on neovascularization *in vivo* or *in vitro*, e.g., inhibiting fundus or retinochoroidal neovascularization, e.g., inhibiting the leakage caused by the neovascularization, e.g., protecting vascular integrity.

The cells for use in any of the *in vitro* assays described above are primary cells or cell lines, including cells naturally expressing or overexpressing Ang2 receptor (e.g., Tie2) or VEGFR (e.g., VEGFR2, i.e., KDR), such as 293 cells overexpressing Tie2 or KDR, e.g., HEK293 or Expi293, or vascular endothelial cells, such as human umbilical vein endothelial cells, e.g., HUVECs.

In some embodiments, the assay can be performed using a marker such as biotin or hFc.

It will be appreciated that any of the above assays can be performed using a combination of the antibody of the present invention and other active agents.

VIII. Immunoconjugate and pharmaceutical composition

In some embodiments, the present invention provides an immunoconjugate comprising any of the VHHs or bispecific binding molecules described herein. Preferably, the immunoconjugate comprises one or more additional therapeutic agents or markers.

In some embodiments, the present invention provides a composition or medicament or formulation comprising any of the VHHs or bispecific binding molecules described herein; preferably, the composition is a pharmaceutical composition. In one embodiment, the composition further comprises a pharmaceutical supplementary material. In one embodiment, the composition, e.g., the pharmaceutical composition, comprises the VHH or bispecific binding molecule of the present invention, and a combination of one or more additional therapeutic agents.

The present invention further comprises a composition (including a pharmaceutical composition) or medicament or formulation comprising the VHH or bispecific binding molecule of the present invention. Such compositions or

medicaments or formulations can further comprise a suitable pharmaceutical supplementary material, such as a pharmaceutical carrier and a pharmaceutical excipient known in the art, including buffers.

As used herein, the "pharmaceutical carrier" includes any and all solvents, dispersion media, isotonic agents and absorption delaying agents, and the like that are physiologically compatible.

For use and application of pharmaceutical supplementary materials, see *Handbook of Pharmaceutical Excipients*, 8th Ed., R. C. Rowe, P. J. Seskey and S. C. Owen, Pharmaceutical Press, London, Chicago.

The composition or medicament or formulation of the present invention may be in a variety of forms. These forms include, for example, liquid, semi-solid, and solid dosage forms, such as liquid solutions (e.g., injectable solutions and eye drops), pulvis or suspensions, liposomes, and suppositories. The preferred form depends on the intended mode of administration and therapeutic use. For example, the composition or medicament or formulation of the present invention may be eye drops.

The medicament or formulation, preferably in the form of a lyophilized formulation or an aqueous solution, comprising the VHH or bispecific binding molecule described herein can be prepared by mixing the VHH or bispecific binding molecule of desired purity of the present invention with one or more optional pharmaceutical supplementary materials.

The composition or medicament or formulation of the present invention may further comprise more than one active ingredient required by a particular indication treated, preferably those having complementarity activity without adversely affecting one another. For example, it is desirable to further provide additional therapeutic agents.

A sustained release formulation can be prepared. Suitable examples of the sustained release formulation include a semipermeable matrix of a solid hydrophobic polymer containing an antibody. The matrix is in the form of a shaped article, such as a film or a microcapsule.

The present invention further provides a pharmaceutical combination or a pharmaceutical combination product comprising the VHH or bispecific binding molecule of the present invention, and one or more additional therapeutic agents. The present invention further provides a kit of parts comprising the pharmaceutical combination, wherein, for example, the kit of parts comprises in the same package:

- a first container containing a pharmaceutical composition comprising the VHH or bispecific binding molecule of the present invention; and
- a second container containing a pharmaceutical composition comprising additional therapeutic agents.

IX. Use and method

In one aspect, the present invention provides a method for preventing or treating an ocular disease in a subject, which comprises administering to the subject an effective amount of the anti-VEGF VHH of the present invention, the anti-Ang2 VHH of the present invention or the bispecific binding molecule of the present invention, or the composition or medicament or formulation comprising same.

In some embodiments, the patient has VEGF, e.g., VEGF A, and/or Ang2 (e.g., at an elevated level, e.g., at a nucleic acid or protein level).

In some embodiments, the ocular disease includes, but is not limited to, ocular diseases associated with angiogenesis, such as ocular diseases associated with corneal neovascularization.

In some embodiments, the ocular disease treatment will benefit from inhibition of VEGF, e.g., VEGF A, and/or Ang2, at a nucleic acid or protein level.

In other aspects, the present invention provides use of the anti-VEGF VHH of the present invention, the anti-Ang2 VHH of the present invention or the bispecific binding molecule of the present invention, or the composition comprising same in the manufacture or preparation of a medicament for the use described herein, e.g., for use in preventing or treating the related diseases or disorders mentioned herein.

In some embodiments, the anti-VEGF VHH of the present invention, the anti-Ang2 VHH of the present invention or the bispecific binding molecule of the present invention, or the composition or medicament or formulation comprising same delays the onset of a disorder and/or a symptom associated with the disorder.

In some embodiments, the anti-VEGF VHH of the present invention, the anti-Ang2 VHH of the present invention or the bispecific binding molecule of the present invention, or the composition or medicament or formulation comprising same can also be administered in combination with one or more other therapies, e.g., therapeutic modalities and/or other therapeutic agents, for the use described herein, e.g., for use in preventing and/or treating the related diseases or disorders mentioned herein.

The route of administration of the anti-VEGF VHH of the present invention, the anti-Ang2 VHH of the present invention or the bispecific binding molecule of the present invention, or the composition or medicament or formulation comprising same is according to known methods, e.g., topical administration, such as intraocular administration or ocular surface administration. In some embodiments, the administration is performed by injection or instillation.

X. Methods and compositions for diagnosis and detection

In certain embodiments, the anti-Ang2 VHH antibody provided herein can be used to detect the presence of Ang2 in a biological sample. In certain embodiments, the anti-VEGFA VHH antibody provided herein can be used to detect the presence of VEGFA in a biological sample. In certain embodiments, the anti-bispecific binding molecule provided herein can be used to detect the presence of Ang2 and/or VEGFA in a biological sample.

The term "detection" used herein includes quantitative and qualitative detections, and exemplary detections may involve immunohistochemistry, immunocytochemistry, flow cytometry (e.g., FACS), magnetic beads complexed with antibody molecules, ELISA, and PCR techniques (e.g., RT-PCR). In certain embodiments, the biological sample is a bodily fluid or an ocular tissue, such as vitreous humor.

In certain embodiments, the method comprises contacting a biological sample with the VHH or bispecific binding molecule described herein under conditions that allow the binding of the VHH or bispecific binding molecule to Ang2 or VEGFA, and detecting the formation of a complex between the VHH or bispecific binding molecule and Ang2 or VEGFA. The formation of the complex indicates the presence of Ang2 or VEGFA. The method may be an *in vitro* or *in vivo* method. In one embodiment, the antibodies of the present invention are used to select a subject suitable for treatment with the VHH or bispecific binding molecule of the present invention, for example, wherein Ang2 or VEGFA is a biomarker for selecting the subject.

In certain embodiments, provided is a labeled VHH or bispecific binding molecule. The label includes, but is not limited to, a label or moiety that is detected directly, e.g., a fluorescent label, a chromophoric label, an electron-dense label, a chemiluminescent label, and a radioactive label, and a moiety that is detected indirectly, such as an

enzyme or a ligand, for example, by enzymatic reaction or molecular interaction. In some embodiments, the label is a marker such as biotin or hFc.

In some embodiments provided herein, the sample is obtained prior to treatment with the VHH or bispecific binding molecule of the present invention. In some embodiments, the sample is obtained prior to application of other therapies. In some embodiments, the sample is obtained during treatment with other therapies, or after treatment with other therapies.

In some embodiments, Ang2 and/or VEGF A is detected prior to treatment, e.g., prior to initial treatment or prior to a treatment after an interval from a certain treatment.

In some embodiments, provided is a method for treating the disease of the present invention, and the method comprises: detecting the presence of Ang2 and/or VEGF A in a subject (e.g., a sample) (e.g., a sample of the subject), thereby determining an Ang2 and/or VEGF A value; comparing the Ang2 and/or VEGF A value to a control value (e.g., a value in a normal individual); and if the Ang2 and/or VEGF A value is greater than the control value, administering to the subject a therapeutically effective amount of the VHH or bispecific binding molecule described herein optionally, in combination with one or more other therapies, thereby treating the disease.

These and other aspects and embodiments of the present invention are illustrated in the drawings (brief description of the drawings follows) and in the following detailed description of the present invention and are described in the following examples. Any or all of the features described above and throughout the present invention may be combined in various embodiments of the present invention. The following examples further illustrate the present invention. However, it should be understood that the examples are described by way of illustration rather than limitation, and various modifications may be made by those skilled in the art.

Example 1. Preparation of Phage Immune Library

Construction of alpaca immune or synthetic library

1.1 Two healthy adult alpacas (Chengdu NBbiolab, Co. Ltd.) were selected, 0.5 mg of recombinant protein antigen VEGFA or Ang2 (Beijing Sino Biological) and Freund's adjuvant were mixed homogeneously according to a ratio of 1:1, and the alpacas were immunized by subcutaneous injection at multiple sites on the back for four times with an immune interval of 2 weeks.

1.2 Alpaca peripheral blood (50 mL) was collected, lymphocytes were isolated, 1 mL of Trizol reagent was added per 2.5×10^7 viable cells, and the total RNA was extracted using a chloroform/isopropanol precipitation method. Reverse transcription was performed using PrimeScript reverse transcription kit (Takara) by taking 10 μg of RNA as a template. The first round of PCR reaction was performed by taking cDNA as a template and using a forward primer Alp-VhL and a reverse primer Alp-2b/2cR to obtain products from the first round of PCR. The second round of PCR reaction was performed by taking the product from the first round of PCR as a template and using a forward primer Alp-VhF and a reverse primer Alp-JHR-SalI to obtain products from the second round of PCR. The pC3-HF vector and the product from the second round of PCR were subjected to double digestion using SacI and SalI (Thermo), respectively, the digested products were reacted with T4 ligase (Thermo), and TG1 competent cells were electrotransformed to construct a VHH antibody library. The bacterial liquid was cryopreserved at -80 °C.

The thawed bacterial liquid was inoculated into 100 mL of YT-AG medium (Shanghai Sangon Biotech), an M13KO7 helper phage was added for infection, and the bacterial cell pellet was resuspended with 2×YT-AK

medium (Shanghai Sangon Biotech) and cultured at 37 °C and at 200 rpm overnight. The culture supernatant was collected, and a recombinant phage was prepared using a PEG/NaCl precipitation method.

1.3 The recombinant phage was subjected to 3 rounds of panning experiments using the biotin-labeled antigen VEGFA (ACRO) or Ang2 (Beijing Sino Biological). 50 μL of M280 magnetic beads (Thermo) and an appropriate amount of biotin-labeled antigens were added to each tube, and the mixture was incubated at room temperature for 30 min. 1×10^{12} cfu of recombinant phage was added, and the mixture was incubated at room temperature for 1 h. The resulting mixture was washed 10 times with 1 mL of PBST, each time for 5 min. Finally, 0.5 mL of glycine buffer with pH of 2.5 was added, the antigen-bound recombinant phage was eluted, TG1 was infected, and the cells were cultured overnight. The recombinant phage was prepared for the next round of panning experiment, and TG1 bacterial clones with positive VHHs were identified.

1.4 The binding activity was detected by Binding ELISA and the clones were sequenced.

VEGF A and Ang2 antigens (Beijing Sino Biological) were taken in advance and diluted to 0.5 μg/mL with PBS buffer to coat a 96-well ELISA plate, and the plate was stored in a refrigerator at 4 °C overnight. The antigen-coated plate was washed 3 times with PBST, a blocking reagent was added to 300 μL/well, and the plate was left to stand at room temperature for 1 h. The plate was washed 3 times with PBST, 80 μL of blocking reagent and 20 μL of expression supernatant of the TG1 strain with positive VHHs identified in step 1.3 described above were added, and the plate was shaken at room temperature for 1 h.

The plate was washed 3 times with PBST, 100 μL of Anti-Flag/HRP secondary antibody (Sigma) diluted with the blocking reagent was added per well, and the plate was shaken at room temperature for 40 min. The plate was washed 6 times with PBST, TMB chromogenic solution was added to 100 μL/well, and the color development was performed in the dark for 5-15 min. Then 100 μL/well of stop solution was added. The plate was read on a microplate reader, the OD450 nm absorbance values were measured, and bacterial clones with a reading value more than 0.5 were selected and sent to Genewiz for sequencing. A single clone of TG1 strain containing each corresponding VHH sequence was selected, and glycerol was added. The mixture was cryopreserved in a refrigerator at -80 °C.

Example 2. Production, Purification and Humanization of Prokaryotic Antibody

According to the present invention, antibody sequences in anti-VEGFA or Ang2 positive phages were obtained by using molecular biology techniques, and the obtained TG1 single clones containing positive VHHs were used to be expressed and purified to obtain VHH antibody proteins.

The TG1 strain containing VHH expression plasmids identified in Example 1 was inoculated into 800 mL of LB-Amp medium and cultured at 37 °C and at 200 rpm until the OD600 value was 0.5-0.6. 1 mM IPTG was added into the bacterial liquid to induce expression, and the strain was cultured overnight at 28 °C and at 200 rpm. The culture supernatant was collected. After centrifugation, 15 mL of PB and 1 mg/mL of polymyxin were added to resuspend the bacteria, and the mixed solution was centrifuged again and filtered through a 0.22 μm filter membrane. The bacterial lysate was passed through a 1 mL Ni Sepharose pre-column, and the column was washed 2 times with PBS. The target protein was eluted with 0.5 M imidazole, and the protein concentration was measured by an ultraviolet method. The protein concentration of the eluted target protein was measured by the ultraviolet method, and the eluted target protein was aliquoted into multiple tubes and stored in a refrigerator at -40 °C. The obtained antibody solution was subsequently referred to as a supernatant.

The amino acid sequences of CDRs and VHHs of 2 anti-VEGF A VHH antibodies (LA42F8 and LA46E11) and 1 anti-Ang2 VHH antibody (LA24C11) obtained in the present invention, as well as sequence numbers are shown in the sequence listing.

Then, the immune library antibodies LA42F8, LA46E11, and LA24C11 obtained above were humanized. The steps are as follows:

(1) determining a CDR loop structure;

(2) searching a human germline sequence database for the closest homologous sequences for each V/J region of the heavy chain;

(3) screening the human germlines for the highest match in heavy and light chains and a minimum quantity of back mutations;

(4) constructing the CDRs of the chimeric antibody onto the framework of a human antibody;

(5) determining the positions of amino acids that maintained the CDR functions in the framework based on the sequences and structural features;

(6) adding back mutations (back to the input amino acids) at important positions identified; and

(7) optimizing amino acids at risk sites.

The amino acid sequences of CDRs and heavy chain variable regions of 3 humanized antibodies obtained in the present invention, namely humanized VHH antibodies LA42F8.5, LA46E11.8, and LA24C11.10 are shown in the attached sequence listing.

The expression and preparation of LA42F8, LA46E11, LA24C11, and humanized antibodies LA42F8.5, LA46E11.8, and LA24C11.10 described above in eukaryotic cells were as follows:

The humanized antibody sequences obtained above were cloned into pcDNA3.1 (Invitrogen), and plasmids containing the antibody sequences were obtained, respectively.

Expi-293 cells (Invitrogen) were passaged according to a desired transfection volume. The cell density was adjusted to 1.5×10^6 cells/mL the day before transfection. The cell density on the day of transfection was approximately 3×10^6 cells/mL. 1/10 of the final volume of F17 medium (Gibco, A13835-01) was taken as a transfection buffer. An appropriate amount of plasmids was added, and the mixture was mixed homogeneously. An appropriate amount of polyethylenimine (PEI) (Polysciences, 23966) was added to the plasmids (the ratio of plasmids to PEI was 1:3 in the 293F cells), mixed and incubated at room temperature for 10 min, resulting in a DNA/PEI mixture. After being resuspended with the DNA/PEI mixture, the cells were introduced at 36.5 °C, 8% CO_2. After 24 h, the cells were supplemented with FEED (Sigma) with 2% of the transfection volume, and were incubated at 36.5 °C, 8% CO_2 at 120 rpm. On Day 6 days of subculture or until a viability fell below 60%, the cell supernatant was collected and purified.

The gravity column for purification was treated with 0.5 M NaOH overnight. Glass containers were washed with distilled water and dried at 180 °C for 4 h, and a purification column was obtained. Before purification, the cell supernatant collected above was centrifuged at 4500 rpm for 30 min, and the cells were discarded. The supernatant was filtered through a 0.22 μL filter. Protein A column (Hitrap Mabselect Sure 5 × 5 mL, GE, 11-0034-95) was equilibrated with 10 mL of binding buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.0). The filtered supernatant was loaded to the purification column, which was then re-equilibrated with 15 mL of binding buffer. 5 mL of eluent buffer (citric acid + sodium citrate 0.1 M, pH 3.5) was added. The eluate was collected, and 80 μL of

Tris-HCl was added per mL of eluate. The buffer of the collected antibodies was changed into PBS (Gibco, 70011-044) by ultrafiltration/diafiltration, and the concentrations were measured. Except for the antibodies of the present invention used for detection in Table 3, FIG. 2, FIG. 4A, and FIG. 5A, the antibodies used in the examples were all expressed and purified using these methods unless the supernatant was specifically mentioned subsequently.

Similarly, the coding nucleic acids of negative control IgG, positive control BI-anti-VEGF, BI836880, and Faricimab (sequences are shown in sequence listing) were cloned into pcDNA3.1, transfected into Expi-293 cells, expressed, and purified, and the methods were same as those in Example 2.

