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Human VASA Amino Acid Sequence

(Accession: NP_077726; SEQ ID NO: 1))

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  1  mgdedweaei nphmssyvpi fekdrysgen qdnfnrtpas ssemddgpsr rdhfmksgfa
 61  sgrnfgnrda gecnkrdnts tmggfgvgks fgnrgfsnsr fedgdssgfw ressndcedn
121  ptrnrgfskr ggyrdgnnse asgpyrrggr gsfrgcrggf glgspnndld pdecmqrtgg
181  lfgsrrpvls gtgngdtsqs rsgsgsergg ykglneevit qsgknswkse aeggessdtq
241  gpkvtyippp ppededsifa hyqtginfdk ydtilvevsg hdappailtf eeanlcqtln
301  nniakagytk ltpvqkysip iilagrdlma caqtgsgkta afllpilahm mhdgitasrf
361  kelqepecii vaptrelvnq iylearkfsf gtcvravviy ggtqlghsir qivqgcnilc
421  atpgrlmdii qkekiglkqi kylvldeadr mldmgfqpem kklisepgmp skeqrqtlmf
481  satfpeeiqr laaeflksny lfvavggvgg acrdvqqtvl qvgqfskrek lveilrnigd
541  ertmvfvetk kkadfiatfl cqekisttsi hgdreqrere qalgdfrfgk cpvlvatsva
601  argldienvq hvinfdlpst ideyvhriqr tgrcgntgra isffdlesdn hlaqplvkvl
661  tdaqqdvpaw leeiafstyi pgfsgstrgn vfasvdtrkg kstlntagfs ssqapnpvdd
721  eswd
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FIG. 1

(57) Abstract: Anti-VASA antibodies (mAbs), particularly humanized mAbs that specifically bind to VASA with high affinity, are disclosed. The amino acid sequences of the CDRs of the light chains and the heavy chains, as well as consensus sequences for these CDRs, of these anti- VASA mAbs are provided. The disclosure also provides nucleic acid molecules encoding the anti-VASA mAbs, expression vectors, host cells, methods for making the anti-VASA mAbs, and methods for expressing the anti-VASA mAbs. Finally, methods of using the anti-VASA mAbs to isolate and/or purify cells expressing VASA are disclosed.

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ANTI-VASA ANTIBODIES, AND METHODS OF PRODUCTION AND USE THEREOF

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 62/051,130, filed September 16, 2014, and U.S. Provisional Application No. 62/089,054, filed December 8, 2014, the entire contents of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present disclosure relates generally to antibodies, their production and use. Specifically, the present disclosure pertains to antibodies which specifically bind to the human VASA protein, methods of producing such antibodies, and diagnostic, therapeutic and clinical methods of using such antibodies.

BACKGROUND

[0003] The VASA protein was identified in *Drosophila* as a component of the germplasm that encodes a DEAD-family ATP-dependent RNA helicase (Liang *et al.* (1994), *Development*, 120:1201-11; Lasko *et al.* (1988), *Nature* 335:611-17). The molecular function of VASA is directed to binding target mRNAs involved in germ cell establishment, oogenesis, and translation onset (Gavis *et al.* (1996), *Development* 110:521-28). VASA is required for pole cell formation and is exclusively restricted to the germ cell lineage throughout development.

[0004] *Vasa* homolog genes have been isolated in various animal species, and VASA can be used as a molecular marker for the germ cell lineage in most animal species (Noce *et al.* (2001), *Cell Structure and Function* 26:131-36). Castrillon *et al.* (2000), *Proc. Natl. Acad. Sci. (USA)* 97(17):958590-9590, for example, demonstrated that the human *Vasa* gene is expressed in ovary and testis but is undetectable in somatic tissues.

[0005] The existence of mammalian female germline stem cells, also known as oogonial stem cells or ovarian stem cells (OSCs) or egg precursor cells, in the somatic tissue of mammalian ovaries was first described in Johnson *et al.* (2004), *Nature* 428:145-50, and has now been confirmed by other research groups (*e.g.*, Zou *et al.* (2009), *Nature Cell Biology,* published online DOI: 10.1038/ncb1869; Telfer & Albertini (2012), *Nature Medicine*

18(3):353-4). The potential use of OSCs to produce oocytes for use in artificial reproduction technologies (ART), including *in vitro* fertilization (IVF), or as sources of highly functional mitochondria for mitochondrial transfer to oocytes, as well as the use of OSCs to treat various symptoms of menopause, have been described in the scientific and patent literature (*e.g.*, Tilly & Telfer (2009), *Mol. Hum. Repro.* 15(7):393-8; Zou *et al.* (2009), *supra*; Telfer & Albertini (2012), *supra*; White *et al.* (2012), *Nature Medicine* 18(3):413-21; WO 2005/121321; U.S. Pat. No. 7,955,846; U.S. Pat. No. 8,652,840; WO2012/142500; U.S. Pat. No. 8,642,329 and U.S. Pat. No. 8,647,869).

[0006] When OSCs were first characterized by Johnson *et al.* (2004), *supra*, it was demonstrated that the cells expressed the VASA protein, and antibodies against the VASA protein have been used to isolate OSCs from ovarian tissue homogenates (*e.g.*, Zou *et al.* (2009), *supra*; White *et al.* (2012), *supra*). Moreover, White *et al.* (2012), *supra*, demonstrated that antibodies to an N-terminal domain of VASA could not be used to isolate viable VASA-expressing OSCs whereas antibodies to a C-terminal domain could effectively isolate the cells, suggesting that the C-terminal domain, but not the N-terminal domain, was extracellular and thus accessible to the antibodies.

[0007] The production of anti-VASA polyclonal antibodies was first described in Castrillon *et al.* (2000), *supra*, and WO01/36445. Polyclonal antibodies directed to the C-terminal portion of human VASA protein are commercially available from Abcam plc (Cambridge, UK; Product Code AB13840), and R&D Systems, Inc. (Minneapolis, MN; Catalog No. AF2030), and a monoclonal antibody directed against the N-terminal portion of human VASA is also commercially available from R&D Systems, Inc. (Minneapolis, MN; Catalog No. AF2030),

[0008] There remains, however, a need for high affinity antibodies directed to the C-terminal extracellular domain of VASA for identifying (*e.g.*, by immunohistochemistry or labeled antibodies) and isolating (*e.g.*, by magnetic or fluorescence activated cell sorting) cells, including but not limited to OSCs, expressing VASA.

SUMMARY

[0009] Anti-VASA antibodies (mAbs), particularly humanized mAbs that specifically bind to VASA with high affinity, are disclosed. The amino acid sequences of the CDRs of the light chains and the heavy chains, as well as consensus sequences for these CDRs, of

these anti-VASA mAbs are provided. The disclosure also provides nucleic acid molecules encoding the anti-VASA mAbs, expression vectors, host cells, methods for making the anti-VASA mAbs, and methods for expressing the anti-VASA mAbs. Finally, methods of using the anti-VASA mAbs to isolate and/or purify cells expressing VASA are disclosed.

[0010] These and other aspects and embodiments of the disclosure are illustrated and described below. Other systems, processes, and features will become apparent to one with skill in the art upon examination of the following drawings and detailed description. It is intended that all such additional systems, processes, and features be included within this description, be within the scope of the present invention, and be protected by the accompanying claims.

BRIEF DESCRIPTION OF THE FIGURES

[0011] FIGURE 1 provides the amino acid sequence of the human VASA protein isoform 1 from GenBank Accession from NP_077726 (SEQ ID NO: 1).

[0012] FIGURE 2 provides the amino acid sequence of the mouse VASA homolog protein isoform 1 from GenBank Accession from NP_001139357 (SEQ ID NO: 2).

[0013] FIGURE 3 provides an amino acid alignment between the C-terminal portion of the human VASA protein (residues 690-724 of SEQ ID NO: 1) and the mouse VASA homolog (residues 691-728 of SEQ ID NO: 2).

[0014] FIGURE 4A shows the region of the C-terminal domains of the VASA/DDX4 polypeptide that is reactive with an antibody of the invention and the control antibody (AB13840, Abcam plc, Cambridge, UK) and FIGURE 4B shows binding of the control antibody to the VASA protein and the V1 and V2 polypeptides.

[0015] FIGURE 5A shows dose response binding curves of the affinity for VASA of 1E9 and 1A12; and FIGURE 5B shows the results of ELISA assays with the VASA, V1 and V2 peptides that suggest that 1E9 binds the same epitope as the commercially available rabbit polyclonal antibody (AB13840, Abcam plc, Cambridge, UK). NC = negative control; VASA = SEQ ID NO: 1 residues 700-724; VASA-1 = V1 or SEQ ID NO: 1 residues 712-721; VASA-2 = V2 or SEQ ID NO: 1 residues 700-709.

[0016] FIGURE 6A shows dose response binding curves of the affinity for VASA of the IgG and scFv-Fc forms of 1E9; and FIGURE 6B shows the results of ELISA assays of the binding of the IgG and scFv-Fc forms of 1E9 with the VASA, V1 and V2 peptides. NC =

negative control; VASA = SEQ ID NO: 1 residues 700-724; VASA-1 = V1 or SEQ ID NO: 1 residues 712-721; VASA-2 = V2 or SEQ ID NO: 1 residues 700-709.

[0017] FIGURE 7A shows the results of binding experiments with three anti-VASA hybridoma antibodies (2M1/1K3, 2M1/1K23 and 2M1/1L5) and two negative controls (2M1/1F5 and 2M1/1H5) which are not VASA-specific; FIGURE 7B shows dose response curves of four VASA-specific hybridoma antibodies (2M1/1K3, 2M1/1K23 and 2M1/1L5) compared to 1E9-lambda; and FIGURE 7C shows dose response curves of the VASA-specific hybridoma antibody 2M1/2K4 compared to 1E9-lambda.

[0018] FIGURE 8 shows the result of subtyping analysis for anti-VASA antibodies from eight hybridomas (2M1/1L20, 2M1/1J20, 1M1/1C9, 2M1/1N3, 2M1/1K23, 1M1/1L5 and 2M1/2K4).

[0019] FIGURES 9A-9B show alignments of some of the VL sequences of the anti-VASA invention. The figure indicates the approximate locations of the three CDR regions (bold, underscore) and the SEQ ID NO corresponding to each sequence.

[0020] FIGURES 10A-10B show alignments of some of the VH sequences of the anti-VASA invention. The figure indicates the approximate locations of the three CDR regions (bold, underscore) and the SEQ ID NO corresponding to each sequence.

[0021] FIGURE 11 shows alignments of the unique CDR sequences of the VL regions of Figure 9.

[0022] FIGURE 12 shows alignments of the unique CDR sequences of the VH regions of Figure 10.

DETAILED DESCRIPTION

[0023] The present disclosure relates to isolated antibodies (Abs), particularly Abs that bind specifically to VASA with high affinity. In certain embodiments, the anti-VASA Abs are derived from particular heavy and light chain sequences and/or comprise particular structural features, such as CDR regions comprising particular amino acid sequences. This disclosure provides isolated anti-VASA Abs, methods of making such anti-VASA Abs, immunoconjugates and bispecific molecules comprising such anti-VASA Abs, and methods of expressing such anti-VASA Abs. This disclosure also relates to methods of using the anti-VASA Abs to isolate and/or purify cells expressing VASA, including mammalian female

germline stem cells or oogonial stem cells (OSCs) or egg precursor cells and their progenitor cells.

[0024] In order that the present disclosure may be more readily understood, certain terms are defined. Additional definitions are set forth throughout the detailed description.

Definitions

[0025] The term "antibody" or abbreviation "Ab," as used herein, includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof, with or without native glycosylation. A complete "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds or an antigen binding portion thereof. Each heavy chain includes a heavy chain variable region (V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_{H1}, C_{H2}, and C_{H3}. Each light chain includes a light chain variable region (V_L) and a light chain constant region with one domain, C_L. The V_H and V_L regions can be further subdivided into complementarity determining regions (CDR) and framework regions (FR). The V_H and V_L regions each include three CDRs, designated CDR1, CDR2 and CDR3, that interact with an antigen (*e.g.*, VASA).

[0026] The term "antigen-binding portion" of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, VASA). Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include a Fab fragment, $F(ab')_2$ fragment, Fab' fragment, Fd fragment, Fv fragment, scFv fragment, dAb fragment, and an isolated CDR.

