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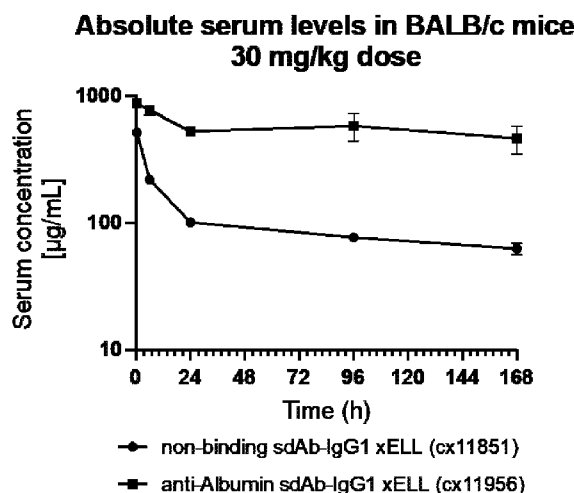


FIG. 9A

(57) Abstract: Provided herein are VHH-containing polypeptides that bind albumin. Uses of the VHH-containing polypeptides are also provided.

ALBUMIN-BINDING POLYPEPTIDES AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of priority of US Provisional Application No. 63/338,629, filed May 5, 2022, and US Provisional Application No. 63/351,362, filed June 11, 2022; each of which is incorporated by reference herein in its entirety for any purpose.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] This application incorporates by reference a Sequence Listing submitted with this application in electronic format entitled 01202-0020-00PCT_ST26, created April 27, 2023, which is 133,294 bytes in size.

FIELD

[0003] The present invention relates to albumin-binding polypeptides, and methods of using albumin-binding polypeptides, for example, to improve the half-life of other molecules.

BACKGROUND

[0004] Plasma proteins are eliminated from circulation by two primary mechanisms: renal filtration of molecules below 60 kDa, and micropinocytosis by endothelial cells. Proteins below the renal threshold are rapidly cleared from circulation resulting in a half-life of 1 day or less, while proteins larger than the renal threshold are primarily cleared through micropinocytosis with half-lives around 3-5 days. Albumin and immunoglobulin G (IgG) are proteins with long plasma half-lives of around 15-30 days due to their large size (66 and 150 kDa respectively) and their ability to recycle from endothelial micropinocytosis through pH dependent binding to the neonatal Fc receptor (FcRn).

[0005] Long plasma half-life is useful for therapeutic agents to accurately and precisely control plasma drug concentrations, optimize efficacy while limiting toxicity, and reduce the frequency and amount of drug needed. Therapeutic proteins with short plasma half-lives due to small size or that lack the ability to recycle through FcRn binding can be fused to an albumin binding entity to prolong plasma half-life. Single-domain antibody VHH domains are ideal albumin binding entities because they are small domains about 12-15 kDa in size, they are single domains composed of a single polypeptide that can be easily fused to another protein or peptide by recombinant means, they are readily humanized to reduce the potential for immunogenicity, and many naturally bind protein A for affinity purification.

[0006] Therefore, there exists a need for VHH domains that bind albumin.

SUMMARY

Embodiment 1. A polypeptide comprising at least one VHH domain that binds albumin, wherein at least one VHH domain that binds albumin comprises a CDR1 sequence selected from SEQ ID NOs: 5-8, a CDR2 sequence selected from SEQ ID NOs: 9-21, and a CDR3 sequence of SEQ ID NO: 22.

Embodiment 2. The polypeptide of embodiment 1, wherein each VHH domain that binds albumin comprises, independently, a CDR1 sequence selected from SEQ ID NOs: 5-8, a CDR2 sequence selected from SEQ ID NOs: 9-21, and a CDR3 sequence of SEQ ID NO: 22.

Embodiment 3. The polypeptide of embodiment 1 or embodiment 2, wherein at least one VHH domain that binds albumin comprises CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 5, 9, and 22; SEQ ID NOs: 5, 10, and 22; SEQ ID NOs: 5, 11, and 22; SEQ ID NOs: 5, 12, and 22; SEQ ID NOs: 5, 13, and 22; SEQ ID NOs: 5, 14, and 22; SEQ ID NOs: 5, 15, and 22; SEQ ID NOs: 6, 15, and 22; SEQ ID NOs: 7, 15, and 22; SEQ ID NOs: 8, 15, and 22; SEQ ID NOs: 6, 16, and 22; SEQ ID NOs: 6, 17, and 22; SEQ ID NOs: 6, 18, and 22; SEQ ID NOs: 6, 19, and 22; SEQ ID NOs: 6, 20, and 22; and SEQ ID NOs: 6, 21, and 22.

Embodiment 4. The polypeptide of embodiment 3, wherein each VHH domain that binds albumin comprises, independently, CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 5, 9, and 22; SEQ ID NOs: 5, 10, and 22; SEQ ID NOs: 5, 11, and 22; SEQ ID NOs: 5, 12, and 22; SEQ ID NOs: 5, 13, and 22; SEQ ID NOs: 5, 14, and 22; SEQ ID NOs: 5, 15, and 22; SEQ ID NOs: 6, 15, and 22; SEQ ID NOs: 7, 15, and 22; SEQ ID NOs: 8, 15, and 22; SEQ ID NOs: 6, 16, and 22; SEQ ID NOs: 6, 17, and 22; SEQ ID NOs: 6, 18, and 22; SEQ ID NOs: 6, 19, and 22; SEQ ID NOs: 6, 20, and 22; and SEQ ID NOs: 6, 21, and 22.

Embodiment 5. The polypeptide of any one of embodiments 1-4, wherein at least one VHH domain that binds albumin is humanized.

Embodiment 6. The polypeptide of embodiment 5, wherein each VHH domain that binds albumin is humanized.

Embodiment 7. The polypeptide of any one of embodiments 1-6, wherein at least one VHH domain that binds albumin comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 23-43 and 71-74.

Embodiment 8. The polypeptide of embodiment 7, wherein each VHH domain that binds albumin comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 23-43 and 71-74.

Embodiment 9. The polypeptide of any one of embodiments 1-7, wherein at least one VHH domain that binds albumin comprises a sequence selected from SEQ ID NOs: 23-43 and 71-74.

Embodiment 10. The polypeptide of any one of embodiments 1-9, wherein each VHH domain that binds albumin comprises a sequence selected from SEQ ID NOs: 23-43 and 71-74.

Embodiment 11. The polypeptide of any one of embodiments 1-10, wherein at least one VHH domain that binds albumin binds human albumin and at least one albumin selected from cynomolgus monkey, mouse, and rat albumin.

Embodiment 12. The polypeptide of any one of embodiments 1-11, wherein each VHH domain that binds albumin binds human albumin and at least one albumin selected from cynomolgus monkey, mouse, and rat albumin.

Embodiment 13. The polypeptide of any one of embodiments 1-12, wherein at least one VHH domain that binds albumin binds human, cynomolgus monkey, mouse, and rat albumin.

Embodiment 14. The polypeptide of any one of embodiments 1-13, wherein each VHH domain that binds albumin binds human, cynomolgus monkey, mouse, and rat albumin.

Embodiment 15. The polypeptide of any one of embodiments 1-14, wherein at least one VHH domain that binds albumin binds human albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Embodiment 16. The polypeptide of any one of embodiments 1-15, wherein at least one VHH domain that binds albumin binds each of human, cynomolgus monkey, mouse, and rat albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Embodiment 17. The polypeptide of any one of embodiments 1-16, wherein each VHH domain that binds albumin binds human albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Embodiment 18. The polypeptide of any one of embodiments 1-17, wherein each VHH domain that binds albumin binds each of human, cynomolgus monkey, mouse, and rat albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.

Embodiment 19. The polypeptide of any one of embodiments 1-18, wherein the each VHH domain that binds albumin does not bind albumin domain 3.

Embodiment 20. The polypeptide of any one of embodiments 1-19, wherein the each VHH domain that binds albumin does not interfere with binding of albumin to FcRn.

Embodiment 21. The polypeptide of any one of embodiments 1-20, wherein the polypeptide comprises at least one binding domain that binds a protein other than albumin.

Embodiment 22. The polypeptide of embodiment 21, wherein at least one binding domain that binds a protein other than albumin is a VHH.

Embodiment 23. The polypeptide of embodiment 22, wherein each binding domain that binds a protein other than albumin is a VHH.

Embodiment 24. The polypeptide of embodiment 21, wherein at least one binding domain that binds a protein other than albumin comprises a heavy chain variable region and a light chain variable region.

Embodiment 25. The polypeptide of embodiment 24, wherein each binding domain that binds a protein other than albumin comprises a heavy chain variable region and a light chain variable region.

Embodiment 26. The polypeptide of any one of embodiments 21-25, wherein at least one binding domain that binds a protein other than albumin is a binding domain of a therapeutic antibody.

Embodiment 27. The polypeptide of embodiment 26, wherein each binding domain that binds a protein other than albumin is a binding domain of a therapeutic antibody.

Embodiment 28. The polypeptide of embodiment 26 or embodiment 27, wherein the therapeutic antibody is useful for treating a disease or disorder selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.

Embodiment 29. The polypeptide of any one of embodiment 1-28, wherein the polypeptide comprises an amino acid sequence of a therapeutic protein.

Embodiment 30. The polypeptide of embodiment 29, wherein the therapeutic protein is useful for treating a disease or disorder selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.

Embodiment 31. The polypeptide of any one of embodiments 1-30, wherein the polypeptide comprises an Fc region.

Embodiment 32. The polypeptide of embodiment 31, wherein the Fc region binds FcRn.

Embodiment 33. The polypeptide of embodiment 31 or embodiment 32, wherein the Fc region is an IgG1 Fc region.

Embodiment 34. The polypeptide of any one of embodiments 31-33, wherein the Fc region comprises one or more substitutions that enhance half-life.

Embodiment 35. The polypeptide of embodiment 34, wherein the Fc region comprises one or more substitutions that enhance FcRn binding at at least one pH and/or reduce dissociation rate of Fc and FcRn.

Embodiment 36. The polypeptide of any one of embodiments 31-35, wherein the Fc region comprises substitutions at one or more amino acid positions selected from 252, 254, 256, 428, or 434.

Embodiment 37. The polypeptide of embodiment 36, wherein the Fc region comprises substitutions at amino acid positions 252, 254, and 256; or amino acid positions 252 and 428; or amino acid positions 428 and 434.

Embodiment 38. The polypeptide of embodiment 37, wherein the Fc region comprises substitutions M252Y, S254T, and T256E; M252Y and M428V; or M428L and N434S.

Embodiment 39. The polypeptide of any one of embodiments 31-38, wherein the Fc region comprises a sequence selected from SEQ ID NOs: 47-68 and 85-87.

Embodiment 40. The polypeptide of any one of embodiments 1-39, wherein the half-life of the polypeptide is greater than the half-life of the same polypeptide lacking a VHH domain that binds albumin.

Embodiment 41. A pharmaceutical composition comprising the polypeptide of any one of embodiments 1-40 and a pharmaceutically acceptable carrier.

Embodiment 42. An isolated nucleic acid that encodes the polypeptide of any one of embodiments 1-40.

Embodiment 43. A vector comprising the nucleic acid of embodiment 42.

Embodiment 44. A host cell comprising the nucleic acid of embodiment 42 or the vector of embodiment 43.

Embodiment 45. A host cell that expresses the polypeptide of any one of embodiments 1-40.

Embodiment 46. A method of producing the polypeptide of any one of embodiments 1-40, comprising incubating the host cell of embodiment 44 or embodiment 45 under conditions suitable for expression of the polypeptide.

Embodiment 47. The method of embodiment 46, further comprising isolating the polypeptide.

Embodiment 48. A method comprising administering to a subject the polypeptide of any one of embodiments 1-40, or the pharmaceutical composition of embodiment 41.

Embodiment 49. A method of treating a disease or disorder comprising administering to a subject with the disease or disorder a pharmaceutically effective amount of the polypeptide of any one of embodiments 1-40, or the pharmaceutical composition of embodiment 41.

Embodiment 50. The method of embodiment 49, wherein the disease or disorder is selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.

BRIEF DESCRIPTION OF THE FIGURES

[0007] FIG. 1A-1C. FIG. 1A is a schematic of a monomeric single-domain antibody that binds albumin comprising an Fc with S364N, Y407N, and K409T mutations (sdAb-NNT-Fc). FIG. 1B-1C shows monovalent binding of 4A01-NNT-hFc (SEQ ID NOs: 23 and 68) to human serum albumin (HSA; 1B) and murine serum albumin (MSA; 1C).

[0008] FIG. 2A-2B. FIG. 2A is a schematic showing the experimental design for biolayer interferometry assessing binding to albumin domain 3. FIG. 2B shows a biolayer interferometry trace showing 4A01-NNT-hFc (SEQ ID NOs: 23 and 68) does not bind to albumin domain 3, while 1C04 does.

[0009] FIG. 3A-3B. FIG. 3A is a schematic showing the experimental design for biolayer interferometry assessing B2M-FcRn binding to albumin. FIG. 3B is a biolayer interferometry trace showing 4A01-NNT-hFc (SEQ ID NOs: 23 and 68) and humanized 4A01v51-NNT-hFc do not block B2m-FcRn binding to albumin, while 1C04 does.

[0010] FIG. 4A-4I. FIG. 4A shows an alignment of certain 4A01 humanized variants (SEQ ID NOs: 24-43). FIG. 4B-4C show human albumin (HSA) binding ELISA of certain 4A01 humanized variants. FIG. 4D-4E show cynomolgus monkey albumin (CSA) binding ELISA of certain 4A01 humanized variants. FIG. 4F-4G show murine albumin (MSA) binding ELISA of certain 4A01 humanized variants. FIG. 4H-4I show rat albumin (RSA) binding ELISA of certain 4A01 humanized variants.

[0011] FIG. 5A-5D show human albumin (HSA; FIG. 5A), cynomolgus monkey albumin (CSA; FIG. 5B), murine albumin (MSA; FIG. 5C), and rat albumin (RSA; FIG. 5D) binding ELISA of humanized hz4A01v51.

[0012] FIG. 6 shows various monospecific and multispecific single-domain antibody formats. Format (i) comprises two albumin-specific VHH domains (black) formatted as VHH-hIgG Fc (hIgG Fc is shown in grey). Format (ii) is a monovalent, albumin-specific VHH domain (black) formatted as VHH-hIgG -NNT-Fc (monomeric Fc, grey). Format (iii) is a bispecific polypeptide consisting of an albumin-binding VHH domain (black) and a non-albumin targeted VHH domain (grey) connected via glycine-serine linker. Format (iv) comprises two albumin-specific VHH domains (black) formatted as hIgG1 Fc-VHH (hIgG Fc is shown in grey). Format (v) is a bispecific polypeptide, bivalent for each target, comprising albumin-specific VHH domains (black) fused to the C-terminus of the heavy chain of a non-albumin antibody

(comprising a light chain and a heavy chain shown in grey), the heavy chain of such polypeptides may be formatted as VH-CH1-hIgG Fc-VHH. Format (vi) is a bispecific polypeptide, bivalent for each target, comprising albumin-specific VHH domains (black) fused to the N-terminus of the heavy chain of a non-albumin antibody (comprising a light chain and a heavy chain shown in grey), the heavy chain of such polypeptides may be formatted as VHH-VH-CH1-hIgG Fc. Format (vii) is a bispecific polypeptide, bivalent for each target, comprising albumin-specific VHH domains (black) fused to the between the CH1 and Fc region of a non-albumin antibody (comprising a light chain and a heavy chain shown in grey), the heavy chain of such polypeptides may be formatted as VH-CH1-VHH-hIgG Fc. Format (viii) is a bispecific polypeptide, bivalent for each target, comprising albumin-specific VHH domains (black) fused to the N-terminus of the light chain of a non-albumin antibody (comprising a light chain and a heavy chain shown in grey), the light chain of such polypeptides may be formatted as VHH-VL-CL. Format (ix) is a bispecific polypeptide, bivalent for each target, comprising albumin-specific VHH domains (black) fused to the C-terminus of the light chain of a non-albumin antibody (comprising a light chain and a heavy chain shown in grey), the light chain of such polypeptides may be formatted as VL-CL-VHH.

[0013] FIG. 7A-7B shows binding of albumin-binding and non-albumin binding sdAb polypeptides to human albumin by ELISA. FIG. 7A shows binding at pH 7.4, while FIG. 7B shows binding at pH 6. Constructs in FIG. 7A-7B are formatted as described in FIG. 6(iii).

[0014] FIG. 8 shows binding of a monovalent sdAb polypeptide (cx5009) to albumin of human, cynomolgus, mouse, or rat by ELISA at pH 7.4. Constructs in FIG. 9 are formatted as described in FIG. 6(ii).

[0015] FIG. 9A-9D shows the *in vivo* pharmacokinetic (PK) profile of an albumin-binding bivalent VHH-hIgG1-xELL-Fc polypeptide (cx11956) compared to a non-targeted bivalent VHH-hIgG1-xELL-Fc polypeptide (cx11851). FIG. 9A and FIG. 9B show the serum PK profile in BALB/c mice dosed with 30 mg/kg of the test articles as determined by ELISA, while FIG. 9C and FIG. 9D show the serum PK profile in mice dosed with 0.3 mg/kg of the test articles. FIG. 9A and FIG. 9C show the absolute serum concentration in µg/mL, while FIG. 9B and FIG. 9D show the data normalized to the concentration 30 min post dosing (cMax). Constructs in FIG. 9A-9D are formatted as described in FIG. 6(i).

[0016] FIG. 10 shows binding of human IgG1 and human IgG1-xELL to recombinant biotinylated human FcγR1 (FcRn)/B2M heterodimers by Bio-Layer Interferometry (BLI). Association and dissociation to FcRn was detected at pH 6 and pH 8.

[0017] FIG. 11A-11B show binding of different monospecific and bispecific albumin-binding single domain antibodies formats to bind recombinant human albumin at neutral (7.4) pH (FIG.

11A), and IL-4R (FIG. 11B) by ELISA. Monospecific molecules (cx12583 and cx12584) are formatted as described in FIG. 6(iv), bispecific molecules are formatted as described in FIG. 6(v) (cx12587 and cx12594) or FIG. 6(vii) (cx12595 and cx12596).

[0018] FIG. 12A-12D show human albumin (HSA) binding ELISA of certain 4A01 humanized variants formatted as monovalent VHH-hIgG1-NNT-Fc polypeptides (FIG. 11A-11B) and bivalent VHH-hIgG1-xELL-Fc polypeptides (FIG. 12C-12D) at pH 6 (FIG. 12A and FIG. 12C) and pH 7.4 (FIG. 12B and 12D).

DETAILED DESCRIPTION

[0019] Embodiments provided herein relate to albumin-binding polypeptides and uses thereof.

Definitions and Various Embodiments

[0020] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0021] All references cited herein, including patent applications, patent publications, and Genbank Accession numbers are herein incorporated by reference, as if each individual reference were specifically and individually indicated to be incorporated by reference in its entirety.

[0022] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, *et al. eds.*, (2003)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor *eds.* (1995)), Harlow and Lane, *eds.* (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R. I. Freshney, *ed.* (1987)); Oligonucleotide Synthesis (M. J. Gait, *ed.*, 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, *ed.*, 1998) Academic Press; Animal Cell Culture (R. I. Freshney, *ed.*, 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, *eds.*, 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, *eds.*); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, *eds.*, 1987); PCR: The Polymerase Chain Reaction, (Mullis *et al.*, *eds.*, 1994); Current Protocols

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[0023] Unless otherwise defined, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context or expressly indicated, singular terms shall include pluralities and plural terms shall include the singular. For any conflict in definitions between various sources or references, the definition provided herein will control.

[0024] In general, the numbering of the residues in an immunoglobulin heavy chain constant region is that of the EU index as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody.

[0025] It is understood that embodiments of the invention described herein include “consisting” and/or “consisting essentially of” embodiments. As used herein, the singular form “a”, “an”, and “the” includes plural references unless indicated otherwise. Use of the term “or” herein is not meant to imply that alternatives are mutually exclusive.

[0026] In this application, the use of “or” means “and/or” unless expressly stated or understood by one skilled in the art. In the context of a multiple dependent claim, the use of “or” refers back to more than one preceding independent or dependent claim.

[0027] The phrase “reference sample”, “reference cell”, or “reference tissue”, denote a sample with at least one known characteristic that can be used as a comparison to a sample with at least one unknown characteristic. In some embodiments, a reference sample can be used as a positive or negative indicator. A reference sample can be used to establish a level of protein and/or mRNA that is present in, for example, healthy tissue, in contrast to a level of protein and/or mRNA present in the sample with unknown characteristics. In some embodiments, the reference sample comes from the same subject, but is from a different part of the subject than that being tested. In some embodiments, the reference sample is from a tissue area surrounding or adjacent to the cancer. In some embodiments, the reference sample is not from the subject being tested, but is a sample from a subject known to have, or not to have, a disorder in question. In some embodiments, the reference sample is from the same subject, but from a point in time

before the subject developed cancer. In some embodiments, the reference sample is from a benign cancer sample, from the same or a different subject. When a negative reference sample is used for comparison, the level of expression or amount of the molecule in question in the negative reference sample will indicate a level at which one of skill in the art will appreciate, given the present disclosure, that there is no and/or a low level of the molecule. When a positive reference sample is used for comparison, the level of expression or amount of the molecule in question in the positive reference sample will indicate a level at which one of skill in the art will appreciate, given the present disclosure, that there is a level of the molecule.

[0028] The terms “benefit”, “clinical benefit”, “responsiveness”, and “therapeutic responsiveness” as used herein in the context of benefiting from or responding to administration of a therapeutic agent, can be measured by assessing various endpoints, *e.g.*, inhibition, to some extent, of disease progression, including slowing down and complete arrest; reduction in the number of disease episodes and/or symptoms; reduction in lesion size; inhibition (that is, reduction, slowing down or complete stopping) of disease cell infiltration into adjacent peripheral organs and/or tissues; inhibition (that is, reduction, slowing down or complete stopping) of disease spread; relief, to some extent, of one or more symptoms associated with the disorder; increase in the length of disease-free presentation following treatment, for example, progression-free survival; increased overall survival; higher response rate; and/or decreased mortality at a given point of time following treatment. A subject or cancer that is “non-responsive” or “fails to respond” is one that has failed to meet the above noted qualifications to be “responsive”.

[0029] The terms “nucleic acid molecule”, “nucleic acid” and “polynucleotide” may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. “Nucleic acid sequence” refers to the linear sequence of nucleotides comprised in the nucleic acid molecule or polynucleotide.

[0030] The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present disclosure, a “polypeptide” refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein

maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0031] “Albumin” as used herein refers to any native, mature albumin that results from processing of an albumin precursor in a cell. The term includes albumin from any vertebrate source, including mammals such as primates (*e.g.*, humans and cynomolgus or rhesus monkeys) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term also includes naturally-occurring variants of albumin, such as splice variants or allelic variants. A nonlimiting exemplary mature human albumin amino acid sequence is shown, *e.g.*, in UniProt Accession No. P02768.2. *See* SEQ ID NO. 1. Nonlimiting exemplary murine, cynomolgus monkey, and rat albumin amino acid sequences are shown in SEQ ID NOs: 2-4.

[0032] The term “specifically binds” to an antigen or epitope is a term that is well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit “specific binding” or “preferential binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. A single-domain antibody (sdAb) or VHH-containing polypeptide “specifically binds” or “preferentially binds” to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, a sdAb or VHH-containing polypeptide that specifically or preferentially binds to an albumin epitope is a sdAb or VHH-containing polypeptide that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other albumin epitopes or non-albumin epitopes. It is also understood by reading this definition that; for example, a sdAb or VHH-containing polypeptide that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding. “Specificity” refers to the ability of a binding protein to selectively bind an antigen.