Example 3. Binding Kinetics of Chimeric Antibodies of the Present Invention for Antigens as Determined by Bio-Layer Interferometry

The equilibrium dissociation constant (KD) for binding of the antibodies of the present invention to human Ang2 was determined by bio-layer interferometry (ForteBio). A ForteBio affinity assay of prior art was performed (Estep, P., et al., High throughput solution Based measurement of antibody-antigen affinity and epitope binning, *MAbs*, 2013.5(2): 270-8).

Half an hour before the experiment, an appropriate number of AMQ (Pall, 1506091) (for sample detection) or AHQ (Pall, 1502051) (for positive control detection) sensors depending on the number of samples were soaked in SD buffer (PBS 1×, BSA 0.1%, Tween-20 0.05%).

100 μL of SD buffer, VHH antibodies prepared above in Example 2, and antigens [including human Ang2 (Beijing Sino Biological) and human VEGF165 (R&D)] were added to a 96-well black polystyrene half-area microplate (Greiner, 675076), respectively. The sensors were arranged according to the positions of the samples. The instrument settings were as follows: the operation procedures were Baseline, Loading -1 nm, Baseline, Association, and Dissociation; the run time of each procedure was dependent on the rates of association and dissociation; the rotation speed was 400 rpm, and the temperature was 30 °C. The K_D values were analyzed by ForteBio analysis software.

In the experiments performed by the assays described above, the affinities of the antibodies are shown in Table 1:

Table 1. Affinity constants (equilibrium dissociation constants) for monovalent bindings of antigens and antibodies by ForteBio assay

Antibody	Antigen	K_D (M)	K_{on} (1/Ms)	k_{dis} (1/s)
LA42F8.5	VEGF 165	ND	ND	ND
LA42F8		6.11E-09	1.89E+05	1.16E-03
LA46E11.8		ND	ND	ND
LA46E11		7.741E-09	1.84E+05	1.42E-03
LA24C11	Ang2	2.18E-09	7.61E+05	1.66E-03
LA24C11.10		ND	ND	ND

ND denotes not detected

Table 2. Affinity constants (equilibrium dissociation constants) for bivalent bindings of antigens and antibodies by ForteBio assay

Antibody	Antigen	K_D (M)	K_{on} (1/Ms)	k_{dis} (1/s)
LA42F8.5	VEGF 165	2.00E-10	1.06E+06	2.13E-04

LA42F8		8.43E-10	2.37E+05	2.00E-04*
LA46E11.8		7.88E-10	4.21E+05	3.32E-04
LA46E11		1.11E-09	1.79E+05	2.00E-04*
LA24C11	Ang2	8.67E-10	2.31E+05	2.00E-04*
LA24C11.10		8.28E-10	2.42E+05	2.00E-04*

* denotes dissociation constants exceeding ForteBio detection limits

Example 4. ELISA Blocking Assay of Anti-VEGF A VHH Antibodies

This example demonstrates the blocking effect of the anti-VEGF A VHH of the present invention on the binding of hVEGF A to a receptor KDR. SA (Thermo, Catalog No. 21125) was diluted to 1 μg/mL, and 100 μL/well of SA was plated in a microplate at 4 °C overnight. The plate was washed 3 times with PBST and blocked with 3% BSA for 1.5 h. The plate was washed 3 times with PBST, 50 ng/mL of biotin-labeled VEGF A165 (ACRO, Catalog No. VE5-H8210) was added, and the plate was incubated for 1.5 h. The LA42F8 supernatant, LA46E11 supernatant, negative control IgG, and positive control BI-anti-VEGF (50 μL) of the antibodies (with an initial concentration of 150 μg/mL, 3-fold serial dilution) prepared in Example 2 were incubated with VEGFR-Fc (Beijing Sino Biological, Catalog No. 10012-H02H, with a final concentration of 0.2 μg/mL) for 20 min in advance, and then the mixtures were added to the plate. The plate was washed 3 times with PBST, an anti-human Fc HRP antibody (Bethyl, Catalog No. A80-104P) (1:10000) was added, and then the plate was incubated for 30 min. The plate was washed 6 times with PBST, the color development was performed with TMB for 5 min, and OD450 nm was read after stopping.

The blocking results of 3 anti-VEGF VHH antibodies obtained in the present invention are shown in FIG. 2. FIG. 2 shows that the candidate molecules LA42F8 and LA46E11 antibodies can completely block the binding of VEGF A to VEGFR2.

Example 5. ELISA Blocking Assay of Anti-Ang2 VHH Antibody

This example demonstrates the blocking effect of the anti-Ang2 VHH antibody of the present invention on the binding of hAng2-biotin (R&D, Catalog No. BT623B/CF) to a Tie2 protein.

Tie2-Fc (Beijing Sino Biological, Catalog No. 10700-H03H) was diluted to 2 μg/mL, and 100 μL/well of Tie2-Fc was plated in a microplate at 4 °C overnight. The plate was washed 3 times with PBST and blocked with 3% BSA for 1.5 h. The plate was washed 3 times with PBST, each of the purified LA24C11.10 and negative control IgG (50 μL) prepared in Example 2 was incubated with hAng2-biotin (with a final concentration of 0.2 μg/mL) for 20 min in advance, and then the mixtures were added to the plate. The plate was washed 3 times with PBST, Avidin HRP (1:2000) was added, and the plate was incubated for 35 min. The plate was washed 6 times with PBST, the color development was performed with TMB for 5 min, and OD450 nm was read after stopping.

The blocking results of the anti-Ang2 VHH antibody obtained in the present invention are shown in Table 3, and the candidate molecule LA24C11 has a blocking effect on the binding of Ang2 to a receptor Tie2.

Table 3. Single-point blocking ELISA of purified antibody

		Ang2-Bio (Sino)	Ang2-Bio (R&D, Catalog No. BT623B/CF)	Ang2-Bio (R&D, Catalog No. BT623B/CF)	

Clone No.	Initial concentration of antibody [1] (mg/mL)	Antibody [1] diluted at 1:10 ratio (OD450 nm)	'Antibody [1] diluted at 1:100 ratio (OD450 nm)	Antibody [1] diluted at 1:10 ratio (OD450 nm)	'Antibody [1] diluted at 1:100 ratio (OD450 nm)	Supernatant [2] (OD450 nm)	Blank (without antibody) (OD450 nm)
24C11	0.556	0.09	0.20	0.07	0.07	0.12	3.45

[1] The antibody refers to the purified prokaryotic supernatant prepared in Example 2, with an initial concentration of 0.556 mg/mL, followed by 1:10 or 1:100 dilution;

[2] The supernatant refers to a prokaryotic supernatant without purification.

Similar to Example 4 (using Ang2-Bio (R&D, Catalog No. BT623B/CF)), the blocking effect of LA24C11.10 on the binding of Ang2 to Tie2 was detected by ELISA blocking experiments. The results are shown in FIG. 3. It can be seen that LA24C11.10 has a blocking effect on the binding of Ang2 to the receptor Tie2.

Example 6. Phosphorylation Assay of Anti-Ang2 VHH Antibodies

This example demonstrates the inhibitory effect of the anti-Ang2 VHH antibodies of the present invention on hAng2-Fc-induced Tie2 phosphorylation.

The hAng2-induced phosphorylation assay was as follows.

In this research, Expi293 cells overexpressing Tie2, 293-Tie2, were co-incubated with the antibody and a recombinant hAng2-Fc protein, and the content of phosphorylated Tie2 in the system was detected, thereby reflecting the inhibitory effect of different antibodies on the hAng2-fc-induced Tie2 phosphorylation.

The pCHO1.0 vector (Invitrogen) carrying a human Tie2 gene (Beijing Sino Biological, Catalog No. HG10700-M) cloned to a multiple cloning site (MCS) was transfected to Expi-293 cells (Thermo) to produce Expi-293 cells overexpressing human Tie2, that is, 293-Tie2 cells.

The 293-Tie2 cells overexpressing human Tie2 were diluted to 2×10^6 cells/mL and added to a 96-well plate at 100 μL per well, the plate was centrifuged at 400 g for 5 min, and the supernatant was discarded.

An experimental medium was prepared using Expi293 medium (Thermo, Catalog No. A1435102): test antibodies (LA24C11 (24C11) and LA24C11.0 (hz24C11.10) prepared in Example 2, negative control IgG, and positive control Nesvacumab (prepared according to CN202010573625.2)) with initial concentrations of 60 μg/mL were added and then serially diluted at a 1:2 ratio; the final concentration of hAng2-Fc (Beijing Sino Biological: 10691-H02H) was 2.5 μg/mL.

The cells were resuspended with 100 μL of experimental medium per well, and incubated at 37 °C for 15 min.

The medium was removed by centrifugation, and 100 μL of NP-40 lysis buffer (Beyotime, Catalog No. P0013F) containing 1% protease (Thermo, Catalog No. 78442) and a phosphatase inhibitor (Thermo, Catalog No. 78442) was added. The plate was placed on ice for 30 min and centrifuged at 2000 g, and the protein supernatant was collected and stored in a refrigerator at -80 °C.

The concentration of pTie2 was detected according to the instruction of a phosphorylated Tie2 ELISA kit (R&D, Catalog No. DYC2720E), and a microplate was coated with a capture antibody in the kit at a concentration of 4 μg/mL and incubated at 4 °C overnight. The plate was washed three times with PBST and blocked with 5% BSA for 1 h. 100 μL of freeze-thawed protein supernatant obtained in the last step and a control pTie2 (R&D, Catalog No. DYC2720E) were added to make a standard curve, and the mixture was incubated at room temperature for 2 h (if the concentration of pTie2 in the sample was too high and exceeded the ELISA detection range, the mixture obtained could be subjected to 2- to 3-fold dilution). The plate was washed three times with PBST, 100 μL of anti-

pTyr antibody conjugated with HRP (R&D, Catalog No. DYC2720E) was added, and the mixture was incubated at room temperature for 2 h. The plate was washed 6 times with PBST, 100 μL of TMB (Solarbio, Catalog No. PR1200) was added for color development, and after 15 min, 100 μL of stop buffer (Solarbio, Catalog No. C1058) was added to stop the reaction. OD450-OD620 were measured using a multimode microplate reader SpectraMax i3. The experimental results are shown in FIG. 4A (24C11 is the purified LA24C11 prokaryotic expression supernatant prepared in Example 2) and FIG. 4B.

Therefore, the anti-Ang2 VHH antibodies LA24C11 and LA24C11.10 of the present invention can effectively inhibit hAng2-Fc-induced 293-Tie2 cell phosphorylation *in vitro*.

Example 7. KDR Reporter Blocking Assay of Anti-VEGF A VHH Antibodies

VEGF A could bind to a related receptor VEGFR2 (KDR), a VEGFR2 signaling pathway was activated, survival, proliferation and migration of vascular endothelial cells were induced, and this research utilized a KDR reporter experiment system and used NFAT-RE-luc2P/KDR HEK293 cells (Promega, Cat. No. CS181401) to detect the blocking effect of the gradiently diluted antibodies on a VEGFA activation-related receptor signaling pathway.

Experimental procedures referred to supplier's (Promega) instructions:

NFAT-RE-luc2P/KDR HEK293 cells which were changed into an experimental medium (DMEM medium containing 10% FBS) 3 days in advance were taken out, and the old medium was removed by pipetting. The medium was washed once with PBS, then 1 mL of accutase solution (Sigma, Catalog No. A6964-500ML) was used to digest the cells until the cells became round and were detached from the wall, and 5 mL of diluted medium was used to stop the reaction. The cells were pipetted into a centrifuge tube and centrifuged at 1000 rpm for 5 min, the medium was discarded, 10 mL of diluted medium (DMEM medium containing 10% FBS) was added to resuspend the cells, and the cells were mixed homogeneously and counted. The cell viability should be more than 90%. The cell density was adjusted to 0.8×10^6 cells/mL with the diluted medium, and the mixture was added to a 96-well white cell culture plate at 50 μL/well according to an experimental layout.

A mixed solution of VEGF A with a concentration of 100 ng/mL and the antibodies to be detected which were gradiently diluted was prepared, and the mixture was left to stand for 30 min and added to the 96-well white cell culture plate containing cells at 50 μL/well. The plate was put into a 37 °C incubator with 5% carbon dioxide and incubated for 6 h. The samples to be detected were as follows: negative control IgG; positive control BI-anti-VEGF; LA42F8; LA46E11; LA42F8.5; LA46E11.8; Blank: only containing the diluted medium, no VEGF A and no antibody; VEGF A 100 ng/mL: only containing 100 ng/mL VEGF A.

The 96-well white cell culture plate incubated for 6 h was taken out from the carbon dioxide incubator, and equilibrated for 10-15 min to room temperature. The Bio-Glo Luciferase Assay System equilibrated to room temperature in advance was added to the 96-well white cell culture plate at 100 μL/well according to the experimental layout, and the plate was incubated at room temperature for 5 min in the dark.

Fluorescence values were read using a multimode microplate reader, the chemiluminescence mode was selected for plate reading mode, the end point method was selected for plate reading type, and the wavelength was set to full wavelength. The fluorescence was collected column by column, and the collection time for each column was 1000 ms.

In the experiments performed by the assays described above, the detection results in FIG. 5 show that the anti-VEGF VHH antibodies LA42F8, LA42F8.5, LA46E11, and LA46E11.8 can all block the activation of VEGF A-induced KDR signaling pathways.

Example 8. Inhibition Assay of VEGF A-Induced HUVEC Survival and Proliferation by Anti-VEGF A VHH

VEGF A could act on VEGFR and other related receptors in vascular endothelial cells, and promote vascular endothelial cell survival, proliferation and migration, thereby inducing neovascularization. This example was based on the VEGF-induced survival and proliferation of human umbilical vein endothelial cells (HUVECs), and the inhibitory effect of the antibodies on the VEGF A-induced survival and proliferation of primary cells was detected. This example determines the survival and proliferation of HUVECs by CCK-8, and the specific method is as follows: HUVECs (Allcells, Catalog No. H-001-CN) were treated one day in advance, and the cells were plated in a 96-well culture plate at 2000 cells/well; the plate was put into in a 37 °C incubator with 5% carbon dioxide and incubated for 24 h.

After the cells were attached to the wall, an experimental medium containing VEGF A with a final concentration of 10 ng/mL and the gradiently diluted antibodies (LA42F8 prepared in Example 2 with an initial concentration of 80 μg/mL and being subjected to serial dilution at a 1:3 ratio, negative control IgG, positive control BI836880, a group (VEGFA) containing only 10 ng/mL of VEGF A, and Blank without VEGF A and antibody) was prepared, the endothelial cell medium in a 96-well plate was replaced by the experimental medium, and the plate was put into a 37 °C incubator with 5% carbon dioxide and incubated for 72 h.

CCK-8 detection solution (Dojindo, Catalog No. CK04) was added at 10 μL/well, and then the plate was put into the 37 °C incubator with 5% carbon dioxide and incubated for 12-24 h.

The absorbance OD_{450}-OD_{620} values were read by using a multimode microplate reader.

In the experiments performed by the assays described above, the detection results are shown in FIG. 6, from which it can be seen that the anti-VEGFA antibody LA42F8 is able to completely inhibit the VEGFA-induced survival and proliferation of HUVEC cells.

Example 9. HEK293-KDR Reporter Blocking Assay of Anti-VEGF A/Ang2 Bispecific Antibodies

Chains of the bispecific binding molecules, IEX04-008, IEX04-010 and IEX04-012 (sequences shown in the sequence listing) of the present invention were constructed into pcDNA3.1 vectors, and the bispecific binding molecules were expressed and purified in 293 cells as described in Example 2.

This example detected the blocking effect of the anti-VEGF A/Ang2 bispecific antibodies on VEGF A by HEK293-KDR reporter assay. Experimental procedures referred to those in Example 7.

The binding molecules or controls used were as follows:

Blank: no VEGF A and no antibody;

VEGF A: 100 ng/mL VEGF A;

Negative control IgG: prepared as described above;

Positive control Faricimab: prepared as described above, with an initial concentration of 13.5 μg/mL and being subjected to gradient dilution at a 1:3 ratio;

Positive control BI-836880: prepared as described above, with an initial concentration of 13.5 μg/mL and being subjected to gradient dilution at a 1:3 ratio; and

IEX04-012: prepared as described above, with an initial concentration of 13.5 μg/mL and being subjected to gradient dilution at a 1:3 ratio.

The detection results in FIG. 7 show that the bispecific binding molecule IEX04-012 inhibits the activation of VEGF-induced KDR signaling pathways, and has better inhibitory ability compared with the control antibody BI836880.

Example 10. HUVEC Proliferation Inhibition Assay of Anti-VEGF A/Ang2 Bispecific Binding Molecules

In this research, the inhibitory effect of the anti-VEGF A/Ang2 bispecific binding molecules on the VEGF A-induced survival and proliferation of HUVEC cells was detected by HEK293-KDR reporter assay.

The experimental procedures were as those in Example 8, and the antibodies used were as follows:

Panel A: IEX04-008, negative control IgG, positive controls Faricimab and BI836880, Blank (i.e., no antibody and VEGF A), and VEGF A group (i.e., only 20 ng/mL of VEGFA added) prepared as described above with initial concentrations of 20 μg/mL and being subjected to serial dilution at a 1:3 ratio;

Panel B: negative control IgG, BI-anti-VEGF, IEX04-010, Blank (i.e., no antibody and VEGF A), and VEGF A group (i.e., only 20 ng/mL of VEGFA added) prepared as described above with initial concentrations of 80 μg/mL and being subjected to serial dilution at a 1:3 ratio; and

Panel C: IEX04-012, negative control IgG, positive controls Faricimab and BI836880, Blank (i.e., no antibody and VEGF A), and VEGF A group (i.e., only 20 ng/mL of VEGFA added) prepared as described above with initial concentrations of 20 nM and being subjected to serial dilution at a 1:3 ratio.

The detection results in FIG. 8 show that the bispecific binding molecules IEX04-008, IEX04-010 and IEX04-012 all inhibit VEGF-induced survival and proliferation of HUVEC cells, and IEX04-012 has lower IC_{50} and better inhibitory ability compared with the control antibodies BI836880 and Faricimab.