[0027] The term "monoclonal antibody" or "monoclonal antibody preparation," as used herein, refers to a preparation of antibody molecules consisting essentially of antibodies having a single heavy chain amino acid sequence and a single light chain amino acid sequence (but which may have heterogeneous glycosylation).

[0028] The term "humanized antibody," as used herein, includes antibodies having constant region and variable region framework regions (FRs) but not CDRs derived from human germline immunoglobulin sequences.

[0029] The term "recombinant antibody," as used herein, includes all antibodies prepared, expressed, created, or isolated by recombinant means. In certain embodiments, recombinant antibodies are isolated from a host cell transformed to express the antibody (*e.g.*,

from a transfectoma). In other embodiments, recombinant antibodies are isolated from a recombinant, combinatorial antibody library, such as a phage display library. Recombinant antibodies may also be prepared, expressed, created, or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences.

[0030] The term "isotype," as used herein, refers to the heavy chain class (*e.g.*, IgA, IgD, IgE, IgG, and IgM for human antibodies) or light chain class (*e.g.*, kappa or lambda in humans) encoded by the constant region genes. The term "subtype" refers to subclasses within the subtype (*e.g.*, IgA_1, IgA_2, IgG_1, IgG_2, IgG_3, IgG_4 in humans).

[0031] The phrase "an antibody specific for" a specified antigen is used interchangeably herein with the phrase "an antibody which specifically binds to" a specified antigen. As used herein, the term "K_a" refers to the association rate and the term "K_d" to the dissociation rate of a particular antibody-antigen complex. The term "K_D" refers to the dissociation constant, which is obtained from the ratio of K_d to K_a and expressed as a molar concentration (M). According to some embodiments, an antibody that "specifically binds to human VASA" is intended to refer to an antibody that binds to human VASA with a K_D of 5×10^{-8} M or less, more preferably 1×10^{-8} M or less.

[0032] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Anti-VASA Antibodies

[0033] The invention provides a variety of new antibodies with high affinity against the human VASA protein, particularly the C-terminal region. The antibodies may comprise the complete VH and VL regions disclosed herein, or may comprise only the CDR sequences disclosed herein. In addition, based upon CDR sequences disclosed herein, sequence motifs for CDR sequences are provided, and the antibodies may comprise CDR sequences defined by the motifs.

[0034] The CDR sequences of the invention (including both the CDRs disclosed in Figures 11 and 12 and the CDRs defined by the sequence motifs disclosed herein) can be combined with other immunoglobulin sequences according to methods well known in the art to produce immunoglobulin molecules with antigen-binding specificity determined by the CDRs of the invention.

[0035] In some embodiments, the CDRs of the invention are combined with framework region (FR) and constant domain (CH or CL) sequences from other antibodies. For example, although some of the CDRs disclosed herein are derived from murine hybridomas and have murine FR and constant domain sequences, they can be recombined with human or other mammalian FR and constant domain sequences to produce humanized or other recombinant antibodies. The production of such recombinant antibodies is well known to those of skill in the art and requires only routine experimentation.

[0036] The type of constant regions included in such recombinant antibodies can be chosen according to their intended use. For example, if the antibodies are intended for therapeutic use to target VASA-expressing cells for destruction, heavy chain constant domains (*i.e.*, Fc regions) of IgG subtypes can be used. If the antibodies are intended only as reagents for labeling cells (*e.g.*, for fluorescence-activated cell sorting (FACS)), a complete antibody, antigen binding fragment (Fab), single-chain variable fragment (Fsc), single domain antibody (sdAb) or even non-antibody immunoglobulin molecule (*e.g.*, an MHC receptor extracellular domain) can be used with the CDRs of the invention.

[0037] The CDRs of the invention can be selected independently such that the CDR1, CDR2 and CDR3 sequences of a given variable light (VL) chain or variable heavy (VH) chain can be chosen from different original VL and VH chains, from different VL and VH CDR motifs, or from a combination of the disclosed CDRs and motifs. However, sequences for light chain CDRs should be selected from the disclosed VL CDRs or VL CDR motifs, and sequences for heavy chain CDRs should be selected from the disclosed VH CDRs or VH CDR motifs. Similarly, the sequences for CDR1 regions should be selected from the disclosed CDR1 or CDR1 motif sequences, the sequences for CDR2 regions should be selected from the disclosed CDR2 or CDR2 motif sequences, and the sequences for CDR3 regions should be selected from the disclosed CDR3 or CDR3 motif sequences, for VL or VH chains as appropriate.

Methods of Using Anti-VASA Antibodies to Detect or Isolate Cells

[0038] The anti-VASA antibodies of the invention can be used in standard methods of immunoaffinity purification, immunohistochemistry and immunotherapy, but with specific application to cells and tissue expressing the VASA protein.

[0039] For example, the anti-VASA antibodies of the invention can be used to isolate cells expressing VASA from a mixed population of cells including only a fraction of cells that express VASA. For example, female germline stem cells or oogonial stem cells or their precursors have been discovered to be present in ovarian tissue at very low proportions. Ovarian tissue (*e.g.*, ovarian surface epithelial and/or cortex) can be excised, dissociated into individual cells, and subjected to techniques such as FACs using fluorescently-labeled anti-VASA antibodies or immunoaffinity purification using immobilized anti-VASA antibodies. The isolated VASA-expressing cells have various utilities in assisted reproductive technologies, as described above.

[0040] Alternatively, immunohistochemistry may be performed using the anti-VASA antibodies of the invention to identify cells or tissues expressing VASA and/or to quantify VASA expression in such cells.

[0041] In addition, the anti-VASA antibodies of the invention can be used therapeutically to target VASA-expressing cells for destruction either by antibody-dependent cell-mediated cytotoxicity (ADCC) or immunotoxins comprising anti-VASA antibodies of the invention conjugated to radio- or chemo-toxic moieties. Antibody-drug conjugates of the anti-VASA antibodies of the invention could also be used to deliver therapeutic drugs to VASA-expressing cells.

Nucleic Acid Molecules Encoding Anti-VASA Antibodies

[0042] The invention also provides nucleic acid molecules encoding the anti-VASA antibodies of the invention. Such nucleic acids can be designed using standard tables for the universal genetic code to choose codons which will encode the desired amino acid sequence, or specialized codon tables can be used that reflect codon biases characteristic of different organisms. Thus, for example, to optimize expression of the anti-VASA antibodies of the invention in CHO cells, a nucleic acid encoding the desired antibody can be designed using a codon table optimized for CHO cells.

[0043] The nucleic acids encoding the anti-VASA antibodies of the invention can be included in a wide variety of vectors known in the art, including cloning vectors (*e.g.*,

bacterial or mammalian cloning vectors), transformation vectors (*e.g.*, homologous recombination, viral integration or autonomously replicating vectors) and expression vectors (*e.g.*, high copy number, inducible or constitutive mammalian expression vectors).

Cells Expressing Anti-VASA Antibodies

[0044] Also provided are host cells expressing heterologous sequences encoding the anti-VASA antibodies of the invention. Such host cells can be useful for commercial production of the anti-VASA antibodies of the invention, and can be produced by transforming appropriate host cells with expression vectors described above.

[0045] In some embodiments the invention provides mammalian cells, including CHO cells, expressing the anti-VASA antibodies of the invention. However, those of skill in the art can express the antibodies in a variety of host cells, including bacterial, yeast, insect and mammalian systems. See, *e.g.*, Verma *et al.* (1998), *J. Immunol. Methods* 216(1-2):165-81, incorporated by reference in its entirety herein.

EXAMPLES

Immunogenic Peptides

[0046] The following peptides were used as immunogens to generate antibodies against the C-terminal domain of human VASA and to screen for antibodies with high affinity binding to VASA:

VASA-1 (V1) immunogen: SQAPNPVDDE (SEQ ID NO: 1 residues 712-721)

VASA-2 (V2) immunogen: GKSTLNTAGF (SEQ ID NO: 1 residues 700-709)

[0047] As shown in Figure 3, these immunogens comprise amino acid sequences from the C-terminal domain of VASA that are highly conserved between the human VASA protein and the mouse VASA homolog.

Hybridoma Generation

[0048] Hybridomas were formed in separate experiments with the VASA peptide immunogens V1 and V2 (above). Peptides were conjugated to carrier proteins by standard methods. Conjugated peptides were used to immunize mice, and to increase the immune response through boosting with the conjugated peptide. Following a period of increased antibody titer in the sera, animals were sacrificed and spleens removed. Splenic B cells were fused to mouse fusion partner cell lines (SP2-0) for isolation and cloning. Hybridomas were

formed by outgrowth at limiting dilution, and clones were developed by cloning titration experiments. The presence of VASA-reactive antibodies was examined by ELISA assays. Hybridomas were derived by outgrowth and stabilization of cells plated at limiting dilution cell cloning.

[0049] The binding of the VASA-reactive antibodies in the region of the C-terminal domains of the VASA/DDX4 polypeptide was compared with the binding control antibodies (AB13840, Abcam plc, Cambridge, UK) to delineate the similarity of the binding epitopes. Exemplary results are shown in Figure 4.

Analysis of hybridomas

[0050] Hybridomas were injected intraperitoneally into mice and, after allowing for a period of growth, ascites fluid was collected and purified, all using standard procedures, and then analyzed by ELISA.

[0051] Binding of the ascites-derived antibodies to the VASA, VASA-1 and VASA-2 polypeptides was used to select antibodies for further analysis. For example, as shown in Figure 7, the binding of four anti-VASA hybridoma antibodies (2M1/1K3, 2M1/1K23, 2M1/1L5 and 2M1/2K4) were compared to two negative controls (2M1/1F5 and 2M1/1H5) which are not VASA-specific and/or to the 1E9-lambda antibody (described below).

Recombinant Library Panning

[0052] As an alternative to hybridoma technology, the generation of antibodies against amino acid residues 700-724 of human VASA/DDX4 was conducted using phage display technology. The phage display library was formed from a pool of normal B cells from ~40 blood donors. Phage were used to display the scFv chain of an antibody

[0053] The results of panning the human naïve scFv library against the VASA/DDX4 700-724 peptide were as shown in Table 1 below:

TABLE 1

Peptide	Round	Titer of output phage (cfu/ml)	Titer of rescued phage (cfu/ml)	ELISA results
VASA	1st	10^7	10^{13}	/
	2nd	10^7	10^{13}	/
	3rd	10^7	10^{12}	No positive clones
	4th	10^7	10^{13}	Two positive clones
	5th	10^7	10^{13}	Several positive clones
	6th	10^7	/	/

[0054] ELISA results of single colonies identified after 3 and 4 rounds of selection are shown in Tables 2-4 below. Two clones were of note: "1A12" (plate 1, row A, column 12) and "1E9" (plate 1, row E, column 9).