[0033] As used herein, the term “inhibit” with regard to the activity of a target protein refers to a decrease in the activity of the protein. In some embodiments, “inhibit” refers to a decrease in activity compared to the protein in the absence of the modulator.

[0034] As used herein, the term “epitope” refers to a site on a target molecule (for example, an antigen, such as a protein, nucleic acid, carbohydrate or lipid) to which an antigen-binding molecule (for example, a sdAb or VHH-containing polypeptide) binds. Epitopes often include a chemically active surface grouping of molecules such as amino acids, polypeptides or sugar side

chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. Epitopes can be formed both from contiguous and/or juxtaposed noncontiguous residues (for example, amino acids, nucleotides, sugars, lipid moiety) of the target molecule. Epitopes formed from contiguous residues (for example, amino acids, nucleotides, sugars, lipid moiety) typically are retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding typically are lost on treatment with denaturing solvents. An epitope may include but is not limited to at least 3, at least 5 or 8-10 residues (for example, amino acids or nucleotides). In some embodiments, an epitope is less than 20 residues (for example, amino acids or nucleotides) in length, less than 15 residues or less than 12 residues. Two antibodies may bind the same epitope within an antigen if they exhibit competitive binding for the antigen. In some embodiments, an epitope can be identified by a certain minimal distance to a CDR residue on the antigen-binding molecule. In some embodiments, an epitope can be identified by the above distance, and further limited to those residues involved in a bond (for example, a hydrogen bond) between a residue of the antigen-binding molecule and an antigen residue. An epitope can be identified by various scans as well, for example an alanine or arginine scan can indicate one or more residues that the antigen-binding molecule can interact with. Unless explicitly denoted, a set of residues as an epitope does not exclude other residues from being part of the epitope for a particular antigen-binding molecule. Rather, the presence of such a set designates a minimal series (or set of species) of epitopes. Thus, in some embodiments, a set of residues identified as an epitope designates a minimal epitope of relevance for the antigen, rather than an exclusive list of residues for an epitope on an antigen.

[0035] A “nonlinear epitope” or “conformational epitope” comprises noncontiguous polypeptides, amino acids and/or sugars within the antigenic protein to which an antigen-binding molecule specific to the epitope binds. In some embodiments, at least one of the residues will be noncontiguous with the other noted residues of the epitope; however, one or more of the residues can also be contiguous with the other residues.

[0036] A “linear epitope” comprises contiguous polypeptides, amino acids and/or sugars within the antigenic protein to which an antigen-binding molecule specific to the epitope binds. It is noted that, in some embodiments, not every one of the residues within the linear epitope need be directly bound (or involved in a bond) by the antigen-binding molecule. In some embodiments, linear epitopes can be from immunizations with a peptide that effectively consisted of the sequence of the linear epitope, or from structural sections of a protein that are relatively isolated from the remainder of the protein (such that the antigen-binding molecule can interact, at least primarily), just with that sequence section.

[0037] The term “antibody” is used in the broadest sense and encompass various polypeptides that comprise antibody-like antigen-binding domains, including but not limited to conventional antibodies (typically comprising at least one heavy chain and at least one light chain) and fragments thereof (*e.g.*, scFv, Fab), single-domain antibodies (sdAbs, comprising at least one VHH domain and an Fc region), VHH-containing polypeptides (polypeptides comprising at least one VHH domain), and fragments of any of the foregoing so long as they exhibit the desired antigen-binding activity. In some embodiments, an antibody comprises a dimerization domain. Such dimerization domains include, but are not limited to, heavy chain constant domains (comprising CH1, hinge, CH2, and CH3, where CH1 typically pairs with a light chain constant domain, CL, while the hinge mediates dimerization) and Fc regions (comprising hinge, CH2, and CH3, where the hinge mediates dimerization).

[0038] The term antibody also includes, but is not limited to, chimeric antibodies, humanized antibodies, and antibodies of various species such as camelid (including llama), shark, mouse, human, cynomolgus monkey, *etc.*

[0039] The term “antigen-binding domain” as used herein refers to a portion of an antibody sufficient to bind antigen. In some embodiments, an antigen binding domain of a conventional antibody comprises three heavy chain CDRs and three light chain CDRs. Thus, in some embodiments, an antigen binding domain comprises a heavy chain variable region comprising CDR1-FR2-CDR2-FR3-CDR3, and any portions of FR1 and/or FR4 required to maintain binding to antigen, and a light chain variable region comprising CDR1-FR2-CDR2-FR3-CDR3, and any portions of FR1 and/or FR4 required to maintain binding to antigen. In some embodiments, an antigen-binding domain of an sdAb or VHH-containing polypeptide comprises three CDRs of a VHH domain. Thus, in some embodiments, an antigen binding domain of an sdAb or VHH-containing polypeptide comprises a VHH domain comprising CDR1-FR2-CDR2-FR3-CDR3, and any portions of FR1 and/or FR4 required to maintain binding to antigen.

[0040] The term “VHH” or “VHH domain” or “VHH antigen-binding domain” as used herein refers to the antigen-binding portion of a single-domain antibody, such as a camelid antibody or shark antibody. In some embodiments, a VHH comprises three CDRs and four framework regions, designated FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. In some embodiments, a VHH may be truncated at the N-terminus or C-terminus such that it comprises only a partial FR1 and/or FR4, or lacks one or both of those framework regions, so long as the VHH substantially maintains antigen binding and specificity.

[0041] The terms “single domain antibody” and “sdAb” are used interchangeably herein to refer to an antibody comprising at least one monomeric domain, such as a VHH domain, without a light chain, and an Fc region. In some embodiments, an sdAb is a dimer of two polypeptides

wherein each polypeptide comprises at least one VHH domain and an Fc region. As used herein, the terms “single domain antibody” and “sdAb” encompass polypeptides that comprise multiple VHH domains, such as a polypeptide having the structure VHH₁-VHH₂-Fc or VHH₁-VHH₂-VHH₃-Fc, wherein VHH₁, VHH₂, and VHH₃ may be the same or different.

[0042] The term “VHH-containing polypeptide” refers to a polypeptide that comprises at least one VHH domain. In some embodiments, a VHH polypeptide comprises two, three, or four or more VHH domains, wherein each VHH domain may be the same or different. In some embodiments, a VHH-containing polypeptide comprises an Fc region. In some such embodiments, the VHH-containing polypeptide may be referred to as an sdAb. Further, in some such embodiments, the VHH polypeptide may form a dimer. Nonlimiting structures of VHH-containing polypeptides, which are also sdAbs, include VHH₁-Fc, VHH₁-VHH₂-Fc, and VHH₁-VHH₂-VHH₃-Fc, wherein VHH₁, VHH₂, and VHH₃ may be the same or different. In some embodiments of such structures, one VHH may be connected to another VHH by a linker, or one VHH may be connected to the Fc by a linker. In some such embodiments, the linker comprises 1-20 amino acids, preferably 1-20 amino acids predominantly composed of glycine and, optionally, serine. Non-limiting linkers are presented in SEQ ID NOs: 88-93. In some embodiments, when a VHH-containing polypeptide comprises an Fc, it forms a dimer. Thus, the structure VHH₁-VHH₂-Fc, if it forms a dimer, is considered to be tetravalent (i.e., the dimer has four VHH domains). Similarly, the structure VHH₁-VHH₂-VHH₃-Fc, if it forms a dimer, is considered to be hexavalent (i.e., the dimer has six VHH domains).

[0043] The term “monoclonal antibody” refers to an antibody (including an sdAb or VHH-containing polypeptide) of a substantially homogeneous population of antibodies, that is, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Thus, a sample of monoclonal antibodies can bind to the same epitope on the antigen. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies may be made by the hybridoma method first described by Kohler and Milstein, 1975, *Nature* 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty *et al.*, 1990, *Nature* 348:552-554, for example.

[0044] The term “CDR” denotes a complementarity determining region as defined by at least one manner of identification to one of skill in the art. In some embodiments, CDRs can be defined in accordance with any of the Chothia numbering schemes, the Kabat numbering scheme, a combination of Kabat and Chothia, the AbM definition, and/or the contact definition. A VHH comprises three CDRs, designated CDR1, CDR2, and CDR3.

[0045] The term “heavy chain constant region” as used herein refers to a region comprising at least three heavy chain constant domains, C_{H1} , hinge, C_{H2} , and C_{H3} . Of course, non-function-altering deletions and alterations within the domains are encompassed within the scope of the term “heavy chain constant region,” unless designated otherwise. Nonlimiting exemplary heavy chain constant regions include γ , δ , and α . Nonlimiting exemplary heavy chain constant regions also include ϵ and μ . Each heavy constant region corresponds to an antibody isotype. For example, an antibody comprising a γ constant region is an IgG antibody, an antibody comprising a δ constant region is an IgD antibody, and an antibody comprising an α constant region is an IgA antibody. Further, an antibody comprising a μ constant region is an IgM antibody, and an antibody comprising an ϵ constant region is an IgE antibody. Certain isotypes can be further subdivided into subclasses. For example, IgG antibodies include, but are not limited to, IgG1 (comprising a γ_1 constant region), IgG2 (comprising a γ_2 constant region), IgG3 (comprising a γ_3 constant region), and IgG4 (comprising a γ_4 constant region) antibodies; IgA antibodies include, but are not limited to, IgA1 (comprising an α_1 constant region) and IgA2 (comprising an α_2 constant region) antibodies; and IgM antibodies include, but are not limited to, IgM1 and IgM2.

[0046] A “Fc region” as used herein refers to a portion of a heavy chain constant region comprising CH_2 and CH_3 . In some embodiments, an Fc region comprises a hinge, CH_2 , and CH_3 . In various embodiments, when an Fc region comprises a hinge, the hinge mediates dimerization between two Fc-containing polypeptides. An Fc region may be of any antibody heavy chain constant region isotype discussed herein. In some embodiments, an Fc region is an IgG1, IgG2, IgG3, or IgG4. In some embodiments, the Fc region is derived from a human Fc region and lacks the C-terminal lysine residue. In some embodiments, the Fc region is derived from a human Fc region and comprises the C-terminal lysine residue. In some embodiments, the C-terminal amino acid of the Fc region is an amino acid other than lysine. In some embodiments, the Fc region is derived from a human Fc region and lacks the C-terminal glycine and lysine residues.

[0047] An “acceptor human framework” as used herein is a framework comprising the amino acid sequence of a heavy chain variable domain (V_H) framework derived from a human immunoglobulin framework or a human consensus framework, as discussed herein. An acceptor human framework derived from a human immunoglobulin framework or a human consensus

framework can comprise the same amino acid sequence thereof, or it can contain amino acid sequence changes. In some embodiments, the number of amino acid changes are fewer than 10, or fewer than 9, or fewer than 8, or fewer than 7, or fewer than 6, or fewer than 5, or fewer than 4, or fewer than 3, across all of the human frameworks in a single antigen binding domain, such as a VHH.

[0048] “Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (for example, an antibody, such as an sdAb, or VHH-containing polypeptide) and its binding partner (for example, an antigen). The affinity or the apparent affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D) or the $K_{D\text{-apparent}}$, respectively. Affinity can be measured by common methods known in the art (such as, for example, ELISA K_D , KinExA, flow cytometry, and/or surface plasmon resonance devices), including those described herein. Such methods include, but are not limited to, methods involving BIAcore®, Octet®, or flow cytometry.

[0049] The term “ K_D ”, as used herein, refers to the equilibrium dissociation constant of an antigen-binding molecule/antigen interaction. When the term “ K_D ” is used herein, it includes K_D and $K_{D\text{-apparent}}$.

[0050] In some embodiments, the K_D of the antigen-binding molecule is measured by flow cytometry using an antigen-expressing cell line and fitting the mean fluorescence measured at each antibody concentration to a non-linear one-site binding equation (Prism Software graphpad). In some such embodiments, the K_D is $K_{D\text{-apparent}}$.

[0051] The term “biological activity” refers to any one or more biological properties of a molecule (whether present naturally as found in vivo, or provided or enabled by recombinant means). Biological properties include, but are not limited to, binding a ligand, inducing or increasing cell proliferation, and inducing or increasing expression of cytokines.

[0052] An “agonist” or “activating” antibody is one that increases and/or activates a biological activity of the target antigen. In some embodiments, the agonist antibody binds to an antigen and increases its biological activity by at least about 20%, 40%, 60%, 80%, 85% or more.

[0053] An “antagonist”, a “blocking” or “neutralizing” antibody is one that inhibits, decreases and/or inactivates a biological activity of the target antigen. In some embodiments, the neutralizing antibody binds to an antigen and reduces its biological activity by at least about 20%, 40%, 60%, 80%, 85% 90%, 95%, 99% or more.

[0054] An “affinity matured” sdAb or VHH-containing polypeptide refers to a sdAb or VHH-containing polypeptide with one or more alterations in one or more CDRs compared to a parent sdAb or VHH-containing polypeptide that does not possess such alterations, such alterations

resulting in an improvement in the affinity of the sdAb or VHH-containing polypeptide for antigen.

[0055] A “humanized VHH” as used herein refers to a VHH in which one or more framework regions have been substantially replaced with human framework regions. In some instances, certain framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized VHH can comprise residues that are found neither in the original VHH nor in the human framework sequences, but are included to further refine and optimize sdAb VHH-containing polypeptide performance. In some embodiments, a humanized sdAb or VHH-containing polypeptide comprises a human Fc region. As will be appreciated, a humanized sequence can be identified by its primary sequence and does not necessarily denote the process by which the antibody was created.

[0056] An “effector-positive Fc region” possesses an “effector function” of a native sequence Fc region. Exemplary “effector functions” include Fc receptor binding; C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (for example B-cell receptor); and B-cell activation, etc. Such effector functions generally require the Fc region to be combined with a binding domain (for example, an antibody variable domain) and can be assessed using various assays.

[0057] A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[0058] A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. In some embodiments, a “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. In some embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, and preferably, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. In some embodiments, the variant Fc region herein will possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, at least about 90% sequence identity

therewith, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity therewith.

[0059] “Fc receptor” or “FcR” describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcγR is a native human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of those receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (See, for example, Daeron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed, for example, in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. For example, the term “Fc receptor” or “FcR” also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, for example, Ghetie and Ward, *Immunol. Today* 18(12):592-598 (1997); Ghetie *et al.*, *Nature Biotechnology*, 15(7):637-640 (1997); Hinton *et al.*, *J. Biol. Chem.* 279(8):6213-6216 (2004); WO 2004/92219 (Hinton *et al.*).

[0060] The term “substantially similar” or “substantially the same,” as used herein, denotes a sufficiently high degree of similarity between two or more numeric values such that one of skill in the art would consider the difference between the two or more values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said value. In some embodiments the two or more substantially similar values differ by no more than about any one of 5%, 10%, 15%, 20%, 25%, or 50%.

[0061] A polypeptide “variant” means a biologically active polypeptide having at least about 80% amino acid sequence identity with the native sequence polypeptide after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Such variants include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the polypeptide. In some embodiments, a variant will have at least about 80% amino acid sequence identity. In some embodiments, a variant will

have at least about 90% amino acid sequence identity. In some embodiments, a variant will have at least about 95% amino acid sequence identity with the native sequence polypeptide.

[0062] As used herein, “percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0063] An amino acid substitution may include but are not limited to the replacement of one amino acid in a polypeptide with another amino acid. Exemplary substitutions are shown in Table 1. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, for example, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 1

Original Residue	Exemplary Substitutions
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe

Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[0064] Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

[0065] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0066] The term “vector” is used to describe a polynucleotide that can be engineered to contain a cloned polynucleotide or polynucleotides that can be propagated in a host cell. A vector can include one or more of the following elements: an origin of replication, one or more regulatory sequences (such as, for example, promoters and/or enhancers) that regulate the expression of the polypeptide of interest, and/or one or more selectable marker genes (such as, for example, antibiotic resistance genes and genes that can be used in colorimetric assays, for example, β -galactosidase). The term “expression vector” refers to a vector that is used to express a polypeptide of interest in a host cell.

[0067] A “host cell” refers to a cell that may be or has been a recipient of a vector or isolated polynucleotide. Host cells may be prokaryotic cells or eukaryotic cells. Exemplary eukaryotic cells include mammalian cells, such as primate or non-primate animal cells; fungal cells, such as yeast; plant cells; and insect cells. Nonlimiting exemplary mammalian cells include, but are not limited to, NSO cells, PER.C6[®] cells (Crucell), and 293 and CHO cells, and their derivatives,

such as 293-6E, CHO-DG44, CHO-K1, CHO-S, and CHO-DS cells. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) as provided herein.

[0068] The term “isolated” as used herein refers to a molecule that has been separated from at least some of the components with which it is typically found in nature or produced. For example, a polypeptide is referred to as “isolated” when it is separated from at least some of the components of the cell in which it was produced. Where a polypeptide is secreted by a cell after expression, physically separating the supernatant containing the polypeptide from the cell that produced it is considered to be “isolating” the polypeptide. Similarly, a polynucleotide is referred to as “isolated” when it is not part of the larger polynucleotide (such as, for example, genomic DNA or mitochondrial DNA, in the case of a DNA polynucleotide) in which it is typically found in nature, or is separated from at least some of the components of the cell in which it was produced, for example, in the case of an RNA polynucleotide. Thus, a DNA polynucleotide that is contained in a vector inside a host cell may be referred to as “isolated”.

[0069] The terms “individual” and “subject” are used interchangeably herein to refer to an animal; for example a mammal. In some embodiments, methods of treating mammals, including, but not limited to, humans, rodents, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets, are provided. In some examples, an “individual” or “subject” refers to an individual or subject in need of treatment for a disease or disorder. In some embodiments, the subject to receive the treatment can be a patient, designating the fact that the subject has been identified as having a disorder of relevance to the treatment, or being at adequate risk of contracting the disorder.

[0070] A “disease” or “disorder” as used herein refers to a condition where treatment is needed and/or desired.

[0071] The term “autoimmune disorder” refers to a disease or disorder typically associated with the nonanaphylactic hypersensitivity reactions (Type II, Type III and/or Type IV hypersensitivity reactions) that generally results from a subject’s own humoral and/or cell-mediated immune response to one or more immunogenic substances of endogenous or exogenous origin.

[0072] The term “inflammatory disorder” refers to disorders associated with inflammation, including, but not limited to, chronic or acute inflammatory diseases, and expressly includes inflammatory autoimmune diseases and inflammatory allergic conditions.

[0073] The term “infection” and “infectious disease or disorder” refer to a disease or disorder caused by an exogenous infectious agent, such as, but not limited to, bacteria, viruses, fungi, protozoa, and parasites.

[0074] The terms “cancer” and “tumor” encompass solid and hematological/lymphatic cancers and also encompass malignant, pre-malignant, and benign growth, such as dysplasia. Exemplary cancers include, but are not limited to: basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.*, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

[0075] In some embodiments, an “increase” or “decrease” refers to a statistically significant increase or decrease, respectively. As will be clear to the skilled person, “modulating” can also involve effecting a change (which can either be an increase or a decrease) in affinity, avidity, specificity and/or selectivity of a target or antigen, for one or more of its ligands, binding partners, partners for association into a homomultimeric or heteromultimeric form, or substrates; effecting a change (which can either be an increase or a decrease) in the sensitivity of the target or antigen for one or more conditions in the medium or surroundings in which the target or antigen is present (such as pH, ion strength, the presence of co-factors, *etc.*); and/or cellular

proliferation or cytokine production, compared to the same conditions but without the presence of a test agent. This can be determined in any suitable manner and/or using any suitable assay known *per se* or described herein, depending on the target involved.

[0076] As used herein, “treatment” is an approach for obtaining beneficial or desired clinical results. “Treatment” as used herein, covers any administration or application of a therapeutic for disease in a mammal, including a human. For purposes of this disclosure, beneficial or desired clinical results include, but are not limited to, any one or more of: alleviation of one or more symptoms, diminishment of extent of disease, preventing or delaying spread (for example, metastasis, for example metastasis to the lung or to the lymph node) of disease, preventing or delaying recurrence of disease, delay or slowing of disease progression, amelioration of the disease state, inhibiting the disease or progression of the disease, inhibiting or slowing the disease or its progression, arresting its development, and remission (whether partial or total). Also encompassed by “treatment” is a reduction of pathological consequence of a proliferative disease. The methods provided herein contemplate any one or more of these aspects of treatment. In-line with the above, the term treatment does not require one-hundred percent removal of all aspects of the disorder.

[0077] “Ameliorating” means a lessening or improvement of one or more symptoms as compared to not administering a therapeutic agent. “Ameliorating” also includes shortening or reduction in duration of a symptom.

[0078] The term “anti-cancer agent” is used herein in its broadest sense to refer to agents that are used in the treatment of one or more cancers. Exemplary classes of such agents include, but are not limited to, chemotherapeutic agents, anti-cancer biologics (such as cytokines, receptor extracellular domain-Fc fusions, and antibodies), radiation therapy, CAR-T therapy, therapeutic oligonucleotides (such as antisense oligonucleotides and siRNAs) and oncolytic viruses.

[0079] The term “biological sample” means a quantity of a substance from a living thing or formerly living thing. Such substances include, but are not limited to, blood, (for example, whole blood), plasma, serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes and spleen.

[0080] The term “control” or “reference” refers to a composition known to not contain an analyte (“negative control”) or to contain an analyte (“positive control”). A positive control can comprise a known concentration of analyte.

[0081] The terms “inhibition” or “inhibit” refer to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. To “reduce” or “inhibit” is to decrease, reduce or arrest an activity, function,

and/or amount as compared to a reference. In some embodiments, by “reduce” or “inhibit” is meant the ability to cause an overall decrease of 10% or greater. In some embodiments, by “reduce” or “inhibit” is meant the ability to cause an overall decrease of 50% or greater. In some embodiments, by “reduce” or “inhibit” is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater. In some embodiments, the amount noted above is inhibited or decreased over a period of time, relative to a control over the same period of time.

[0082] As used herein, “delaying development of a disease” means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0083] “Preventing,” as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease. Unless otherwise specified, the terms “reduce”, “inhibit”, or “prevent” do not denote or require complete prevention over all time, but just over the time period being measured.

[0084] A “therapeutically effective amount” of a substance/molecule, agonist or antagonist may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, agonist or antagonist to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the substance/molecule, agonist or antagonist are outweighed by the therapeutically beneficial effects. A therapeutically effective amount may be delivered in one or more administrations. A therapeutically effective amount refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic and/or prophylactic result.

[0085] The terms “pharmaceutical formulation” and “pharmaceutical composition” are used interchangeably and refer to a preparation which is in such form as to permit the biological activity of the active ingredient(s) to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations may be sterile.

[0086] A “pharmaceutically acceptable carrier” refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a “pharmaceutical composition” for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and are compatible with other ingredients of the

formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed.

[0087] Administration “in combination with” one or more further therapeutic agents includes simultaneous (concurrent) and sequential administration in any order.

[0088] The term “concurrently” is used herein to refer to administration of two or more therapeutic agents, where at least part of the administration overlaps in time, or where the administration of one therapeutic agent falls within a short period of time relative to administration of the other therapeutic agent, or wherein the therapeutic effect of both agents overlap for at least a period of time.

[0089] The term “sequentially” is used herein to refer to administration of two or more therapeutic agents that does not overlap in time, or wherein the therapeutic effects of the agents do not overlap.

[0090] As used herein, “in conjunction with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in conjunction with” refers to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

[0091] The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0092] An “article of manufacture” is any manufacture (for example, a package or container) or kit comprising at least one reagent, for example, a medicament for treatment of a disease or disorder (for example, cancer), or a probe for specifically detecting a biomarker described herein. In some embodiments, the manufacture or kit is promoted, distributed, or sold as a unit for performing the methods described herein.