Example 11. Ang2 Blocking Assay of Anti-VEGF A/Ang2 Bispecific Antibodies

Ang2 could bind to its native receptor Tie2, and this research detected the blocking effect of the anti-VEGF A/Ang2 bispecific binding molecules on the binding between Ang2 and Tie2 by ELISA assay and FACS assay.

(1) ELISA

The ability of IEX04-008, IEX04-010, IEX04-012, and control antibodies BI-836880 and Faricimab to block the binding of human Ang2 to hTie2 was detected by ELISA.

The hTie2 protein (Beijing Sino Biological) was resuspended and dissolved to a concentration of 2 μg/mL with PBS, and a microplate was coated with the protein and incubated overnight. The plate was blocked with 5% BSA for 1 h, and a biotinylated antigen (Recombinant Biotinylated hAngiopoietin-2 protein (R&D)) was diluted to 600 μg/mL and added to the plate at 50 μL/well. The antibodies prepared as described above (IEX04-008, IEX04-010, IEX04-012, positive control antibodies BI836880 and Faricimab, and negative control IgG) were each diluted serially at a 1:2 ratio starting from the maximum concentration of 300 nM for a total of 8 or 12 dilution gradients. The diluted antibodies were added at 50 μL/well and incubated in PBS on ice for 30 min, and the biotinylated antigen had a final concentration of 300 ng/mL. The antigen-antibody mixture obtained as described above was incubated in the microplate for 90 min and washed three times with PBS, and the supernatant was discarded. 100 μL of Avidin-HRP (Invitrogen) diluted at a 1:10000 ratio was added to each well, incubated at room temperature for 30 min, and washed six times with PBS. A TMB chromogenic solution (solarbio) was added to the plate at 100

μL/well for color development for 1 min, and the reaction was stopped by adding a stop solution (Solarbio) at 100 μL/well.

The plate was read on a microplate reader at OD_{450} and OD_{620} per well.

The experimental results (see FIG. 9) show that IEX04-008, IEX04-010, IEX04-012, and the control antibody BI836880 all have a complete blocking effect, and that IC_{50} of the bispecific binding molecules of the present invention are significantly smaller than that of the positive control antibody.

(2) Flow cytometry assay (FACS)

The ability of IEX04-008, IEX04-010, IEX04-012, positive control antibodies BI-836880 and Faricimab, and negative control IgG to block the binding of human Ang2-hFc to Tie2 on the cell surface was detected by FACS.

The antigen hAng2-Fc protein (Beijing Sino Biological, Catalog No. 10691-H02H) was diluted to 4 μg/mL and added at 50 μL/well. The antibodies prepared as described above (IEX04-008, IEX04-010, IEX04-012, positive control antibodies BI-836880 and Faricimab, and negative control IgG) were each 2-fold diluted gradiently starting from the maximum concentration of 800 nM for a total of 12 dilution gradients. The diluted antibodies were added at 50 μL/well and incubated in PBS on ice for 30 min, the antigen hAng2-Fc protein had a final concentration of 2 μg/mL, and each antibody had a maximum final concentration of 400 nM. 293-Tie2 cells prepared as described above were adjusted to 2×10^5 cells/well and added at 100 μL/well. The cells were centrifuged at 300 g for 5 min. The supernatant was discarded. Then the cells were resuspended in an antigen-antibody mixture. The mixture was incubated on ice for 30 min. PBS was added at 100 μL/well. After centrifugation at 300 g for 5 min, the mixture was washed once with PBS. 100 μL of goat anti-human IgG-PE (Southern Biotech) diluted at a 1:200 ratio was added to each well. After a 20-min ice bath, PBS was added at 100 μL/well. The mixture was centrifuged at 300 g for 5 min, prior to one wash with PBS. The cells were resuspended with 100 μL of PBS. The cell fluorescence signal values were measured by a flow cytometer (BD Biosciences). According to MFI, the concentration-dependent curve was fitted with GraphPad. The results are shown in FIG. 10. It is shown that the bispecific binding molecules IEX04-008, IEX04-010, and IEX04-012 can all effectively block the binding of human Ang2-hFc to Tie2, and have IC_{50} lower than that of the positive control.

Example 12. Ang2 Phosphorylation Inhibition Assay of Anti-VEGF A/Ang2 Bispecific Binding Molecules

This example demonstrates the inhibitory effect of the bispecific binding molecules of the present invention on hAng2-Fc-induced Tie2 phosphorylation by using hAng2-induced phosphorylation assay.

In this research, Expi293 cells overexpressing Tie2, 293-Tie2, were co-incubated with the bispecific binding molecules and a recombinant hAng2-Fc protein, and the content of phosphorylated Tie2 in the system was detected, thereby reflecting the inhibitory effect of different antibodies on the hAng2-Fc-induced Tie2 phosphorylation.

The overexpressed 293-Tie2 cells prepared above were diluted to 2×10^6 cells/mL and added to a 96-well plate at 100 μL per well, the plate was centrifuged at 400 g for 5 min, and the supernatant was discarded.

An experimental medium was prepared using Expi293 medium (Thermo, Catalog No. A1435102), in which test antibodies (IEX04-012 prepared above, positive controls BI-836880 and Faricimab, and negative control IgG) with maximum final concentration of 60 μg/mL were each added and then serially diluted at a 1:2 ratio; the final concentration of hAng2-Fc (Beijing Sino Biological, Catalog No. 10691-H02H) was 2.5 μg/mL.

The cells were resuspended with 100 μL of the experimental medium per well and incubated at 37 °C for 15 min. The medium was removed by centrifugation. 100 μL of NP-40 lysis buffer containing 1% protease and a

phosphatase inhibitor was added, and the plate was left to stand on ice for 30 min. The plate was centrifuged at 2000 g, and the protein supernatant was collected and stored in a refrigerator at -80 °C.

The concentration of pTie2 was detected according to the instruction of a phosphorylated Tie2 ELISA kit (R&D, DYC2720E), and a microplate was coated with a capture antibody at a concentration of 4 μg/mL and incubated at 4 °C overnight. The plate was washed three times with PBST and blocked with 5% BSA for 1 h. 100 μL of the sample to be detected and a control pTie2 (R&D, DYC2720E) were added to make a standard curve, and the mixture was incubated at room temperature for 2 h (if the concentration of pTie2 in the sample was too high and exceeded the ELISA detection range, the mixture obtained could be subjected to 2- to 3-fold dilution). The plate was washed three times with PBST, 100 μL of anti-pTyr antibody conjugated with HRP (R&D, Catalog No. DYC2720E) was added, and the mixture was incubated at room temperature for 2 h. The plate was washed 6 times with PBST, 100 μL of TMB was added for color development, and after 15 min, 100 μL of stop buffer was added to stop the reaction. OD450-OD620 of each well was measured using a spectrophotometer.

The experimental results are shown in FIG. 11. The antibody IEX04-012 of the present invention can effectively inhibit hAng2-Fc-induced 293-Tie2 phosphorylation *in vitro*, and IC_{50} is superior to that of the positive control.

Example 13. Inhibition Assay of Ang2 Vascular Endothelial Cell Leakage by Anti-VEGF A/Ang2 Bispecific Binding Molecules

This research identified the effect and function of anti-VEGF A/Ang2 bispecific binding molecules on vascular endothelial cell leakage by HUVEC-Tie2 leakage assay.

HUVEC-Tie2 cells overexpressing Tie2 were obtained by transfecting HUVEC cells (Allcells, Catalog No. H-001-CN) with lentivirus.

300 μL of EGM-2 medium was plated on the bottom layer of a mini-well 96-well insert culture dish, the cells were digested with accutase (Sigma) and HUVEC-Tie2 was obtained. The cells were resuspended to 1×10^7 cells/mL using the EGM-2 medium and plated at 100 μL/well on the top layer of the culture dish. The lower chamber medium (EGM-2 medium) was replaced every 24 h, and after 24 h, the lower chamber medium was replaced by an experimental medium, wherein the experimental medium contained the following components:

Blank: EGM-2 medium (Lonza, Catalog No. CC-5035);

VEGF A group: EGM-2 Medium + 20 ng/mL VEGF (R&D, Catalog No. 293-VE);

IgG group (VEGF A + IgG): EGM-2 medium + 20 ng/mL VEGF + 10 μg/mL IgG;

Ang1 group (VEGF A + Ang1): EGM-2 medium + 20 ng/mL VEGF (R&D, Catalog No. 293-VE) + 200 ng/mL Ang1 (R&D, Catalog No. 923-AN);

IEX04-012 group (VEGF A + IEX04-012): EGM-2 medium + 20 ng/mL VEGF + 10 μg/mL IEX04-012;

BI-836880 group (VEGF A + BI-836880): EGM-2 medium + 20 ng/mL VEGF + 10 μg/mL BI-836880; and

Faricimab group (VEGF A + Faricimab): EGM-2 medium + 20 ng/mL VEGF + 10 μg/mL Faricimab.

The experimental medium described above was placed and incubated at 37 °C with 5% CO_2.

After 24 h, 1 μL of FITC-Dextran (Sigma, Catalog No. FD2000S-1G) (4 mg/mL) was added to the experimental medium per well. The mixture was placed at 37 °C with 5% CO_2. After 30 min, the lower chamber medium was taken out, diluted at a 1:10 ratio with PBS, and detected by a multimode microplate reader at an exciting wavelength of 488 nm and an emitting wavelength of 535 nm.

The experimental results are shown in FIG. 12, showing that the antibody IEX04-012 of the present invention can effectively reduce the VEGF-induced vascular endothelial cell permeability.

Example 14. Laser-Induced Choroidal Neovascularization Pharmacodynamic Test

This assay adopted a rhesus monkey laser-induced choroidal neovascularization model to determine the neovascularization resistance of the bispecific binding molecule IEX04-012 of the present invention.

Rhesus monkey:

Species: a rhesus monkey; grade: a normal grade; weight: 3.30-4.20 kg of body weight when purchased, and 3.35-4.35 kg of body weight during molding; source: Sichuan Hengshu Bio-Technology; production license number: SCXK (Sichuan) 2019-029; laboratory animal quality certification number: No.0023356.

In this test, laser photocoagulation was performed around the macular fovea of the fundus of the rhesus monkey to induce the choroidal neovascularization in the fundus and establish an animal model similar to the human choroidal neovascularization. Before and after 20 days of photocoagulation, fundus fluorescence angiography was performed to judge the molding condition. 20 rhesus monkeys (half male and half female) which were successfully molded were selected to be divided into 5 groups, namely a model control group, an IEX04-012 low-dose group, an IEX04-012 high-dose group, an Eylea group, and a Faricimab group, wherein each group contained 4 monkeys, half male and female.

On day 21 after photocoagulation, each group of monkeys was administered individually according to the doses in the table. IEX04-012, Eylea (Bayer), or Faricimab (all dissolved in 0.9% sodium chloride injectable solution) was administered by intravitreal injection at both eyes, and the model control was given an equal volume of 0.9% sodium chloride injectable solution. Color fundus photography, fundus fluorescence angiography (leak spot statistics and leak area measurements) (Robin J Goody, Wenzheng Hu, Afshin Shafiee et al., Optimization of laser-induced choroidal neovascularization in African green monkeys. Experimental Eye Research, Exp Eye Res. 2011 92(6):464-72), and optical coherence tomography (OCT, Wang Q, Lin X, Xiang W et al., Assessment of laser induction of Bruch's membrane disruption in monkey by spectral-domain optical coherence tomography. British Journal of Ophthalmology, 2015, 99(1):119-24) were performed on each group of animals on day 7, 14, 21, and 28 after the administration to observe the inhibition effect of the test sample on the choroidal neovascularization. The animals were euthanized on day 29 after the administration, and then both eyes were taken out for histological examination with immunohistochemical (HE) staining.

Experimental design table

Group	Dose	Administration volume	Concentration	Route of administration
Control	/	/	X mg/ml	
IEX04-012 Low dose	10 μg/eye	50 μL/eye	0.2mg/ml	Intravitreal administration
IEX04-012 High dose	30 μg/eye	50 μL/eye	0.6mg/ml	Intravitreal administration
Eylea	10 μg/eye	50 μL/eye	0.2mg/ml	Intravitreal administration
Faricimab	30 μg/eye	50 μL/eye	0.6mg/ml	Intravitreal administration

The results in FIGs. 13-15 show that the bispecific binding molecule of the present invention showed significant anti-neovascularization after 28 days of administration, and the statistics of the number of grade four leakage spots

(FIG. 13A) and the number of grade three to four leakage spots (FIG. 13B) showed that the treated animals of the IEX04-012 group had significantly fewer high-leakage spots than those of the control and positive control administration groups. The OCT results showed a significant decrease in retinal thickness in the animals of the IEX04-012 treatment group, suggesting that the retinal edema degree is reduced and the effect is superior to that of the control (FIG. 14). The fundus fluorescence angiographic results show that the fundus leakage area of the IEX04-012 treatment group was significantly reduced, and the effect was superior to those of positive controls Eylea and Faricimab (FIG. 15), indicating that the antibody of the present invention can significantly inhibit the leakage caused by neovascularization. In conclusion, it is verified that the antibody of the present invention in combination with the anti-VEGF inhibitor has a significant inhibitory effect on the laser-induced fundus neovascularization, and has the function of protecting the integrity of blood vessels.

29 days after the administration, rhesus monkeys were anesthetized with pentobarbital sodium according to the body weight (at about 30 mg/kg by intravenous injection, and the dose could be adjusted according to health conditions of the animals) and euthanized by bleeding from abdominal aorta or femoral artery. The rhesus monkeys were roughly observed, and bilateral eyeballs were taken.

Some animal eyes were fixed in a modified Davidson's fixative solution and embedded in paraffin, and laser-molded areas were selected for conventional HE staining such as CD31 IHC staining to perform the histopathological examination.

In the pathological section of the antibody group of the present invention, the retinopathy area was significantly reduced, the retinal edema was alleviated, and the tissue hyperplasia of the laser injury area was reduced compared with the anti-VEGF single treatment. The results show better retina morphological improvement (see FIG. 16), the inhibition of the retinochoroidal neovascularization, and the enhancement of the blood vessel integrity function (FIG. 17).

SEQUENCE LISTING

SEQ ID NO	Antibody name	LA42F8 (VEGFA VHH)
1	HCDR1	GFNLDYYPIG
2	HCDR2	CISSVGSTNYADSVKG
3	HCDR3	DPLCSALILPPPFLS
4	VH	QLQLVESGGGLVQPGGSLRLSCAASGFNLDYYPIGWFRQA PGKEREGVSCISSVGSTNYADSVKGRFTISRDNAKNTVYLQ MNSLKPEDTAVYYCAADPLCSALILPPPFLSWGQGTQVTVS S
	Antibody name	LA42F8.5
1	HCDR1	GFNLDYYPIG
2	HCDR2	CISSVGSTNYADSVKG
3	HCDR3	DPLCSALILPPPFLS

5	VH	EVQLLESGGGLVQPGGSLRLSCAASGFNLDYYPIGWFRQA PGKGLEGVSCISSVGSTNYADSVKGRFTISRDNSKNTVYLQ MNSLRAEDTAVYYCAADPLCSALILPPPFLSWGQGTQVTVS S
	Antibody name	LA46E11 (VEGFA VHH)
6	HCDR1	GSIFSINAMG
7	HCDR2	TMIGGSSTFYADSVNG
8	HCDR3	DVTGRSGTSIWNKRDDY
9	VH	QVQLEESGGGLVQPGGSLRLSCVASGSIFSINAMGWHRQAP GSQRELVATMIGGSSTFYADSVNGRFTISRDNAKNTVYLQM NSLKLEDTAVYYCYADVTGRSGTSIWNKRDDYWGQGTLV TVSS
	Antibody name	LA46E11.8 (VEGFA VHH)
6	HCDR1	GSIFSINAMG
10	HCDR2	TMIGGSSTFYAESVQG
8	HCDR3	DVTGRSGTSIWNKRDDY
11	VH	EVQLLESGGGLVQPGGSLRLSCAASGSIFSINAMGWHRQAP GKQRELVATMIGGSSTFYAESVQGRFTISRDNSKNTVYLQM NSLRAEDTAVYYCYADVTGRSGTSIWNKRDDYWGQGTQV TVSS
	Antibody name	LA24C11 (Ang2 VHH)
16	HCDR1	GFALDYYAIG
17	HCDR2	CISSGDGSTYYADSVKG
18	HCDR3	DSRGDDVACEGLRRNEYDY
19	VH	QVQLVESGGGLVQPGGSLRLSCAASGFALDYYAIGWFRQA PGKEREGVSCISSGDGSTYYADSVKGRFTISRDNAKNTVYL QMNSLKPEDTAVYYCATDSRGDDVACEGLRRNEYDYWGQ GTQVTVSS
	Antibody name	LA24C11.10 (Ang2 VHH)
16	HCDR1	GFALDYYAIG
20	HCDR2	CISSGEGSTYYADSVKG
18	HCDR3	DSRGDDVACEGLRRNEYDY
21	VH	*EVQLLESGGGLVQPGGSLRLSCAASGFALDYYAIGWFRQAPGK GLEGVSCISSGEGSTYYADSVKGRFTISRDNSKNTVYLQMNSLR AEDTAVYYCATDSRGDDVACEGLRRNEYDYWGQGTLVTVSS*
	Antibody name	IEX04-008 LA46E11.8 (VHH) (underlined) + linker (bold) + LA46E11.8 (VHH) (underlined) + LA24C11.10 (italic)