TABLE 2

plate 1												
	3 rounds						4 rounds					
	VASA peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.062	0.061	0.057	0.063	0.065	0.092	0.059	0.059	0.059	0.060	0.059	0.550
B.	0.055	0.058	0.056	0.056	0.064	0.073	0.060	0.057	0.060	0.58	0.063	0.059
C.	0.065	0.058	0.060	0.063	0.069	0.072	0.069	0.063	0.066	0.061	0.070	0.063
D.	0.072	0.064	0.067	0.066	0.061	0.062	0.069	0.069	0.070	0.070	0.117	0.071
E.	0.778	0.058	0.055	0.071	0.056	0.059	0.057	0.056	0.458	0.064	0.060	0.059
F.	0.057	0.059	0.059	0.060	0.059	0.062	0.063	0.057	0.059	0.057	0.059	0.056
G.	0.058	0.055	0.056	0.082	0.061	0.066	0.061	0.057	0.056	0.058	0.068	0.055
H.	0.044	0.058	0.058	0.056	0.053	0.096	0.056	0.052	0.056	0.054	0.054	0.056
	non-relevant peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.085	0.063	0.062	0.069	0.056	0.089	0.054	0.059	0.056	0.057	0.057	0.061
B.	0.062	0.053	0.054	0.06	0.09	0.066	0.063	0.054	0.054	0.058	0.058	0.062
C.	0.064	0.063	0.071	0.069	0.069	0.067	0.062	0.06	0.057	0.062	0.064	0.057
D.	0.094	0.063	0.067	0.069	0.069	0.067	0.071	0.067	0.067	0.066	0.135	0.061
E.	0.078	0.058	0.059	0.116	0.055	0.057	0.054	0.064	0.061	0.054	0.056	0.059
F.	0.062	0.056	0.056	0.056	0.055	0.064	0.063	0.057	0.062	0.056	0.054	0.058
G.	0.057	0.06	0.059	0.066	0.056	0.064	0.057	0.057	0.057	0.055	0.077	0.055
H.	0.061	0.066	0.061	0.054	0.058	0.111	0.057	0.054	0.057	0.058	0.052	0.054

TABLE 3

plate 2-after 4 round of selection												
	VASA peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.052	0.045	0.053	0.045	0.051	0.045	0.046	0.044	0.049	0.044	0.045	0.050
B.	0.049	0.051	0.051	0.045	0.042	0.054	0.046	0.045	0.055	0.045	0.048	0.053
C.	0.048	0.047	0.048	0.054	0.051	0.047	0.047	0.045	0.047	0.052	0.051	0.055
D.	0.062	0.050	0.048	0.047	0.059	0.056	0.059	0.063	0.048	0.057	0.052	0.061
E.	0.047	0.042	0.042	0.045	0.051	0.041	0.047	0.042	0.044	0.052	0.050	0.054
F.	0.047	0.049	0.040	0.042	0.046	0.043	0.046	0.042	0.052	0.045	0.051	0.054
G.	0.047	0.052	0.045	0.041	0.039	0.051	0.048	0.049	0.052	0.043	0.054	0.050
H.	0.055	0.048	0.054	0.042	0.043	0.048	0.048	0.049	0.051	0.051	0.048	0.054
	non-relevant peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.047	0.053	0.050	0.042	0.053	0.053	0.041	0.043	0.042	0.053	0.053	0.054
B.	0.052	0.053	0.054	0.054	0.053	0.043	0.043	0.045	0.053	0.045	0.055	0.054
C.	0.052	0.047	0.054	0.053	0.055	0.045	0.045	0.043	0.053	0.055	0.057	0.053
D.	0.047	0.049	0.054	0.056	0.047	0.049	0.054	0.051	0.056	0.062	0.065	0.062
E.	0.052	0.045	0.042	0.045	0.041	0.051	0.040	0.047	0.041	0.056	0.053	0.054
F.	0.052	0.053	0.041	0.045	0.052	0.053	0.054	0.052	0.533	0.049	0.045	0.053
G.	0.051	0.053	0.049	0.050	0.051	0.043	0.049	0.052	0.053	0.053	0.054	0.051
H.	0.055	0.052	0.054	0.053	0.045	0.051	0.051	0.051	0.052	0.062	0.054	0.053

TABLE 4

plate 3-after 4 rounds of selection												
	VASA peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.074	0.052	0.058	0.076	0.052	0.063	0.052	0.055	0.040	0.052	0.054	0.072
B.	0.047	0.041	0.052	0.064	0.072	0.051	0.059	0.048	0.053	0.048	0.054	0.053
C.	0.051	0.042	0.042	0.044	0.053	0.056	0.052	0.048	0.044	0.048	0.060	0.056
D	0.057	0.049	0.045	0.051	0.053	0.046	0.067	0.047	0.046	0.046	0.059	0.058
E.	0.054	0.046	0.042	0.126	0.041	0.047	0.051	0.040	0.042	0.043	0.048	0.073
F.	0.077	0.045	0.040	0.047	0.042	0.040	0.042	0.039	0.041	0.053	0.051	0.051
G.	0.178	0.056	0.044	0.041	0.051	0.050	0.055	0.042	0.042	0.051	0.044	0.052
H.	0.054	0.042	0.045	0.041	0.049	0.039	0.045	0.089	0.050	0.051	0.061	0.055
	non-relevant peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.050	0.056	0.055	0.049	0.053	0.055	0.051	0.059	0.051	0.044	0.047	0.054
B.	0.058	0.075	0.061	0.064	0.073	0.061	0.053	0.054	0.059	0.056	0.059	0.063
C.	0.076	0.056	0.053	0.054	0.056	0.053	0.053	0.053	0.057	0.063	0.049	0.061
D.	0.069	0.052	0.052	0.058	0.056	0.048	0.059	0.059	0.056	0.052	0.051	0.056
E.	0.047	0.056	0.050	0.118	0.063	0.067	0.052	0.053	0.054	0.053	0.056	0.054
F.	0.053	0.054	0.054	0.052	0.054	0.054	0.053	0.053	0.043	0.056	0.046	0.056
G.	0.063	0.056	0.054	0.045	0.045	0.049	0.050	0.053	0.053	0.052	0.055	0.053
H.	0.058	0.055	0.054	0.047	0.053	0.048	0.050	0.051	0.054	0.053	0.053	0.058

[0055] ELISA results of single colonies identified after 5 rounds of selection are shown in Tables 5-7 below. Clones of note included 1A11, 1B4, 1B7, 1D4, 1D5, 1E2, 1E3, 1F7, 1G3, 1G12, 2B8, 2C7, 2E11, 2F1, 2G8, 2G10, 2H9, 3B2, 3B5, 3B7, 3D11, 3E5, 3E12, 3F6 and 3H11.

TABLE 5

plate 1-after 5 rounds of selection												
	VASA peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.049	0.049	0.122	0.135	0.050	0.129	0.051	0.089	0.077	0.084	0.227	0.077
B.	0.051	0.197	0.056	0.212	0.067	0.099	0.280	0.109	0.122	0.094	0.049	0.053
C.	0.181	0.168	0.062	0.059	0.105	0.051	0.127	0.098	0.101	0.093	0.061	0.080
D.	0.057	0.186	0.143	0.408	0.527	0.057	0.178	0.061	0.124	0.060	0.061	0.077
E.	0.159	0.342	0.230	0.046	0.047	0.042	0.120	0.119	0.053	0.119	0.126	0.064
F.	0.160	0.177	0.160	0.086	0.048	0.134	0.248	0.053	0.079	0.054	0.159	0.052
G.	0.167	0.119	0.246	0.085	0.049	0.050	0.050	0.052	0.050	0.102	0.053	0.458
H.	0.126	0.136	0.096	0.050	0.048	0.049	0.060	0.049	0.058	0.104	0.066	0.052
	non-relevant peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.053	0.054	0.051	0.052	0.053	0.054	0.054	0.050	0.051	0.044	0.050	0.052
B.	0.056	0.054	0.053	0.053	0.052	0.052	0.062	0.053	0.052	0.053	0.054	0.053
C.	0.056	0.055	0.056	0.056	0.056	0.053	0.053	0.052	0.052	0.051	0.054	0.053
D.	0.060	0.060	0.060	0.057	0.065	0.059	0.058	0.061	0.052	0.056	0.057	0.055
E.	0.052	0.083	0.051	0.053	0.043	0.043	0.042	0.039	0.043	0.050	0.053	0.057
F.	0.052	0.052	0.050	0.050	0.041	0.040	0.048	0.043	0.050	0.053	0.052	0.052
G.	0.051	0.051	0.048	0.049	0.052	0.043	0.054	0.046	0.052	0.051	0.053	0.061
H.	0.052	0.048	0.046	0.049	0.044	0.050	0.050	0.049	0.049	0.051	0.051	0.052

TABLE 6

plate 2-after 5 rounds of selection												
	VASA peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.075	0.051	0.067	0.050	0.049	0.069	0.150	0.094	0.081	0.050	0.043	0.103
B.	0.136	0.054	0.107	0.075	0.059	0.052	0.120	0.318	0.159	0.095	0.152	0.052
C.	0.103	0.056	0.055	0.052	0.140	0.053	0.210	0.056	0.116	0.054	0.140	0.114
D.	0.098	0.141	0.058	0.114	0.104	0.057	0.070	0.077	0.079	0.049	0.138	0.054
E.	0.071	0.065	0.058	0.077	0.044	0.050	0.121	0.051	0.050	0.049	0.212	0.083
F.	0.210	0.051	0.046	0.110	0.043	0.063	0.043	0.056	0.052	0.057	0.051	0.062
G.	0.054	0.078	0.064	0.060	0.053	0.051	0.054	0.475	0.055	0.272	0.076	0.061

H.	0.050	0.050	0.050	0.054	0.050	0.054	0.051	0.050	0.290	0.055	0.061	0.056
	non-relevant peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.040	0.041	0.044	0.041	0.040	0.048	0.046	0.047	0.040	0.045	0.044	0.045
B.	0.039	0.052	0.039	0.047	.042	0.050	0.052	0.060	0.053	0.042	0.045	0.043
C.	0.036	0.043	0.051	0.041	0.042	0.051	0.053	0.062	0.052	0.053	0.050	0.040
D.	0.047	0.055	0.048	0.046	0.047	0.051	0.049	0.058	0.048	0.052	0.054	0.052
E.	0.051	0.051	0.040	0.039	0.043	0.041	0.040	0.040	0.040	0.043	0.067	0.046
F.	0.054	0.051	0.046	0.045	0.47	0.040	0.043	0.050	0.043	0.049	0.048	0.040
G.	0.038	0.050	0.047	0.040	0.039	0.039	0.045	0.060	0.041	0.048	0.050	0.044
H.	0.039	0.058	0.039	0.040	0.049	0.048	0.050	0.049	0.058	0.048	0.044	0.049

TABLE 7

plate 3-after 5 rounds of selection												
	VASA peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.047	0.122	0.105	0.176	0.177	0.102	0.040	0.164	0.104	0.109	0.169	0.081
B	0.048	0.218	0.094	0.054	0.314	0.155	0.287	0.146	0.052	0.166	0.054	0.054
C.	0.199	0.059	0.052	0.105	0.060	0.054	0.118	0.152	0.054	0.145	0.055	0.053
D.	0.053	0.096	0.066	0.056	0.058	0.077	0.055	0.048	0.196	0.155	0.259	0.133
E.	0.139	0.052	0.052	0.046	0.471	0.089	0.199	0.052	0.049	0.042	0.173	0.244
F.	0.055	0.051	0.068	0.046	0.093	0.412	0.083	0.041	0.129	0.052	0.053	0.053
G.	0.101	0.056	0.058	0.039	0.051	0.050	0.075	0.046	0.042	0.044	0.070	0.052
H.	0.135	0.083	0.062	0.052	0.052	0.050	0.056	0.071	0.073	0.094	0.200	0.050
	non-relevant peptide											
	1	2	3	4	5	6	7	8	9	10	11	12
A.	0.055	0.056	0.053	0.051	0.054	0.056	0.054	0.45	0.049	0.053	0.055	0.053
B.	0.057	0.057	0.054	0.055	0.059	0.056	0.056	0.044	0.058	0.052	0.054	0.055
C.	0.057	0.055	0.056	0.054	0.049	0.052	0.043	0.052	0.055	0.055	0.050	0.055
D.	0.060	0.062	0.059	0.058	0.061	0.058	0.057	0.047	0.059	0.058	0.061	0.059
E.	0.056	0.045	0.048	0.055	0.071	0.048	0.046	0.043	0.048	0.056	0.056	0.059
F.	0.054	0.045	0.055	0.047	0.053	0.070	0.044	0.052	0.053	0.053	0.054	0.055
G.	0.052	0.055	0.049	0.049	0.041	0.047	0.044	0.046	0.054	0.053	0.053	0.051
H.	0.053	0.052	0.057	0.041	0.046	0.044	0.051	0.051	0.052	0.052	0.048	0.050

[0056] Clones shown in bold were PCR amplified.

Conversion to scFv-Fc fusion and expression in mammalian cells

[0057] After 5 rounds of panning, DNA digestion patterns showed that many clones from the 5th round of panning were the same, indicating that additional rounds of selection and ELISA analysis were not needed.

[0058] Two unique clones (1A12, 1E9) were selected for conversion to scFv-Fc fusions for expression in mammalian cells and for ELISA and FACS analysis. Figure 5A shows dose response binding curves that indicated that 1E9 had an EC50 of 0.02779 nM and 1A12 had an EC50 of 0.2156 nM. In addition, Figure 5B shows the results of ELISA assays with the V1 and V2 VASA peptides which suggest that 1E9 binds the same epitope as the commercially available rabbit polyclonal antibody (AB13840, Abcam plc, Cambridge, UK).