[0093] The terms “label” and “detectable label” mean a moiety attached, for example, to an antibody or antigen to render a reaction (for example, binding) between the members of the specific binding pair, detectable. The labeled member of the specific binding pair is referred to as “detectably labeled.” Thus, the term “labeled binding protein” refers to a protein with a label incorporated that provides for the identification of the binding protein. In some embodiments, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, for example, incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (for example, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to,

the following: radioisotopes or radionuclides (for example, ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , or ^{153}Sm); chromogens, fluorescent labels (for example, FITC, rhodamine, lanthanide phosphors), enzymatic labels (for example, horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (for example, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunoassays include moieties that produce light, for example, acridinium compounds, and moieties that produce fluorescence, for example, fluorescein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety.

Exemplary albumin-binding polypeptides

[0094] Single-domain antibodies (sdAbs) including VHH domains that bind albumin are provided herein. In some embodiments, a VHH domain that binds albumin does not interfere with albumin binding to FcRn. In some embodiments, a VHH domain that binds albumin does not bind domain 3 of albumin. In some embodiments, a VHH domain that binds albumin binds with an affinity (K_D) between 0.01 nM and 5 nM, or between 0.01 nM at 2 nM, or between 0.01 nM and 1 nM, between 0.01 nM and 0.5 nM, 0.05 nM and 5 nM, or between 0.05 nM at 2 nM, or between 0.05 nM and 1 nM, or between 0.05 nM and 0.5 nM.

[0095] In various embodiments, a polypeptide comprising at least one VHH domain that binds albumin is provided. In some embodiments, a polypeptide comprising one, two, three, four, five, six, seven, or eight VHH domains that bind albumin is provided. In some embodiments, a polypeptide provided herein comprises one, two, three, or four VHH domains that bind albumin. Such polypeptides may comprise one or more additional VHH domains that bind one or more target proteins other than albumin.

[0096] In various embodiments, a polypeptide that comprises one or more VHH domains that bind albumin also comprises a therapeutic antigen-binding domain and/or a therapeutic polypeptide. Such therapeutic antigen-binding domains include, but are not limited to, traditional antibody antigen-binding domains, which comprise a heavy chain variable region and a light chain variable region, and single-domain antibody antigen-binding domains, such as VHH domains. Nonlimiting formats of polypeptides comprising one or more VHH domains that bind albumin and one or more traditional antibody domains are provided in FIG. 6(v)-(ix). Other non-limiting exemplary therapeutic polypeptides include, for example, receptor extracellular domains, enzymes, and ligands. In various embodiments, the polypeptide comprising at least one VHH domain that binds albumin has a longer half-life *in vivo* than the

same polypeptide without the at least one VHH domain that binds albumin. In some embodiments, the half-life is at least 1.5x, at least 2x, at least 3x, at least 4x, or at least 5x longer than the half-life of the polypeptide without the VHH domain that binds albumin.

[0097] In some embodiments, a polypeptide that comprises at least one VHH domain that binds albumin comprises an Fc region. In some embodiments, a polypeptide provided herein comprises one, two, three, or four VHH domains that bind albumin and an Fc region. In some embodiments, an Fc region mediates dimerization of the polypeptide at physiological conditions.

[0098] In various embodiments, a VHH domain that binds albumin comprises a CDR1 sequence selected from SEQ ID NOs: 5-8, a CDR2 sequence selected from SEQ ID NOs: 9-21, and a CDR3 sequence of SEQ ID NO: 22. In various embodiments, a VHH domain that binds albumin comprises CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 5, 9, and 22; SEQ ID NOs: 5, 10, and 22; SEQ ID NOs: 5, 11, and 22; SEQ ID NOs: 5, 12, and 22; SEQ ID NOs: 5, 13, and 22; SEQ ID NOs: 5, 14, and 22; SEQ ID NOs: 5, 15, and 22; SEQ ID NOs: 6, 15, and 22; SEQ ID NOs: 7, 15, and 22; SEQ ID NOs: 8, 15, and 22; SEQ ID NOs: 6, 16, and 22; SEQ ID NOs: 6, 17, and 22; SEQ ID NOs: 6, 18, and 22; SEQ ID NOs: 6, 19, and 22; SEQ ID NOs: 6, 20, and 22; and SEQ ID NOs: 6, 21, and 22.

[0099] In some embodiments, a VHH domain that binds albumin comprises an amino acid sequence that is a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 23-43 and 71-74. In some embodiments, a VHH domain that binds albumin comprises an amino acid sequence selected from SEQ ID NOs: 23-43 and 71-74.

[00100] In some embodiments, a VHH domain that binds albumin is provided, which competes for binding to albumin with a VHH domain comprising an amino acid sequence selected from SEQ ID NOs: 23-43 and 71-74.

[00101] In some embodiments, a VHH domain that binds albumin may be humanized. Humanized antibodies (such as sdAbs or VHH-containing polypeptides) are useful as therapeutic molecules because humanized antibodies reduce or eliminate the human immune response to non-human antibodies, which can result in an immune response to an antibody therapeutic, and decreased effectiveness of the therapeutic. Generally, a humanized antibody comprises one or more variable domains in which CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (for example, the antibody

from which the CDR residues are derived), for example, to restore or improve antibody specificity or affinity.

[00102] Humanized antibodies and methods of making them are reviewed, for example, in Almagro and Fransson, (2008) *Front. Biosci.* 13: 1619-1633, and are further described, for example, in Riechmann *et al.*, (1988) *Nature* 332:323-329; Queen *et al.*, (1989) *Proc. Natl Acad. Sci. USA* 86: 10029-10033; US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, (2005) *Methods* 36:25-34; Padlan, (1991) *Mol. Immunol.* 28:489-498 (describing “resurfacing”); Dall’Acqua *et al.*, (2005) *Methods* 36:43-60 (describing “FR shuffling”); and Osbourn *et al.*, (2005) *Methods* 36:61-68 and Klimka *et al.*, (2000) *Br. J. Cancer*, 83:252-260 (describing the “guided selection” approach to FR shuffling).

[00103] Human framework regions that can be used for humanization include but are not limited to: framework regions selected using the “best-fit” method (see, for example, Sims *et al.* (1993) *J. Immunol.* 151 :2296); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of heavy chain variable regions (see, for example, Carter *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; and Presta *et al.* (1993) *J. Immunol.* 151:2623); human mature (somatically mutated) framework regions or human germline framework regions (see, for example, Almagro and Fransson, (2008) *Front. Biosci.* 13:1619-1633); and framework regions derived from screening FR libraries (see, for example, Baca *et al.*, (1997) *J. Biol. Chem.* 272: 10678-10684 and Rosok *et al.*, (1996) *J. Biol. Chem.* 271 :22611-22618). Typically, the FR regions of a VHH are replaced with human FR regions to make a humanized VHH. In some embodiments, certain FR residues of the human FR are replaced in order to improve one or more properties of the humanized VHH. VHH domains with such replaced residues are still referred to herein as “humanized.”

[00104] As provided herein, sdAbs including VHH domains that bind albumin can slow the clearance of molecules they are linked to, including molecules comprising a human Fc region. In various embodiments, an Fc region included in an albumin-binding polypeptide is a human Fc region, or is derived from a human Fc region. Non-limiting sdAb formats comprising an Fc region are shown in FIG. 6.

[00105] In some embodiments, an Fc region included in an albumin-binding polypeptide is derived from a human Fc region, and comprises a three amino acid deletion in the lower hinge corresponding to IgG1 E233, L234, and L235, herein referred to as “Fc xELL.” Fc xELL polypeptides do not engage FcγRs and thus are referred to as “effector silent” or “effector null”, however in some embodiments, xELL Fc regions bind FcRn and therefore have extended half-life and transcytosis associated with FcRn mediated recycling.

[00106] In some embodiments, the Fc region included in an albumin-binding polypeptide is derived from a human Fc region and comprises mutations M252Y and M428V, which may be referred to as “YV”. In some embodiments, such mutations enhance binding to FcRn at the acidic pH of the endosome (near 6.5), while losing detectable binding at neutral pH (about 7.2), allowing for enhanced FcRn mediated recycling and extended half-life. In some embodiments, the Fc region included in an albumin-binding polypeptide is derived from a human Fc region and comprises mutations M252Y, S254T, and T256E, which may be referred to as “YTE”. In some embodiments, such mutations extend serum half-life in humans by significantly reducing the dissociation rate of Fc and FcRn. In some embodiments, the Fc region included in an albumin-binding polypeptide is derived from a human Fc region and comprises mutations M428L and N434S, which may be referred to as “LS”. In some embodiments, such mutations extend serum half-life in humans by increasing the binding affinity of Fc for FcRn at pH6 and reducing the dissociation rate. Various Fc mutations that enhance circulating half-life are described, for example, in Saunders, *Front. Immunol.* doi.org/10.3389/fimmu.2019.01296 (2019).

[00107] In some embodiments, the Fc region included in an albumin-binding polypeptide is derived from a human Fc region and comprises mutations designed for heterodimerization, herein referred to as “knob” and “hole”. In some embodiments, the “knob” Fc region comprises the mutation T366W. In some embodiments, the “hole” Fc region comprises mutations T366S, L368A, and Y407V. In some embodiments, Fc regions used for heterodimerization comprise additional mutations, such as the mutation S354C on a first member of a heterodimeric Fc pair that forms an asymmetric disulfide with a corresponding mutation Y349C on the second member of a heterodimeric Fc pair. In some embodiments, one member of a heterodimeric Fc pair comprises the modification H435R or H435K to prevent protein A binding while maintaining FcRn binding. In some embodiments, one member of a heterodimeric Fc pair comprises the modification H435R or H435K, while the second member of the heterodimeric Fc pair is not modified at H435. In various embodiments, the hold Fc region comprises the modification H435R or H435K (referred to as “hole-R” in some instances when the modification is H435R), while the knob Fc region does not. In some instances, the hole-R mutation improves purification of the heterodimer over homodimeric hole Fc regions that may be present.

[00108] Nonlimiting exemplary Fc regions that may be used in an albumin-binding polypeptide include Fc regions comprising the amino acid sequences of SEQ ID NOs: 47-68 and 85-87.

Exemplary activities of albumin-binding polypeptides

[00109] In various embodiments, the albumin-binding polypeptides provided herein bind to an epitope of albumin outside of domain 3. In some embodiments, the albumin-binding polypeptides provided herein do not interfere with (i.e., does not inhibit) albumin binding to FcRn. Methods of determining whether an albumin-binding polypeptide interferes with albumin binding to FcRn are known in the art; nonlimiting exemplary methods are also provided herein.

[00110] In some embodiments, a polypeptide comprising an albumin binding domain provided herein has a longer half-life *in vivo* than the polypeptide lacking the albumin binding domain. In various embodiments, a polypeptide comprising an albumin binding domain provided herein has a half-life that is at least 1.5x, at least 2x, at least 3x, at least 4x, or at least 5x longer than the half-life of the polypeptide without the albumin binding domain.

Polypeptide Expression and Production

[00111] Nucleic acid molecules comprising polynucleotides that encode a polypeptide comprising an albumin-binding domain are provided. In some embodiments, the nucleic acid molecule may also encode a leader sequence that directs secretion of the polypeptide comprising an albumin-binding domain, which leader sequence is typically cleaved such that it is not present in the secreted polypeptide. The leader sequence may be a native heavy chain (or VHH) leader sequence, or may be another heterologous leader sequence.

[00112] Nucleic acid molecules can be constructed using recombinant DNA techniques conventional in the art. In some embodiments, a nucleic acid molecule is an expression vector that is suitable for expression in a selected host cell.

[00113] Vectors comprising nucleic acids that encode a polypeptide comprising an albumin-binding domain are provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, *etc.* In some embodiments, a vector is selected that is optimized for expression of polypeptides in a desired cell type, such as CHO or CHO-derived cells, or in NSO cells. Exemplary such vectors are described, for example, in Running Deer *et al.*, *Biotechnol. Prog.* 20:880-889 (2004).

[00114] In some embodiments, a polypeptide comprising an albumin-binding domain may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells (such as yeast), plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44, Lec13 CHO cells, and FUT8 CHO cells; PER.C6[®] cells (Crucell); and NSO cells. In some embodiments, the polypeptides may be expressed in yeast. *See, e.g.*, U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on

its ability to make desired post-translational modifications to the polypeptide. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[00115] Introduction of one or more nucleic acids (such as vectors) into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, *etc.* Nonlimiting exemplary methods are described, for example, in Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method.

[00116] Host cells comprising any of the nucleic acids or vectors described herein are also provided. In some embodiments, a host cell that expresses a polypeptide comprising an albumin-binding domain described herein is provided. The polypeptides expressed in host cells can be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include the ROR1 ECD and agents that bind Fc regions. For example, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind the Fc region and to purify a polypeptide that comprises an Fc region. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also be suitable for purifying some polypeptides such as antibodies. Ion exchange chromatography (for example anion exchange chromatography and/or cation exchange chromatography) may also be suitable for purifying some polypeptides such as antibodies. Mixed-mode chromatography (for example reversed phase/anion exchange, reversed phase/cation exchange, hydrophilic interaction/anion exchange, hydrophilic interaction/cation exchange, *etc.*) may also be suitable for purifying some polypeptides such as antibodies. Many methods of purifying polypeptides are known in the art.

[00117] In some embodiments, the polypeptide is produced in a cell-free system. Nonlimiting exemplary cell-free systems are described, for example, in Sitaraman *et al.*, *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); Endo *et al.*, *Biotechnol. Adv.* 21: 695-713 (2003).

[00118] In some embodiments, a polypeptide comprising an albumin-binding domain prepared by the methods described above are provided. In some embodiments, the polypeptide is prepared in a host cell. In some embodiments, the polypeptide is prepared in a cell-free system. In some embodiments, the polypeptide is purified. In some embodiments, a cell culture media comprising a polypeptide is provided.

[00119] In some embodiments, compositions comprising antibodies prepared by the methods described above are provided. In some embodiments, the composition comprises a polypeptide comprising an albumin-binding domain prepared in a host cell. In some embodiments, the composition comprises a polypeptide prepared in a cell-free system. In some embodiments, the composition comprises a purified polypeptide.

Exemplary methods of treating diseases using albumin-binding polypeptides

[00120] In some embodiments, methods of treating disease in an individual are provided, comprising administering a therapeutic polypeptide comprising an albumin-binding domain provided herein. Such diseases include any disease that would benefit from treatment with the therapeutic polypeptide. Nonlimiting exemplary diseases that may be treated with therapeutic polypeptides comprising an albumin-binding domain provided herein include infectious diseases, autoimmune diseases or disorders, inflammatory diseases or disorders, and cancer. The method comprises administering to the individual an effective amount of a therapeutic polypeptide comprising an albumin-binding domain provided herein. Such methods of treatment may be in humans or animals. In some embodiments, methods of treating humans are provided.

[00121] The therapeutic polypeptides comprising an albumin-binding domain provided herein can be administered as needed to subjects. Determination of the frequency of administration can be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like. In some embodiments, an effective dose of a therapeutic polypeptide is administered to a subject one or more times. In some embodiments, an effective dose of a therapeutic polypeptide is administered to the subject daily, semiweekly, weekly, every two weeks, once a month, etc. An effective dose of a therapeutic polypeptide is administered to the subject at least once. In some embodiments, the effective dose of a therapeutic polypeptide may be administered multiple times, including multiple times over the course of at least a month, at least six months, or at least a year.

[00122] In some embodiments, pharmaceutical compositions are administered in an amount effective for treating disease. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In general, antibodies may be administered in an amount in the range of about 0.05 mg/kg body weight to about 100 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 10 µg/kg body weight to about 100 mg/kg body weight per

dose. In some embodiments, antibodies may be administered in an amount in the range of about 50 µg/kg body weight to about 5 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 100 µg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 100 µg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 0.5 mg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 0.05 mg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 0.05 mg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, antibodies may be administered in an amount in the range of about 5 mg/kg body weight or lower, for example less than 4, less than 3, less than 2, or less than 1 mg/kg of the antibody.

[00123] In some embodiments, therapeutic polypeptides can be administered *in vivo* by various routes, including, but not limited to, intravenous, intra-arterial, parenteral, intraperitoneal or subcutaneous. The appropriate formulation and route of administration may be selected according to the intended application.

Pharmaceutical compositions

[00124] In some embodiments, compositions comprising polypeptides comprising albumin-binding domains are provided in formulations with a wide variety of pharmaceutically acceptable carriers (see, for example, Gennaro, Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus, 20th ed. (2003); Ansel *et al.*, Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th ed., Lippencott Williams and Wilkins (2004); Kibbe *et al.*, Handbook of Pharmaceutical Excipients, 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are available. Moreover, various pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available. Non-limiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

[00125] In some embodiments, a pharmaceutical composition comprises a polypeptide comprising an albumin-binding domain at a concentration of at least 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL, 100 mg/mL, 125 mg/mL, 150 mg/mL, 175 mg/mL, 200 mg/mL, 225 mg/mL, or 250 mg/mL.

Nonlimiting exemplary methods of diagnosis and treatment

[00126] In some embodiments, the methods described herein are useful for evaluating a subject and/or a specimen from a subject (*e.g.* a cancer patient). In some embodiments, evaluation is one or more of diagnosis, prognosis, and/or response to treatment.

[00127] In some embodiments, the methods described herein comprise evaluating a presence, absence, or level of a protein. In some embodiments, the methods described herein comprise evaluating a presence, absence, or level of expression of a nucleic acid. The compositions described herein may be used for these measurements. In some embodiments, the evaluation may direct treatment (including treatment with the polypeptides described herein).

Kits

[00128] Also provided are articles of manufacture and kits that include any of the polypeptides comprising an albumin-binding domain as described herein, and suitable packaging. In some embodiments, the invention includes a kit with (i) a polypeptide comprising an albumin-binding domain, and (ii) instructions for using the kit to administer the polypeptide to an individual.

[00129] Suitable packaging for compositions described herein are known in the art, and include, for example, vials (*e.g.*, sealed vials), vessels, ampules, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed. Also provided are unit dosage forms comprising the compositions described herein. These unit dosage forms can be stored in a suitable packaging in single or multiple unit dosages and may also be further sterilized and sealed. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable. The instructions relating to the use of the antibodies generally include information as to dosage, dosing schedule, and route of administration for the intended treatment or industrial use. The kit may further comprise a description of selecting an individual suitable or treatment.

[00130] The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. For example, kits may also be provided that contain sufficient dosages of molecules disclosed herein to provide effective treatment for an individual for an extended period, such as about any of a week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, or more. Kits may also include multiple unit doses of molecules and instructions for use and packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies. In some embodiments, the kit includes a dry (*e.g.*, lyophilized) composition that

can be reconstituted, resuspended, or rehydrated to form generally a stable aqueous suspension of antibody.

EXAMPLES

[00131] The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Development of anti-albumin single domain antibodies (sdAbs)

[00132] Single domain antibodies targeting human albumin were generated via immunization of llamas and alpaca with a recombinant version of human serum albumin (SEQ ID NO: 1).

[00133] Following the development of specific anti-albumin antibody titers, llama/alpaca peripheral blood mononuclear cells (PBMCs) were isolated from 500mL of blood from the immunized animal and total mRNA was isolated using the Qiagen RNeasy Maxi Kit and subsequently converted to first strand cDNA using Thermo Superscript IV Reverse Transcriptase and oligo-dT priming. VHH sequences were specifically amplified via PCR using the cDNA as template and cloned into a yeast surface display vector as VHH-Fc-AGA2 fusion proteins. The Fc was a human IgG1 Fc or, in some cases, a variant IgG1 Fc with reduced effector function.

[00134] Yeast libraries displaying the VHH-Fc-AGA2 fusion proteins were enriched using recombinant forms of human albumin via magnetic bead isolation followed by fluorescence activated cell sorting (FACS). Sorted yeast were plated out and isolated colonies were picked into 96-well blocks and an induction of yeast cell surface expression of VHH-Fc-AGA2 fusion protein was conducted. Biotinylated recombinant human albumin or irrelevant biotinylated protein (albumin negative) were directly applied to induced yeast, washed, treated with fluorophore labelled streptavidin, and analyzed by 96-well flow cytometry.

[00135] Nucleic acid sequences encoding VHHs that bound to biotinylated recombinant human albumin and not to irrelevant biotinylated protein were cloned in-frame with a human Fc xELL encoding region into mammalian expression vectors, and expressed by transient transfection in HEK293 Freestyle cells (293F cells) or CHO cells using

polyethylenimine. Supernatant was collected after 3-7 days, secreted recombinant protein was purified by protein A chromatography, and concentration was calculated from the absorbance at 280 nm and extinction coefficient.

[00136] Anti-albumin sdAb 4A01 was selected for humanization.

Example 2: Monovalent binding of anti-albumin sdAb 4A01 to human and mouse albumin

[00137] Monomeric anti-albumin sdAb 4A01 was made by fusing the 4A01 VHH to a human Fc comprising S364N, Y407N, and K409T mutations (4A01-NNT-hFc; SEQ ID NOs: 23 and 68; FIG. 1A). Binding of monomeric anti-albumin sdAb 4A01 was assessed by ELISA by titrating monomeric sdAb onto immobilized albumin protein from the indicated species (Medisorp plate), and detecting using anti-human Fc (HRP), as follows.

[00138] Plates were coated with the albumin protein of the indicated species (Sigma) at 2 µg/ml, 50 µl/well, 4°C overnight. 1x Fish Gelatin (blocking agent, Biotium) was added to the coated wells and incubated for 1 hour at RT. Titrations of 4A01-NNT-hFc (starting at 100nM, 1:3 dilutions, last well blank) were added and then incubated for 1 hour at RT. Plates were then washed 3 times with 0.1% D-PBST before adding of anti-human Fc HRP antibody (1:2000 in 0.1% D-PBST, Jackson). Plates were incubated for 30 minutes at RT and then washed 3 times with 0.1% D-PBST. TMB substrate was then added and the absorbance at 650nm read on a plate reader (Molecular Devices). Data were plotted using equation one site – total binding ($Y = B_{max} * X / (K_d + X) + NS * X + \text{Background}$, GraphPad Prism).

[00139] As shown in FIG. 1B and 1C, anti-albumin sdAb 4A01 bound to both human and mouse albumin with comparable affinity. The K_D for human albumin was 0.23 nM and the K_D for mouse albumin was 0.20 nM.

Example 3: Anti-albumin sdAb 4A01 does not bind albumin domain 3

[00140] Albumin binds to the beta-2 microglobulin FcRn complex primarily through domain 3, and that binding is believed to be involved in the improved half-life of proteins fused to anti-albumin antibodies, or fused to albumin itself. To determine whether anti-albumin sdAb 4A01 binds to albumin domain 3, binding of 4A01-NNT-hFc was assayed by biolayer interferometry, as follows.

[00141] Albumin domain 3 (mouse Fc tagged) was immobilized on anti-mouse IgG Fc capture biosensor. All buffers/protein formulations were in MBST5 (50nM MES pH5, 150mM NaCl, 0.025% Tween)). A baseline was established with buffer only. Mouse Fc-tagged human albumin domain III (10 µg/ml) was loaded onto the anti-mouse IgG Fc capture biosensors

(ForteBio). Anti-albumin sdAbs 4A01 (4A01-NNT-hFc) and 1C04 (similar format) were then loaded and allowed to associate with the captured biotin domain 3, followed by dissociation with MBST5. sdAb 1C04 is known to bind to albumin domain 3, and was used as a positive control. See FIG. 2A.

[00142] As shown in FIG. 2B, 1C04, but not 4A01, bound to the immobilized albumin domain 3.