22	Full length	EVQLLESGGGLVQPGGSLRLSCAASGSIFSINAMGWHRQAP GKQRELVATMIGGSSTFYAESVQGRFTISRDNSKNTVYLQM NSLRAEDTAVYYCYADVTGRSGTSIWNKRDDYWGQGTQV TVSS**GGGGSGGGGS**EVQLLESGGGLVQPGGSLRLSCAASG SIFSINAMGWHRQAPGKQRELVATMIGGSSTFYAESVQGRF TISRDNSKNTVYLQMNSLRAEDTAVYYCYADVTGRSGTSI WNKRDDYWGQGTQVTVSS**GGGGSGGGGS***EVQLLESGGG* *LVQPGGSLRLSCAASGFALDYYAIGWFRQAPGKGLEGVSCISSG* *EGSTYYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCAT* *DSRGDDVACEGLRRNEYDYWGQGTLVTVSS*
	Antibody name	IEX04-010 VEGFR extracellular domain + Fc + linker (underlined) + LA24C11.10 (italic)
24	Full length	SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKK FPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGH LYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTART ELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLS TLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGS*EVQL* *LESGGGLVQPGGSLRLSCAASGFALDYYAIGWFRQAPGKGLEG* *VSCISSGEGSTYYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTA* *VYYCATDSRGDDVACEGLRRNEYDYWGQGTLVTVSS***
	Name	Aflibercept (VEGFR extracellular domain (underlined) + Fc (italic + bold), wherein FLT1 domain 2 is underlined, and KDR domain 3 is underlined and bold)

25	Full length	SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKK FPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGH LYKTNYLTHRQTNTII**DVVLSPSHGIELSVGEKLVLNCTAR TELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMK KFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVH EK*DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG***
26	VEGFR extracellular domain	SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKK FPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGH LYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTART ELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLS TLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK
27	Fc	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPG
\	Antibody name	IEX04-012 Ranibizumab scFv containing disulfide bond mutations + linker (bold) + LA24C11.10 (italic)
28	Full length	DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPG KAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYSTVPWTFGCGTKVEIK**GGGGSGGGGSGGGGS GGGGS**EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGM NWVRQAPGKCLEWVGWINTYTGEPTYAADFKRRFTFSLD TSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDV WGQGTLVTVSS**GGGGSGGGGSGGGGS***EVQLLESGGGLVQ PGGSLRLSCAASGFALDYYAIGWFRQAPGKGLEGVSCISSGEGS TYYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCATDSR GDDVACEGLRRNEYDYWGQGTLVTVSS****
		Ranibizumab scFv containing disulfide bond mutations

29	scFv	DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPG KAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYSTVPWTFGCGTKVEIK**GGGGSGGGGSGGGGS GGGGS**EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGM NWVRQAPGKCLEWVGWINTYTGEPTYAADFKRRFTFSLD TSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDV WGQGTLVTVSS
30	VL	DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPG KAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYSTVPWTFGCGTKVEIK
31	LCDR1	QDISNYLN
32	LCDR2	FTSSLHS
33	LCDR3	QQYSTVPWT
34	VH	EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQ APGKCLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTA YLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT LVTVSS
35	HCDR1	GYDFTHY
36	HCDR2	NTYTGE
37	HCDR3	YPYYYGTSHWYFDV
23	Linker	$(GGGGS)_n$, wherein n = 1, 2, 3, or 4
		Positive control antibody BI836880 (VEGFA terminal)
12	VH	DVQLVESGGGLVQPGGSLRLSCAASGRTFSSYSMGWFRQA PGKEREFVVAISKGGYKYDAVSLEGRFTISRDNAKNTVYLQ INSLRPEDTAVYYCASSRAYGSSRLRLADTYEYWGQGTLVT VSS
		Positive control antibody Faricimab

13	HC 1	EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQ APGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTA YLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLAQDWL NGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNA YTQKSLSLSPGK
40	LC1	DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPG KAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC
14	HC 2	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVR QAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSIS TAYMELSRLRSDDTAVYYCARSPNPYYYDSSGYYYPGAFDI WGQGTMVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMASRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLAQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFS CSVMHEALHNAYTQKSLSLSPGK
41	LC 2	SYVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQ APVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEA DYYCQVWDSSSDHWVFGGGTKLTVLSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSC
		Positive control antibody BI836880 (VEGFA/HSA/Ang2)

15	VH	DVQLVESGGGLVQPGGSLRLSCAASGRTFSSYSMGWFRQA PGKEREFVVAISKGGYKYDAVSLEGRFTISRDNAKNTVYLQ INSLRPEDTAVYYCASSRAYGSSRLRLADTYEYWGQGTLVT VSS**GGGGSGGGS**EVQLVESGGGLVQPGNSLRLSCAASGFT FSSFGMSWVRQAPGRGLEWVSSISGSGSDTLYADSVKGRFT ISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV TVSS**GGGGSGGGS**EVQLVESGGGLVQPGGSLRLSCAVSGI TLDDYAIGWFRQAPGKEREGVSAIRSSGGSTYYADSVKGRF TISSDNSKNTVYLQMNSLRPEDTAVYYCAAVPAGRLRYGE QWYPIYEYDAWGQGTLVTVSS
		Negative control IgG
38	Heavy chain	EVRLLESGGGLVQPGGSLRLSCAASGFTFSNYAMGWVRQA PGKGLEWVSAISGSGGSTYYADSVKGRFTTSRDDSKNALY LQMNSLRAEDTAVYYCARGGPGWYAADVWGQGTTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
42	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQADLPAFAFGGGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
		Positive control antibody BI-anti-VEGF
39		DVQLVESGGGLVQPGGSLRLSCAASGRTFSSYSMGWFRQA PGKEREFVVAISKGGYKYDAVSLEGRFTISRDNAKNTVYLQ INSLRPEDTAVYYCASSRAYGSSRLRLADTYEYWGQGTLVT VSS

CLAIMS

1. An anti-Ang2 VHH antibody, comprising 3 CDRs, HCDR1, HCDR2, and HCDR3 as follows, wherein

the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 16;

the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 17 or 20; and

the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 18.

2. The VHH antibody according to claim 1, wherein the VHH

(1) comprises or consists of an amino acid sequence set forth in SEQ ID NO: 19 or 21, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 19 or 21; or

(2) comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 19 or 21, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.

3. A bispecific binding molecule binding to VEGF A and Ang2, comprising a first target-binding region specifically binding to VEGF A and a second target-binding region specifically binding to Ang2, wherein the second target-binding region is the VHH antibody according to claim 1 or 2,

optionally, wherein the first target-binding region is selected from:

a VHH specifically binding to VEGF A;

an antigen-binding fragment of an antibody, e.g., an scFv, specifically binding to VEGF A, for example, the antibody is a fully human or humanized antibody; or

a VEGF receptor (VEGF R) specifically binding to VEGF A or an extracellular domain thereof or a fusion protein comprising the extracellular domain thereof, e.g., a fusion protein of the extracellular domain thereof with Fc.

4. The bispecific binding molecule according to claim 3, being a bispecific antibody.

5. The bispecific binding molecule according to claim 3 or 4, being divalent, trivalent, or tetravalent.

6. The bispecific binding molecule according to claim 4 or 5, having the following structure:

light chain variable region VL of the anti-VEGF antibody-linker-heavy chain variable region VH of the anti-VEGF antibody-linker-anti-Ang2 VHH or

heavy chain variable region VH of the anti-VEGF antibody-linker-light chain variable region VL of the anti-VEGF antibody-linker-anti-Ang2 VHH.

7. The bispecific binding molecule according to claim 6, wherein:

the light chain variable region VL of the anti-VEGF antibody comprises LCDR1, LCDR2, and LCDR3, wherein the LCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 31; the LCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 32; and the LCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 33; and/or

the heavy chain variable region VH of the anti-VEGF antibody comprises HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 35; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 36; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 37.

8. The bispecific binding molecule according to claim 6 or 7, wherein

the heavy chain variable region VH of the anti-VEGF antibody comprises or consists of an amino acid sequence set forth in SEQ ID NO: 34, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 34; or the heavy chain variable region VH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 34, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements;

the light chain variable region VL of the anti-VEGF antibody comprises or consists of an amino acid sequence set forth in SEQ ID NO: 30, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 30; or the light chain variable region VL comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 30, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.

9. The bispecific binding molecule according to any one of claims 6 to 8, wherein

(1) the binding molecule comprises or consists of an amino acid sequence set forth in SEQ ID NO: 28, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 28; or

(2) the bispecific binding molecule comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 28, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.

10. The bispecific binding molecule according to claim 4 or 5, having the following structure:

first anti-VEGF VHH-linker-second anti-VEGF VHH-linker-anti-Ang2 VHH,

wherein the first anti-VEGF VHH is the same as or different from the second anti-VEGF VHH.

11. The bispecific binding molecule according to claim 10, wherein the first anti-VEGF VHH or the second anti-VEGF VHH comprises HCDR1, HCDR2, and HCDR3, wherein

the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 1; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 2; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 3;

or

the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 6; the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 7 or 10; and the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 8.

12. The bispecific binding molecule according to claim 10 or 11, wherein the first anti-VEGF VHH or the second anti-VEGF VHH comprises or consists of an amino acid sequence set forth in SEQ ID NO 4, 5, 9, or 11, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO 4, 5, 9, or 11; or the VHH comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.

13. The bispecific binding molecule according to any one of claims 10 to 12, wherein

(1) the binding molecule comprises or consists of an amino acid sequence set forth in SEQ ID NO: 22, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 22; or

(2) the bispecific binding molecule comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 22, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.

14. The bispecific binding molecule according to claim 3, comprising one or two of the following chains:

VEGF R extracellular domain-Fc-linker-anti-Ang2 VHH.

15. The bispecific binding molecule according to claim 14, wherein the VEGFR extracellular domain is an extracellular domain of VEGFR from a human; preferably, the VEGFR extracellular domain comprises a second antibody-like domain of VEGFR1 and a third antibody-like domain of VEGFR2; more preferably, the VEGFR extracellular domain comprises a second antibody-like domain of human VEGFR1 and a third antibody-like domain of human VEGFR2.

16. The bispecific binding molecule according to claim 14 or 15, wherein the VEGFR extracellular domain comprises or consists of an amino acid sequence set forth in SEQ ID NO: 26, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 26.

17. The bispecific binding molecule according to any one of claims 14 to 16, wherein the Fc is an Fc derived from human IgG1, IgG2, IgG3, or IgG4, preferably the Fc comprises or consists of an amino acid sequence set forth in SEQ ID NO: 27, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 27.

18. The bispecific binding molecule according to any one of claims 14 to 17, wherein the VEGF R extracellular domain-Fc is a fusion protein of the VEGFR extracellular domain and the Fc, such as aflibercept or a derivative thereof; for example, the VEGF R extracellular domain-Fc comprises or consists of an amino acid sequence set forth in SEQ ID NO: 25, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 25.

19. The bispecific binding molecule according to any one of claims 14 to 18, wherein

(1) the binding molecule comprises or consists of an amino acid sequence set forth in SEQ ID NO: 24, or comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 24; or

(2) the bispecific binding molecule comprises an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 24, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.

20. The bispecific binding molecule according to any one of claims 3 to 19, wherein the linker comprises or consists of an amino acid sequence set forth in SEQ ID NO: 23.

21. An anti-VEGF A VHH antibody, comprising 3 CDRs, HCDR1, HCDR2, and HCDR3, as follows, wherein

the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 1;
the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 2; and
the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 3;
or
the HCDR1 comprises or consists of a sequence set forth in SEQ ID NO: 6;
the HCDR2 comprises or consists of a sequence set forth in SEQ ID NO: 7 or 10; and
the HCDR3 comprises or consists of a sequence set forth in SEQ ID NO: 8.

22. The VHH antibody according to claim 21,

(1) comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11, or comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11; or

(2) comprising an amino acid sequence having one or several (preferably no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) mutations compared to an amino acid sequence set forth in SEQ ID NO: 4, 5, 9, or 11, wherein the mutations are, for example, replacements, deletions, or additions, preferably replacements, such as conservative replacements.

23. A nucleic acid molecule encoding the VHH antibody according to any one of claims 1, 2, 21, and 22 or the bispecific binding molecule according to any one of claims 3 to 20 or a chain of the binding molecule.

24. An expression vector comprising the nucleic acid molecule according to claim 23, wherein preferably, the expression vector is pcDNA3.1.

25. A host cell comprising the nucleic acid molecule according to claim 23 or the expression vector according to claim 24, wherein preferably, the host cell is prokaryotic or eukaryotic, for example, a bacterium such as an *E.coli* cell, e.g., TG1, or a 293 cell such as a 293F or Expi-293 cell.

26. A method for preparing the VHH antibody according to any one of claims 1, 2, 21, and 22 or the bispecific binding molecule according to any one of claims 3 to 20, comprising culturing the host cell according to claim 25 under conditions suitable for expressing the VHH antibody or bispecific binding molecule or the chain thereof, and optionally recovering the VHH or bispecific binding molecule from the host cell (or the host cell medium).

27. An immunoconjugate comprising the VHH antibody according to any one of claims 1, 2, 21, and 22 or the bispecific binding molecule according to any one of claims 3 to 20

28. A pharmaceutical composition or formulation comprising the VHH antibody according to any one of claims 1, 2, 21, and 22 or the bispecific binding molecule according to any one of claims 3 to 20, and optionally one or more additional therapeutic agents and optionally a pharmaceutical supplementary material.

29. A method for preventing or treating an ocular disease in a subject, comprising administering to the subject an effective amount of the VHH antibody according to any one of claims 1, 2, 21, and 22 or the bispecific binding molecule according to any one of claims 3 to 20, the immunoconjugate according to claim 27, or the pharmaceutical composition or formulation according to claim 28.

30. The method according to claim 29, wherein the ocular disease is selected from ocular diseases associated with angiogenesis, such as ocular diseases associated with corneal neovascularization.

31. The method according to claim 29 or 30, wherein the patient has VEGF, e.g., VEGF A, and/or Ang2 (e.g., at an elevated level, e.g., at a nucleic acid or protein level).

32. The method according to any one of claims 29 to 31, wherein the method further comprises administering to the patient one or more therapies, such as therapeutic modalities and/or additional therapeutic agents.

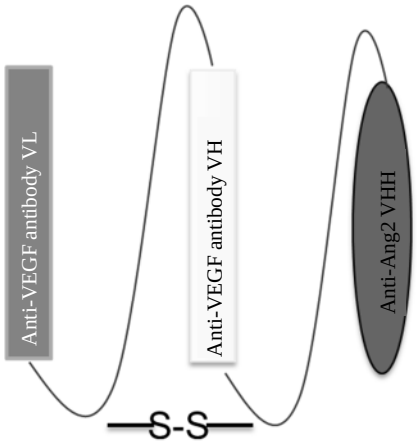


FIG. 1A

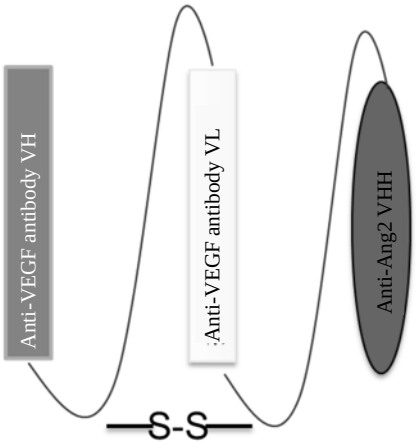


FIG. 1B

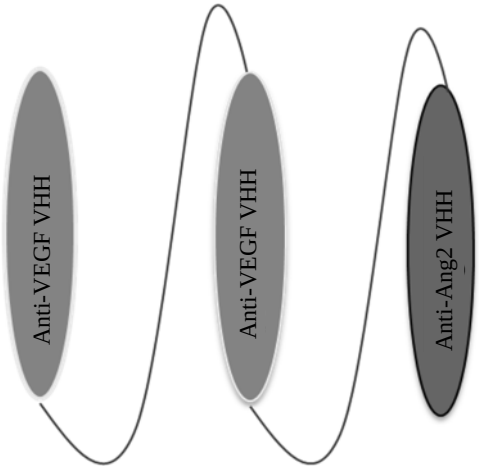


FIG. 1C

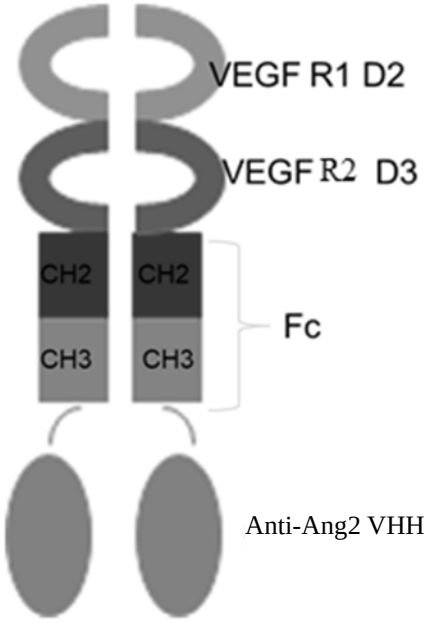
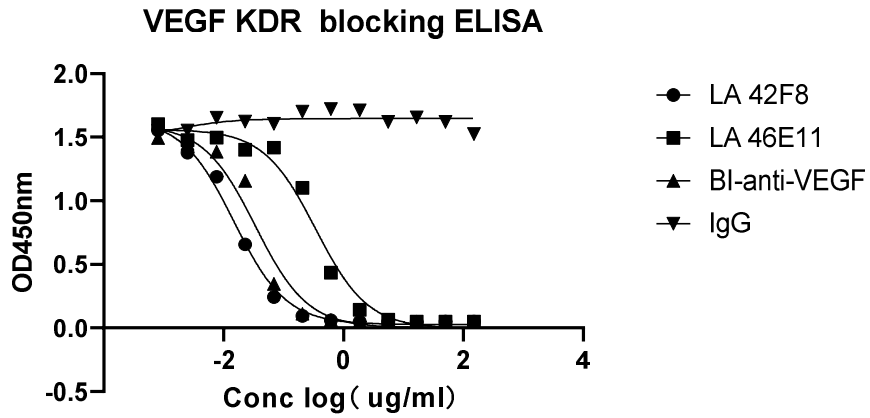