[0059] Two different forms of the 1E9 antibody were compared: IgG and scFv-Fc. As shown in Figure 6A, 1E9 IgG had an EC50 of 0.08919 nM and the 1E9 scFv-Fc had an EC50 of 0.3072 nM. In addition, as shown in Figure 6B, both forms were specific towards the VASA-1 epitope.

Synthetic Antibody Gene Production

[0060] The following steps were employed to produce synthetic antibody genes:

[0061] (1) Subtype determination of hybridoma antibodies. The IgG subtypes of the hybridoma antibodies were determined using commercially available kits according to manufacturer's protocols (e.g., Mouse Monoclonal Antibody Isotyping Kit, Catalog No. MMT1, AbDSerotech, Kidlington, UK). Figure 8 shows the result of subtyping analysis for anti-VASA antibodies from eight hybridomas (2M1/1L20, 2M1/1J20, 1M1/1C9, 2M1/1N3, 2M1/1K23, 1M1/1L5 and 2M1/2K4). All of the antibodies were IgG1, IgG2a or IgG2b.

[0062] (2) Degenerate primer synthesis. Based on the subtype information for the eight hybridoma antibodies tested, degenerate primers for mouse IgG VH and VL were designed using sequence information from a mouse IgG database (*i.e.*, the International Immunogenetics Information System® or IMGT database; see Lefranc *et al.* (2003), *Leukemia* 17:260-266, and Alamyar *et al.* (2012), *Methods Mol. Biol.* 2012;882:569-604). Ten degenerate forward primers were designed and synthesized for the VH chain and ten for the VL chain (9 for kappa and one for lambda chains). In addition, two degenerate reverse primers for the VH chain (one for the IgG1 and IgG2b subtypes, and one for the IgG2a subtype) and five for the VL chain (four for kappa and one for lambda chains) were designed and synthesized.

[0063] (3) RNA extraction, amplification, cloning and sequencing. RNA was extracted from hybridoma cells by standard techniques, first strand cDNA synthesis was performed by standard techniques using gene-specific and oligo(dT) primers, and the cDNA was amplified using gene-specific primers. The amplified DNA was then ligated into a commercially

available bacterial cloning vector (pMD18-T, Sino Biological, Inc., Beijing, China). Standard methodologies were conducted to transform the ligation products into *E. coli* DH5a, and to sequence positive clones.

Antibody Sequence Analyses

[0064] Clones producing potentially useful anti-Vasa antibodies were DNA sequenced and the corresponding amino acid sequences were deduced. Sequences are disclosed for eight antibodies derived from the hybridomas described above (*i.e.*, 1N23, 1K23, 2K4, 1C9, 1J20, 1L20, 1K3, 1L5), four additional antibodies derived from hybridomas produced under contract (*i.e.*, CTA4/5, CTB4/11, CTC2/6, CTD2/6) and two antibodies derived from phage display (*i.e.*, 1A12 and 1E9).

Variable Light Chain Sequences

[0065] VL of 1N23. Positive VL clones from the 1N23 hybridoma were sequenced and six were found to encode functional VL chains. These six clones were designated 1N23VL5-5, 1N23VL5-8_0816, 1N23VL1-8, 1N23VL1-2_0820, 1N23VL1-4_0820 and 1N23VL1-2.

[0066] VL of 1K23. Positive VL clones from the 1K23 hybridoma were sequenced and four were found to encode functional VL chains. These four clones were designated 1K23VL2-5, 1K23VL2-6, 1K23VL2-8_0822 and 1K23VL2-3_0829.

[0067] VL of 2K4. Positive VL clones from the 2K4 hybridoma were sequenced and eight were found to encode functional VL chains. These eight clones were designated 2K4VL1-3_0820, 2K4VL1-4, 2K4VL1-1, 2K4VL1-6_0820, 2K4VL2-5_0816, 2K4VL2-4, 2K4VL2-6_0816 and 2K4VL2-5.

[0068] VL of 1C9. Positive VL clones from the 1C9 hybridoma were sequenced and three were found to encode functional VL chains. These three clones were designated 1C9VL2-4, 1C9VL2-6 and 1C9VL2-3_0816.

[0069] VL of 1J20. Positive VL clones from the 1J20 hybridoma were sequenced and three were found to encode functional VL chains. These three clones were designated 1J20VL5-2_0907, 1J20VL5-6_0907 and 1J20VL4-3_0907.

[0070] VL of 1L20. Positive VL clones from the 1L20 hybridoma were sequenced and one was found to encode a functional VL chain. That clone was designated 1L20VL5-0912_091.

[0071] VL of 1K3. Positive VL clones from the 1K3 hybridoma were sequenced and four were found to encode functional VL chains. These four clones were designated 1K3VL2-5, 1K3VL2-5, 1K3VL2-3 and 1K3VL2-4.

[0072] VL of 1L5. Positive VL clones from the 1L5 hybridoma were sequenced and two were found to encode functional VL chains. These two clones were designated 1L5VL2-4 and 1L5VL3-1.

[0073] Additional VLs. VL sequences were obtained for four additional hybridoma antibodies designated CTA4_VL, CTB4_VL, CTC6_VL, CTD6_VL.

[0074] VL Sequence Alignments. Alignments of all of the VL sequences described above are shown in Figure 9. The figure indicates the approximate locations of the three CDR regions (bold, underscore) and the SEQ ID NO corresponding to each sequence.

[0075] Unique VL CDR Sequences. Alignments of the unique CDR sequences of the VLs of Figure 9 are shown in Figure 11. Of the 34 VL sequences, there are only 5 unique CDR1 sequences, 6 unique CDR2 sequences and 8 unique CDR3 sequences, as shown in Figure 11.

[0076] VL CDR Consensus Sequences. Based on the sequences disclosed in Figure 11, as well as structure/function characteristics of the naturally occurring amino acids, consensus sequences for the VL CDRs can be determined.

[0077] One consensus sequence is VL CDR1 Motif 1:

$X_1\ X_2\ X_3\ X_4\ X_5\ X_6\ X_7\ X_8\ X_9\ X_{10}\ X_{11}$ (SEQ ID NO:132)

where X_1 is Q, N, K, R, S or T; X_2 is S, T, C, N or Q; X_3 is I, L, V, M or A; X_4 is V, L, I, M, A or absent; X_5 is H, K, R or absent; X_6 is S, T, C or absent; X_7 is N, Q or absent; X_8 is G, A or absent; X_9 is N or Q; X_{10} is T, S, C, N or Q; and X_{11} is Y, F or W. In some embodiments, X_1 is limited to Q, K or S; and/or X_2 is limited to S or N; and/or X_3 is limited to I or L; and/or X_4 is limited to V, L or absent; and/or X_5 is limited to H or absent; and/or X_6 is limited to S or absent; and/or X_7 is limited to N or absent; and/or X_8 is limited to G or absent; and/or X_9 is limited to N; and/or X_{10} is limited to T, S or N; and/or X_{11} is limited to Y or F. In some embodiments, the subsequence $X_1\ X_2\ X_3$ is limited to Q N I; in some embodiments, the subsequence $X_1\ X_2\ X_3$ is limited to Q S L; and in some embodiments, the subsequence $X_1\ X_2\ X_3$ is limited to K S L. In addition, in some embodiments, when $X_1\ X_2\ X_3$ is Q S L or Q N I, then X_4 is V; whereas in other embodiments, when $X_1\ X_2\ X_3$ is K S L, then X_4 is L. In some embodiments, when $X_9\ X_{10}$ is N T, then X_{11} is Y.

[0078] Noting in particular that the VL CDR1 sequences of SEQ ID NOs: 86-88 are quite distinct from the others in Figure 11, an alternative consensus sequence is VL CDR1 Motif 2:

$X_1\ X_2\ X_3\ X_4\ X_5\ X_6\ X_7\ X_8\ X_9\ X_{10}\ X_{11}$ (SEQ ID NO:133)

where X_1 is Q, N, K or R; X_2 is S, T, C, N or Q; X_3 is I, L, V, M or A; X_4 is V, L, I, M or A; X_5 is H, K or R; X_6 is S, T or C; X_7 is N or Q; X_8 is G or A; X_9 is N or Q; X_{10} is T, S or C; and X_{11} is Y, F or W. In some embodiments, X_1 is limited to Q or K; and/or X_2 is limited to S or N; and/or X_3 is limited to I or L; and/or X_4 is limited to V or L; and/or X_5 is limited to H; and/or X_6 is limited to S; and/or X_7 is limited to N; and/or X_8 is limited to G; and/or X_9 is limited to N; and/or X_{10} is limited to T; and/or X_{11} is limited to Y. In some embodiments, the subsequence $X_1\ X_2\ X_3$ is limited to Q N I; in some embodiments, the subsequence $X_1\ X_2\ X_3$ is limited to Q S L; and in some embodiments, the subsequence $X_1\ X_2\ X_3$ is limited to K S L. In addition, in some embodiments, when $X_1\ X_2\ X_3$ is Q S L or Q N I, then X_4 is V; whereas in other embodiments, when $X_1\ X_2\ X_3$ is K S L, then X_4 is L. In some embodiments, when $X_9\ X_{10}$ is N T, then X_{11} is Y.

[0079] For the VL CDR2, one consensus sequence is VL CDR2 Motif 1:

$Y_1\ Y_2\ Y_3$ (SEQ ID NO: 134)

where Y_1 is K, R or H; Y_2 is V, I, L, M, A, T, S or C; and Y_3 is S, T, C, N or Q. In some embodiments, Y_2 is limited to V, I, M or T; and/or Y_3 is limited to S or N.

[0080] Noting in particular that the VL CDR2 sequences of SEQ ID NO: 94 is quite distinct from the others in Figure 11, an alternative consensus sequence is VL CDR2 Motif 2:

$Y_1\ Y_2\ Y_3$ (SEQ ID NO: 135)

where Y_1 is D or E; Y_2 is N or Q; and Y_3 is N or Q. In some embodiments, Y_1 is limited to D; and/or Y_2 is limited to N; and/or Y_3 is limited to N.

[0081] Similarly, noting that the VL CDR2 sequences of SEQ ID NO: 95 is quite distinct from the others in Figure 11, an alternative consensus sequence is VL CDR2 Motif 3:

$Y_1\ Y_2\ Y_3$ (SEQ ID NO: 136)

where Y_1 is Q or N; Y_2 is D or E; and Y_3 is K, R or H. In some embodiments, Y_1 is limited to Q; and/or Y_2 is limited to D; and/or Y_3 is limited to K.

[0082] For the VL CDR3, one consensus sequence is VL CDR3 Motif 1:

$Z_1\ Z_2\ Z_3\ Z_4\ Z_5\ Z_6\ Z_7\ Z_8\ Z_9\ Z_{10}$ (SEQ ID NO: 137)

where Z_1 is S, T, C, F, Y, M, L, V, I or A; Z_2 is Q, N, S, T or C; Z_3 is S, T, C, G, A, H, K, R, Q, N, Y, F or W; Z_4 is A, G, S, T, C, L, I, V, M, D or E; Z_5 is H, K, R, E, D, S, T or C; Z_6 is

V, L, I, M, A, Y, F, W, S, T or C; Z_7 is P, S, T, C or absent; Z_8 is S, T, C or absent; Z_9 is W, P, L, I, V, M, A, F, or Y; and Z_{10} is T, S, C, V, L, I, M, A. In some embodiments, Z_1 is limited to S, F, M or L; and/or Z_2 is limited to Q or S; and/or Z_3 is limited to S, G, H, Q or Y; and/or Z_4 is limited to A, S, T, L, or D; and/or Z_5 is limited to H, E, D or S; and/or Z_6 is limited to V, Y, F, or S; and/or Z_7 is limited to P, S or absent; and/or Z_8 is limited to S or absent; and/or Z_9 is limited to W, P, L or F; and/or Z_{10} is limited to T or V.