[00143] Anti-albumin sdAb 4A01 (4A01-NNT-hFc), hz4A01v51, and 1C04 were then tested for interference with albumin-FcRn binding, as follows. Binding was assessed by biolayer interferometry using biotinylated recombinant FcRn-B2M immobilized on a streptavidin biosensor. The immobilized FcRn-B2M was then complexed with recombinant human albumin. All buffers/protein formulations were in MBST5 (50mM MES pH5, 150mM NaCl, 0.025% Tween). A baseline was established with buffer only. Biotinylated FcRn-B2M (10µg/ml, Acro Biosystems) was loaded onto the streptavidin biosensors (ForteBio), and a further baseline determined. 50µM recombinant human albumin (Sigma) was then added and allowed to associate with the immobilized FcRn-B2M. Anti-albumin sdAbs 4A01 and 4A01v51, and sdAb 1C04 were then loaded and allowed to associate with the captured biotin domain 3, followed by dissociation with MBST5. See FIG. 3A

[00144] As shown in FIG. 2B, 4A01 and hz4A01v51 both bound to albumin associated with FcRn-B2M, but 1C04 did not.

Example 4: Humanization of anti-albumin sdAb 4A01 and species cross-reactivity

[00145] Various humanized forms of sdAb 4A01 were made based on the human heavy chain frameworks VH3-23*04. Certain amino acids were back-mutated to the donor amino acid, and certain mutations were tested, for example, in CDR2. FIG. 4A shows an alignment of the human heavy chain acceptor sequence with the humanized forms of 4A01.

[00146] Binding of monomeric anti-albumin sdAbs 4A01 ("Im4A01") and its humanized versions to human serum albumin, cynomolgus serum albumin, murine serum albumin, and rat serum albumin was determined by ELISA as follows. Medisorp plates were coated with albumin protein at 2µg/ml, 50µl/well at 4°C overnight (human, murine, and rat albumin – Sigma, cynomolgus monkey albumin – Abcam). 1x Fish Gelatin (blocking agent, Bethyl Laboratories) was added to albumin-coated wells followed by a 1 hour incubation at RT. Titrations of sdAb fusion proteins (starting at 100nM, across 1:3 or down 1:4) were added and incubated for 1 hour at RT. Plates were washed 3 times with 0.1% D-PBST, and then anti-human Fc HRP antibody (1:2000 in 0.1% D-PBST, Jackson) was added and incubated for 30 minutes at RT. Plates were washed 3 times with 0.1% D-PBST, and then TMB substrate was added. Absorbance at 650nm

was read on a plate reader (Molecular Devices) and data were plotted using equation one site – total binding (Model: $Y=B_{max} \cdot X/(K_D+X) + NS \cdot X + \text{Background}$, GraphPad Prism).

[00147] Binding of 4A01 and humanized forms of 4A01 to human albumin is shown in FIG. 4B-4C. All of the sdAbs bound human albumin with a K_D between 0.10 and 0.43 nM. Binding of 4A01 and humanized forms of 4A01 to cynomolgus monkey albumin is shown in FIG. 4D-4E. All of the sdAbs bound cynomolgus monkey albumin with a K_D between 0.11 and 0.34 nM. Binding of 4A01 and humanized forms of 4A01 to murine albumin is shown in FIG. 4F-4G. All of the sdAbs bound murine albumin with a K_D between 0.11 and about 0.25 nM. Binding of 4A01 and humanized forms of 4A01 to rat albumin is shown in FIG. 4H-4I. All of the sdAbs bound rat albumin with a K_D between 0.14 and about 0.33 nM.

[00148] FIG. 5A-5D show binding of 4A01 and humanized hz4A01v51 to human (5A), cynomolgus monkey (5B), murine (5C), and rat (5D) albumin. 4A01 and all of the humanized variants tested bound all four species of albumin with an affinity of less than 1 nM. Humanized hz4A01v51 bound all four species of albumin with an affinity of less than 0.3 nM, and achieved maximal binding of greater than 90%.

Example 5: Binding of single domain antibody polypeptides to human albumin

[00149] Binding of humanized single domain antibody (sdAb) polypeptides to human albumin at neutral (7.4) or endosomal (6) pH was tested by ELISA. 96-well ELISA plates were coated with 2 µg/mL recombinant albumin in PBS overnight at 4°C, washed with PBS/0.05% Tween-20 (PBS-T) and then blocked with 5% milk powder in PBS-T for 2 h at room temperature. Serial dilutions of test articles were prepared in PBS pH 7.4 or a buffer containing 20mM His, 150 mM NaCl, pH 6 and added to the plates. Plates were incubated at 4°C for 1 h. After the incubation cells were washed in the respective buffer and then incubated for 30 min at room temperature with a horse radish peroxidase (HRP)-conjugated anti-idiotypic antibody detecting the sdAbs. Plates were washed in their respective buffers and TMB substrate was added. The HRP-TMB reaction was allowed to develop for 6 min and was then stopped with an equal volume of HCl-based stop solution. Absorbance at 450 nm was measured with a 96 well plate reader. The data were plotted and analyzed using GraphPad Prism analysis software. The results are shown in FIG. 7.

[00150] As shown in FIG. 7A and 7B, a bivalent bispecific sdAb polypeptide comprising an albumin binding domain of SEQ ID NO: 43 (hz4A01v51 VHH) and a non-mammalian targeted binding domain, formatted as shown in FIG. 6(iii) (cx11917) binds albumin with low nanomolar to sub-nanomolar affinity. The apparent affinity is only mildly affected by pH with a K_d of 0.7 nM at a neutral pH (7.4) compared to a K_d of 2 nM at pH 6. Binding is only mediated by the

monovalent albumin-targeting sdAb subunit, as an sdAb polypeptide comprising two non-mammalian targeted binding domains formatted as described in FIG. 6(iii) (cx11916) did not bind albumin with appreciable affinity at any pH tested.

Example 6: Species cross-reactivity of albumin-binding single domain antibody polypeptides

[00151] Binding of a humanized single domain antibody (sdAb) polypeptide (cx5009, SEQ ID 69), comprising hz4A01v51 VHH (SEQ ID NO: 43) and a monomeric Fc region (Fc region with S364N, Y407N, and K409T substitutions, SEQ ID NO: 68) to recombinant human, cynomolgus, mouse or rat albumin at neutral (7.4) pH was tested by ELISA. 96-well ELISA plates were coated with 2 µg/mL recombinant albumin in PBS overnight at 4°C, washed with PBS/0.05% Tween-20 (PBS-T) and then blocked with 1x fish gelatin for 1 h at room temperature. Serial dilutions of test articles were prepared in PBS-T pH 7.4 and added to the plates. Plates were incubated for 1 h at room temperature. After the incubation cells were washed in PBS-T and then incubated for 30 min at room temperature with an HRP-conjugated secondary antibody specific to human IgG1. Plates were then washed before addition of a TMB substrate. The HRP-TMB reaction was allowed to develop and absorbance at 650 nm was measured with a 96 well plate reader. The data were plotted and analyzed using GraphPad Prism analysis software. The results are shown in FIG. 3.

[00152] As shown in FIG. 8, cx5009, a monovalent albumin-specific sdAb, hz4A01v51 VHH-hIgG1-xELL-NNT-Fc (SEQ ID NO: 69), formatted as shown in FIG. 6(ii), binds to albumin from human, cynomolgus, mouse, and rat. Apparent affinities at a neutral pH (7.4) are similar across species with K_{ds} in the sub-nanomolar range (~0.2nM).

Example 7: *In vivo* pharmacokinetic profile of albumin-binding single domain antibody polypeptides

[00153] The ability of albumin-binding single domain antibodies (sdAb) to extend the serum exposure of human IgG was tested in healthy mice. The pharmacokinetic (PK) profile of a humanized bivalent sdAb polypeptide cross-reactive to mouse albumin and formatted as hz4A01v51 VHH-hIgG1-xELL-Fc (cx11956, SEQ ID NO: 70) was compared to that of a bivalent sdAb polypeptide not cross-reactive to mouse but formatted in the same VHH-hIgG1-xELL-Fc structure (cx11851). The xELL variation of human IgG1 reduces Fc gamma receptor binding but does not affect FcRn binding. This was confirmed *in vitro* using biolayer interferometry. Human IgG1 is cross-reactive to mouse FcRn, enabling FcRn-mediated recycling of human antibodies in mice.

[00154] To determine the PK profile of sdAb-hIgG xELL-Fc test articles, BALB/c mice were injected intravenously with either 30 mg/kg or 0.3 mg/kg single doses and serum samples were drawn 30 min, 6 h, 24 h, 96 h and 168 h after the test article injection. Test article concentrations in mouse serum were determined by ELISA. For the PK ELISA, human FcRn/B2M heterodimeric protein (His-tag, Acro Biosystem) was immobilized on 96-well ELISA plates by incubating 4 µg/mL of a protein solution in PBS for 12h at 4°C. The next day plates were blocked with a 3% BSA TBS-T buffer for 2 h before incubation of the serum samples on these plates for 2 h. Binding of test article in the serum samples to the FcRn immobilized on the ELISA plates was detected using an HRP-conjugated secondary anti-idiotypic detection antibody able to bind the sdAb. The secondary antibody was incubated on the plates for 1 h and binding was visualized using a TMB substrate solution followed by addition of stop solution (1 M H₂SO₄) and measuring the absorbance at 450 nm on an Emax spectrophotometer (Molecular Devices). Absorbance values were converted into test article concentrations in SoftMax Pro using standard curves from proteins with known concentrations. 4-parameter logistic regression was used to fit the standard curve. Data were exported and graphed using GraphPad Prism analysis software.

[00155] As shown in FIG. 9, the albumin-targeting sdAb polypeptide can slow the clearance of human IgG1 and extend the serum exposure when attached to the IgG1. Absolute concentrations of anti-albumin hz4A01v51 VHH-IgG1 xELL-Fc (cx11956) in serum after single doses of 30 mg/kg (FIG. 9A) or 0.3 mg/kg (FIG. 9C) are significantly higher than concentrations of a non-targeted VHH-IgG1 xELL-Fc of equivalent size that does not bind albumin (cx11851). Despite injection of an equal amount of protein cx11851, cMax levels 30 min after the injection are already lower than those of cx11956. Further, the more rapid clearance of the non-targeted construct (cx11851) continues within the first 6h after injection as shown in the normalized plots (FIG. 9B and FIG. 9D). Relative to the cMax (30 min) time point, non-targeted cx11851 concentrations drop by almost 60% in the 30 mg/kg dose level cohort by 6 hours, whereas albumin-binding cx11956 concentrations only drop by about 12% (FIG. 10B). Similarly, at the low dose level (0.3 mg/kg), concentration of albumin-binding cx11956 dropped to only about 87% of Cmax, compared to about 72% of cMax for non-albumin-binding cx11851.

[00156] These findings demonstrate that an albumin-binding VHH domain can enhance the serum exposure of IgG antibodies. Without intending to be bound by any particular theory, the enhanced serum exposure may result from an albumin/FcRn recycling pathway that is independent of IgG/FcRn mediated recycling.

[00157] To confirm equivalent binding of human IgG1-xELL and wild-type human IgG to FcRn, binding of an sdAb polypeptide formatted with either of the two IgG1 Fc variants to

recombinant human FcRn/B2M was tested by Bio-Layer Interferometry using an Octet96 Red reader (Sartorius). In brief, biotinylated human FcRn/B2m was loaded onto a streptavidin biosensor. Association of the test articles was measured for 60 seconds by dipping the sensor in 100 nM test article dilutions. Test articles were diluted either in a buffer containing 50 mM MES, 150 mM NaCl and 0.025% Tween-20 at pH 6 or a buffer with 50 mM Tris, 150 mM NaCl and 0.025% Tween-20 at pH 8. Test article dissociation was followed for 300 seconds by dipping the sensor in the respective pH buffer without test articles. Association and dissociation curves were exported using the Forte Data Analysis software.

[00158] The results are shown in FIG. 10. FcRn binding of the IgG1 xELL Fc used in these studies is comparable to that of wild type IgG1 Fc. Both molecules show similar association rates at pH 6 and have no appreciable affinity for FcRn at pH 8. Accordingly, an albumin-binding VHH domain is expected to enhance serum exposure of molecules with wild type IgG1 Fc domains, as well as molecules with IgG1 xELL domains.

Example 8: Binding of various single-domain antibody formats to human albumin

[00159] The ability of different monospecific and bispecific albumin-binding single domain antibody formats to bind recombinant human albumin at neutral (7.4) pH, and a second target (IL-4R) was evaluated by ELISA. Monospecific antibodies comprising an albumin-targeting sdAb (hz4A01v51 VHH) linked to the C-terminal end of an xELL Fc region via a glycine-serine linker of 6 or 12 residues, bispecific antibodies comprising the Fab domains (VL-CL (SEQ ID NO: 77 and VH-CH1 (SEQ ID NO: 76) of an IL-4R-targeting antibody (dupilumab), an IgG1 or IgG4 Fc region, and an albumin-targeting sdAb (hz4A01v51 VHH) positioned at different locations, and monospecific control IL-4R targeting molecules lacking the albumin-targeting sdAb were evaluated. The test article designations and general structure of the polypeptides used in this study are summarized in Table 2. For the ELISA 96-well ELISA plates (MaxiSorb, Biolegend), were coated with human albumin or IL4R at 1 ug/mL (100uL/well) overnight at 4°C in PBS. The plates were washed 3x times in 0.05% PBST (150uL/well) and then blocked with Casein in 0.05% PBST (200uL/well) for 2 hours at RT. The plates were washed 3x times in 0.05% PBST, and 100uL of titrated test articles in 0.05% PBST were added to the wells of the plate (starting 100nM, 1:3 dilutions, 11-point titration) and incubated at 4°C for 1 hour. After another wash, plates were then incubated for 30 min at room temperature with an HRP-conjugated secondary antibody in 0.05% PBST (100uL/well) specific to human IgG1 (Jackson ImmunoResearch). Plates were then washed again before addition of TMB substrate (100uL/well) that was allowed to reach RT before addition to the plate. The HRP-TMB reaction was allowed to develop for approximately 10 minutes, then TMB stop buffer was added

(100uL/well) and the absorbance was measured at 450 nm on a plate reader with absorbance at 650 nm subtracted (Molecular Devices). The data were plotted and analyzed using GraphPad Prism analysis software. The results are shown in FIG. 11A-11B.

Table 2

Designation	General Structure†	Type of Fc Region	SEQ ID NO:
cx12583 [^]	Fc Region-GS6-VHH	Human IgG1 xELL	77
cx12584 [^]	Fc Region-GS12-VHH	Human IgG1 xELL	78
cx12585*	VL-CL (light chain)	n/a	75
	VH-CH1-Fc Region (heavy chain)	Human IgG1 xELL	79
cx12587*	VL-CL (light chain)	n/a	75
	VH-CH1-Fc Region-VHH (heavy chain)	Human IgG1 xELL	80
cx12596*	VL-CL (light chain)	n/a	75
	VH-CH1-VHH-Fc Region (heavy chain)	Human IgG1 xELL	81
cx12590*	VL-CL (light chain)	n/a	75
	VH-CH1-Fc Region (heavy chain)	Human IgG4 (PYV)	82
cx12594*	VL-CL (light chain)	n/a	75
	VH-CH1-Fc Region-VHH (heavy chain)	Human IgG4 (PYV)	83
cx12595*	VL-CL (light chain)	n/a	75
	VH-CH1-VHH-Fc Region (heavy chain)	Human IgG4 (PYV)	84
VHH = albumin binding VHH 4A01v51 VL-CL = light chain of dupilumab VH-CH1 = variable heavy chain & constant region 1 of dupilumab [^] one chain of homodimer shown; <i>see, e.g.</i> , FIG. 6(iii) * one light chain and one heavy chain of tetramer having two light chains and two heavy chains is shown; <i>see, e.g.</i> , FIG. 6(v) and 6(vii)			

[00160] As shown in FIG. 11A, all the molecules comprising an anti-albumin VHH exhibited binding to albumin with the polypeptides comprising the VHH between the CH1 and Fc region exhibiting slightly higher affinity for albumin than the molecules with the anti-albumin VHH C-terminal to the Fc region. No binding was observed for the molecules lacking an anti-albumin VHH (cx12585 and cx12590). As shown in FIG. 11B, all the molecules comprising a binding domain for the second target (*i.e.*, the Fab domain of dupilumab) exhibited binding to the second target, IL-4R with very similar affinity demonstrating that the presence of an albumin-binding domain does not interfere with binding to a second target. No binding was observed for molecules lacking a binding domain for the second target (cx12583 and cx12584). These data, together with the data presented in Example 7 show that the albumin-targeting sdAb can mediate albumin binding when located at the N-terminus, C-terminus, or within a polypeptide (e.g., between domains) of a molecule. Accordingly, an albumin-binding VHH domain is expected to enhance serum exposure of molecules, including molecules comprising Fc regions, regardless of where the albumin-binding VHH domain is located.

Example 9: Modification of anti-albumin sdAb Hz4A01v51

[00161] The framework regions of Hz4A01v51 were further modified, including by back-mutating certain residues to the donor amino acid and/or introducing alternative charged residues. The modified VHHs (Hz4A01v51.9, Hz4A01v51.11, Hz4A01v51.12, and Hz4A01v51.13), were used to generate monovalent (VHH-fused to Fc NTT) anti-albumin binding molecules having the general structure shown in FIG. 6(ii). Several (Hz4A01v51.9, Hz4A01v51.12, and Hz4A01v51.13) were also used to generate bivalent (VHH-fused to Fc xELL) anti-albumin binding molecules having the general structure shown in FIG. 6(i). Binding of the monovalent and bivalent anti-albumin molecules and the monovalent Hz4A01v51-NNT-hFc to human serum albumin at pH 6.0 and at pH 7.0 was determined by ELISA as follows. Plates (MaxiSorb, Biolegend) were coated with albumin at 2ug/mL (100uL) overnight at 4°C in PBS, or with albumin at 2ug/mL (100uL) overnight at 4°C in 20mM His-HCl, 150mM NaCl pH 6. The plates were washed 3x times in 0.05% PBST or with the pH 6 buffer and then blocked with 5% Milk PBST for 2 hours at RT. The plates were washed 3x times in 0.05% PBST or with the pH 6 buffer and titrations of the test articles in PBST or the pH 6 buffer were added to the plates (starting 100nM, 1:5 dilutions) and incubated at 4°C for 1 hour. After another wash, plates were then incubated for 30 min at room temperature with an HRP-conjugated secondary antibody specific to human IgG1. Plates were then washed before addition of a TMB substrate. The HRP-TMB reaction was allowed to develop for six minutes, TMB stop buffer was added and the absorbance was measured at 450nm on a plate reader (Molecular Devices). The data were plotted and analyzed using GraphPad Prism analysis software. The 0.16 nM titration point was not tested for the negative control and ELL samples.

[00162] In the monovalent format the modified anti-albumin antibodies exhibit similar binding profiles at pH 6 (FIG. 12A), and pH 7.4 (FIG. 12B) and all exhibited improved binding over that observed for Hz4A01v51, particularly at pH 6. In the bivalent format, Hz4A01v51.9 and Hz4A01v51.13 exhibited similar binding profiles that were improved over Hz4A01v51 at both pH 6 (FIG. 12C) and pH 7.4 (FIG. 12D).

[00163] The disclosure may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the disclosure. Scope of the disclosure is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

Table of Certain Sequences

SEQ ID NO	Description	Sequence
1	Human serum albumin (HSA); UniProtKB/Swiss-Prot: P02768.2; mature form: amino acids 25-609	MKWVTFISLL FLFSSAYSRG VFRRAHAKSE VAHRFKDLGE ENFKALVLIA FAQYLQQCPF EDHVKLNVNEV TEFAKTCVAD ESAENCDKSL HTLFGDKLCT VATLRETYGE MADCCAKQEP ERNECFLQHK DDNPNLPLRV RPEVDVMCTA FHDNEETFLK KYLVEIARRH PYFYAPELLF FAKRYKAAFT ECCQAADKAA CLLPKLDELRL DEGKASSAKQ RLKASLQKF GERAFAKAWAV ARLSQRFPKA EFAEVSKLVT DLTQVHTECC HGDLLLECADD RADLAKYICE NQDSISSKLK ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF LGMFLYEYAR RHPDYSVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE FKPLVEEPQN LIKQNCLEFE QLGEYKFQNA LLVRYTKKVP QVSTPTLVEV SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV LNQLCVLHEK TPVSDRVTKC CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL SEKERQIKKQ TALVELVKHK PKATKEQLKA VMDDFAAFVE KCCKADDKET CFAEEGKKLV AASQAALGL
2	Murine serum albumin (MSA); UniProtKB/Swiss-Prot: P07724.3; mature form: amino acids 25-608	MKWVTFLLLL FVSGSAFSRG VFRREAHKSE IAHRYNLGE QHFKGLVLIA FSQYLQKCSY DEHAKLVQEV TDFAKTCVAD ESAANCDKSL HTLFGDKLCA IPNLRENYGE LADCCAKQEP ERNECFLQHK DDNPPLPFE RPEAEAMCTS FKENPTTFMG HYLHEVARRH PYFYAPELLY YAEQYNEILT QCCAEADKES CLTPKLDGVK EKALVSSVRQ RMKCSSMQKF GERAFAKAWAV ARLSQTFPNA DFAEITKLAT DLTQVNECC HGDLLLECADD RAELAKYMCE NQATISSKLQ TCCDKPLLK AHCLSEVEHD TMPADLPAIA ADFVEDQEV KNYAEAKDVF LGTFLYEYSR RHPDYSVSL LRLAKKYEAT LEKCCAEANP PACYGTVLAE FQPLVEEPKN LVKTNCDLYE KLGEYGFQNA ILVRYTQKAP QVSTPTLVEA ARNLGRVGTK CCTLPEDQRL PCVEDYLSAI LNRVCLLHEK TPVSEHVTCK CSGSLVERRP CSALTVDTE YVPKEFKAET FTFHSDICTL PEKEKQIKKQ TALAELVKHK PKATAEQLKT VMDDFAQFLD TCCKAADKDT CFSTEGPNLV TRCKDALA
3	Cynomolgus monkey serum albumin (CSA); mature form: amino acids 25-608	MKWVTFISLL FLFSSAYSRG VFRRDTHKSE VAHRFKDLGE EHFKGLVLVA FSQYLQQCPF EEHVKLNVNEV TEFAKTCVAD ESAENCDKSL HTLFGDKLCT VATLRETYGE MADCCAKQEP ERNECFLQHK DDNPNLPLV RPEVDVMCTA FHDNEATFLK KYLVEVARRH PYFYAPELLF FAARYKAAFA ECCQAADKAA CLLPKLDELRL DEGKASSAKQ RLKASLQKF GDRAFAKAWAV ARLSQKFPKA EFAEVSKLVT DLTQVHTECC HGDLLLECADD RADLAKYMCE NQDSISSKLK ECCDKPLLEK SHCLAEVEND EMPADLPSLA ADYVESKDVC KNYAEAKDVF LGMFLYEYAR RHPDYSVLL LRLAKAYEAT LEKCCAAADP HECYAKVFDE FQPLVEEPQN LVKQNCLEFE QLGEYKFQNA LLVRYTKKVP QVSTPTLVEV SRNLGKVGAK CCKLPEAKRM PCAEDYLSVV LNRLCVLHEK TPVSEKVTCK CTESLVNRRP CFSALELDEA YVPKAFNAET FTFHADMCTL SEKEKQVKKQ TALVELVKHK PKATKEQLKG VMDNFAAFVE KCCKADDKEA CFAEEGPKFV AASQAALA
4	Rat serum albumin (RSA); UniProtKB/Swiss-Prot: P02770.2; mature form: amino acids 25-608	MKWVTFLLLL FISGSASFSG VFRREAHKSE IAHRFKDLGE QHFKGLVLIA FSQYLQKCPY EEHIKLVQEV TDFAKTCVAD ENAENCDKSI HTLFGDKLCA IPKL RDNYGE LADCCAKQEP ERNECFLQHK DDNPNLPPFQ RPEAEAMCTS FQENPTSFLG HYLHEVARRH PYFYAPELLY YAEKYNEVLT QCCTESDKAA CLTPKLDVAV EKALVAARVQ RMKCSSMQRF GERAFAKAWAV ARMSQRFPA EFAEITKLAT DVTKINKECC HGDLLLECADD