FIG. 1D



	LA 42F8	LA 46E11	BI-anti-VEGF	IgG
EC50	0.01490	0.3435	0.03401	0.002084

FIG. 2

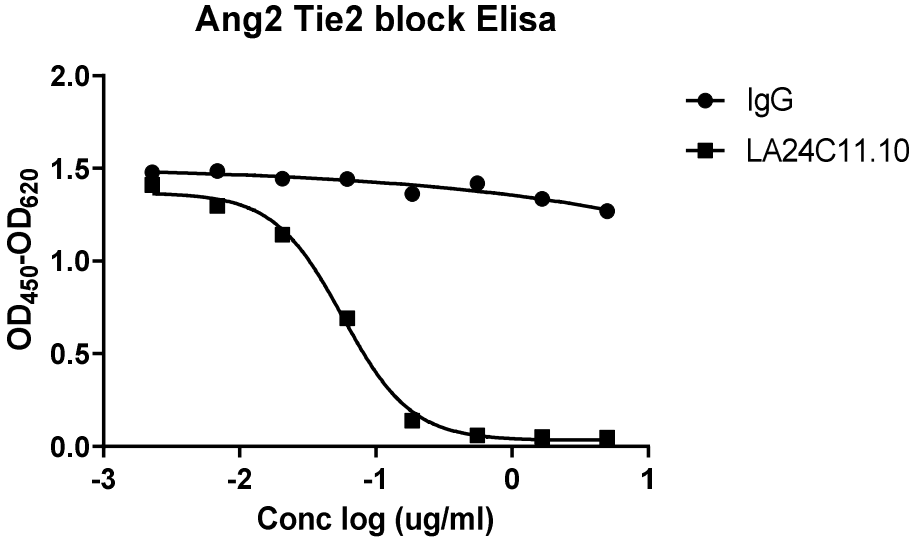
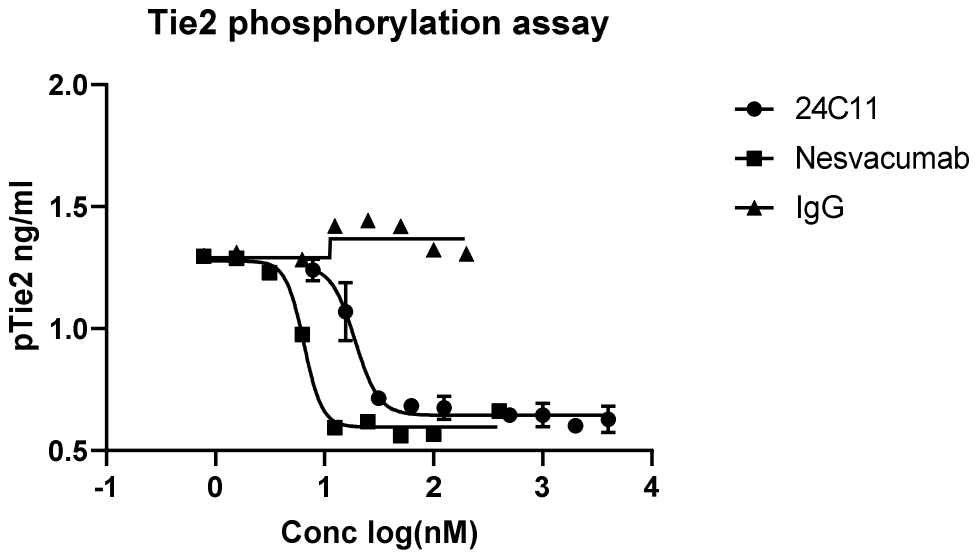


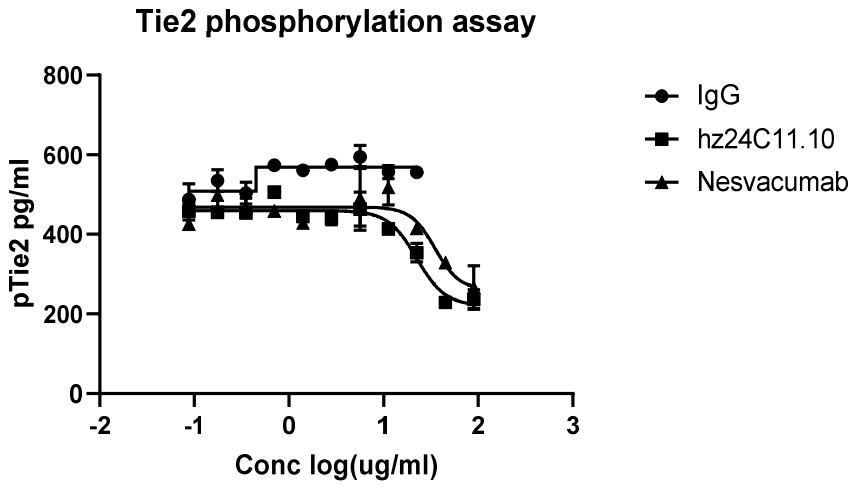
FIG. 3

A



	24C11	Nesvacumab	IgG
IC50	19.08	6.485	~ 11.25

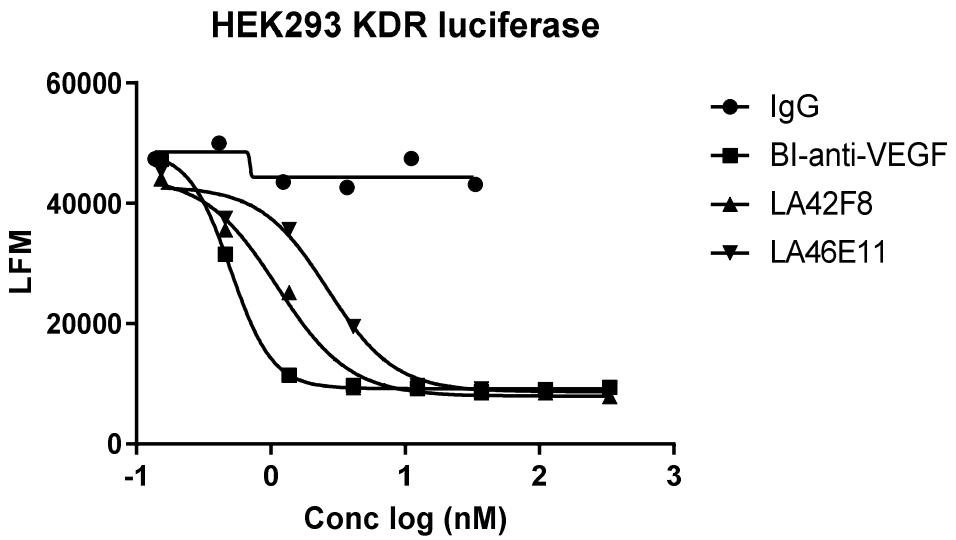
B



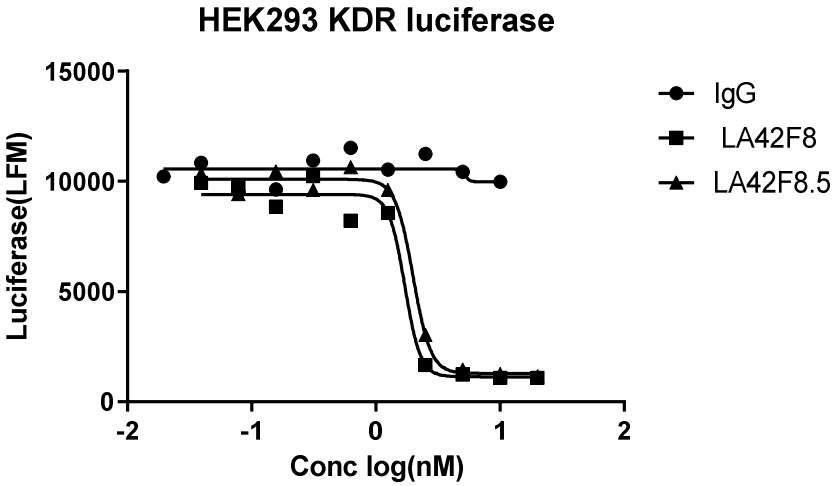
	IgG	hz24C11.10	Nesvacumab
IC50	~ 0.4502	22.39	34.94

FIG. 4

A


HEK293 KDR luciferase
60000
40000
20000
0
LFM
-1
0
1
2
3
Conc log (nM)
IgG
BI-anti-VEGF
LA42F8
LA46E11

B


HEK293 KDR luciferase
15000
10000
5000
0
Luciferase(LFM)
-2
-1
0
1
2
Conc log(nM)
IgG
LA42F8
LA42F8.5

C

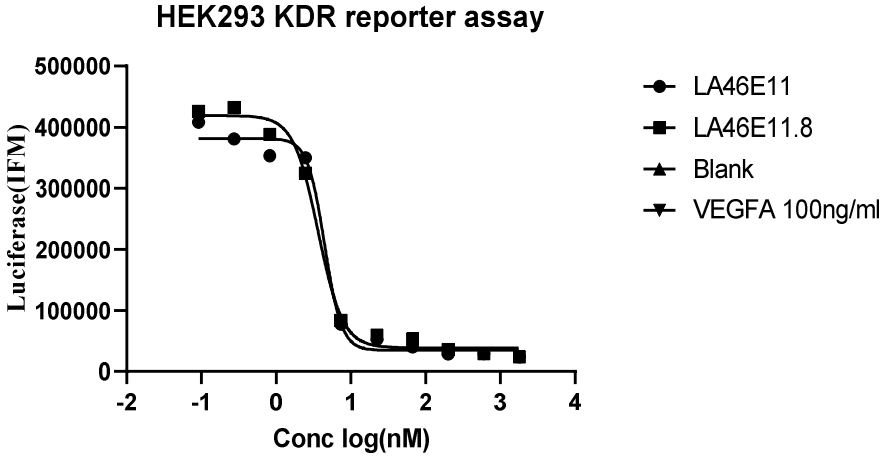
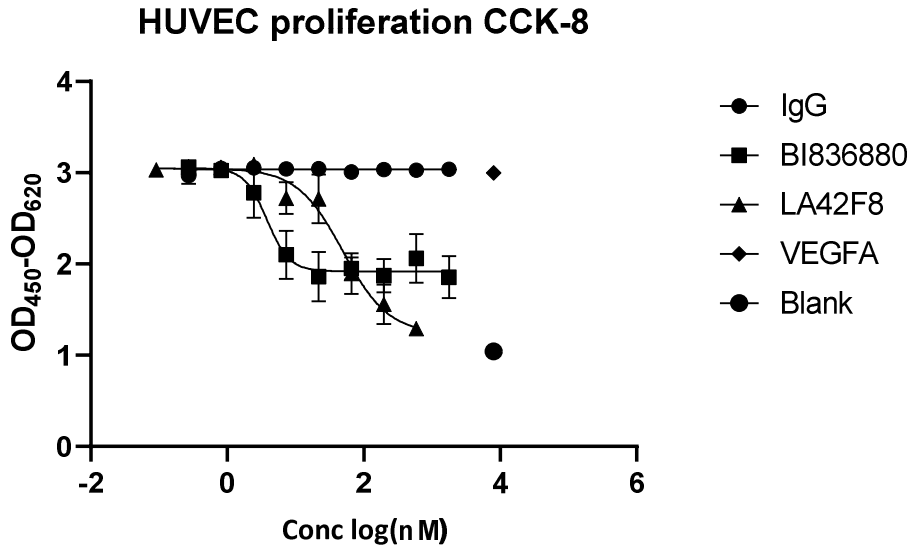
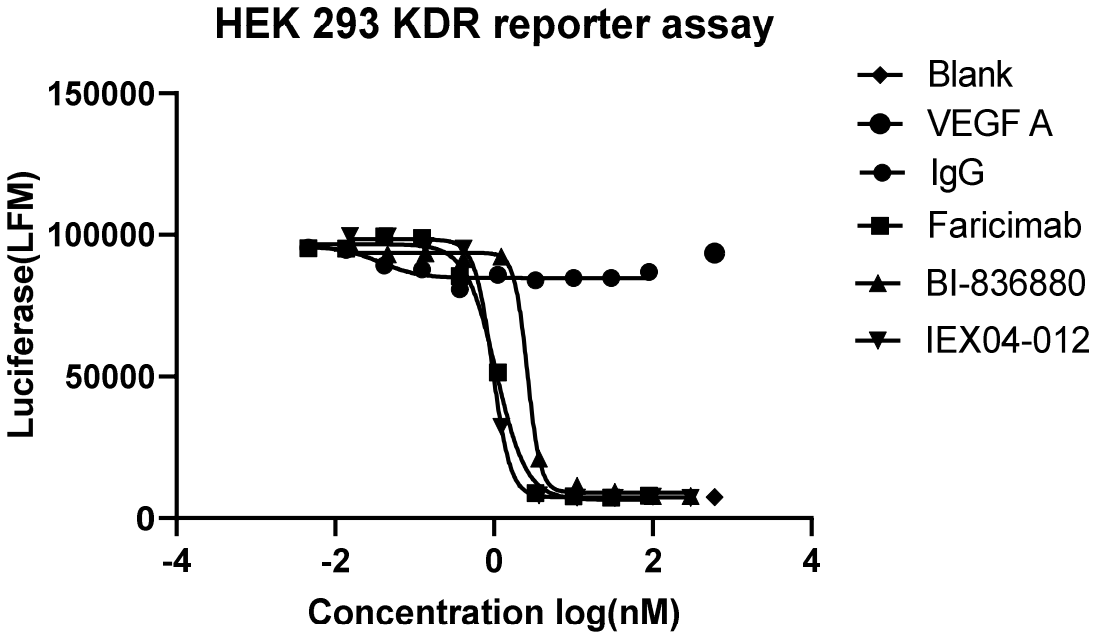

HEK293 KDR reporter assay
500000
400000
300000
200000
100000
0
Luciferase(IFM)
-2
-1
0
1
2
3
4
Conc log(nM)
LA46E11
LA46E11.8
Blank
VEGFA 100ng/ml

FIG. 5



	IgG	BI836880	LA42F8
IC50	~ 0.2425	3.812	49.41

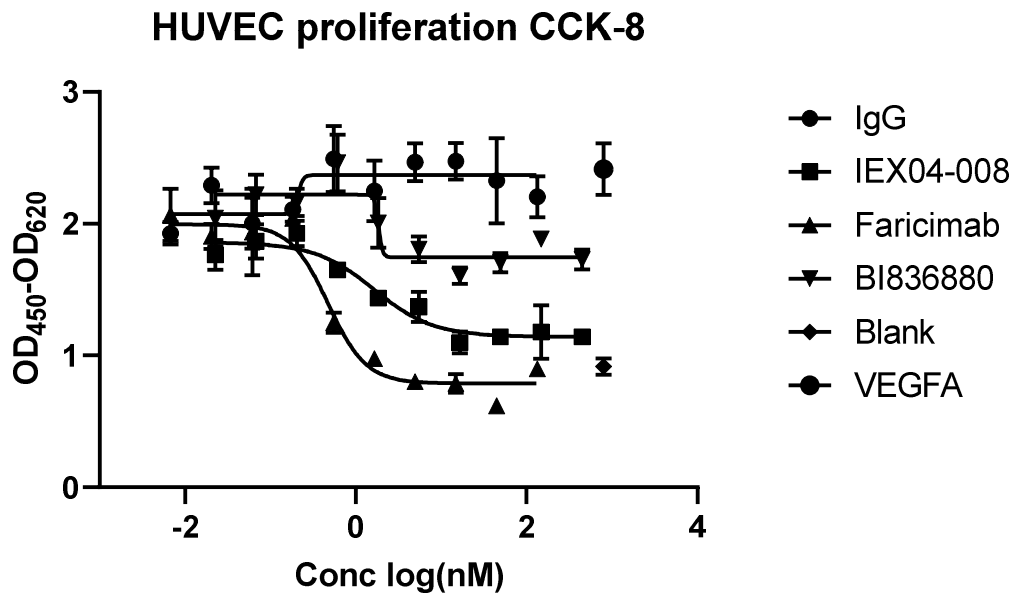
Fig. 6



	IgG	Faricimab	BI-836880	IEX04-012
IC50	0.03672	1.064	2.631	0.9586

FIG. 7

A



	IgG	IEX04-008	Faricimab	BI836880
IC50	~ 0.2100	1.637	0.4744	~ 1.848

B

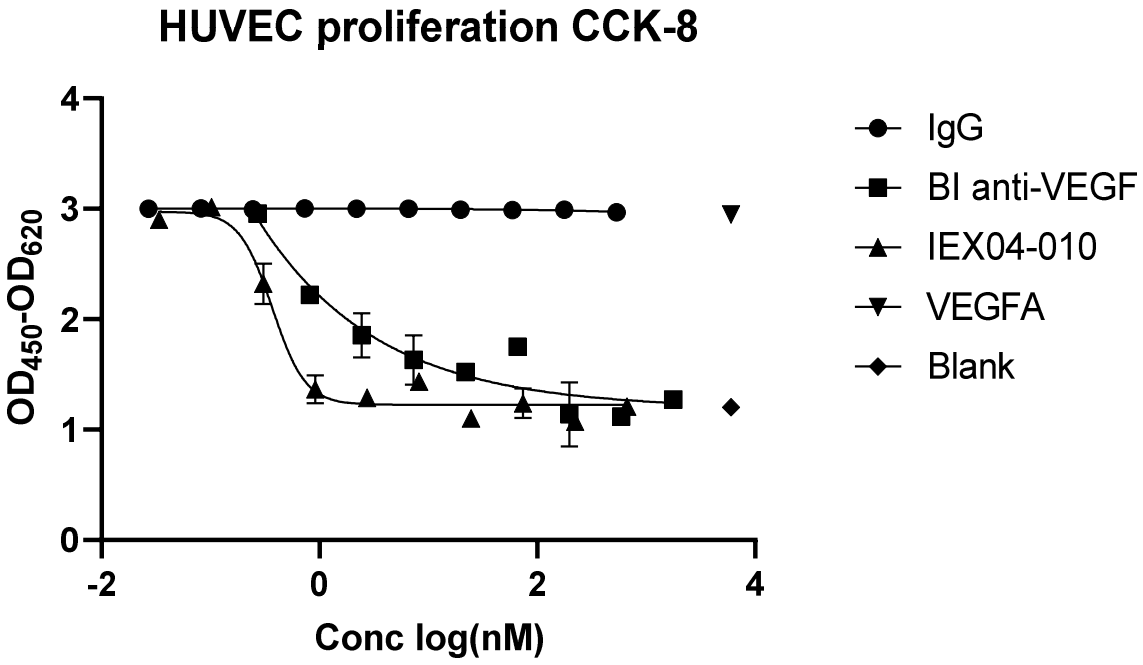
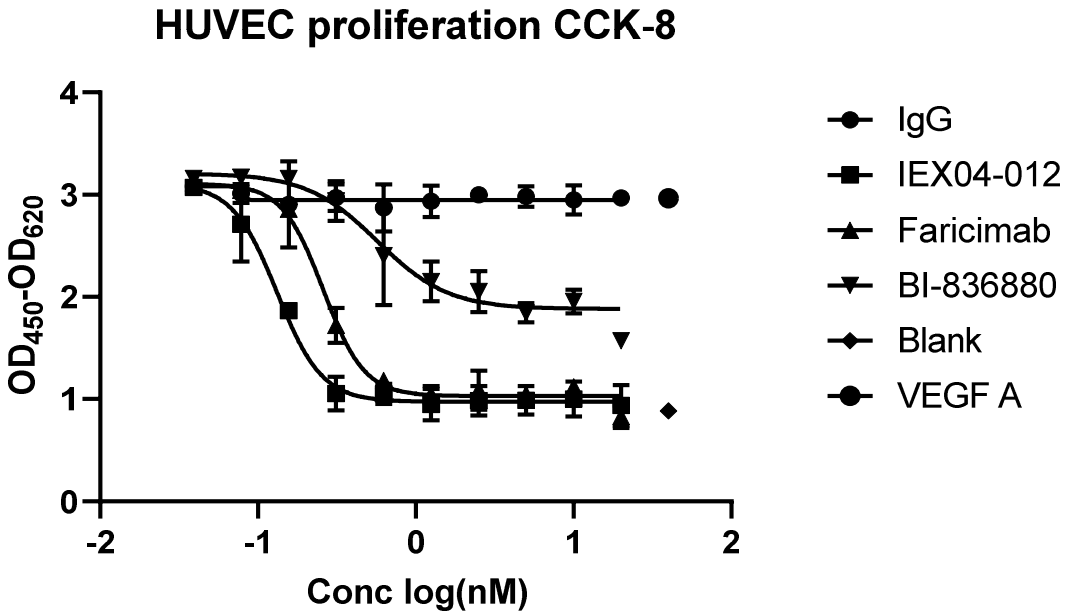