[0083] Noting in particular that the VL CDR3 sequences of SEQ ID NOs: 96-98 have a positive charge at position Z_5 whereas the others in Figure 11 do not, an alternative consensus sequence is VL CDR3 Motif 2:

$Z_1\ Z_2\ Z_3\ Z_4\ Z_5\ Z_6\ Z_7\ Z_8\ Z_9\ Z_{10}$ (SEQ ID NO:138)

where Z_1 is S, T, C, F or Y; Z_2 is Q or N; Z_3 is S, T, C, G or A; Z_4 is A, G, S, T or C; Z_5 is H, K or R; Z_6 is V, L, I, M or A; Z_7 is P or absent; Z_8 is absent; Z_9 is W, P, L, I, V, M, A, F or Y; and Z_{10} is T, S, or C. In some embodiments, Z_1 is limited to S or F; and/or Z_2 is limited to Q; and/or Z_3 is limited to S or G; and/or Z_4 is limited to A, S or T; and/or Z_5 is limited to H; and/or Z_6 is limited to V; and/or Z_7 is limited to P or absent; and/or Z_8 is limited to absent; and/or Z_9 is limited to W, P, L or F; and/or Z_{10} is limited to T.

[0084] Noting in particular that the VL CDR3 sequences of SEQ ID NOs: 99-102 have a negative charge at position Z_5 whereas the others in Figure 11 do not, an alternative consensus sequence is VL CDR3 Motif 3:

$Z_1\ Z_2\ Z_3\ Z_4\ Z_5\ Z_6\ Z_7\ Z_8\ Z_9\ Z_{10}$ (SEQ ID NO:139)

where Z_1 is M, C, L, I, V, A; Z_2 is Q or N; Z_3 is H, K, R, Q, N, G, A, Y or F; Z_4 is L, I, V, M, A, D or E; Z_5 is E or D; Z_6 is Y or F; Z_7 is P; Z_8 is absent; Z_9 is W, P, L, I, V, M, A, F or Y; and Z_{10} is T, S, or C. In some embodiments, Z_1 is limited to M or L,; and/or Z_2 is limited to Q; and/or Z_3 is limited to H, Q, G or Y; and/or Z_4 is limited to L or D; and/or Z_5 is limited to E or D; and/or Z_6 is limited to Y or F; and/or Z_7 is limited to P; and/or Z_8 is limited to absent; and/or Z_9 is limited to W, P, L or F; and/or Z_{10} is limited to T.

[0085] Noting in particular that the VL CDR3 sequence of SEQ ID NO: 103 is quite distinct from the others in Figure 11, an alternative consensus sequence is VL CDR3 Motif 4:

$Z_1\ Z_2\ Z_3\ Z_4\ Z_5\ Z_6\ Z_7\ Z_8\ Z_9\ Z_{10}$ (SEQ ID NO:140)

where Z_1 is S, T or C; Z_2 is S, T or C; Z_3 is Y or F; Z_4 is T, S, or C; Z_5 is S, T or C; Z_6 is S, T or C; Z_7 is S, T or C; Z_8 is S, T or C; Z_9 is W, P, F or Y; and Z_{10} is V, L, I, M, A, T, S or C. In some embodiments, Z_1 is limited to S or T; and/or Z_2 is limited to S or T; and/or Z_3 is

limited to Y; and/or Z_4 is limited to T or S; and/or Z_5 is limited to S or T; and/or Z_6 is limited to S or T; and/or Z_7 is limited to S or T; and/or Z_8 is limited to S or T; and/or Z_9 is limited to W, P or F; and/or Z_{10} is limited to V, L, I, T or S. In some embodiments, Z_1 is limited to S; and/or Z_2 is limited to S; and/or Z_3 is limited to Y; and/or Z_4 is limited to T; and/or Z_5 is limited to S; and/or Z_6 is limited to S; and/or Z_7 is limited to S; and/or Z_8 is limited to S; and/or Z_9 is limited to W; and/or Z_{10} is limited to V.

[0086] Finally, noting in particular that the VL CDR3 sequence of SEQ ID NO: 104 is quite distinct from the others in Figure 11, an alternative consensus sequence is VL CDR3 Motif 5:

$Z_1\ Z_2\ Z_3\ Z_4\ Z_5\ Z_6\ Z_7\ Z_8\ Z_9\ Z_{10}$ (SEQ ID NO:141)

where Z_1 is Q or N; Z_2 is A or G; Z_3 is W, Y or F; Z_4 is D or E; Z_5 is S, T or C; Z_6 is R, K or H; Z_7 is T, S or C; Z_8 is V, I, L, M or A; Z_9 is V, I, L, M or A; and Z_{10} is I, L, V, M or A. In some embodiments, Z_1 is limited to Q; and/or Z_2 is limited to A; and/or Z_3 is limited to W; and/or Z_4 is limited to D; Z_5 is limited to S; and/or Z_6 is limited to R; and/or Z_7 is limited to T; and/or Z_8 is limited to V; and/or Z_9 is limited to V; and/or Z_{10} is limited to I.

Variable Heavy Chain Sequences

[0087] VH of 1N23. Positive VH clones from the 1N23 hybridoma were sequenced and all four were found to encode functional VH chains. These four clones were designated 1N23VH3-5, 1N23VH3-7, 1N23VH2-1 and 1N23VH1-5.

[0088] VH of 1K23. Positive VH clones from the 1K23 hybridoma were sequenced and six were found to encode functional VH chains. These six clones were designated 1K23VH2-1_0910, 1K23VH1-4_0907, 1K23VH1-10_0907, 1K23VH8-4_0907, 1K23VH8-5_0907 and 1K23VH8-9_0907.

[0089] VH of 2K4. Positive VH clones from the 2K4 hybridoma were sequenced and four were found to encode functional VH chains. These four clones were designated 2K4VH3-8, 2K4VH2-8, 2K4VH1-1 and 2K4VH1-4.

[0090] VH of 1C9. Positive VH clones from the 1C9 hybridoma were sequenced and eight were found to encode functional VL chains. These eight clones included four unique sequences which are designated 1C9VH2-404-8_1024, 1C9VH2-405-12_1024, 1C9VH2-411-1_1024 and 1C9VH2-406-4_1024.

[0091] VH of 1J20. Positive VH clones from the 1J20 hybridoma were sequenced and two were found to encode functional VH chains. These two clones were designated 1J20VH1-7_0910 and 1J20VH1-1-6_0829.

[0092] VH of 1L20. Positive VH clones from the 1L20 hybridoma were sequenced and three were found to encode functional VH chains. These three clones were designated 1L20VH2-3_0903, 1L20VH2-1_0907 and 1L20VH2-3_0910.

[0093] VH of 1K3. Positive VH clones from the 1K3 hybridoma were sequenced and five were found to encode functional VH chains. These five clones were designated 1K3VH6-7, 1K3VH6-8_0816, 1K3VH3-4, 1K3VH3-4 and 1K3VH3-3_0816.

[0094] VH of 1L5. Positive VH clones from the 1L5 hybridoma were sequenced and nine were found to encode functional VH chains. These nine clones were designated 1L5VH003-5-8_0907, 1L5VH003-6-3_0907, 1L5VH001-7-6_0907, 1L5VH001-6-5_0907, 1L5VH001-6-11_0907, 1L5VH003-6-2_0910, 1L5VH001-6-12_0907, 1L5VH003-3-4_0907 and 1L5VH003-3-8_0907.

[0095] Additional VHs. VH sequences were obtained for four additional hybridoma antibodies designated CTA5_VH, CTB11_VH, CTC2_VH, CTD2_VH.

[0096] VH Sequence Alignments. Alignments of all of the VH sequences described above are shown in Figure 10. The figure indicates the approximate locations of the three CDR regions (bold, underscore) and the SEQ ID NO corresponding to each sequence.

[0097] Unique VH CDR Sequences. Alignments of the unique CDR sequences of the VHs of Figure 10 are shown in Figure 12. Of the 43 VH sequences, there are only 8 unique CDR1 sequences, 9 unique CDR2 sequences and 10 unique CDR3 sequences, as shown in Figure 12.

[0098] VH CDR Consensus Sequences. Based on the sequences disclosed in Figure 12, as well as structure/function characteristics of the naturally occurring amino acids, consensus sequences for the VH CDRs can be determined.

[0099] For the VH CDR1, one consensus sequence is VH CDR1 Motif 1:

$X_1\ X_2\ X_3\ X_4\ X_5\ X_6\ X_7\ X_8$ (SEQ ID NO:142)

where X_1 is G or A; X_2 is Y, F, W, D or E; X_3 is T, S, C or M; X_4 is F, Y, W, V, L, I, M or A; X_5 is T, S, C, N, or Q; X_6 is S, T, C, A or G; X_7 is Y, F, W, N, Q, G or A; and X_8 is W, A, G, Y or F. In some embodiments, X_1 is limited to G; and/or X_2 is limited to Y, F or D; and/or X_3 is limited to T or S; and/or X_4 is limited to F or V; and/or X_5 is limited to T, S or N; and/or

X_6 is limited to S, T or A; and/or X_7 is limited to Y, F, N or G; and/or X_8 is limited to W, A or Y. In some embodiments, the subsequence X_1 X_2 X_3 is limited to G Y T; and in some embodiments, the subsequence X_1 X_2 X_3 is limited to G F T. In addition, in some embodiments, the subsequence X_1 X_7 X_8 is limited to S Y W.

[0100] Noting in particular that the VH CDR1 sequence of SEQ ID NOs: 109-110 and 112 are quite distinct from the others in Figure 12, an alternative consensus sequence is VH CDR1 Motif 2:

X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 (SEQ ID NO: 143)

where X_1 is G or A; X_2 is Y, F or W; X_3 is T, S, C or M; X_4 is F, Y or W; X_5 is T, S or C; X_6 is S, T or C; X_7 is Y, F or W; and X_8 is W. In some embodiments, X_1 is limited to G; and/or X_2 is limited to Y or F; and/or X_3 is limited to T or S; and/or X_4 is limited to F; and/or X_5 is limited to T or S; and/or X_6 is limited to S or T; and/or X_7 is limited to Y or F; and/or X_8 is limited to W. In some embodiments, the subsequence X_1 X_2 X_3 is limited to G Y T; and in some embodiments, the subsequence X_1 X_2 X_3 is limited to G F T. In addition, in some embodiments, the subsequence X_1 X_7 X_8 is limited to S Y W.

[0101] For the VH CDR2, one consensus sequence is VH CDR2 Motif 1:

Y_1 Y_2 Y_3 Y_4 Y_5 Y_6 Y_7 Y_8 Y_9 Y_{10} (SEQ ID NO: 144)

where Y_1 is I, L, V, M or A; Y_2 is Y, F, H, R, K, S or T; Y_3 is P, S, T, Y, F, R, K or H; Y_4 is G, A, S, T, K, R, H, D or E; Y_5 is T, S or absent; Y_6 is R, K, H or absent; Y_7 is N, Q, D, E, G, A or absent; Y_8 is G, A, S, T, Y or F; Y_9 is D, E, A, G, N or Q; and Y_{10} is T, S, I, L, V, M, A, K, R or H. In some embodiments, Y_1 is limited to I; and/or Y_2 is limited to Y, H, R, K or S; and/or Y_3 is limited to P, S, Y or R; and/or Y_4 is limited to G, S, K or D; and/or Y_5 is limited to T or absent; and/or Y_6 is limited to R or absent; and/or Y_7 is limited to N, D, G or absent; and/or Y_8 is limited to G, A, S or Y; and/or Y_9 is limited to D, E, A or N; and/or Y_{10} is limited to T, I or K.

[0102] Noting in particular that the VH CDR2 sequence of SEQ ID NO: 120-121 are quite distinct from the others in Figure 12, an alternative consensus sequence is VH CDR2 Motif 2:

Y_1 Y_2 Y_3 Y_4 Y_5 Y_6 Y_7 Y_8 Y_9 Y_{10} (SEQ ID NO:145)

where Y_1 is I, L, V, M or A; Y_2 is Y, F, H, R, K, S or T; Y_3 is P, S, T, Y or F; Y_4 is G, A, S, T, K, R or H; Y_5 is T, S or absent; Y_6 is R, K, H or absent; Y_7 is N, Q, D, E or absent; Y_8 is G, A, S, T, Y or F; Y_9 is D, E, A, G, N or Q; and Y_{10} is T, S, I, L, V, M or A. In some

embodiments, Y_1 is limited to I; and/or Y_2 is limited to Y, H, R or S; and/or Y_3 is limited to P, S or Y; and/or Y_4 is limited to G, S or K; and/or Y_5 is limited to T or absent; and/or Y_6 is limited to R or absent; and/or Y_7 is limited to N, D or absent; and/or Y_8 is limited to G, A, S or Y; and/or Y_9 is limited to D, E, A or N; and/or Y_{10} is limited to T or I.