		RAELAKYMCE NQATISSKLQ ACCDKPVLQK SQCLAEIEHD NIPADLPSIA ADFVEDKEVC KNYAEAKDVF LGTFLYEYSR RHPDYSVSL LRLAKKYEAT LEKCCAEGDP PACYGTVLAE FQPLVEEPKN LVKTNCELYE KLGEYGFQNA VLVRYTQKAP QVSTPTLVEA ARNLGRVGTK CCTLPEAQRL PCVEDYLSAI LNRLCVLHEK TPVSEKVT KC CSGSLVERRP CFSALTVD ET YVPKEFKAET FTFHSDICTL PDKEKQIKKQ TALAEVLKHK PKATEDQLKT VMGDFAQFVD KCCKAADKDN CFATEGPNLV ARSKEALA
5	4A01, Hz4A01v1, Hz4A01v3, Hz4A01v4, Hz4A01v5, Hz4A01v6, Hz4A01v8, Hz4A01v9, Hz4A01v3-5, Hz4A01v3-7, Hz4A01v3-8 CDR1	GFAFSTSGMV
6	Hz4A01v3-1, Hz4A01v3-7.1k-RITA, Hz4A01v3-7.1k-RITI, Hz4A01v3-7.1k-RITL, Hz4A01v3-7.1k-RITS, Hz4A01v3-7.1k-RITT, Hz4A01v3-7.1k-RITV, Hz4A01v51, Hz4A01v51.9, Hz4A01v51.11, Hz4A01v51.12, Hz4A01v51.13 CDR1	GFTFSTSGMV
7	Hz4A01v3-3 CDR1	GFAFSTSGMA
8	Hz4A01v3-4 CDR1	GFAFSTSGMS
9	4A01, Hz4A01v1 CDR2	TISDDSRIR
10	Hz4A01v4 CDR2	TISDESRIIR
11	Hz4A01v5 CDR2	TISDEGRIIR
12	Hz4A01v6 CDR2	TISDSGRIIR
13	Hz4A01v8 CDR2	TISDNARIIR
14	Hz4A01v9 CDR2	TISDDTRIIR
15	Hz4A01v3 Hz4A01v3-1, Hz4A01v3-3, Hz4A01v3-4, Hz4A01v3-5, Hz4A01v3-7, Hz4A01v3-8 CDR2	TISDDARIIR
16	Hz4A01v3-7.1k-RITA CDR2	TISDDARITA
17	Hz4A01v3-7.1k-RITI CDR2	TISDDARITI
18	Hz4A01v3-7.1k-RITL CDR2	TISDDARITL
19	Hz4A01v3-7.1k-RITS CDR2	TISDDARITS

20	Hz4A01v3-7.1k-RITT CDR2	TISDDARITT
21	Hz4A01v3-7.1k-RITV, Hz4A01v51, Hz4A01v51.9, Hz4A01v51.11, Hz4A01v51.12, Hz4A01v51.13 CDR2	TISDDARITV
22	4A01, Hz4A01v1, Hz4A01v3, Hz4A01v4, Hz4A01v5, Hz4A01v6, Hz4A01v8, Hz4A01v9, Hz4A01v3-1, Hz4A01v3-3, Hz4A01v3-4, Hz4A01v3-5, Hz4A01v3-7, Hz4A01v3-8, Hz4A01v3-7.1k-RITA, Hz4A01v3-7.1k-RITI, Hz4A01v3-7.1k-RITL, Hz4A01v3-7.1k-RITS, Hz4A01v3-7.1k-RITT, Hz4A01v3-7.1k-RITV, Hz4A01v51, Hz4A01v51.9, Hz4A01v51.11, Hz4A01v51.12, Hz4A01v51.13 CDR3	YRAGSRIGSYEDYY
23	4A01 VHH	QVQLQESGGGLVQPGGSLRLSCAASGFAFSTSGMVWVRQATGKG LEWVSTISDDSRIRYADSVNGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTQVTVSS
24	Hz4A01v1	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWVRQAPGKG LEWVSTISDDSRIRYAESVKGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTLLVTVKP
25	Hz4A01v3	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWVRQAPGKG LEWVSTISDDARIIRYAESVKGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTLLVTVKP
26	Hz4A01v4	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWVRQAPGKG LEWVSTISDESRIIRYAESVKGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTLLVTVKP
27	Hz4A01v5	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWVRQAPGKG LEWVSTISDEGRIIRYAESVKGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTLLVTVKP
28	Hz4A01v6	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWVRQAPGKG LEWVSTISDSGRIIRYAESVKGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTLLVTVKP
29	Hz4A01v8	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWVRQAPGKG LEWVSTISDNARIIRYAESVKGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTLLVTVKP
30	Hz4A01v9	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWVRQAPGKG LEWVSTISDDTRIIRYAESVKGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTLLVTVKP
31	Hz4A01v3-1	EVQLVESGGGEVQPGGSLRLSCAASGFTFSTSGMVWVRQAPGKG LEWVSTISDDARIIRYAESVKGRFTISRDNKNTLYLQMNLSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGTLLVTVKP

32	H _z 4A01v3-3	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMAVWRQAPGKG LEWVSTISDDARIIRYAESVKGRFTISRDNKNTLYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
33	H _z 4A01v3-4	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMSVWRQAPGKG LEWVSTISDDARIIRYAESVKGRFTISRDNKNTLYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
34	H _z 4A01v3-5	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWRQAPGKG LEWVSTISDDARIIRYAESVKGRFTISRDNKNTLYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
35	H _z 4A01v3-7	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWRQAPGKG LEWVSTISDDARIIRYAESVKGRFTISRDNKNTLYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
36	H _z 4A01v3-8	EVQLVESGGGEVQPGGSLRLSCAASGFAFSTSGMVWRQAPGKG LEWVSTISDDARIIRYAESVKGRFTISRDNKNTLYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYWGQGT LVTVKP
37	H _z 4A01v3-7.1k-RITA	EVQLVESGGGEVQPGKSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITAYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
38	H _z 4A01v3-7.1k-RITI	EVQLVESGGGEVQPGKSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITIIYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
39	H _z 4A01v3-7.1k-RITL	EVQLVESGGGEVQPGKSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITLYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
40	H _z 4A01v3-7.1k-RITS	EVQLVESGGGEVQPGKSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITSYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
41	H _z 4A01v3-7.1k-RITT	EVQLVESGGGEVQPGKSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITTYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
42	H _z 4A01v3-7.1k-RITV	EVQLVESGGGEVQPGKSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITVYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
43	H _z 4A01v51 VHH	EVQLVESGGGEVQPGGSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITVYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKP
71	H _z 4A01v51.9 VHH	QVQLLES GGGEVQPGGSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITVYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVEP
72	H _z 4A01v51.11 VHH	QVQLLES GGGKVQPGGSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITVYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVEP
73	H _z 4A01v51.12 VHH	EVQLLES GGGKVQPGGSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITVYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVEP
74	H _z 4A01v51.13 VHH	EVQLVESGGGEVQPGGSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITVYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVRP
69	cx5009	EVQLVESGGGEVQPGGSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITVYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKPGGGGPSVFL FPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLT VLVHQLDNLNGKEYCKVSNKALPAP PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVNL TCVLKGFYFP SDIAVEWESNGQPENNYKTT PPVLDSDGSFFLNSLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGSGGGGS
70	cx11956 (one chain of the homodimer shown)	EVQLVESGGGEVQPGGSLRLSCAASGFTFSTSGMVWRQAPGKG LEWVSTISDDARITVYAESVKGRFTISRDNKNTVYLQMNSLRA EDTAVYYCATYRAGSRIGSYEDYYRGQGT LVTVKPGGGGDKTHT CPPCPAPGGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLVHQLDNLNG KEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK

		NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
75	dupilumab VL-CL (light chain in cx12585, cx12587, cx12596, cx12590, cx12594, cx12595)	DIVMTQSPSLSLPVTGPGEPAISCRSSQSLLYSIGNYLDWYLQK SGQSPQLLIYLGNSRASGVDPDRFSGSGSGTDFTLKISRVEAEDV GFYYCMQALQTPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKKS GTASVVCCLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
76	dupilumab VH-CH1	EVQLVESGGGLEQPGGSLRLSCAGSGGFTFRDYAMTWVRQAPGKG LEWVSSISGSGGNTYYADSVKGRFTISRDNKNTLYLQMNLSRA EDTAVYYCAKDRLSITIRPRYYGLDVWGQGTITVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKVD KRV
77	cx12583 (one chain of the homodimer shown)	GGGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP PVLDSDGSEFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQK SLSLSPGSGGGSEVQLVESGGGEVQPGGSLRLSCAASGFTFSTS GMVWVRQAPGKGLEWVSTISDDARITVYAESVKGRFTISRDNK NTVYLQMNLSRAEDTAVYYCATYRAGSRIGSYEDYYRGQGTLT VKP
78	cx12584 (one chain of the homodimer shown)	GGGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP PVLDSDGSEFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQK SLSLSPGSGGGSEVQLVESGGGEVQPGGSLRLSCAASG FTFSTSGMVWVRQAPGKGLEWVSTISDDARITVYAESVKGRFTI SRDNAKNTVYLQMNLSRAEDTAVYYCATYRAGSRIGSYEDYYRG QGTLTVTVPK
79	cx12585 heavy chain (one chain of the tetramer shown)	EVQLVESGGGLEQPGGSLRLSCAGSGGFTFRDYAMTWVRQAPGKG LEWVSSISGSGGNTYYADSVKGRFTISRDNKNTLYLQMNLSRA EDTAVYYCAKDRLSITIRPRYYGLDVWGQGTITVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKVD KRVDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKS LSLSP
80	cx12587 heavy chain (one chain of the tetramer shown)	EVQLVESGGGLEQPGGSLRLSCAGSGGFTFRDYAMTWVRQAPGKG LEWVSSISGSGGNTYYADSVKGRFTISRDNKNTLYLQMNLSRA EDTAVYYCAKDRLSITIRPRYYGLDVWGQGTITVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKVD KRVDKTHTCPPCPAPGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKS LSLSPGSGGGSEVQLVESGGGEVQPGGSLRLSCAASGFTFSTSG MVWVRQAPGKGLEWVSTISDDARITVYAESVKGRFTISRDNK NTVYLQMNLSRAEDTAVYYCATYRAGSRIGSYEDYYRGQGTLT VTKP
81	cx12596 heavy chain (one chain of the tetramer shown)	EVQLVESGGGLEQPGGSLRLSCAGSGGFTFRDYAMTWVRQAPGKG LEWVSSISGSGGNTYYADSVKGRFTISRDNKNTLYLQMNLSRA EDTAVYYCAKDRLSITIRPRYYGLDVWGQGTITVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKVD KRVGGSGGSEVQLVESGGGEVQPGGSLRLSCAASGFTFSTSGM VVRQAPGKGLEWVSTISDDARITVYAESVKGRFTISRDNKNTV

		YLQMNSLRAEDTAVYYCATYRAGSRIGSYEDYYRGQGLTVTKP GGGGDKTHTCPPCPAPGGPSVFLFPPKPKDTLMI SRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PVLDSGDSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQK SLSLSP
82	cx12590 heavy chain (one chain of the tetramer shown)	EVQLVESGGGLEQPGGSLRLSCAGSGFTFRDYAMTWVRQAPGKG LEWVSSISGSGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRA EDTAVYYCAKDRLSITIRPRYYGLDVWGQGT TTVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVH TFFPAVLQSSGLYSLSSVTVPSSSLG TKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLYISRTPEV TCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV SVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVHEALHNH YTQKSLSLSLG
83	cx12594 heavy chain (one chain of the tetramer shown)	EVQLVESGGGLEQPGGSLRLSCAGSGFTFRDYAMTWVRQAPGKG LEWVSSISGSGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRA EDTAVYYCAKDRLSITIRPRYYGLDVWGQGT TTVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVH TFFPAVLQSSGLYSLSSVTVPSSSLG TKTYTCNV DHKPSNTKVD KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLYISRTPEV TCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV SVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVHEALHNH YTQKSLSLSLGSGGSEVQLVESGGGEVQPGGSLRLSCAASGFT FSTSGMVWVRQAPGKGLEWVSTISDDARITVYAESVKGRFTISR DNAKNTVYLQMNSLRAEDTAVYYCATYRAGSRIGSYEDYYRGQG TLTVTKP
84	cx12595 heavy chain (one chain of the tetramer shown)	EVQLVESGGGLEQPGGSLRLSCAGSGFTFRDYAMTWVRQAPGKG LEWVSSISGSGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRA EDTAVYYCAKDRLSITIRPRYYGLDVWGQGT TTVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVH TFFPAVLQSSGLYSLSSVTVPSSSLG TKTYTCNV DHKPSNTKVD KRVGGSGGSEVQLVESGGGEVQPGGSLRLSCAASGFTFSTSGMV WVRQAPGKGLEWVSTISDDARITVYAESVKGRFTISRDN AKNTV YLQMNSLRAEDTAVYYCATYRAGSRIGSYEDYYRGQGLTVTKP GGGESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLYISRTPE VTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVHEALHN HYTQKSLSLSLG
47	human IgG1 Fc region	DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PVLDSGDSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQK SLSLSPGK
48	human IgG1 Fc xELL	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYT QKSLSLSPGK
49	IgG1 Fc region M252Y and M428V (YV) S354C T366W knob	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTT PP

		VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVHEALHNHYTQKSLSLSPGK
50	IgG1 Fc region M252Y, M428V, H435R (YVR) T366S, L368A, Y407V hole	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLP PSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVHEALHNRYTQKSLSLSPGK
51	IgG1 Fc region xELL H435R	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVHEALHNRYTQKSLSLSPGK
52	IgG1 Fc region xELL M252Y and M428V (YV)	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVHEALHNHYTQKSLSLSPGK
53	IgG1 Fc region xELL M252Y and M428L (YL)	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHNHYTQKSLSLSPGK
54	IgG1 Fc region xELL M252Y, M428L, H435R (YLR)	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHNRYTQKSLSLSPGK
55	IgG1 Fc region xELL M252Y, M428V, H435R (YVR)	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVHEALHNRYTQKSLSLSPGK
56	IgG1 Fc region xELL S354C T366W knob	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVHEALHNHYTQKSLSLSPGK
57	IgG1 Fc region xELL H435R S354C T366W knob	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVHEALHNRYTQKSLSLSPGK
58	IgG1 Fc region xELL M252Y and M428V (YV) S354C T366W knob	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVHEALHNHYTQKSLSLSPGK
59	IgG1 Fc region xELL M252Y and M428L (YL) S354C T366W knob	DKTHTCPPCPAPGPGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHNHYTQKSLSLSPGK

60	IgG1 Fc region xELL M252Y, M428L, H435R (YLR) S354C T366W knob	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVVFSCSVLHEALHNRYTQKSLSL SPGK
61	IgG1 Fc region xELL M252Y, M428V, H435R (YVR) S354C T366W knob	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVVFSCSVVHEALHNRYTQKSLSL SPGK
62	IgG1 Fc region xELL T366S, L368A, Y407V hole	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMIISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSR DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSL SPGK
63	IgG1 Fc region xELL H435R, T366S, L368A, Y407V hole	DKTHTCPPCPAPGGPSVFLFPPKPKDTLMIISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSR DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVVFSCSVMHEALHNRYTQKSLSL SPGK
64	IgG1 Fc region xELL M252Y and M428V (YV) T366S, L368A, Y407V hole	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSR DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVVFSCSVVHEALHNHYTQKSLSL SPGK
65	IgG1 Fc region xELL M252Y and M428L (YL) T366S, L368A, Y407V hole	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSR DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVVFSCSVLHEALHNHYTQKSLSL SPGK
66	IgG1 Fc region xELL M252Y, M428L, H435R (YLR) T366S, L368A, Y407V hole	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSR DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVVFSCSVLHEALHNRYTQKSLSL SPGK
67	IgG1 Fc region xELL M252Y, M428V, H435R (YVR) T366S, L368A, Y407V hole	DKTHTCPPCPAPGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSR DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVVFSCSVVHEALHNRYTQKSLSL SPGK
68	IgG1 Fc region S364N, Y407N, and K409T (NNT)	GGPSVFLFPPKPKDTLMIISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVNLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLNSTLT VDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPG
85	Human IgG4 Fc region	ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMIISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT LPSSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVVFSCSVMHEALHNHYTQ KSLSLSLGK
86	IgG4 Fc region S228P (P)	ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMIISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT LPSSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT

		PPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQ KSLSLSLGK
87	IgG4 Fc region S228P, M252Y and M428V (PYV)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLYISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQ KSLSLSLGK
88	Linker 1	GGGG
89	Linker 2	GSGGGS
90	Linker 3	GSGGGS
91	Linker 4	GSGSGGSGGGS
92	Linker 5	GSGGGS
93	Linker 6	GSGGSGSGGGS
94	VH3-23*04	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKG LEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRA EDTAVYYC

What is claimed is:

1. A polypeptide comprising at least one VHH domain that binds albumin, wherein at least one VHH domain that binds albumin comprises a CDR1 sequence selected from SEQ ID NOs: 5-8, a CDR2 sequence selected from SEQ ID NOs: 9-21, and a CDR3 sequence of SEQ ID NO: 22.
2. The polypeptide of claim 1, wherein each VHH domain that binds albumin comprises, independently, a CDR1 sequence selected from SEQ ID NOs: 5-8, a CDR2 sequence selected from SEQ ID NOs: 9-21, and a CDR3 sequence of SEQ ID NO: 22.
3. The polypeptide of claim 1 or claim 2, wherein at least one VHH domain that binds albumin comprises CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 5, 9, and 22; SEQ ID NOs: 5, 10, and 22; SEQ ID NOs: 5, 11, and 22; SEQ ID NOs: 5, 12, and 22; SEQ ID NOs: 5, 13, and 22; SEQ ID NOs: 5, 14, and 22; SEQ ID NOs: 5, 15, and 22; SEQ ID NOs: 6, 15, and 22; SEQ ID NOs: 7, 15, and 22; SEQ ID NOs: 8, 15, and 22; SEQ ID NOs: 6, 16, and 22; SEQ ID NOs: 6, 17, and 22; SEQ ID NOs: 6, 18, and 22; SEQ ID NOs: 6, 19, and 22; SEQ ID NOs: 6, 20, and 22; and SEQ ID NOs: 6, 21, and 22.
4. The polypeptide of claim 3, wherein each VHH domain that binds albumin comprises, independently, CDR1, CDR2, and CDR3 sequences selected from: SEQ ID NOs: 5, 9, and 22; SEQ ID NOs: 5, 10, and 22; SEQ ID NOs: 5, 11, and 22; SEQ ID NOs: 5, 12, and 22; SEQ ID NOs: 5, 13, and 22; SEQ ID NOs: 5, 14, and 22; SEQ ID NOs: 5, 15, and 22; SEQ ID NOs: 6, 15, and 22; SEQ ID NOs: 7, 15, and 22; SEQ ID NOs: 8, 15, and 22; SEQ ID NOs: 6, 16, and 22; SEQ ID NOs: 6, 17, and 22; SEQ ID NOs: 6, 18, and 22; SEQ ID NOs: 6, 19, and 22; SEQ ID NOs: 6, 20, and 22; and SEQ ID NOs: 6, 21, and 22.
5. The polypeptide of any one of claims 1-4, wherein at least one VHH domain that binds albumin is humanized.
6. The polypeptide of claim 5, wherein each VHH domain that binds albumin is humanized.
7. The polypeptide of any one of claims 1-6, wherein at least one VHH domain that binds albumin comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 23-43 and 71-74.
8. The polypeptide of claim 7, wherein each VHH domain that binds albumin comprises a sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a sequence selected from SEQ ID NOs: 23-43 and 71-74.
9. The polypeptide of any one of claims 1-7, wherein at least one VHH domain that binds albumin comprises a sequence selected from SEQ ID NOs: 23-43 and 71-74.

10. The polypeptide of any one of claims 1-9, wherein each VHH domain that binds albumin comprises a sequence selected from SEQ ID NOs: 23-43 and 71-74.
11. The polypeptide of any one of claims 1-10, wherein at least one VHH domain that binds albumin binds human albumin and at least one albumin selected from cynomolgus monkey, mouse, and rat albumin.
12. The polypeptide of any one of claims 1-11, wherein each VHH domain that binds albumin binds human albumin and at least one albumin selected from cynomolgus monkey, mouse, and rat albumin.
13. The polypeptide of any one of claims 1-12, wherein at least one VHH domain that binds albumin binds human, cynomolgus monkey, mouse, and rat albumin.
14. The polypeptide of any one of claims 1-13, wherein each VHH domain that binds albumin binds human, cynomolgus monkey, mouse, and rat albumin.
15. The polypeptide of any one of claims 1-14, wherein at least one VHH domain that binds albumin binds human albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.
16. The polypeptide of any one of claims 1-15, wherein at least one VHH domain that binds albumin binds each of human, cynomolgus monkey, mouse, and rat albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.
17. The polypeptide of any one of claims 1-16, wherein each VHH domain that binds albumin binds human albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.
18. The polypeptide of any one of claims 1-17, wherein each VHH domain that binds albumin binds each of human, cynomolgus monkey, mouse, and rat albumin with an affinity of less than 5 nM, less than 2 nM, less than 1 nM, or less than 0.5 nM.
19. The polypeptide of any one of claims 1-18, wherein the each VHH domain that binds albumin does not bind albumin domain 3.
20. The polypeptide of any one of claims 1-19, wherein the each VHH domain that binds albumin does not interfere with binding of albumin to FcRn.
21. The polypeptide of any one of claims 1-20, wherein the polypeptide comprises at least one binding domain that binds a protein other than albumin.
22. The polypeptide of claim 21, wherein at least one binding domain that binds a protein other than albumin is a VHH.
23. The polypeptide of claim 22, wherein each binding domain that binds a protein other than albumin is a VHH.

24. The polypeptide of claim 21, wherein at least one binding domain that binds a protein other than albumin comprises a heavy chain variable region and a light chain variable region.
25. The polypeptide of claim 24, wherein each binding domain that binds a protein other than albumin comprises a heavy chain variable region and a light chain variable region.
26. The polypeptide of any one of claims 21-25, wherein at least one binding domain that binds a protein other than albumin is a binding domain of a therapeutic antibody.
27. The polypeptide of claim 26, wherein each binding domain that binds a protein other than albumin is a binding domain of a therapeutic antibody.
28. The polypeptide of claim 26 or claim 27, wherein the therapeutic antibody is useful for treating a disease or disorder selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.
29. The polypeptide of any one of claim 1-28, wherein the polypeptide comprises an amino acid sequence of a therapeutic protein.
30. The polypeptide of claim 29, wherein the therapeutic protein is useful for treating a disease or disorder selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.
31. The polypeptide of any one of claims 1-30, wherein the polypeptide comprises an Fc region.
32. The polypeptide of claim 31, wherein the Fc region binds FcRn.
33. The polypeptide of claim 31 or claim 32, wherein the Fc region is an IgG1 Fc region.
34. The polypeptide of any one of claims 31-33, wherein the Fc region comprises one or more substitutions that enhance half-life.
35. The polypeptide of claim 34, wherein the Fc region comprises one or more substitutions that enhance FcRn binding at at least one pH and/or reduce dissociation rate of Fc and FcRn.
36. The polypeptide of any one of claims 31-35, wherein the Fc region comprises substitutions at one or more amino acid positions selected from 252, 254, 256, 428, or 434.
37. The polypeptide of claim 36, wherein the Fc region comprises substitutions at amino acid positions 252, 254, and 256; or amino acid positions 252 and 428; or amino acid positions 428 and 434.
38. The polypeptide of claim 37, wherein the Fc region comprises substitutions M252Y, S254T, and T256E; M252Y and M428V; or M428L and N434S.