FIG. 8

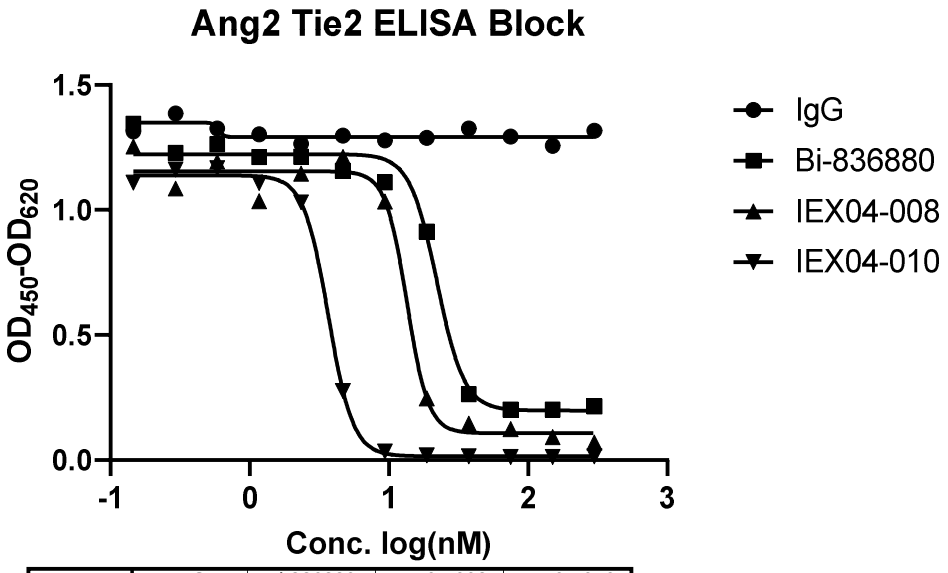
C



	IgG	IEX04-012	Faricimab	BI-836880
IC50	~ 0.07786	0.1323	0.2592	0.5703

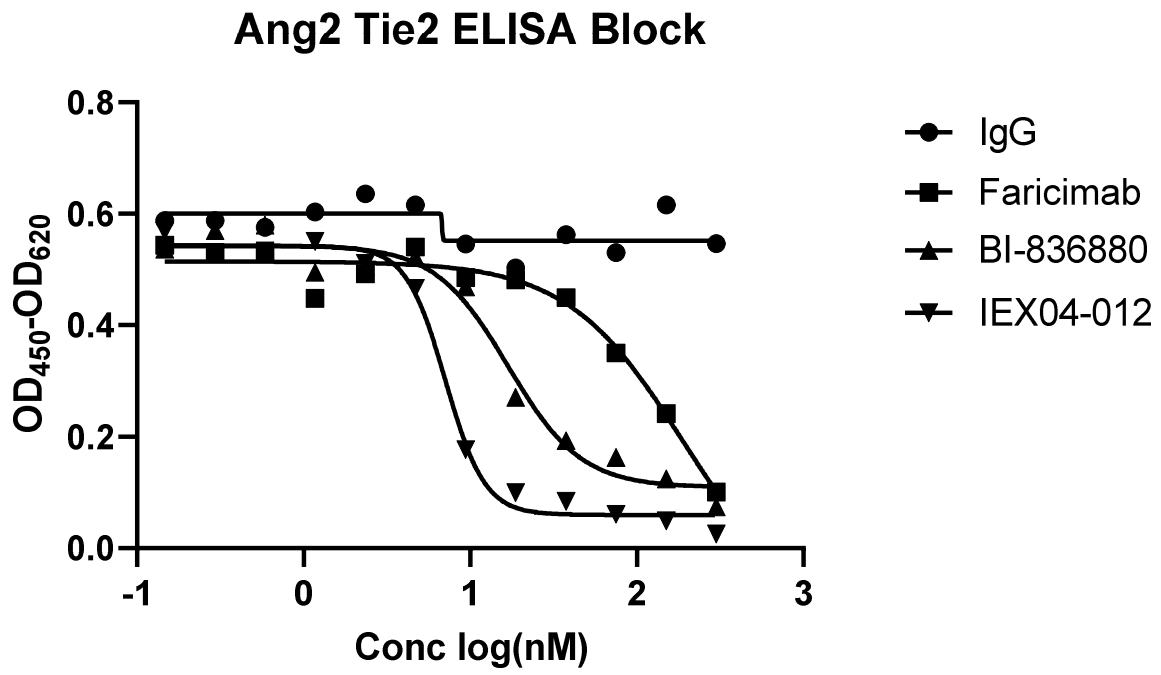
FIG. 8 (continued)

A



	IgG	Bi-836880	IEX04-008	IEX04-010
IC50	~ 0.5959	22.21	13.48	3.677

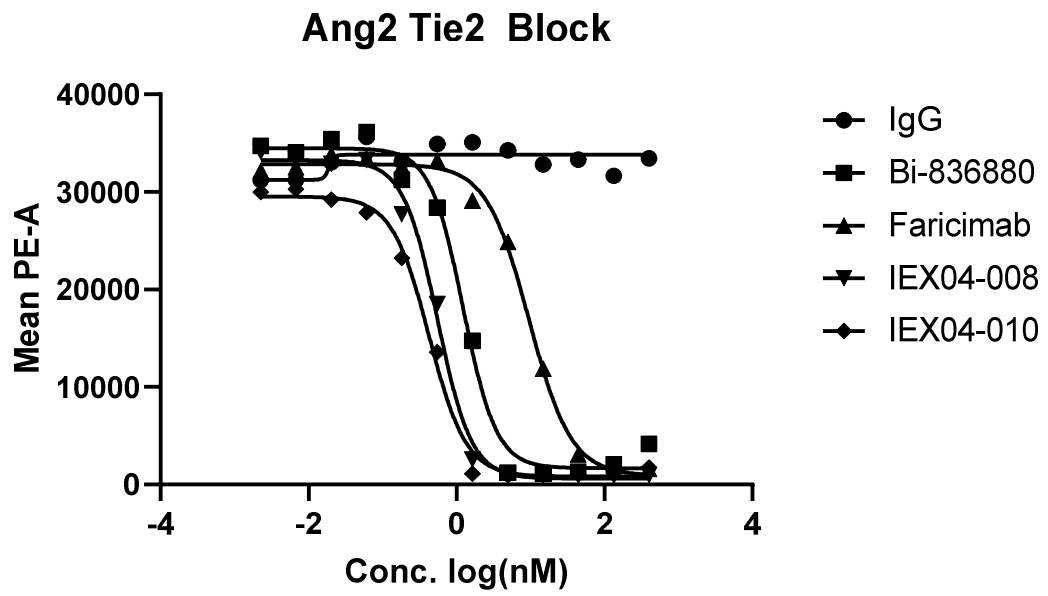
B



	IgG	Faricimab	BI-836880	IEX04-012
IC50	~ 6.786	188.1	16.87	7.096

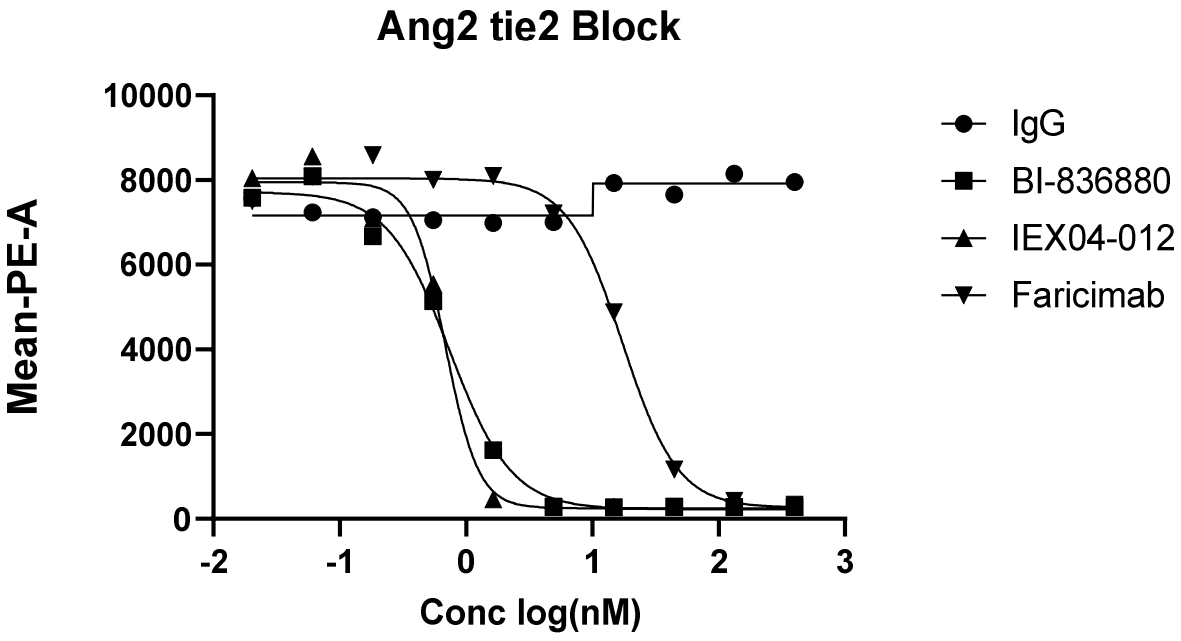
FIG. 9

A



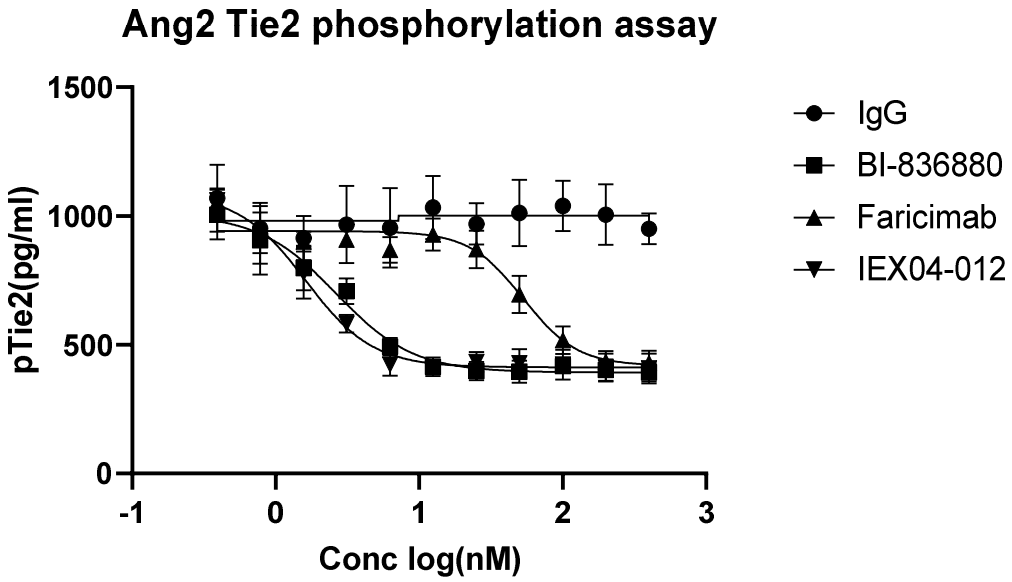
	IgG	Bi-836880	Faricimab	IEX04-008	IEX04-010
IC50	~ 0.01896	1.232	9.550	0.5541	0.4275

B



	IgG	BI-836880	IEX04-012	Faricimab
IC50	~ 10.14	0.7415	0.6855	17.35

FIG. 10



BI-836880	Faricimab	IEX04-012
2.632	53.17	1.614

FIG. 11

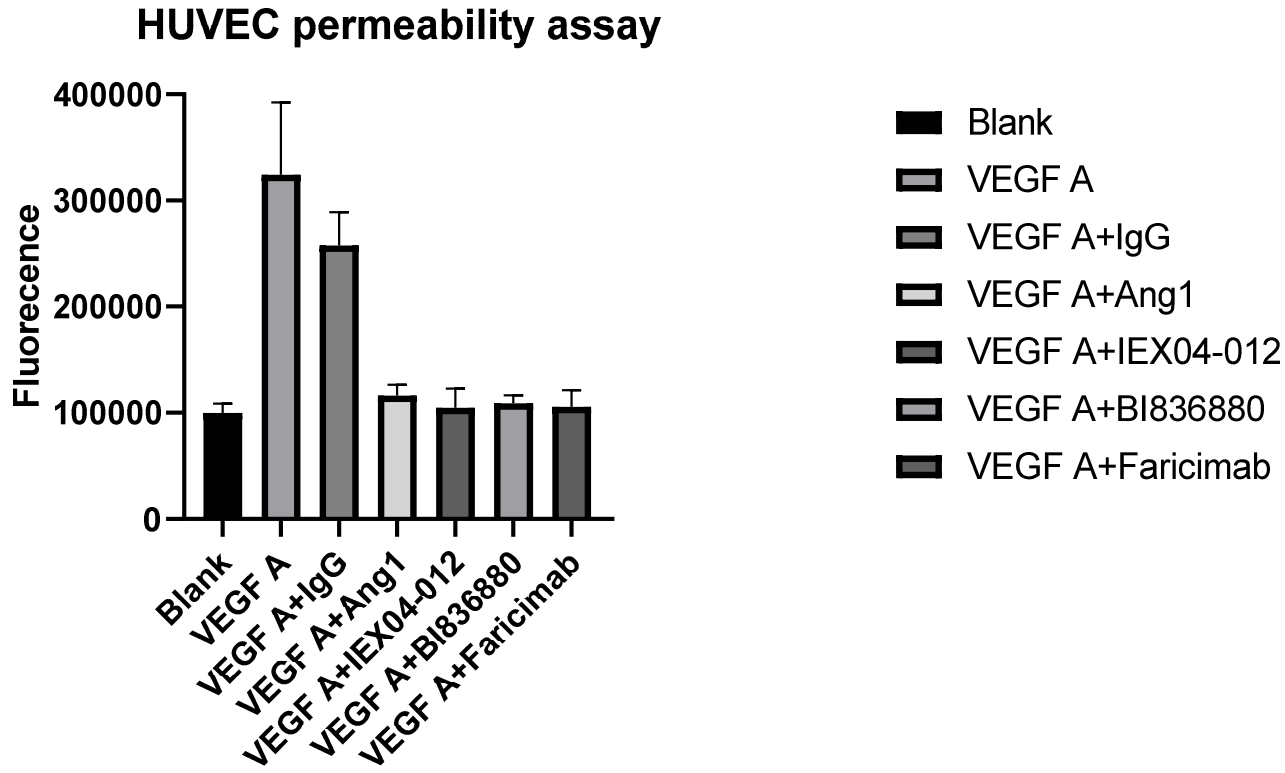
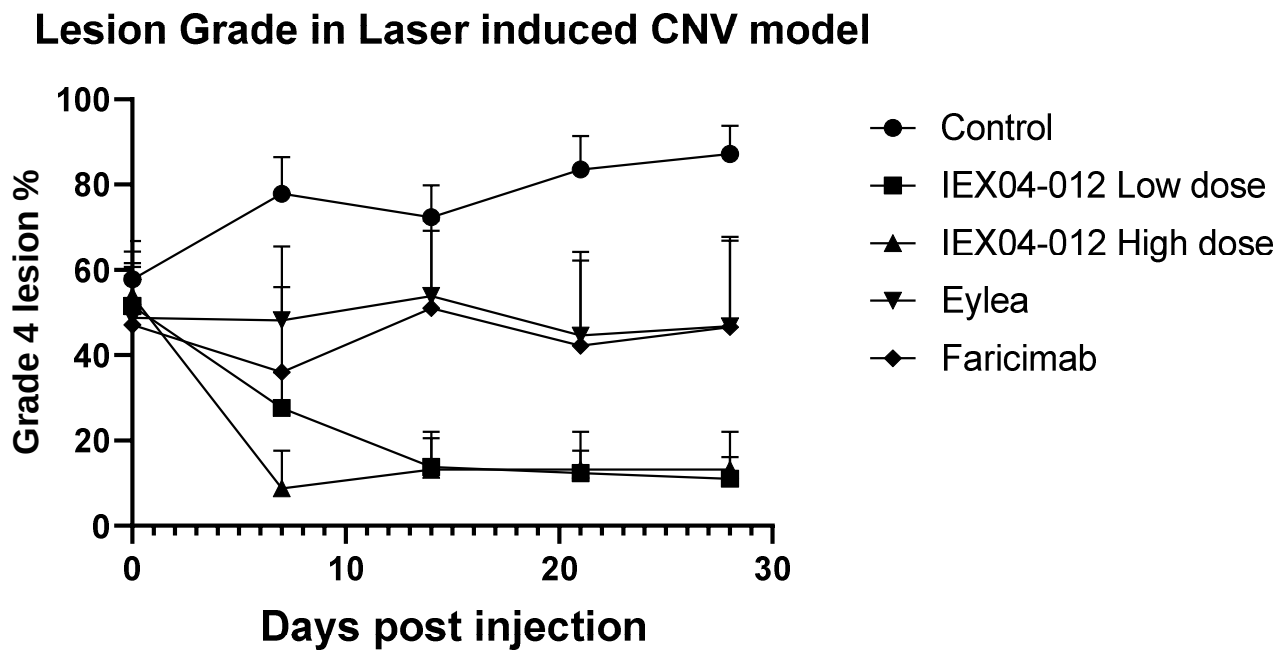