[0103] For the VH CDR3, one consensus sequence is VH CDR3 Motif 1:

$Z_1\ Z_2\ Z_3\ Z_4\ Z_5\ Z_6\ Z_7\ Z_8\ Z_9\ Z_{10}\ Z_{11}\ Z_{12}\ Z_{13}\ Z_{14}\ Z_{15}$ (SEQ ID NO:146)

where Z_1 is A, G, V, L, I or M; Z_2 is R, K, H, C or M; Z_3 is G, A, R, K, H, S, T, Y, F, W, D, E or absent; Z_4 is Y, F, W, N, Q, G, A, R, K, H or absent; Z_5 is S, T, N, Q, E, D or absent; Z_6 is D, E or absent; Z_7 is L, I, V, M, A, S, T or absent; Z_8 is L, I, V, M, A or absent; Z_9 is G, A, R, K, H or absent; Z_{10} is I, L, V, M, A, N, Q, R, K, H or absent; Z_{11} is A, M, F, Y, W, S, T, G or absent; Z_{12} is W, Y, F, A, G or absent; Z_{13} is F, Y, W, G, A, M or C; Z_{14} is A, G, M, D, E, W, Y or F; and Z_{15} is Y, F, W, G, A or V. In some embodiments, Z_1 is limited to A or V; and/or Z_2 is limited to R, K or C; and/or Z_3 is limited to G, R, S, Y, D or absent; and/or Z_4 is limited to Y, N, G, R or absent; and/or Z_5 is limited to S, N, E or absent; and/or Z_6 is limited to D or absent; and/or Z_7 is limited to L, S or absent; and/or Z_8 is limited to L or absent; and/or Z_9 is limited to G, R or absent; and/or Z_{10} is limited to I, N, R, L or absent; and/or Z_{11} is limited to A, F, S, G or absent; and/or Z_{12} is limited to W, Y, A or absent; and/or Z_{13} is limited to F, Y, G or M; and/or Z_{14} is limited to A, D, W or Y; and/or Z_{15} is limited to Y, F, W or G.

[0104] Although the disclosed subject matter has been described and illustrated in the foregoing exemplary embodiments, it is understood that the present disclosure has been made only by way of example, and that numerous changes in the details of implementation of the disclosed subject matter may be made without departing from the spirit and scope of the disclosed subject matter, which is limited only by the claims which follow.

CLAIMS

We claim:

1. An antibody that specifically binds to a human VASA protein comprising an immunoglobulin heavy chain and an immunoglobulin light chain,

a) wherein the variable region of said light chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 83-88;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-95; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 96-104; and

b) wherein the variable region of said heavy chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 105-112;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 113-121; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 122-131.

2. An antibody preparation comprising: an antibody that specifically binds to a human VASA protein comprising an immunoglobulin heavy chain and an immunoglobulin light chain,

a) wherein the variable region of said light chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 83-88;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-95; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 96-104; and

b) wherein the variable region of said heavy chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 105-112;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 113-121: and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 122-131.

3. The antibody preparation of claim 2 wherein said preparation is a monoclonal antibody preparation.

4. The antibody preparation of claim 2 wherein said preparation is a mixture of at least two monoclonal antibody preparations.

5. An isolated nucleic acid molecule encoding a heavy chain or light chain of an antibody that specifically binds to a human VASA protein comprising an immunoglobulin heavy chain and an immunoglobulin light chain,

a) wherein the variable region of said light chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 83-88:

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-95; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 96-104; and

b) wherein the variable region of said heavy chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 105-112;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 113-121; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 122-131.

6. The isolated nucleic acid of claim 5 wherein said nucleic acid is selected from the group consisting of a cloning vector, an expression vector, a heterologous recombination vector and a viral integration vector.

7. A method of isolating a cell expressing a VASA protein comprising:

(A) obtaining a population of cells;

(B) contacting the population of cells with a multiplicity of antibodies comprising an antibody that specifically binds to a human VASA protein comprising an immunoglobulin heavy chain and an immunoglobulin light chain,

a) wherein the variable region of said light chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 83-88;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-95; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 96-104; and

b) wherein the variable region of said heavy chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 105-112;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 113-121; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 122-131; and

(C) separating cells in the population that specifically bind the antibodies from cells in the population that do not specifically bind the antibodies.

8. The method of claim 7 wherein the cells are separated by fluorescence activated cell sorting.

9. The method of claim 7 wherein the cells are separated using an immobilized secondary antibody by fluorescence activated cell sorting.

10. A cell transformed with a nucleic acid molecule encoding a heavy chain or light chain of an antibody that specifically binds to a human VASA protein comprising an immunoglobulin heavy chain and an immunoglobulin light chain,

a) wherein the variable region of said light chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 83-88;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-95; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 96-104; and

b) wherein the variable region of said heavy chain comprises:

(i) a CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 105-112;

(ii) a CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 113-121; and

(iii) a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 122-131.

11. The cell of claim 10, wherein said nucleic acid molecule is selected from the group consisting of a cloning vector, an expression vector, a heterologous recombination vector, and a viral integration vector.

12. The cell of claim 10, wherein said cell is a mammalian cell.

13. The cell of claim 12, wherein said cell is a rodent cell.

14. The cell of claim 12, wherein said cell is a Chinese Hamster Ovary (CHO) cell.

15. The cell of claim 12, wherein said cell is a human cell.

Human VASA Amino Acid Sequence

(Accession: NP_077726; SEQ ID NO: 1))

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  1  mgdedweaei nphmssyvpi fekdrysgen qdnfnrtpas ssemddgpsr rdhfmksgfa
 61  sgrnfgnrda gecnkrdnts tmggfgvgks fgnrgfsnsr fedgdssgfw ressndcedn
121  ptrnrgfskr ggyrdgnnse asgpyrrggr gsfrgcrggf glgspnndld pdecmqrtgg
181  lfgsrrpvls gtgngdtsqs rsgsgsergg ykglneevit gsgknswkse aeggessdtq
241  gpkvtyippp ppededsifa hyqtginfdk ydtilvevsg hdappailtf eeanlcqtln
301  nniakagytk ltpvqkysip iilagrdlma caqtgsgkta afllpilahm mhdgitasrf
361  kelqepecii vaptrelvnq iylearkfsf gtcvravviy ggtqighsir qivqgcnilc
421  atpgrlmdii gkekiglkqi kylvldeadr mldmgfgpem kkliscpgmp skeqrqtlmf
481  satfpeeiqr laaeflksny lfvavgqvgg acrdvqqtvl qvgqfskrek lveilrnigd
541  ertmvfvetk kkadfiatfl cqekisttsi hgdreqrere qalgdfrfgk cpvlvatsva
601  argldienvq hvinfdlpst ideyvhrigr tgrcgntgra isffdlesdn hlaqplvkvl
661  tdaqqdvpaw leeiafstyi pgfsgstrgn vfasvdtrkg kstlntagfs ssqapnpvdd
721  eswd
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FIG. 1

Mouse VASA Homolog Amino Acid Sequence

(Accession: NP_001139357, SEQ ID NO: 2))

```
  1  mgdedweaei lkphvssyvp vfekdkyssg angdtfnrts assemedgps grddfmrsgf
 61  psgrslgsrd igesskkent sttggfgrgk gfgnrgflnn kfeegdssgf wkesnndced
121  nqtrsrgfsk rggcqdgnds easgpfrrgg rgsfrgcrgg fglgrpnses dqdqgtqrgg
181  glfgsrkpaa sdsgngdtyq srsgsgrggy kglneevvtg sgknswkset eggessdsqg
241  pkvtyipppp pededsifah yqtginfdky dtilvevsgh dappailtfe eanlcqtlnn
301  niakagytkl tpvqkysipi vlagrdlmac aqtgsgktaa fllpilahmm rdgitasrfk
361  elqepeciiv aptrelinqi ylearkfsfg tcvravviyg gtqfghsvrq ivqgcnilca
421  tpgrlmdiig kekiglkqvk ylvldeadrm ldmgfgpemk kliscpgmps keqrqtllfs
481  atfpeeiqrl agdflkssyl fvavgqvgga crdvqqtilq vgqyskrekl veilrnigde
541  rtmvfvetkk kadfiatflc qekisttsih gdreqrereq algdfrcgkc pvlvatsvaa
601  rgldienvqh vinfdlpsti deyvhrigrt grcgntgrai sffdtdsdnh laqplvkvls
661  daqqdvpawl eeiafstyvp psfssstrgg avfasvdtrk nyqgkhtlnt agisssqapn
721  pvddeswd
```

FIG. 2

Human ...nvfasvdtrk gkstlntagfsssqapnpvddeswd
(SEQ ID NO: 1 residues 690-724)
Mouse ...avfasvdtrknyqgkhtlntagisssqapnpvddeswd
(SEQ ID NO: 2 residues 691-728)

FIG. 3

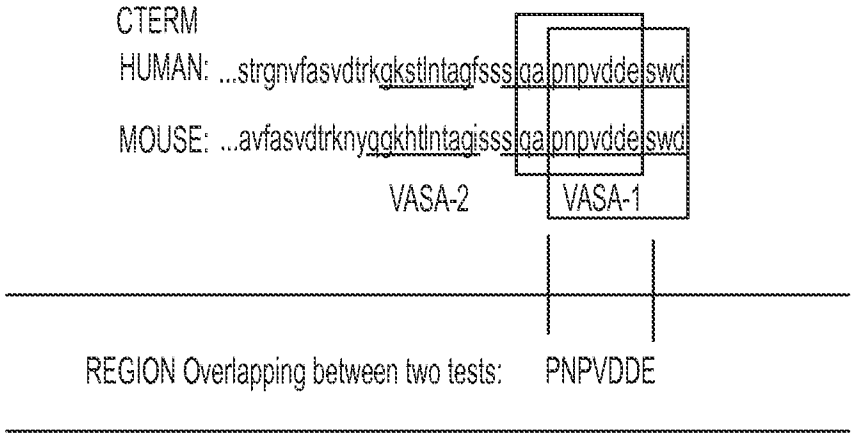


FIG. 4A

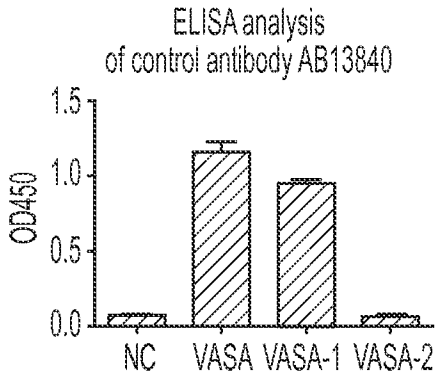
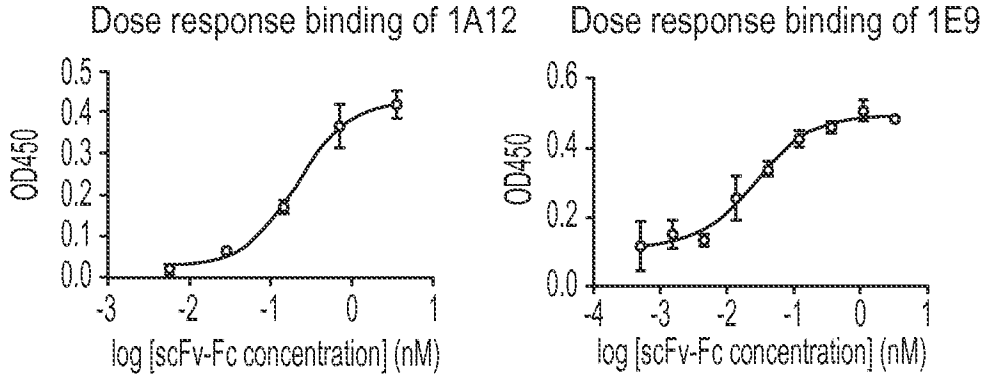