39. The polypeptide of any one of claims 31-38, wherein the Fc region comprises a sequence selected from SEQ ID NOs: 47-68 and 85-87.

40. The polypeptide of any one of claims 1-39, wherein the half-life of the polypeptide is greater than the half-life of the same polypeptide lacking a VHH domain that binds albumin.

41. A pharmaceutical composition comprising the polypeptide of any one of claims 1-40 and a pharmaceutically acceptable carrier.

42. An isolated nucleic acid that encodes the polypeptide of any one of claims 1-40.

43. A vector comprising the nucleic acid of claim 42.

44. A host cell comprising the nucleic acid of claim 42 or the vector of claim 43.

45. A host cell that expresses the polypeptide of any one of claims 1-40.

46. A method of producing the polypeptide of any one of claims 1-40, comprising incubating the host cell of claim 44 or claim 45 under conditions suitable for expression of the polypeptide.

47. The method of claim 46, further comprising isolating the polypeptide.

48. A method comprising administering to a subject the polypeptide of any one of claims 1-40, or the pharmaceutical composition of claim 41.

49. A method of treating a disease or disorder comprising administering to a subject with the disease or disorder a pharmaceutically effective amount of the polypeptide of any one of claims 1-40, or the pharmaceutical composition of claim 41.

50. The method of claim 49, wherein the disease or disorder is selected from an autoimmune disease or disorder, an inflammatory disease or disorder, an infection, and cancer.

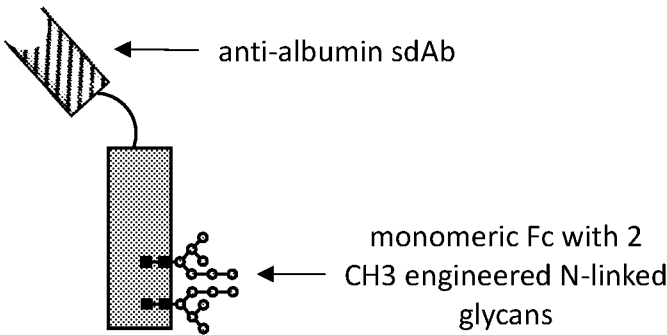
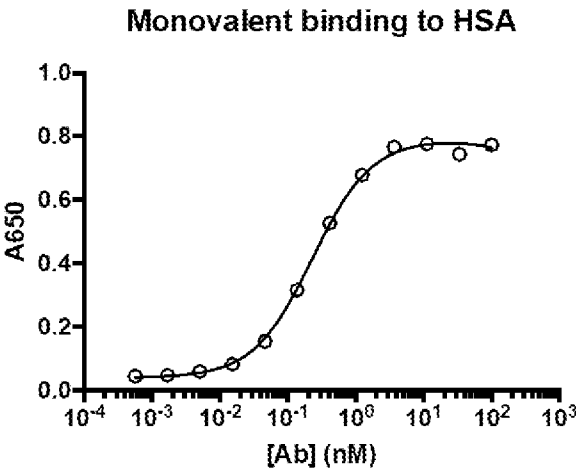
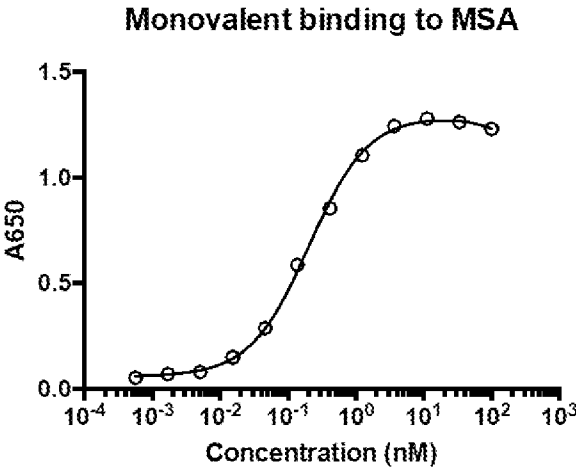


FIG. 1A



	4A01
Kd	0.2298

FIG. 1B



	4A01
Kd	0.2037

FIG. 1C

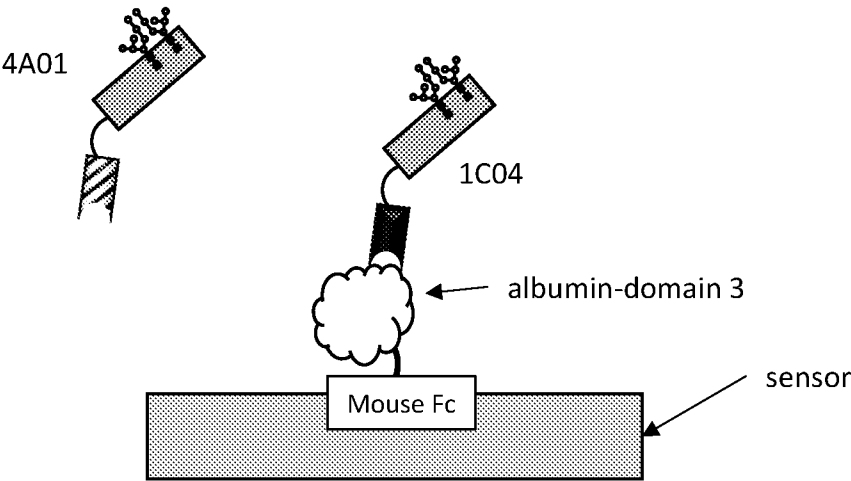


FIG. 2A

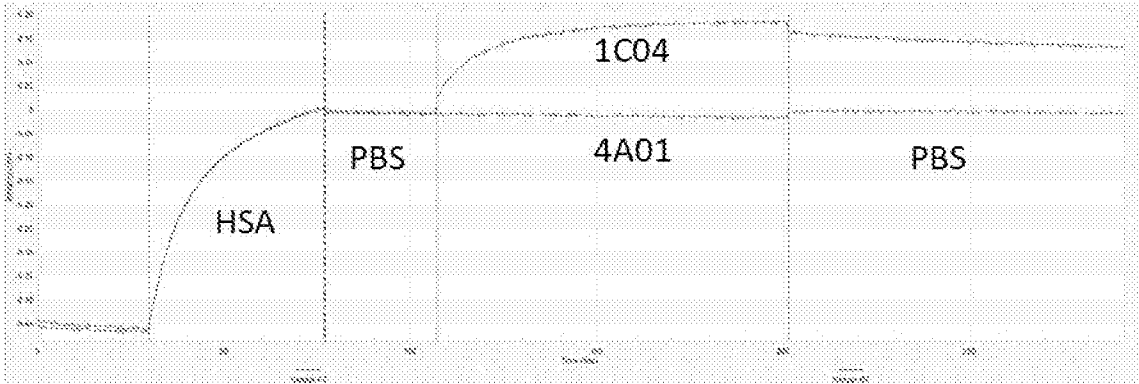


FIG. 2B

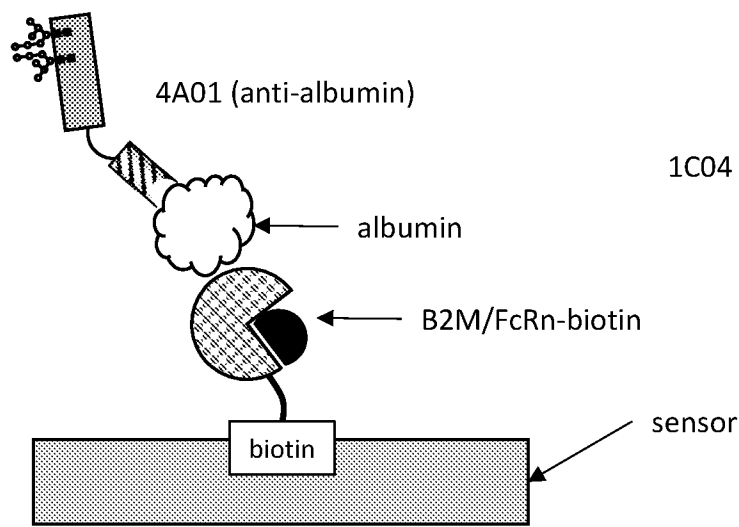


FIG. 3A

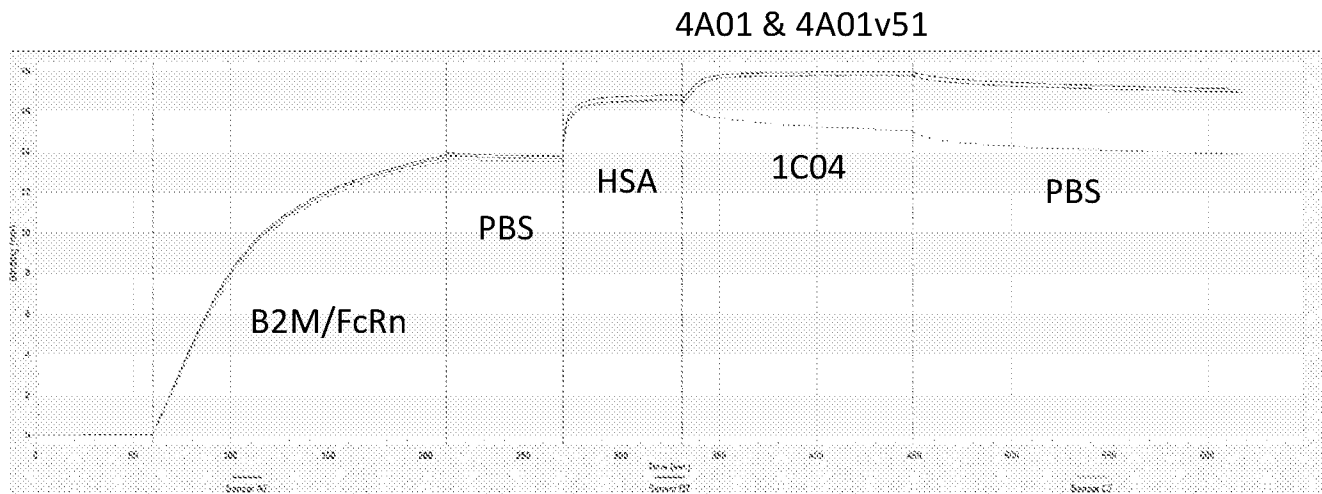


FIG. 3B

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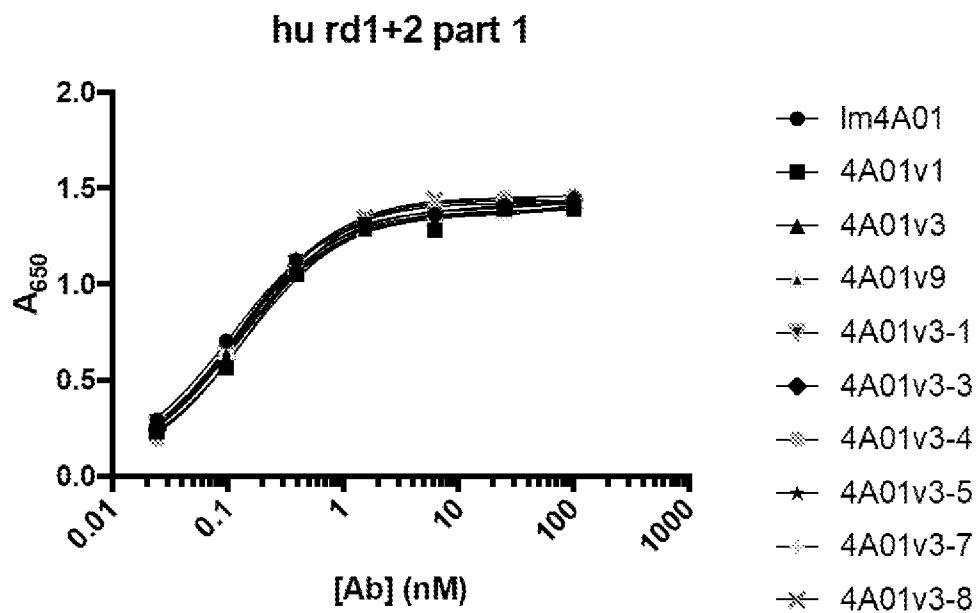
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4A01v3~7.1k~RITL      EVQLVESGGGGEVQPGKSLRLSCAASGFTTFSTSGMVAWVRQAPGKGLWVSTISDDARITLY
4A01v3~7.1k~RITS      EVQLVESGGGGEVQPGKSLRLSCAASGFTTFSTSGMVAWVRQAPGKGLWVSTISDDARITSY
4A01v3~7.1k~RITT      EVQLVESGGGGEVQPGKSLRLSCAASGFTTFSTSGMVAWVRQAPGKGLWVSTISDDARITTY
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4A01v4                EVQLVESGGGGEVQPGGSLRLSCAASGFAFSTSGMVAWVRQAPGKGLWVSTISDSERIIRY
4A01v5                EVQLVESGGGGEVQPGGSLRLSCAASGFAFSTSGMVAWVRQAPGKGLWVSTISDSERIIRY
4A01v6                EVQLVESGGGGEVQPGGSLRLSCAASGFAFSTSGMVAWVRQAPGKGLWVSTISDSERIIRY
4A01v1                EVQLVESGGGGEVQPGGSLRLSCAASGFAFSTSGMVAWVRQAPGKGLWVSTISDSERIIRY
4A01v9                EVQLVESGGGGEVQPGGSLRLSCAASGFAFSTSGMVAWVRQAPGKGLWVSTISDDTRIIRY
4A01v8                EVQLVESGGGGEVQPGGSLRLSCAASGFAFSTSGMVAWVRQAPGKGLWVSTISDNARIIRY
4A01v3                EVQLVESGGGGEVQPGGSLRLSCAASGFAFSTSGMVAWVRQAPGKGLWVSTISDDARIIRY
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4A01v3~7              EVQLVESGGGGEVQPGGSLRLSCAASGFAFSTSGMVAWVRQAPGKGLWVSTISDDARIIRY
4A01v3~1              EVQLVESGGGGEVQPGGSLRLSCAASGFTTFSTSGMVAWVRQAPGKGLWVSTISDDARIIRY
VH3~23*04             EVQLVESGGGGLVQPGGSLRLSCAASGFTTFSSYAMSWVRQAPGKGLWVSAISGSGGSTYY
*****  ****  *****;***; . * * *****;***. *

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4A01v3~7.1k~RITI      AESVKGRFTISRDNAKNTVYLQMNSLRAEDTAVYYCATYRAGSRIGSYEDYYRCQCTLVT
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4A01v1                AESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCATYRAGSRIGSYEDYYRCQCTLVT
4A01v9                AESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCATYRAGSRIGSYEDYYRCQCTLVT
4A01v8                AESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCATYRAGSRIGSYEDYYRCQCTLVT
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4A01v3~8              AESVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCATYRAGSRIGSYEDYYRCQCTLVT
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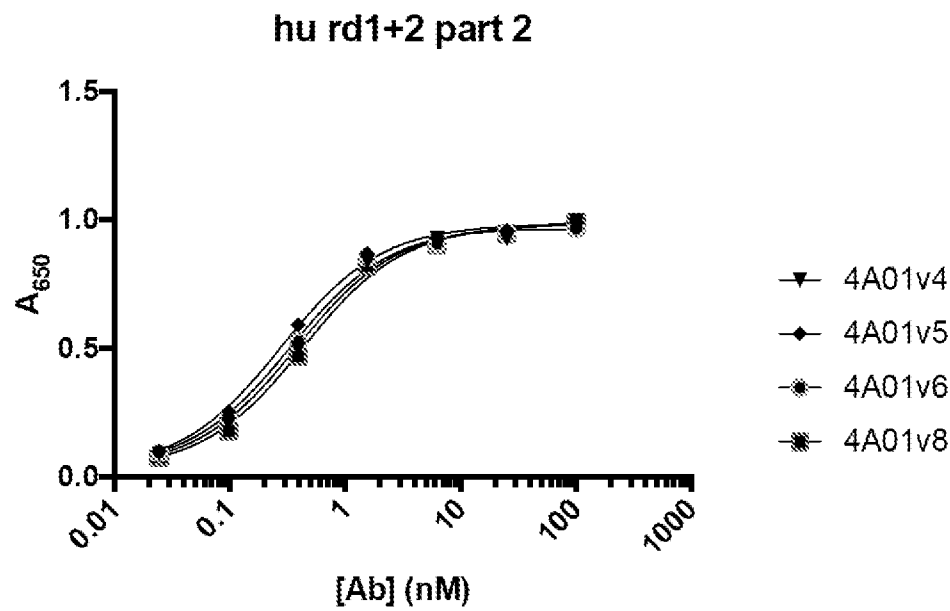
FIG. 4A

5/23



	Bmax	Kd
Im4A01	1.341	0.1047
4A01v1	1.338	0.1391
4A01v3	1.321	0.1245
4A01v9	1.351	0.1145
4A01v3-1	1.321	0.123
4A01v3-3	1.362	0.1287
4A01v3-4	1.427	0.1515
4A01v3-5	1.394	0.1215
4A01v3-7	1.398	0.1213
4A01v3-8	1.414	0.1355

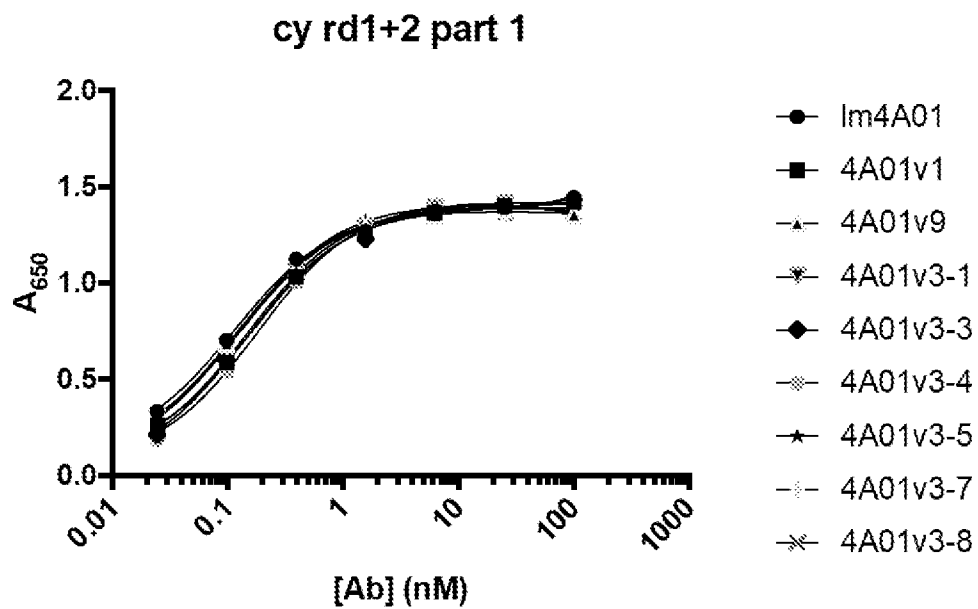
FIG. 4B



	Bmax	Kd
4A01v4	0.947	0.3783
4A01v5	0.9567	0.2763
4A01v6	0.9403	0.3321
4A01v8	0.9545	0.4283

FIG. 4C

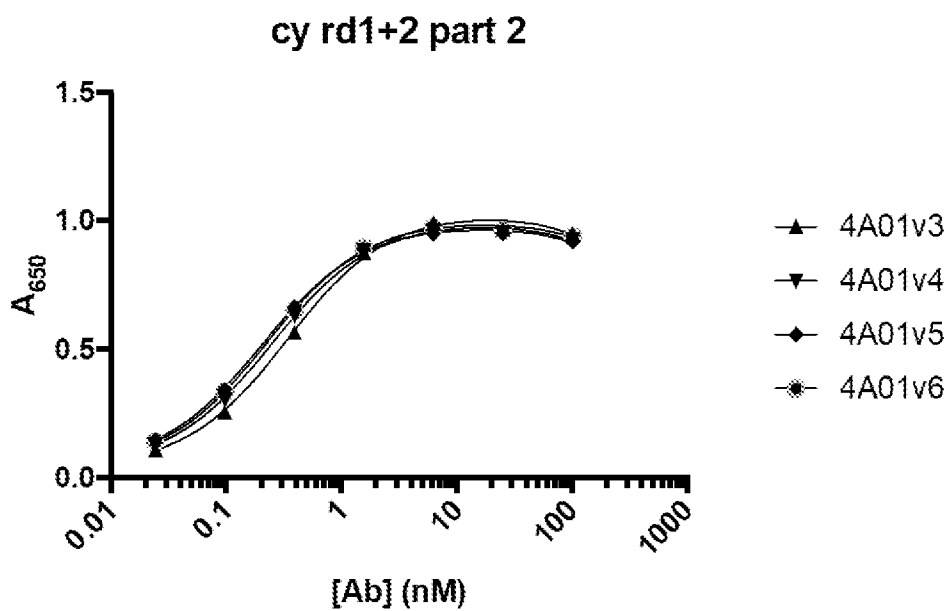
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	Bmax	Kd
Im4A01	1.268	0.1126
4A01v1	1.309	0.1572
4A01v9	1.294	0.117
4A01v3-1	1.295	0.1272
4A01v3-3	1.35	0.1417
4A01v3-4	1.37	0.181
4A01v3-5	1.34	0.1262
4A01v3-7	1.312	0.1178
4A01v3-8	1.347	0.1467

FIG. 4D

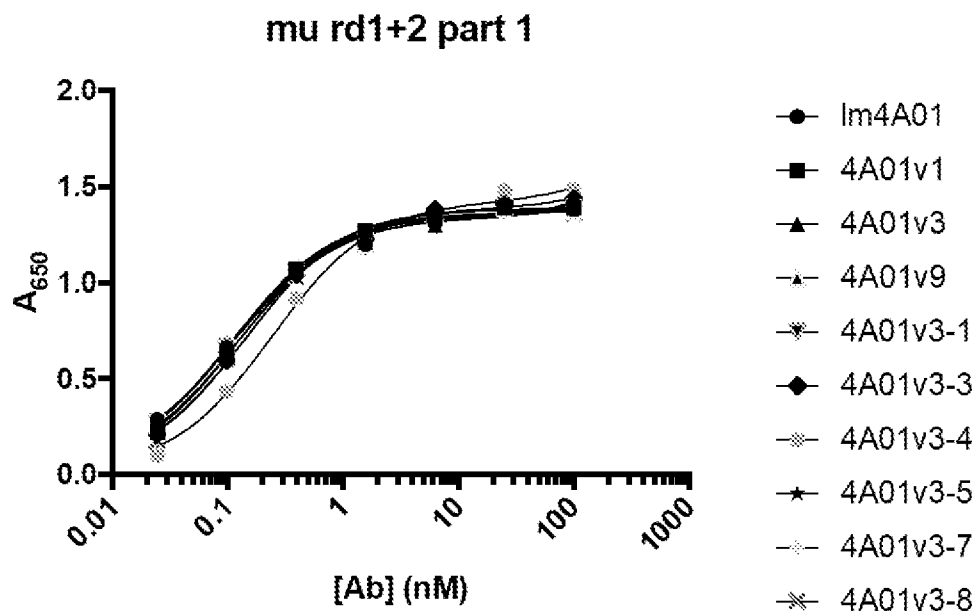
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	Bmax	Kd
4A01v3	0.9943	0.3381
4A01v4	0.9542	0.2474
4A01v5	0.9393	0.2032
4A01v6	0.9607	0.2253