FIG. 12

A



B

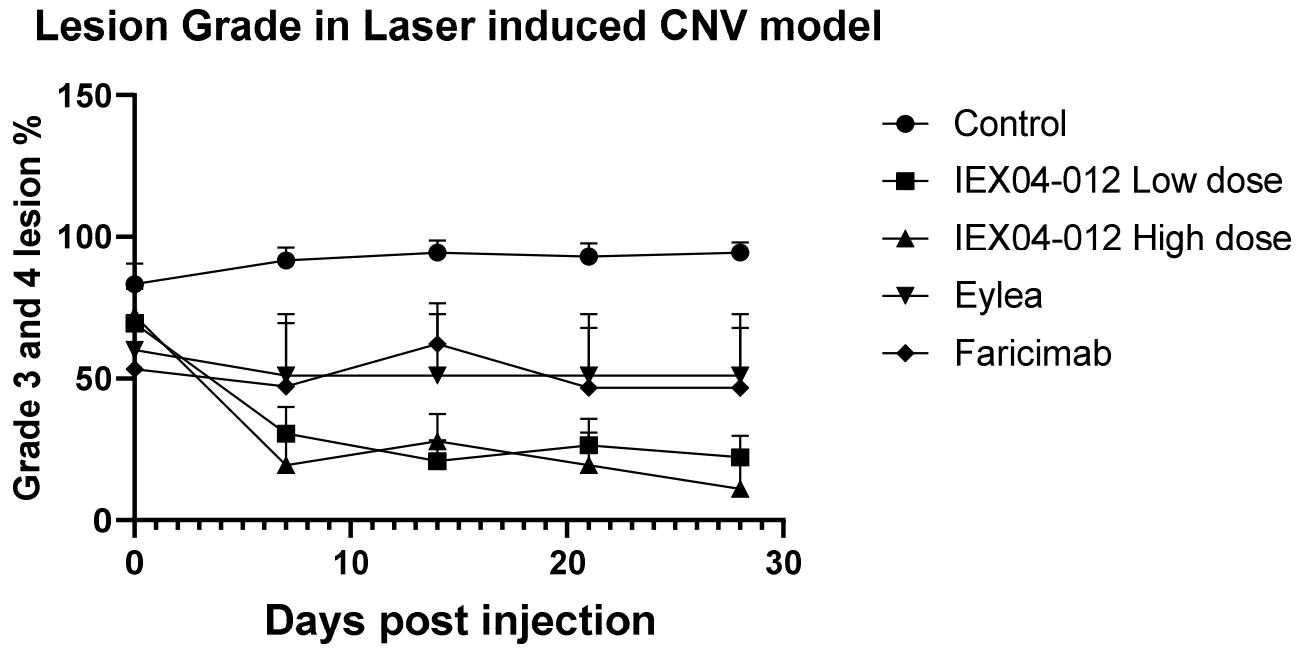


FIG. 13

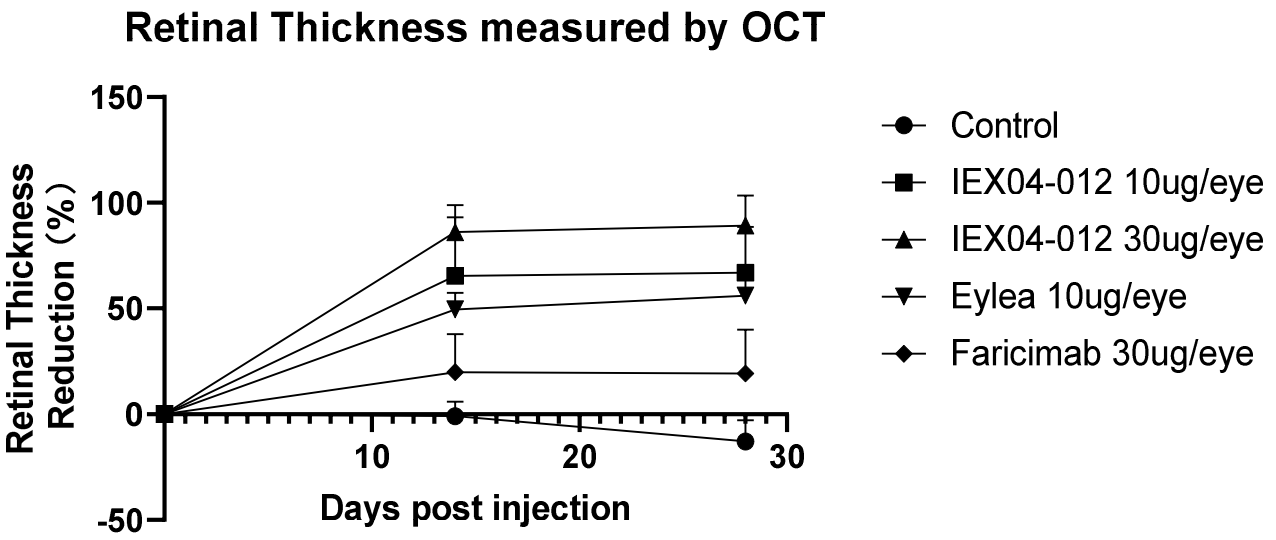

Retinal Thickness measured by OCT
Retinal Thickness Reduction (%)
150
100
50
0
-50
10
20
30
Days post injection
Control
IEX04-012 10ug/eye
IEX04-012 30ug/eye
Eylea 10ug/eye
Faricimab 30ug/eye

FIG. 14

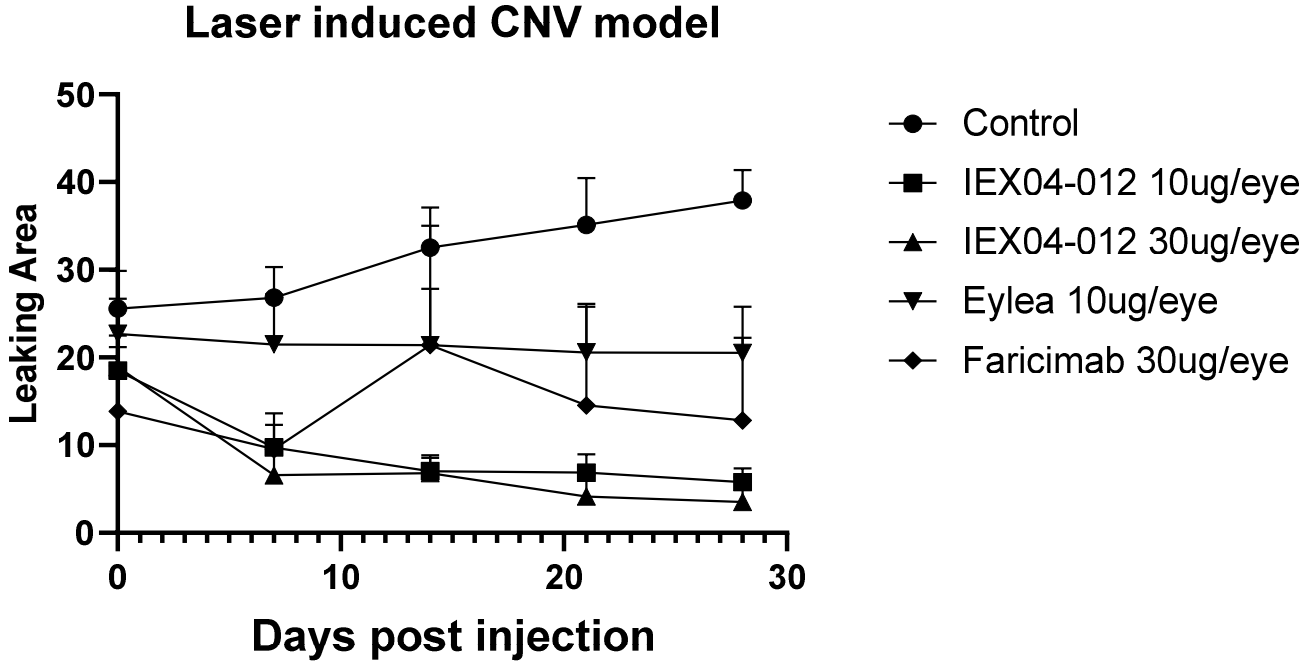
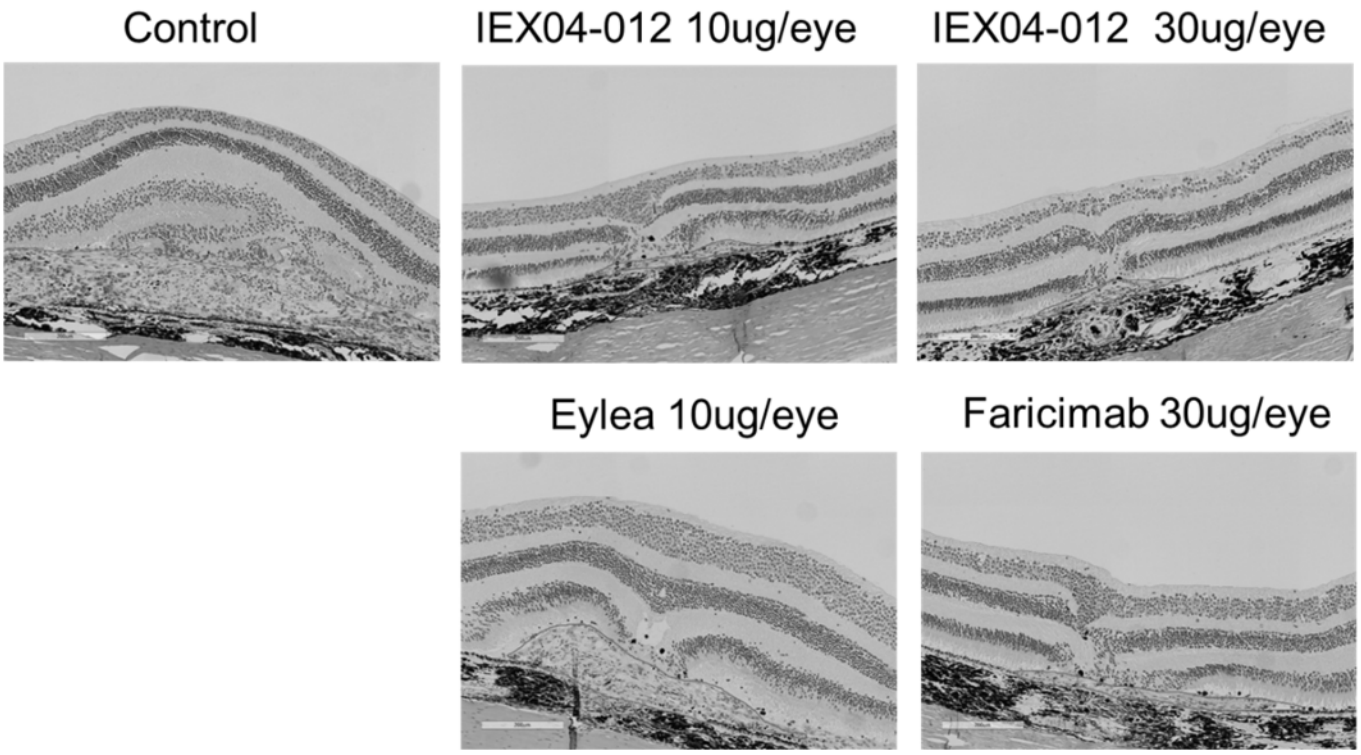

Laser induced CNV model
Leaking Area
50
40
30
20
10
0
0
10
20
30
Days post injection
Control
IEX04-012 10ug/eye
IEX04-012 30ug/eye
Eylea 10ug/eye
Faricimab 30ug/eye

FIG. 15

A


Control
IEX04-012 10ug/eye
IEX04-012 30ug/eye
Eylea 10ug/eye
Faricimab 30ug/eye

B

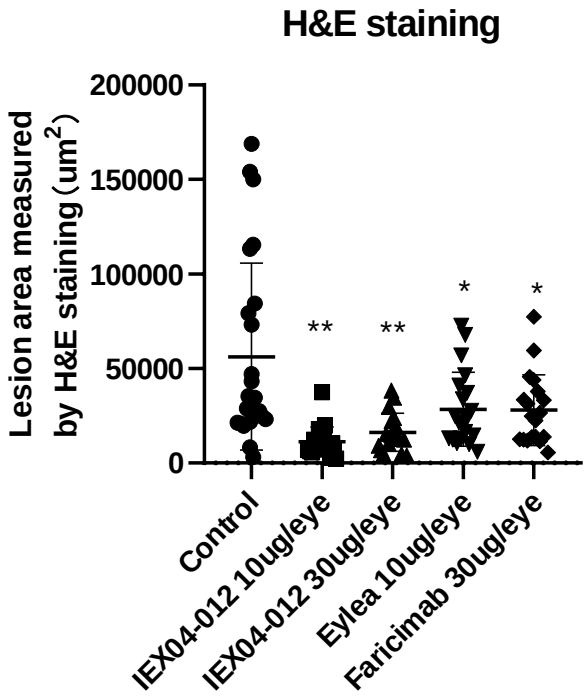
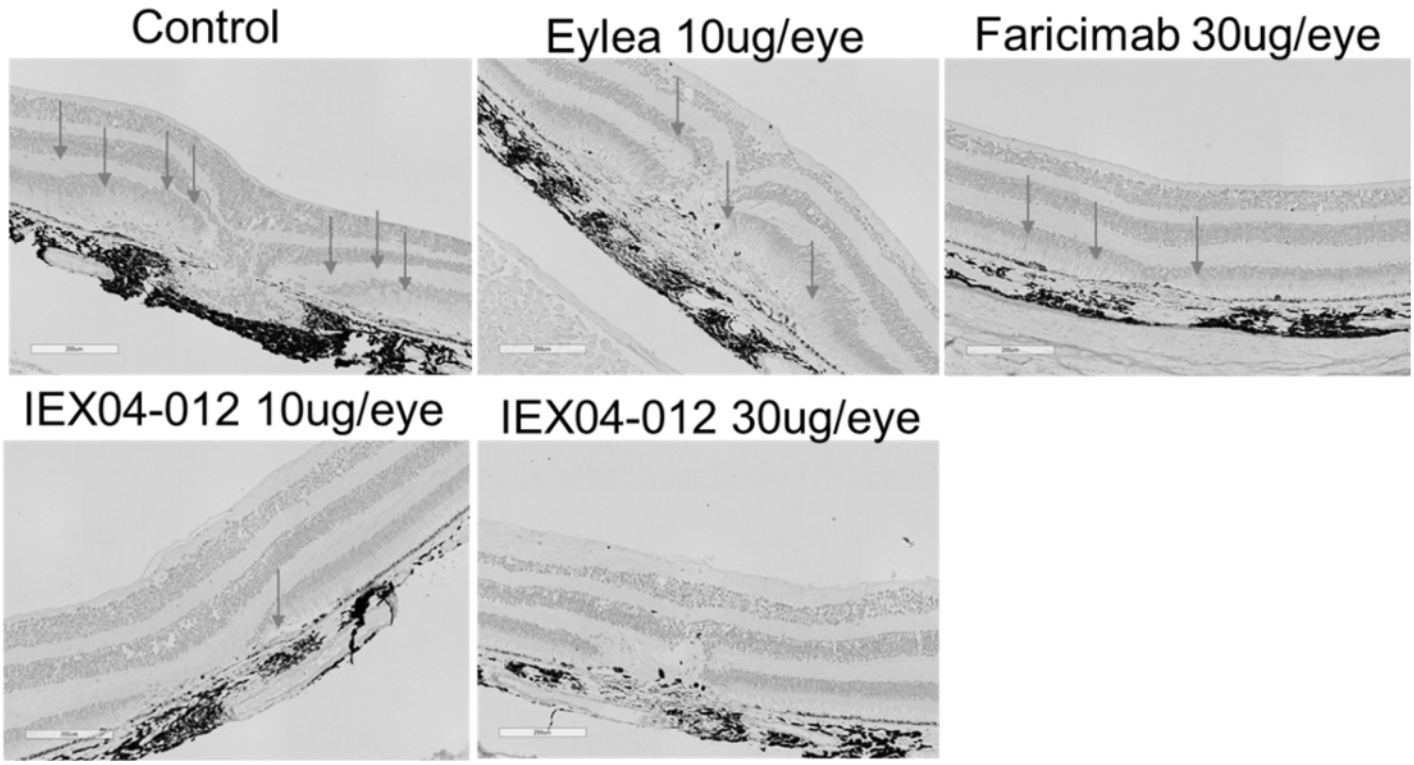