FIG. 4B



Best-fit values	
BOTTOM	0.02413
TOP	0.4334
LOGEC50	-0.6664
HILLSLOPE	1.275
EC50	0.2156

Goodness of Fit	
Degrees of Freedom	6
R2	0.9669

Best-fit values	
BOTTOM	0.1137
TOP	0.4941
LOGEC50	-1.556
HILLSLOPE	1.020
EC50	0.02779

Goodness of Fit	
Degrees of Freedom	14
R2	0.9282

FIG. 5A

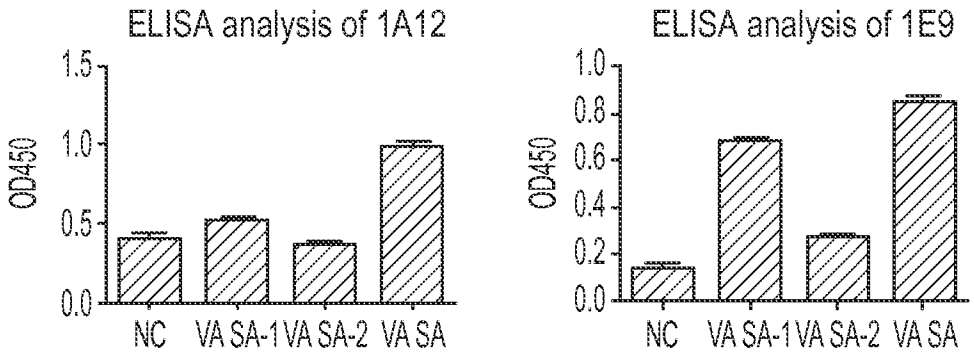
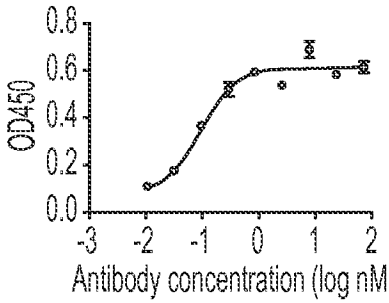


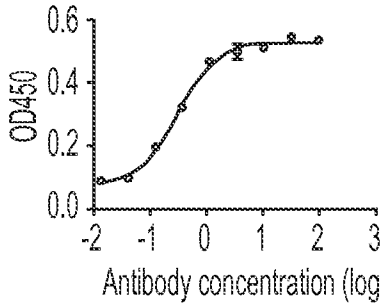
FIG. 5B

Dose response binding of 1E9 IgG



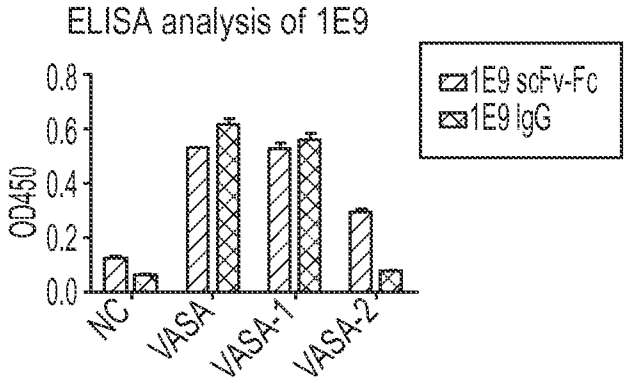
Best-fit values	
BOTTOM	0.07817
TOP	0.6084
LOGEC50	-1.050
HILLSLOPE	1.374
EC50	0.08919

Dose response binding of 1E9 scFv-Fc



Best-fit values	
BOTTOM	0.07416
TOP	0.5299
LOGEC50	-0.5126
HILLSLOPE	1.254
EC50	0.3072

FIG. 6A



VASA peptide sequence: GKSTLNTAGFSSSQAPNPVDDESWD
VASA-1 peptide sequence: SQAPNPVDDE
VASA-2 peptide sequence: GKSTLNTAGF

FIG. 6B

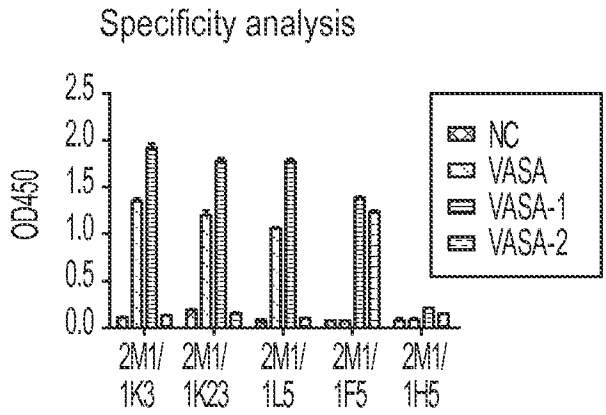
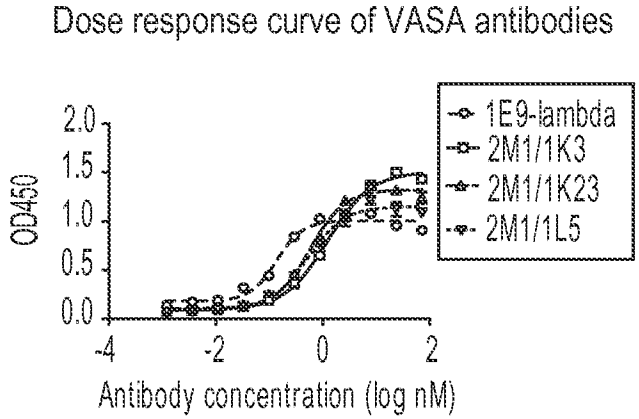
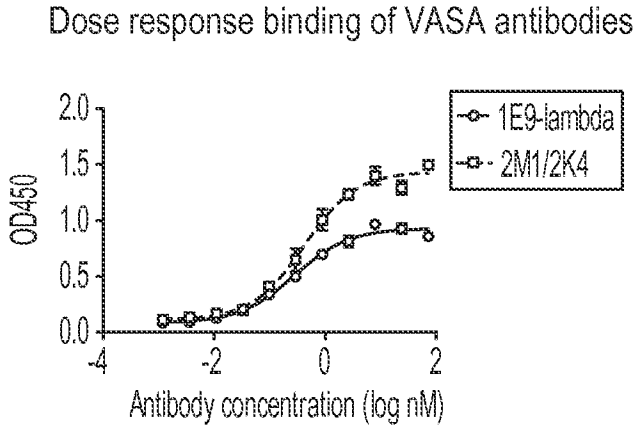


FIG. 7A



	1E9-lambda	2M1/1K3	2M1/1K23	2M1/1L5
Best-fit values				
BOTTOM	0.1822	0.09026	0.1057	0.08715
TOP	1.007	1.509	1.326	1.154
LOGEC50	-0.8650	0.09294	-0.2333	-0.2855
HILLSLOPE	1.763	1.072	1.338	1.258
EC50	0.1365	1.239	0.5844	0.5182

FIG. 7B



	1E9-lambda	2M1/2K4
Best-fit values		
BOTTOM	0.08445	0.1134
TOP	0.9319	1.437
LOGEC50	-0.5576	-0.3812
HILLSLOPE	0.8795	0.9339
EC50	0.2770	0.4157

FIG. 7C

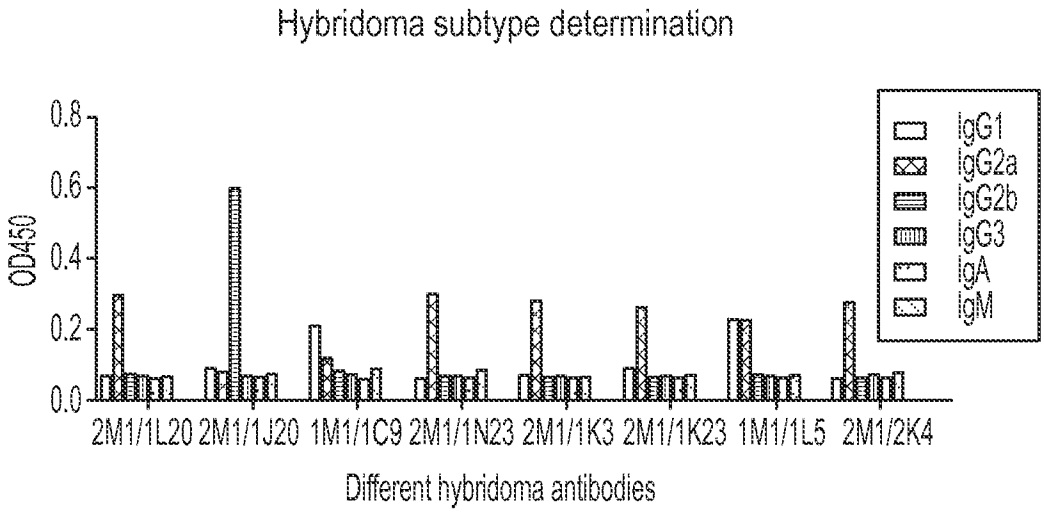


FIG. 8

Light Chain Variable Region Sequence Alignments

SEQ ID NO.	CLONE NAME	CDR1	CDR2
3	1N23VL5-5	FTVMTQTPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
4	1N23VL5-8_0816	STVMTQTPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
5	1N23VL1-8_	LVLMTQTPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
6	1N23VL1-2_0820	SVLMTQTPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
7	1N23VL1-4_0820	YVLMTQTPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
8	1K23VL2-5_	QVLMTQAPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
9	1N23VL1-2	RFQVSQTPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
10	1K23VL2-6	VFVMTQAPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
11	1K23VL2-8_0822	LIVMTQAPLSLPVSLGDQASTSCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
12	1K23VL2-3_0829	STVMTQAPLSLPVSLGDQASISCR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRF	60
13	2K4VL1-3_0820	SVLMTQTPLSLPVSLGDQASISSR-SSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKISNRF	60
14	2K4VL1-4_	LVLMTQTPLSLPVSLGDQASISCR-SSQNLVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRF	60
15	2K4VL1-1	LVLMTQTPLSLPVSLGDQASISCR-SSQNLVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRF	60
16	2K4VL1-6_0820	LVLMTQTPLSLPVSLGDQASISCR-SSQNLVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRF	60
17	1C9VL2-4_	LIVMTQAAPSVPVTPGESVSISCR-STKSLLHSNGNTYLSWFLQRPGQSPQLLIYRMSNLA	60
18	1C9VL2-6	YIVMTQAAPSVPVTPGESVSISCR-STKSLLHSNGNTYLSWFLQRPGQSPQLLIYRMSNLA	60
19	1C9VL2-3_0816	SGLMTQAAPSVPVTPGESVSISCR-STKSLLHSNGNTYLSWFLQRPGQSPQLLIYRMSNLA	60
20	2K4VL2-5_0816	PGLMTQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA	60
21	2K4VL2-4_	SIVMTQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA	60
22	2K4VL2-6_0816	STVMTQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA	60
23	1J20VL5-2_0907	DIVMTQSAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA	60

FIG. 9A

```
24 1U20VL5-6_0907    DIVMTQSAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA 60
25 1U20VL4-3_0907    DIVLTQSAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLVYRMSNLA 60
26 1L20VL5-0912_0917 DGVMTQSAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA 60
27 1K3VL2-5           LIVMTQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA 60
28 1K3VL2-3           LIVMTQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA 60
29 1K3VL2-4           SIVMTQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA 60
30 2K4VL2-5           VFVMTQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA 60
31 1L5VL2-4           DIVMTQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA 60
32 1L5VL3-1           LIVITQAAPSVPVTPGESVSISCR-SSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLA 60
33 CTC6_VL            DIVMTQAAPSVSVTPGESVSISCR-STKSLLHSNGNTYLYWLLQRPGQSPQRLIYHMSNLA 60
34 CTD6_VL            DIVMTQAAPSVSVTPGESVSISCR-STKSLLHSNGNTYLYWLLQRPGQSPQRLIYHMSNLA 60
35 CTA4_VL            DIKMTQSPSSVFASLGERVTITCK-ASQ----NINSFLTWFHQKPGKSPTTLIYRTNRLL 55
36 CTB4_VL            DIKMTQSPSSVFASLGERVTITCK-ASQ----NINSFLTWFHQKPGKSPTTLIYRTNRLL 55
37 1E9_VL             SYVLTQ-PPSVSAAPGQKVTISCSGSSSNI----GNNYVSWYQQLPGTAPKLLIYDNNKRP 56
38 1A12_VL            SYVLTQ-PPSVSVSPGQTASVTCSGD-KL----GNKYASWYQQKPGQSPVLVIYQDKKRP 55
```

FIG. 9A Cont.