FIG. 4E

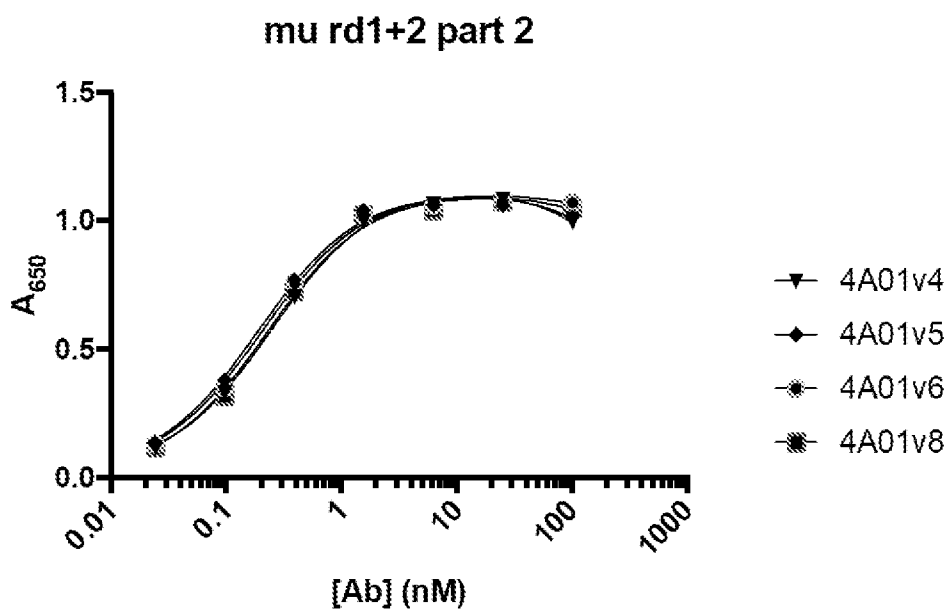
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	Bmax	Kd
Im4A01	1.264	0.1141
4A01v1	1.352	0.1285
4A01v3	1.296	0.1207
4A01v9	1.303	0.1121
4A01v3-1	1.289	0.109
4A01v3-3	1.35	0.1417
4A01v3-4	1.401	0.2409
4A01v3-5	1.31	0.1117
4A01v3-7	1.317	0.1105
4A01v3-8	1.357	0.1427

FIG. 4F

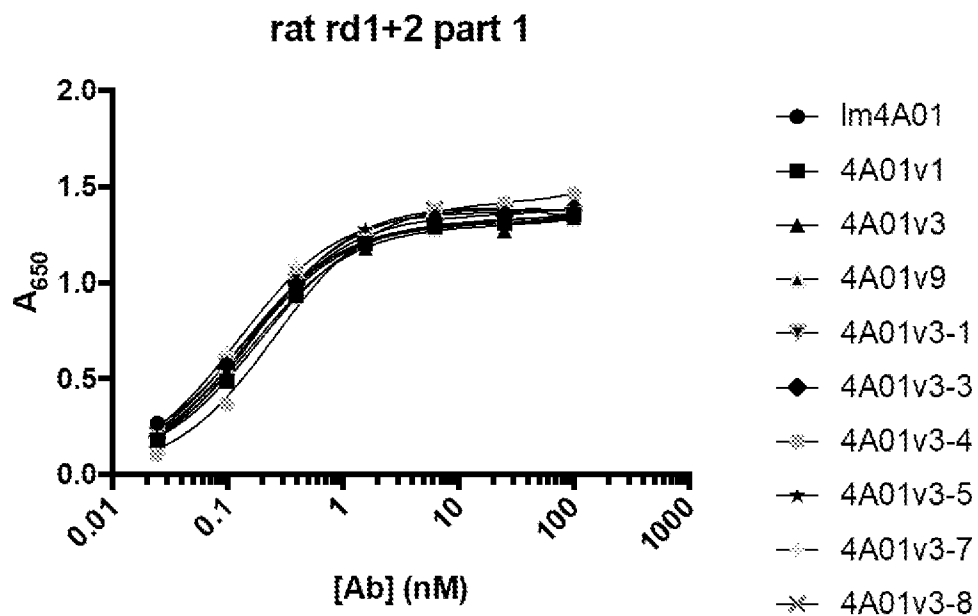
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	Bmax	Kd
4A01v4	1.103	0.2503
4A01v5	1.095	0.1888
4A01v6	1.089	0.2064
4A01v8	1.097	0.2257

FIG. 4G

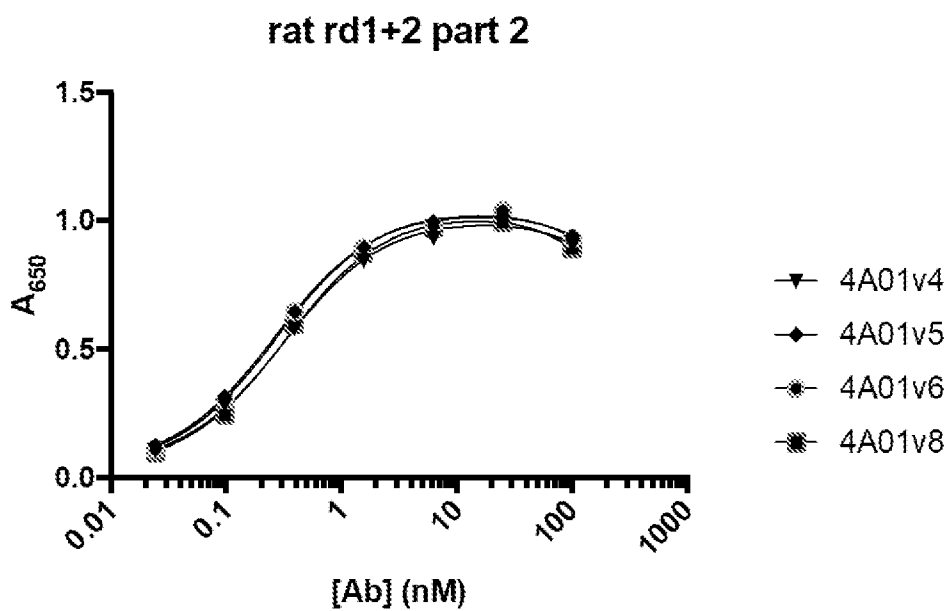
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	Bmax	Kd
Im4A01	1.241	0.1444
4A01v1	1.285	0.1836
4A01v3	1.25	0.1617
4A01v9	1.269	0.1441
4A01v3-1	1.276	0.1296
4A01v3-3	1.32	0.1516
4A01v3-4	1.407	0.2518
4A01v3-5	1.347	0.1621
4A01v3-7	1.347	0.1226
4A01v3-8	1.389	0.1689

FIG. 4H

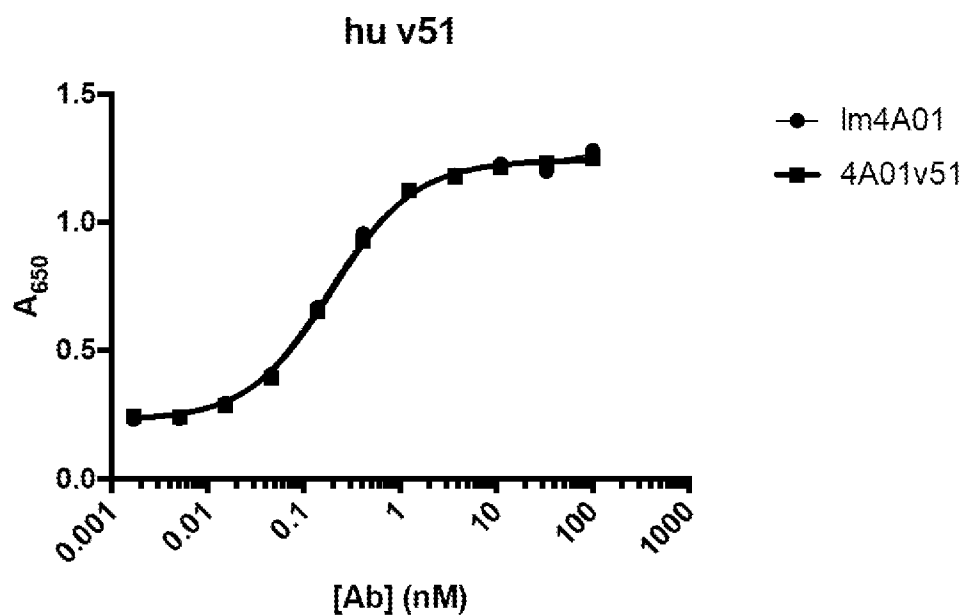
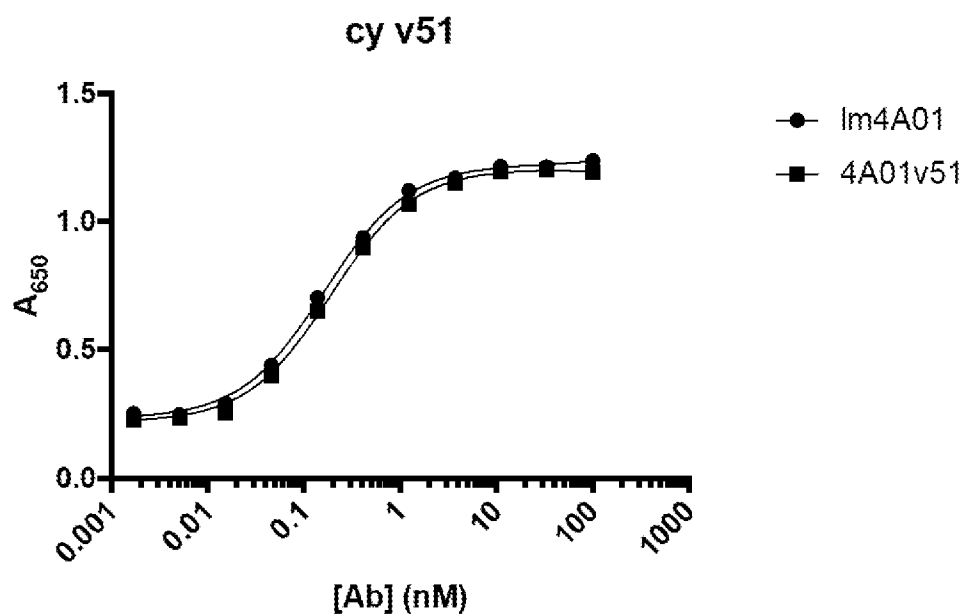
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	Bmax	Kd
4A01v4	0.9724	0.3203
4A01v5	1.005	0.2599
4A01v6	1.016	0.2731
4A01v8	1.006	0.3279