H&E staining
Lesion area measured by H&E staining (um2)
200000
150000
100000
50000
0
**
**
*
*
Control
IEX04-012 10ug/eye
IEX04-012 30ug/eye
Eylea 10ug/eye
Faricimab 30ug/eye

FIG. 16

A


Control
Eylea 10ug/eye
Faricimab 30ug/eye
IEX04-012 10ug/eye
IEX04-012 30ug/eye

B

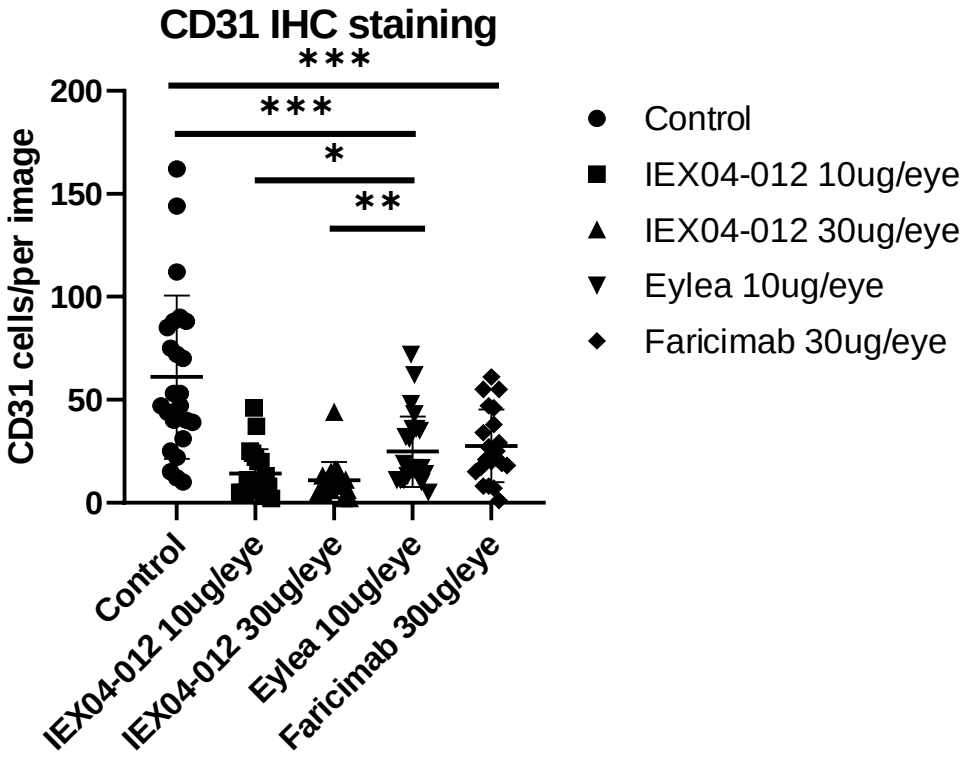

CD31 IHC staining
CD31 cells/per image
200
150
100
50
0
***
***
*
**
Control
IEX04-012 10ug/eye
IEX04-012 30ug/eye
Eylea 10ug/eye
Faricimab 30ug/eye

FIG. 17

SEQUENCE LISTING

<110> INNOVENT BIOLOGICS (SUZHOU) CO., LTD.

<120> BISPECIFIC BINDING MOLECULE BINDING TO VEGF AND ANG2 AND USE THEREOF

<130> PF 220440PCT

<150> CN202110623779.2
<151> 2021-06-04

<160> 42

<170> PatentIn version 3.3

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<220>
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1 5 10

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<211> 16
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<400> 2

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

<210> 3

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<400> 3

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1 5 10 15

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<211> 123
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<400> 4

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

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

Pro Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Val Gly Ser Thr Asn Tyr Ala Asp Ser Val Lys
50 55 60

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

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Ala Asp Pro Leu Cys Ser Ala Leu Ile Leu Pro Pro Pro Phe Leu Ser
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

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<220>
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<400> 5

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

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

Pro Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Val Gly Ser Thr Asn Tyr Ala Asp Ser Val Lys
50 55 60

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

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

Ala Asp Pro Leu Cys Ser Ala Leu Ile Leu Pro Pro Pro Phe Leu Ser

100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

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<400> 6

Gly Ser Ile Phe Ser Ile Asn Ala Met Gly
1 5 10

<210> 7
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<400> 7

Thr Met Ile Gly Gly Ser Ser Thr Phe Tyr Ala Asp Ser Val Asn Gly
1 5 10 15

<210> 8
<211> 17
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Asp Val Thr Gly Arg Ser Gly Thr Ser Ile Trp Asn Lys Arg Asp Asp
1 5 10 15

Tyr

<210> 9
<211> 125
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<220>
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<400> 9

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

Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Ser Ile Phe Ser Ile Asn
20 25 30

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

Ala Thr Met Ile Gly Gly Ser Ser Thr Phe Tyr Ala Asp Ser Val Asn
50 55 60

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

Gln Met Asn Ser Leu Lys Leu Glu Asp Thr Ala Val Tyr Tyr Cys Tyr
85 90 95

Ala Asp Val Thr Gly Arg Ser Gly Thr Ser Ile Trp Asn Lys Arg Asp
100 105 110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

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<211> 16
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<220>
<223> Synthetic construct

<400> 10

Thr Met Ile Gly Gly Ser Ser Thr Phe Tyr Ala Glu Ser Val Gln Gly
1 5 10 15

<210> 11
<211> 125
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<220>
<223> Synthetic construct

<400> 11

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe Ser Ile Asn
20 25 30

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

Ala Thr Met Ile Gly Gly Ser Ser Thr Phe Tyr Ala Glu Ser Val Gln
50 55 60

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

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

Ala Asp Val Thr Gly Arg Ser Gly Thr Ser Ile Trp Asn Lys Arg Asp
100 105 110

Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 12
<211> 125
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 12

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

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

Ser Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Val Ala Ile Ser Lys Gly Gly Tyr Lys Tyr Asp Ala Val Ser Leu Glu
50 55 60

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

Gln Ile Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Ser Ser Arg Ala Tyr Gly Ser Ser Arg Leu Arg Leu Ala Asp Thr Tyr
100 105 110

Glu Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 13
<211> 453
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 13

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

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

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

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
50 55 60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala 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 Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
100 105 110

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

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

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

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

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
180 185 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
195 200 205

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
210 215 220

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
225 230 235 240

Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ala Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
290 295 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu Ala Gln Asp Trp Leu
305 310 315 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala
325 330 335

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

Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln
355 360 365

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

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
385 390 395 400

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

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
420 425 430

Val Met His Glu Ala Leu His Asn Ala Tyr Thr Gln Lys Ser Leu Ser
435 440 445

Leu Ser Pro Gly Lys
450

<210> 14
<211> 463
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 14

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

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

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

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

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

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

Ala Arg Ser Pro Asn Pro Tyr Tyr Tyr Asp Ser Ser Gly Tyr Tyr Tyr
100 105 110

Pro Gly Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
115 120 125

Ser Ala Ser Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His
225 230 235 240

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
245 250 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ala Ser Arg Thr
260 265 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
275 280 285

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
290 295 300

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305 310 315 320

Val Leu Thr Val Leu Ala Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
325 330 335

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

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro
355 360 365

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala
370 375 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385 390 395 400

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

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

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
435 440 445

His Asn Ala Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<210> 15
<211> 387
<212> PRT
<213> Artificial

<220>
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<400> 15

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

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

Ser Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Val Ala Ile Ser Lys Gly Gly Tyr Lys Tyr Asp Ala Val Ser Leu Glu
50 55 60

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

Gln Ile Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Ser Ser Arg Ala Tyr Gly Ser Ser Arg Leu Arg Leu Ala Asp Thr Tyr
100 105 110

Glu Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly
130 135 140

Leu Val Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
145 150 155 160

Phe Thr Phe Ser Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly
165 170 175

Arg Gly Leu Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr
180 185 190

Leu Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
195 200 205

Ala Lys Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp
210 215 220

Thr Ala Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser
225 230 235 240

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
245 250 255

Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro
260 265 270

Gly Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Thr Leu Asp
275 280 285

Asp Tyr Ala Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu
290 295 300

Gly Val Ser Ala Ile Arg Ser Ser Gly Gly Ser Thr Tyr Tyr Ala Asp
305 310 315 320

Ser Val Lys Gly Arg Phe Thr Ile Ser Ser Asp Asn Ser Lys Asn Thr
325 330 335

Val Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr

340 345 350

Tyr Cys Ala Ala Val Pro Ala Gly Arg Leu Arg Tyr Gly Glu Gln Trp
355 360 365

Tyr Pro Ile Tyr Glu Tyr Asp Ala Trp Gly Gln Gly Thr Leu Val Thr
370 375 380

Val Ser Ser
385

<210> 16
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 16

Gly Phe Ala Leu Asp Tyr Tyr Ala Ile Gly
1 5 10

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

<220>
<223> Synthetic construct

<400> 17

Cys Ile Ser Ser Gly Asp Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 18
<211> 19
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 18

Asp Ser Arg Gly Asp Asp Val Ala Cys Glu Gly Leu Arg Arg Asn Glu
1 5 10 15

Tyr Asp Tyr

<210> 19
<211> 128
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 19

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

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

Ala Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Gly Asp Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

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

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Asp Ser Arg Gly Asp Asp Val Ala Cys Glu Gly Leu Arg Arg
100 105 110

Asn Glu Tyr Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 20
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 20

Cys Ile Ser Ser Gly Glu Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 21
<211> 128
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 21

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

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

Ala Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu Glu Gly Val
35 40 45

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

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val 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 Thr Asp Ser Arg Gly Asp Asp Val Ala Cys Glu Gly Leu Arg Arg
100 105 110

Asn Glu Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 22
<211> 398
<212> PRT
<213> Artificial

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<400> 22

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Ile Phe Ser Ile Asn
            20   25   30

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

Ala Thr Met Ile Gly Gly Ser Ser Thr Phe Tyr Ala Glu Ser Val Gln
    50   55   60

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

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

Ala Asp Val Thr Gly Arg Ser Gly Thr Ser Ile Trp Asn Lys Arg Asp
            100   105   110

Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly
        115   120   125

Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser Gly Gly
    130   135   140

Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser
145   150   155   160

Gly Ser Ile Phe Ser Ile Asn Ala Met Gly Trp His Arg Gln Ala Pro
                165   170   175

Gly Lys Gln Arg Glu Leu Val Ala Thr Met Ile Gly Gly Ser Ser Thr
            180   185   190

Phe Tyr Ala Glu Ser Val Gln Gly Arg Phe Thr Ile Ser Arg Asp Asn
195 200 205

Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
210 215 220

Thr Ala Val Tyr Tyr Cys Tyr Ala Asp Val Thr Gly Arg Ser Gly Thr
225 230 235 240

Ser Ile Trp Asn Lys Arg Asp Asp Tyr Trp Gly Gln Gly Thr Gln Val
245 250 255

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val
260 265 270

Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
275 280 285

Arg Leu Ser Cys Ala Ala Ser Gly Phe Ala Leu Asp Tyr Tyr Ala Ile
290 295 300

Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu Glu Gly Val Ser Cys
305 310 315 320

Ile Ser Ser Gly Glu Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly
325 330 335

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln
340 345 350

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Thr
355 360 365

Asp Ser Arg Gly Asp Asp Val Ala Cys Glu Gly Leu Arg Arg Asn Glu

370 375 380

Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
385 390 395

<210> 23
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<220>
<221> REPEAT
<222> (1)..(5)
<223> repeat 1 to 4 times

<400> 23

Gly Gly Gly Gly Ser
1 5

<210> 24
<211> 574
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 24

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
1 5 10 15

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
20 25 30

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr

```
        35                  40                  45

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
    50                  55                  60

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
65                  70                  75                  80

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
                85                  90                  95

Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile
            100                 105                 110

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

Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys
    130                 135                 140

His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly
145                 150                 155                 160

Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr
                165                 170                 175

Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met
            180                 185                 190

Thr Lys Lys Asn Ser Thr Phe Val Arg Val His Glu Lys Asp Lys Thr
        195                 200                 205

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
    210                 215                 220
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Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
225 230 235 240

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
245 250 255

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
260 265 270

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
275 280 285

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
290 295 300

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
305 310 315 320

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
325 330 335

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
340 345 350

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
355 360 365

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
370 375 380

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
385 390 395 400

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
405 410 415

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly
420 425 430

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val
435 440 445

Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
450 455 460

Arg Leu Ser Cys Ala Ala Ser Gly Phe Ala Leu Asp Tyr Tyr Ala Ile
465 470 475 480

Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu Glu Gly Val Ser Cys
485 490 495

Ile Ser Ser Gly Glu Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly
500 505 510

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln
515 520 525

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Thr
530 535 540

Asp Ser Arg Gly Asp Asp Val Ala Cys Glu Gly Leu Arg Arg Asn Glu
545 550 555 560

Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
565 570

<210> 25
<211> 431
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 25

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
1 5 10 15

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
20 25 30

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
35 40 45

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
50 55 60

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
65 70 75 80

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
85 90 95

Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile
100 105 110

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

Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys
130 135 140

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His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly
145                 150                 155                 160

Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr
                165                 170                 175

Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met
            180                 185                 190

Thr Lys Lys Asn Ser Thr Phe Val Arg Val His Glu Lys Asp Lys Thr
        195                 200                 205

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
    210                 215                 220

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
225                 230                 235                 240

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
                245                 250                 255

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
            260                 265                 270

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
        275                 280                 285

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
    290                 295                 300

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
305                 310                 315                 320
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Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
325 330 335

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
340 345 350

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
355 360 365

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
370 375 380

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
385 390 395 400

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
405 410 415

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
420 425 430

<210> 26
<211> 205
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 26

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
1 5 10 15

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
20 25 30

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
35 40 45

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
50 55 60

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
65 70 75 80

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
85 90 95

Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile
100 105 110

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

Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys
130 135 140

His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly
145 150 155 160

Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr
165 170 175

Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met
180 185 190

Thr Lys Lys Asn Ser Thr Phe Val Arg Val His Glu Lys
195 200 205

<210> 27
<211> 226
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 27

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1 5 10 15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35 40 45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130 135 140

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

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
195 200 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
210 215 220

Pro Gly
225

<210> 28
<211> 393
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 28

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

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

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

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

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

Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser
            100   105   110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
        115   120   125

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser
    130   135   140

Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr Gly
145   150   155   160

Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val Gly
                165   170   175

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe Lys
            180   185   190

Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr Leu
        195   200   205

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
    210   215   220

Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val Trp
225 230 235 240

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
245 250 255

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser
260 265 270

Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
275 280 285

Ala Ser Gly Phe Ala Leu Asp Tyr Tyr Ala Ile Gly Trp Phe Arg Gln
290 295 300

Ala Pro Gly Lys Gly Leu Glu Gly Val Ser Cys Ile Ser Ser Gly Glu
305 310 315 320

Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
325 330 335

Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg
340 345 350

Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Thr Asp Ser Arg Gly Asp
355 360 365

Asp Val Ala Cys Glu Gly Leu Arg Arg Asn Glu Tyr Asp Tyr Trp Gly
370 375 380

Gln Gly Thr Leu Val Thr Val Ser Ser
385 390

<210> 29
<211> 250
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 29

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

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

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

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

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

Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser
100 105 110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
115 120 125

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser
130 135 140

Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Asp Phe Thr His Tyr Gly
145 150 155 160

Met Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val Gly
165 170 175

Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe Lys
180 185 190

Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr Leu
195 200 205

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
210 215 220

Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val Trp
225 230 235 240

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
245 250

<210> 30
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 30

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

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

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

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

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

Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 31
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 31

Gln Asp Ile Ser Asn Tyr Leu Asn
1   5

<210> 32
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 32

Phe Thr Ser Ser Leu His Ser
1 5

<210> 33
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 33

Gln Gln Tyr Ser Thr Val Pro Trp Thr
1 5

<210> 34
<211> 123
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 34

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

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

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

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Ala Asp Phe
50 55 60

Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala 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 Lys Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
100 105 110

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

<210> 35
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 35

Gly Tyr Asp Phe Thr His Tyr
1 5

<210> 36
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 36

Asn Thr Tyr Thr Gly Glu
1 5

<210> 37

<211> 14
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 37

Tyr Pro Tyr Tyr Tyr Gly Thr Ser His Trp Tyr Phe Asp Val
1 5 10

<210> 38
<211> 449
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 38

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

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

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

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Thr Ser Arg Asp Asp Ser Lys Asn Ala 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 Gly Gly Pro Gly Trp Tyr Ala Ala Asp Val Trp Gly Gln Gly
100 105 110

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

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

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

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

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

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

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

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

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

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

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

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

Lys

<210> 39
<211> 125
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 39

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

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

Ser Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Val Ala Ile Ser Lys Gly Gly Tyr Lys Tyr Asp Ala Val Ser Leu Glu
50 55 60

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

Gln Ile Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Ser Ser Arg Ala Tyr Gly Ser Ser Arg Leu Arg Leu Ala Asp Thr Tyr
100 105 110

Glu Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> 40
<211> 214
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 40

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

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

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

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

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

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

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

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

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 41
<211> 213
<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 41

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

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

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

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser

50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Arg Val Glu Ala Gly
65 70 75 80

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

Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala Ser
100 105 110

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

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
130 135 140

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
145 150 155 160

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

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
180 185 190

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
195 200 205

Glu Pro Lys Ser Cys
210

<210> 42
<211> 214

<212> PRT
<213> Artificial

<220>
<223> Synthetic construct

<400> 42

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

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

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

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

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

Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

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

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

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

145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210