FIG. 4I

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**FIG. 5A****FIG. 5B**

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mu v51

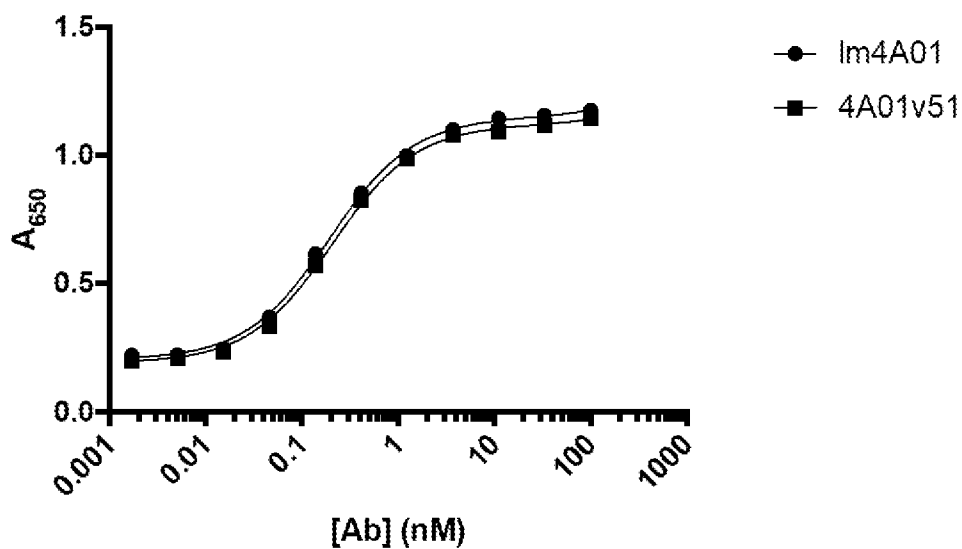


FIG. 5C

rat v51

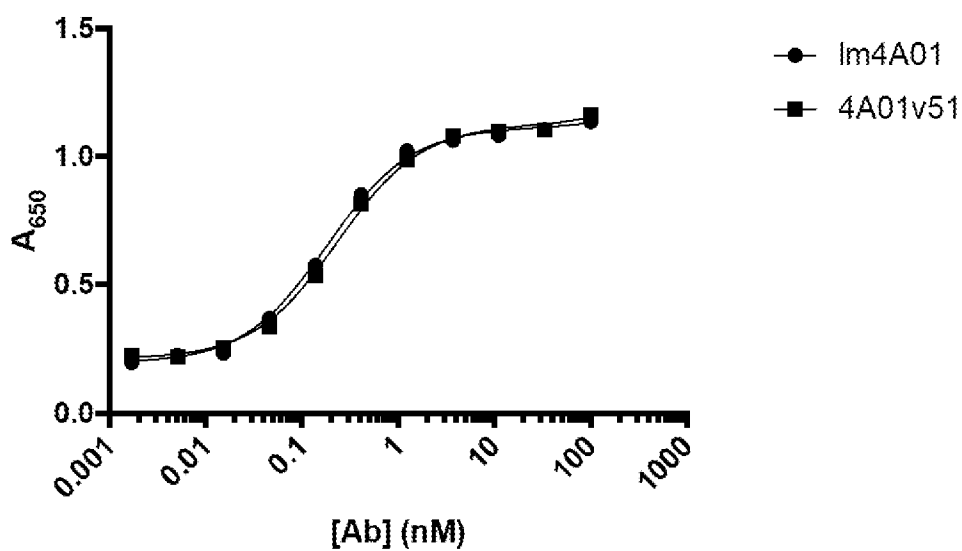


FIG. 5D

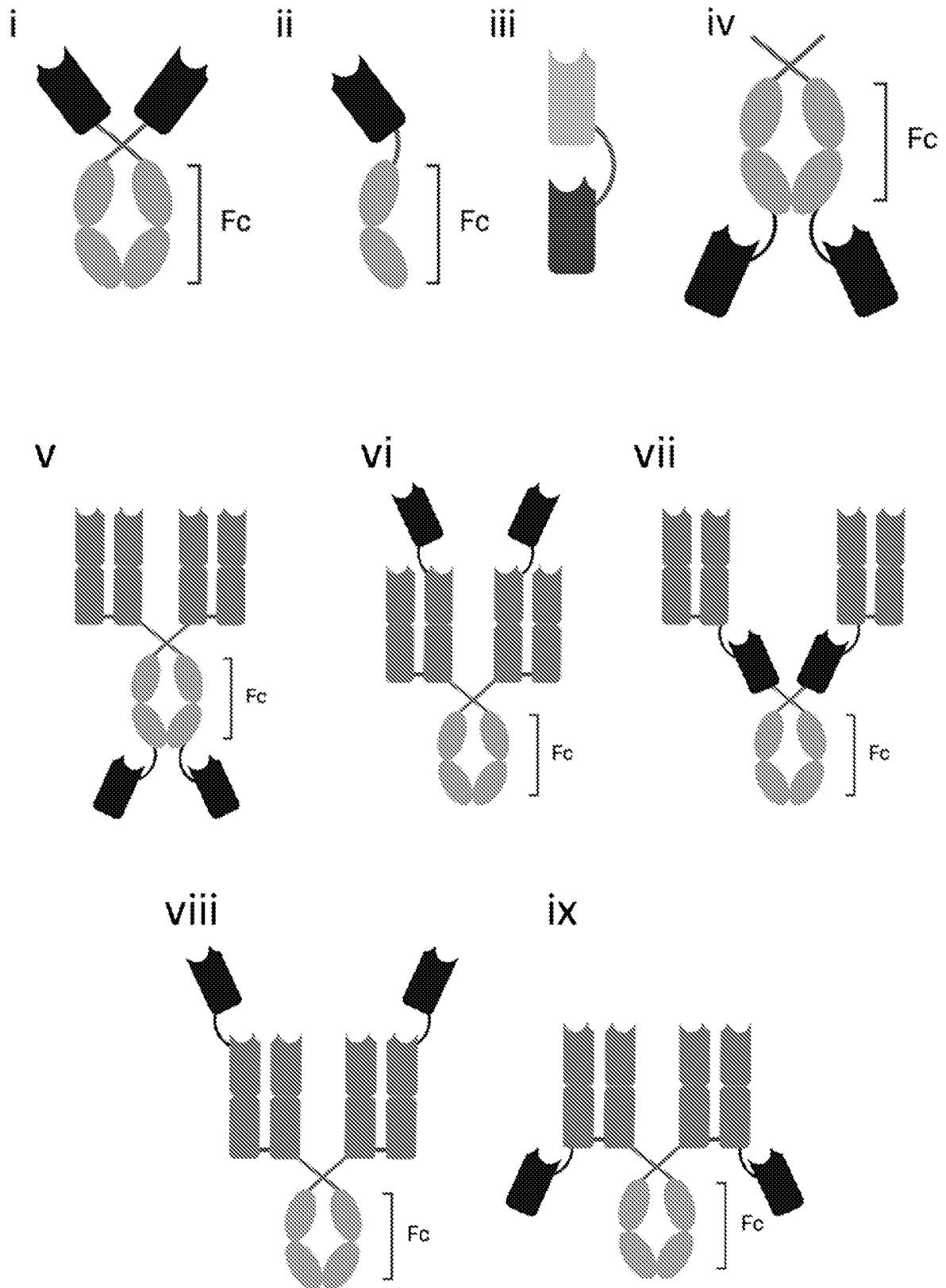


FIG. 6

Albumin Binding at pH 7.4

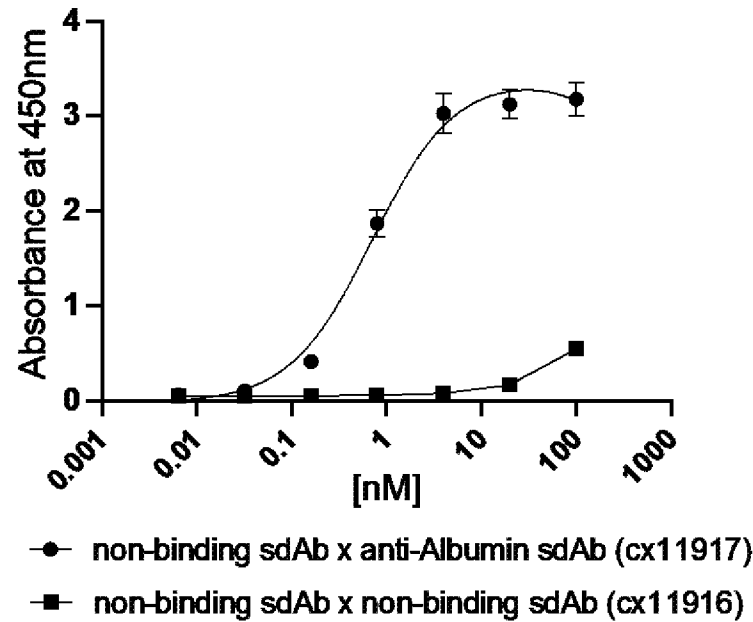


FIG. 7A

Albumin Binding at pH 6

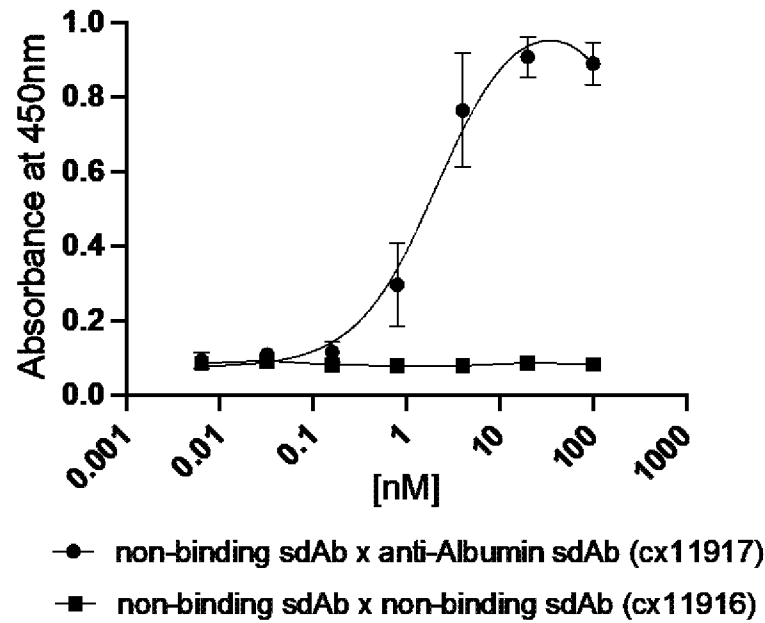


FIG. 7B

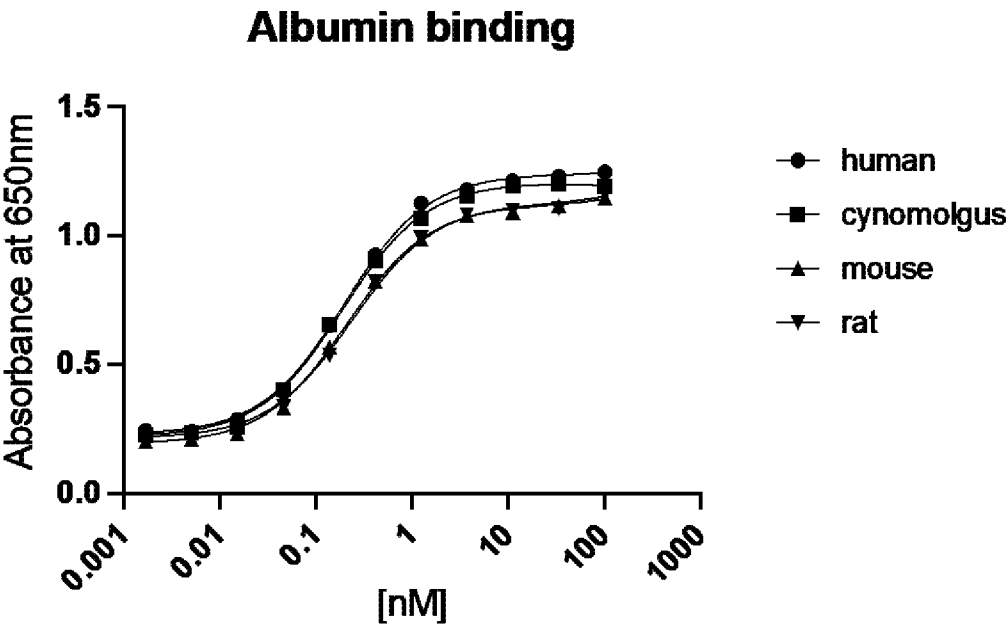


FIG. 8

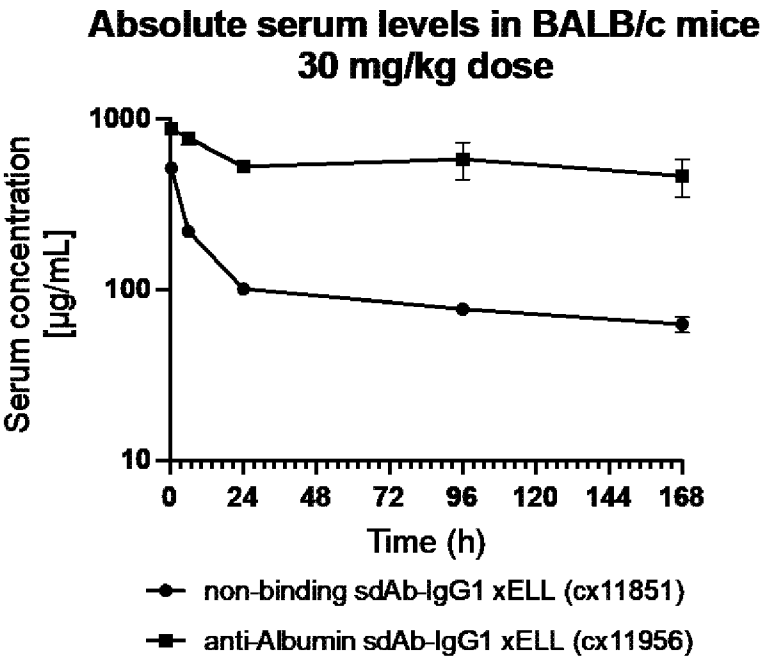


FIG. 9A

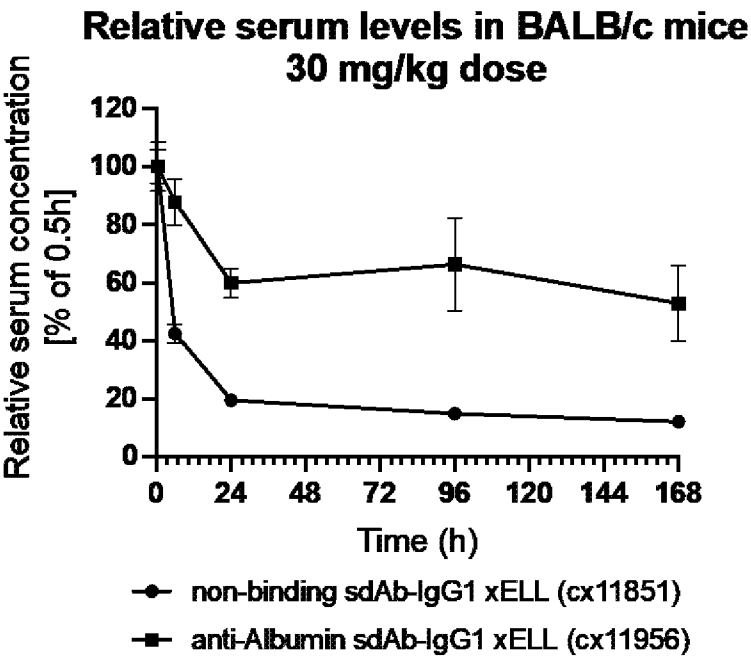


FIG. 9B

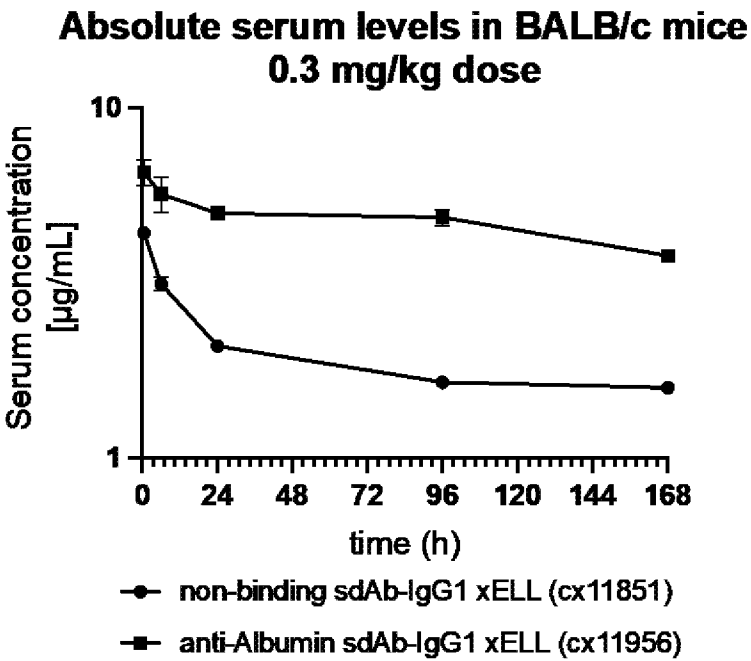


FIG. 9C

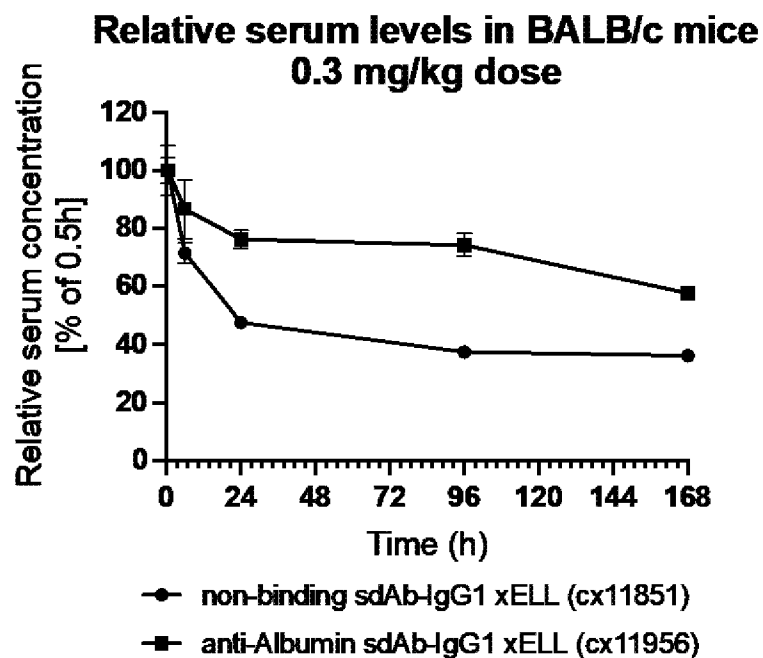


FIG. 9D

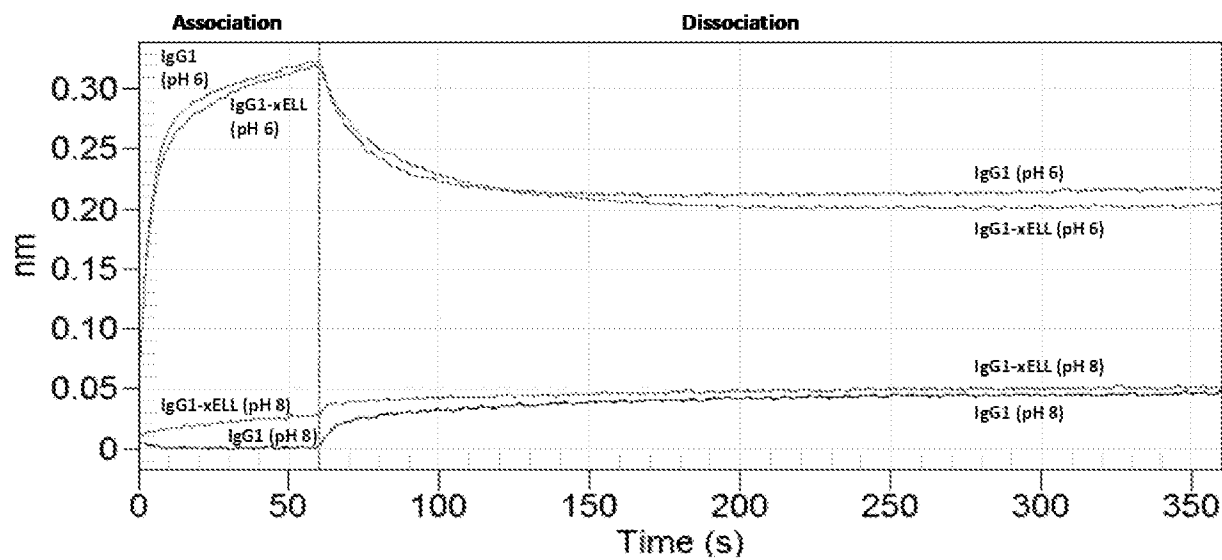
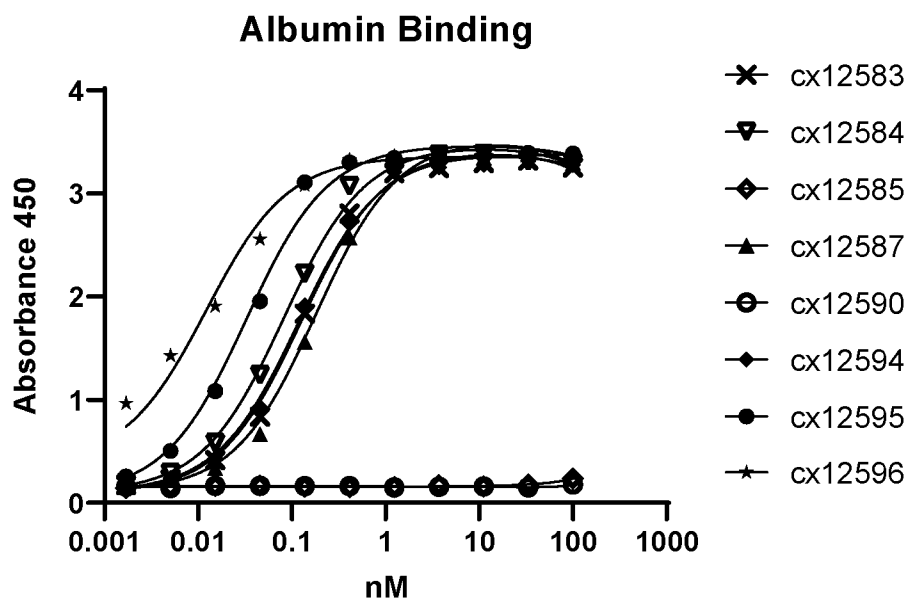
IgG binding to human FcRn (Bio-layer interferometry)

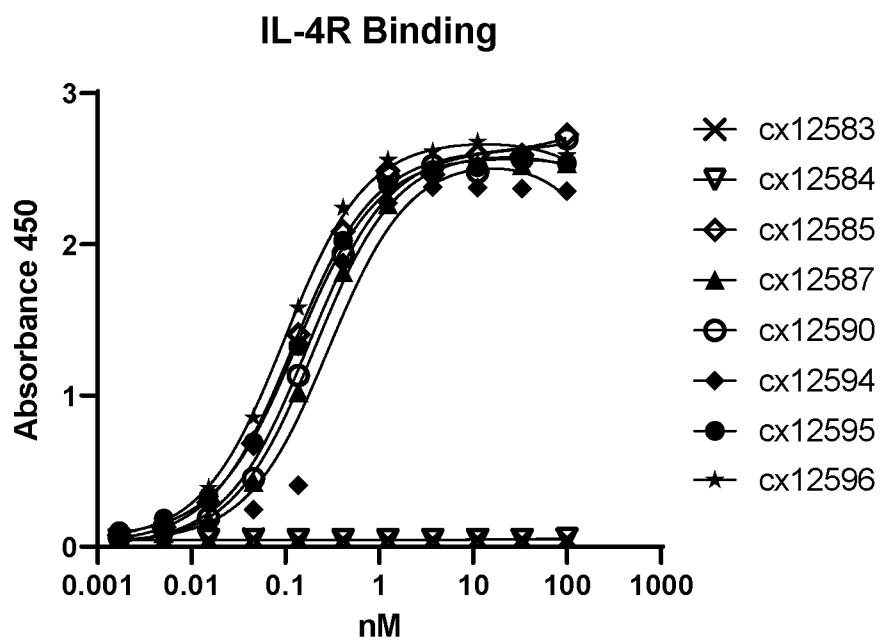
FIG. 10



	Bmax	Kd
cx12587	3.441	0.1801
cx12594	3.299	0.1176
cx12595	3.396	0.03256
cx12596	2.981	0.01221
cx12583	3.339	0.1255
cx12584	3.371	0.08132

FIG. 11A

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	Bmax	Kd
cx12585	2.597	0.1195
cx12587	2.606	0.2064
cx12590	2.620	0.1716
cx12594	2.560	0.2892
cx12595	2.528	0.1307
cx12596	2.661	0.09747

FIG. 11B

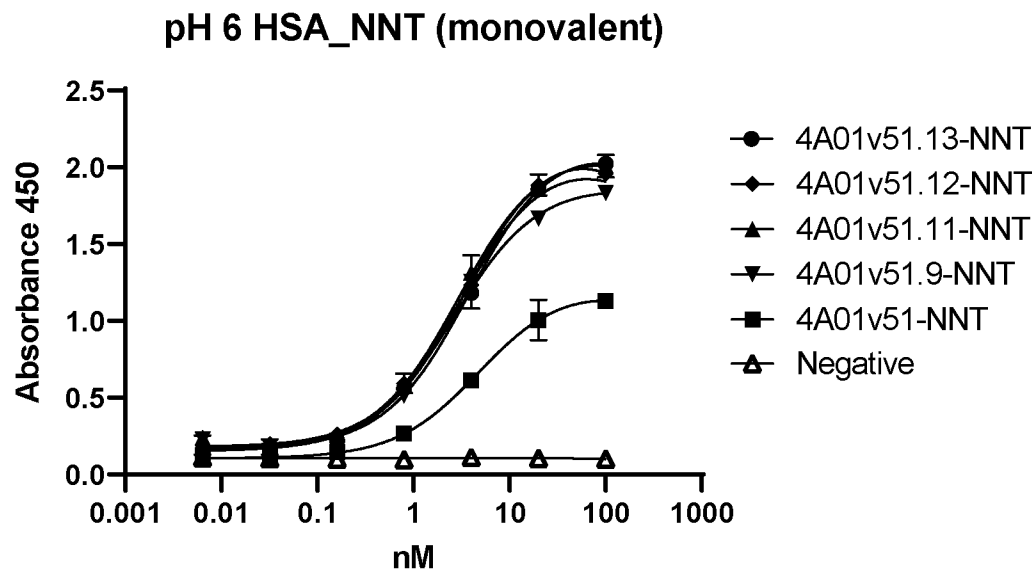


FIG. 12A

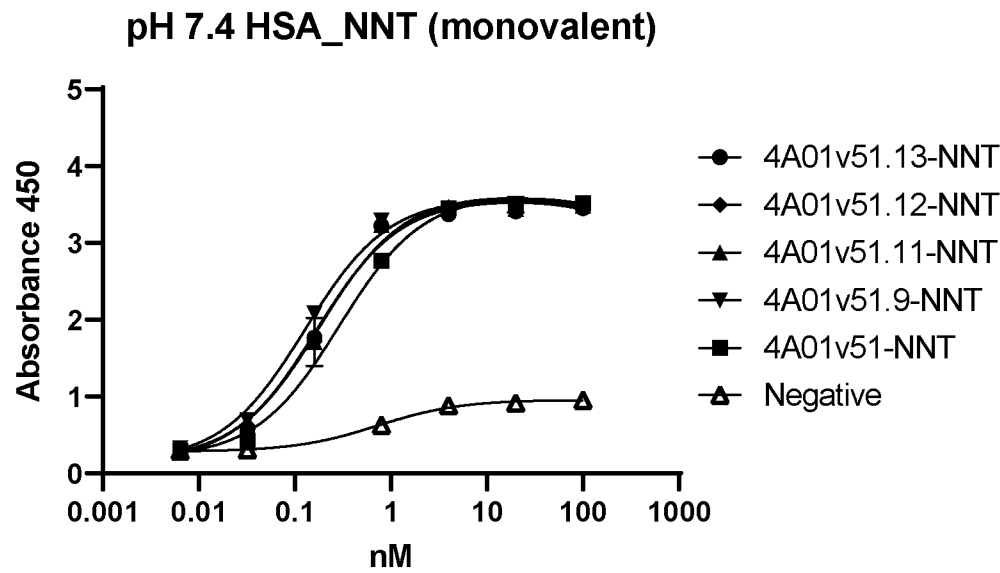


FIG. 12B

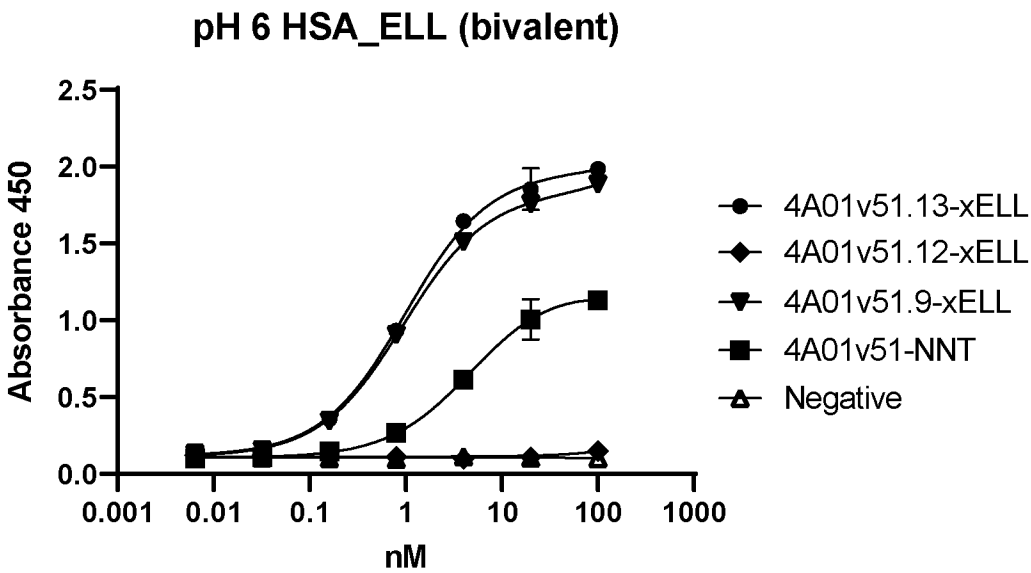


FIG. 12C

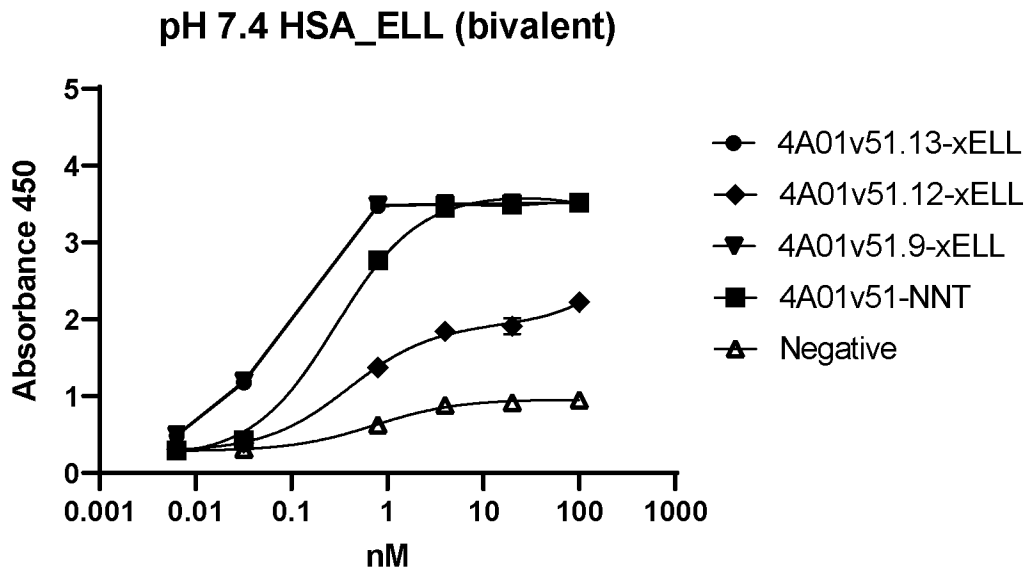


FIG. 12D

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2023/066580

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/18 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEN XIAOLE ET AL: "A Dual Target-Directed Single Domain-Based Fusion Protein Against Interleukin-6 Receptor Decelerate Experimental Arthritis Progression Via Modulating JNK Expression", INFLAMMATION, PLENUM PRESS, NEW YORK, NY, US, vol. 44, no. 4, 10 March 2021 (2021-03-10), pages 1620-1628, XP037511256, ISSN: 0360-3997, DOI: 10.1007/S10753-021-01446-7 [retrieved on 2021-03-10]	1, 2, 5-8, 11-21, 29, 30, 40-46, 48-50
Y	abstract; figures 1-4; compounds VHH-0031	31-40
A	page 1623 - page 1626 ----- -/-	3, 4, 9, 10



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search

31 July 2023

Date of mailing of the international search report

08/08/2023

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2

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Fax: (+31-70) 340-3016

Authorized officer

Schulz, Regine

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2023/066580

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/172528 A1 (ANWITA BIOSCIENCES INC [US]) 27 August 2020 (2020-08-27)	1, 2, 5-8, 11-21, 29, 30, 40-46, 48-50
Y	abstract; claims 1, 6, 7, 16-29, 31-38,	31-40
A	43-50; figures 1-5; examples 1-9; tables 4, 5, 7-13; compounds A19, C1 - C4, C6, C7 -----	3, 4, 9, 10
X	US 2007/178082 A1 (SILENCE KAREN [BE] ET AL) 2 August 2007 (2007-08-02)	1, 2, 5-8, 11-30, 40-46, 48-50
Y	abstract; figures 2, 5, 11, 14; examples	31-39
A	1- 17, 25-30; tables 2, 3, 5; compounds MSA21, MSA24, MSA21VHH#3E, MSA21/MSA21/TNF3E -----	3, 4, 9, 10
Y	MALAUQUIAS ANGELA DONATO MAIA ET AL: "A review of plant-based expression systems as a platform for single-domain recombinant antibody production", INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, ELSEVIER BV, NL, vol. 193, 23 October 2021 (2021-10-23), pages 1130-1137, XP086894865, ISSN: 0141-8130, DOI: 10.1016/J.IJBIOMAC.2021.10.126 [retrieved on 2021-10-23] abstract; figures 1F, G; table 2 page 1134, paragraph 4.1 -----	31-40
Y	DE VLIENER DORIEN ET AL: "Single-Domain Antibodies and Their Formatting to Combat Viral Infections", ANTIBODIES, vol. 8, no. 1, 20 December 2018 (2018-12-20), page 1, XP055811729, DOI: 10.3390/antib8010001 abstract; table 1 page 13, paragraph 1st - paragraph 2nd page 16, paragraph 1st full page 5, paragraph 1st full ----- -/--	31-40

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2023/066580

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LAFAYE PIERRE ET AL: "Use of camel single-domain antibodies for the diagnosis and treatment of zoonotic diseases", COMPARATIVE IMMUNOLOGY, MICROBIOLOGY AND INFECTIOUS DISEASES, PERGAMON PRESS, OXFORD, GB, vol. 60, 25 September 2018 (2018-09-25), pages 17-22, XP085530731, ISSN: 0147-9571, DOI: 10.1016/J.CIMID.2018.09.009 abstract; figure 1 page 17 - page 19, left-hand column, paragraph first full -----	31-40

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/066580

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2023/066580

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020172528 A1	27-08-2020	CN 113508134 A	15-10-2021
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		ES 2551682 T3	23-11-2015
		ES 2655912 T3	22-02-2018
		PT 2316852 E	23-06-2014
		US 2007178082 A1	02-08-2007