Light Chain Variable Region Sequence Alignments (continued)

SEQ ID NO.	CLONE NAME	CDR3	
3	1N23VL5-5	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEIK-	112
4	1N23VL5-8_0816	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEIK-	112
5	1N23VL1-8	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEDW-	112
6	1N23VL1-2_0820	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEDW-	112
7	1N23VL1-4_0820	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEIK-	112
8	1K23VL2-5	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEIK-	112
9	1N23VL1-2	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEIK-	112
10	1K23VL2-6	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEIK-	112
11	1K23VL2-8_0822	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKLEIK-	112
12	1K23VL2-3_0829	SGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFC**SQSAHVP-WTF**GGGTKKTGS-	112
13	2K4VL1-3_0820	SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC**SQSTHVP-PTF**GGGTKLEIK-	112
14	2K4VL1-4	SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC**FQSSHV--LTF**GGGTKLEIK-	111
15	2K4VL1-1	SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC**FQSSHV--LTF**GGGTKLEIK-	111
16	2K4VL1-6_0820	SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC**FQSSHV--LTF**GGGTKLEIK-	111
17	1C9VL2-4	SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC**MQHLEYP-LTF**GAGTKLEIK-	112
18	1C9VL2-6	SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC**MQHLEYP-LTF**GAGTKLEIK-	112
19	1C9VL2-3_0816	SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC**MQHLEYP-LTF**GAGTKLEIK-	112
20	2K4VL2-5_0816	SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC**MQHLEYP-LTF**GAGTKLEIK-	112
21	2K4VL2-4	SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC**MQHLEYP-LTF**GAGTKLEIK-	112

FIG. 9B

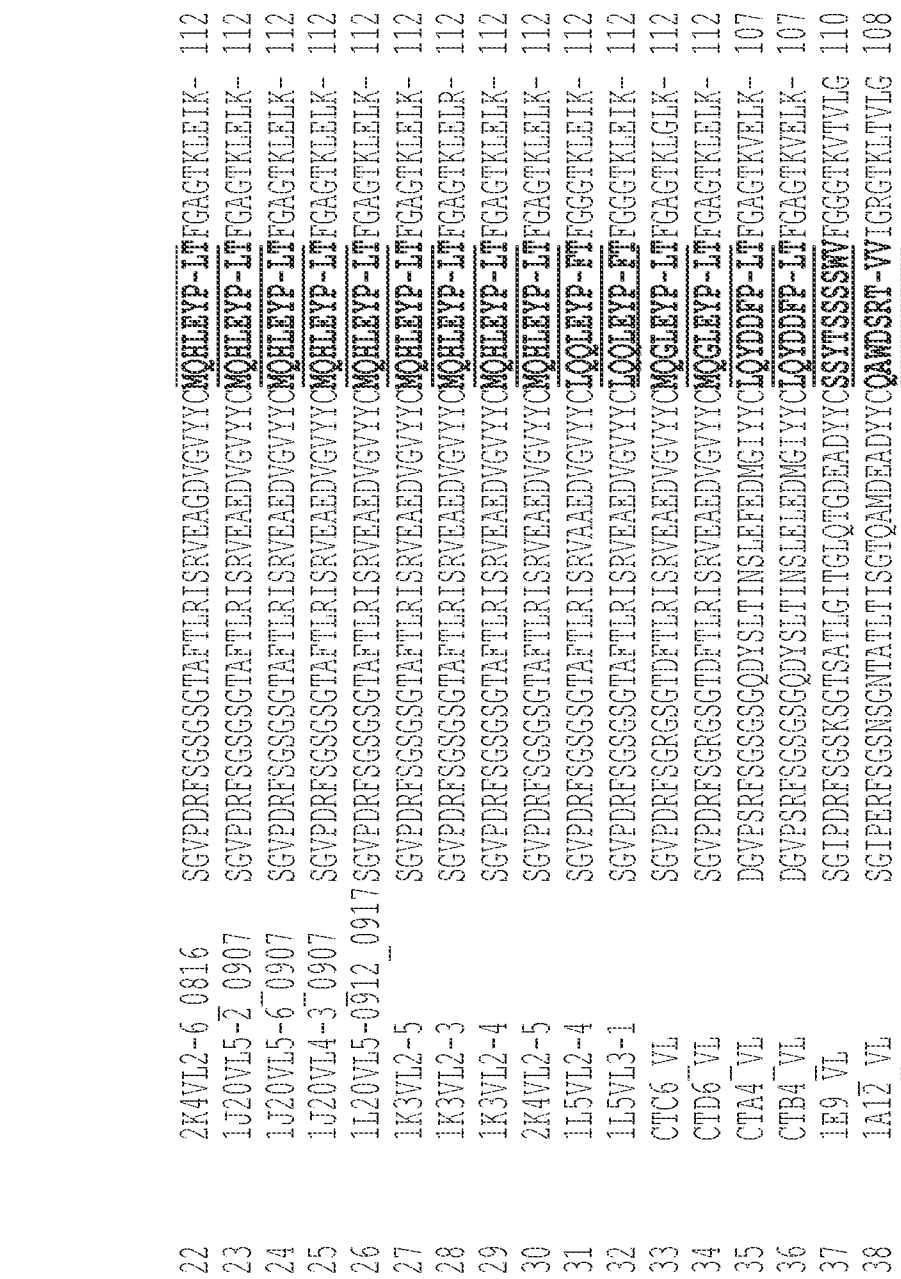

22 2K4VL2-6_0816 SGVPDRFSGSGSGTAFTLRISRVEAGDVGVYYCMQHLEYP-LTFGAGTKLEIK- 112
23 1J20VL5-2_0907 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYP-LTFGAGTKLELK- 112
24 1J20VL5-6_0907 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYP-LTFGAGTKLELK- 112
25 1J20VL4-3_0907 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYP-LTFGAGTKLELK- 112
26 1L20VL5-0912_0917 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYP-LTFGAGTKLELK- 112
27 1K3VL2-5 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYP-LTFGAGTKLELK- 112
28 1K3VL2-3 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYP-LTFGAGTKLELR- 112
29 1K3VL2-4 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYP-LTFGAGTKLELK- 112
30 2K4VL2-5 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYP-LTFGAGTKLELK- 112
31 1L5VL2-4 SGVPDRFSGSGSGTAFTLRISRVAAEDVGVYYCLQQLEYP-FTFGGGTKLEIK- 112
32 1L5VL3-1 SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQQLEYP-FTFGGGTKLEIK- 112
33 CTC6_VL SGVPDRFSGRGSGTDFTLRISRVEAEDVGVYYCMQGLEYP-LTFGAGTKLGLK- 112
34 CTD6_VL SGVPDRFSGRGSGTDFTLRISRVEAEDVGVYYCMQGLEYP-LTFGAGTKLELK- 112
35 CTA4_VL DGVPSRFSGSGSGQDYSLTINSLEFEDMGIYYCLQYDDFP-LTFGAGTKVELK- 107
36 CTB4_VL DGVPSRFSGSGSGQDYSLTINSLELEDMGIYYCLQYDDFP-LTFGAGTKVELK- 107
37 1E9_VL SGIPDRFSGSKSGTSATLGITGLQTGDEADYYCSSYTSSSSWVFGGGTKVTVLG 110
38 1A12_VL SGIPERFSGSNSGNTATLTISGTQAMDEADYYCQAWDSRT-VVIGRGTKLTVLG 108

FIG. 9B Cont.

Heavy Chain Variable Region Sequence Alignments

SEQ ID NO.	CLONE NAME	CDR1	CDR2	
39	1K3VH6-7	EVQLQQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
40	1K3VH6-8_0816	EVQLQQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
41	1K3VH3-8_	EVQLKQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
42	2K4VH3-8	EVQLKQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
43	1K3VH3-4	SVQLKQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
44	1K3VH3-3_0816	SVQLKQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
45	2K4VH2-8_	RSQLKESGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
46	2K4VH1-1	SVKLQESGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
47	2K4VH1-4	SVKLQESGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
48	1C9_VH404-8_1024	QVQLQPSGAELARPGASVKLSCKASGFTFTNYWMQWIKQRPGQGLEWIGAIYPG--NGET		58
49	1C9_VH405-12_1024	QVQLQPSGAELARPGASVKLSCKASGFTFTNYWMQWIKQRPGQGLEWIGAIYPG--DGET		58
50	1C9_VH411-1_1024	QVQLQPSGAELARPGAPVKLSCKASGFTFTNYWMQWIKQRPGQGLEWIGAIYPG--DGET		58
51	1C9_VH406-4_1024	QVQLQPSGAELARPGASVKLSCKASGFTFTNYWMQWIKQRPGQGLEWIGAIYPG--DGET		58
52	1L20VH2-3_0903	QVQLKESGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
53	1L20VH2-1_0907	QVQLKESGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPS--NGDT		58
54	1L20VH2-3_0910	QVQLKESGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
55	1J20VH1-7_0910	DVKLQESGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
56	1J20VH1-1-6_0829	QVQLQQSGAELARPGASVKLSRKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG--NGDT		58
57	1L5VH003-5-8_0907	EVQLQQSGAALVRPGASVKLSCKASGYSFTSYWMNWVKQRPGLGLEWIGMIHPS--DSET		58
58	1L5VH003-6-3_0907	EVQLQQSGAALVRPGASVKLSCKASGYSFTSYWMNWVKQRPGLGLEWIGMIHPS--DSET		58
59	1L5VH001-7-6_0907	EVQLQQSGAALVRPGASVKLSCKASGYSFTSYWMNWVKQRPGLGLEWIGMIHPS--DSET		58

FIG. 10A

FIG. 10A Cont.

Heavy Chain Variable Region Sequence Alignments (continued)

SEQ ID NO.	CLONE NAME	CDR3	
39	1K3VH6-7	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
40	1K3VH6-8_0816	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
41	1K3VH3-8	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
42	2K4VH3-8	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
43	1K3VH3-4	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
44	1K3VH3-3_0816	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
45	2K4VH2-8	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
46	2K4VH1-1	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
47	2K4VH1-4	RYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ARG----GIAWFAY**WGQGTLVTVSA	117
48	1C9_VH404-8_1024	RHTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ASGYP-----YFAY**WGQGTLVTVSA	116
49	1C9_VH405-12_1024	RHTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ASGYP-----YFAY**WGQGTLVTVSA	116
50	1C9_VH411-1_1024	RHTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYC**ASGYP----YFAY**WGQGTLVTVSA	116
51	1C9_VH406-4_1024	RHTQKFKGKATLSADKSSSTAYMQLSSLASEDSAVYYC**ASGYP-----YFAY**WGQGTLVTVSA	116
52	1L20VH2-3_0903	RYTQKFKGKATLTADKSSSTANMQLSSLASEDSAVYYC**AKGD---GNFWFAY**WGQGTLVTVSA	118
53	1L20VH2-1_0907	RYTQKFKGKATLTADKSSSTANMQLSSLASEDSAVYYC**AKGD---GNFWFAY**WGQGTLVTVSA	118
54	1L20VH2-3_0910	RYTQKFKGKATLTADKSSSTANMQLSGLASEDSAVYYC**AKGD---GNFWFAY**WGQGTLVTVSA	118
55	1J20VH1-7_0910	RYTQKFKGKATLTADKSSSTANMQLSSLASEDSAVYYC**AKGD---GNFWFAY**WGQGTLVTVSA	118
56	1J20VH1-1-6_0829	RYTQKFKGKATLTADKSSSTANMQLSSLASEDSAVYYC**AKGD---GNFWFAY**WGQGTLVTVSA	118
57	1L5VH003-5-8_0907	RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYC**ACRY---DRSYFDY**WGQGTTLTVSS	118
58	1L5VH003-6-3_0907	RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYC**ACRY---DRSYFDY**WGQGTTLTVSS	118
59	1L5VH001-7-6_0907	RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYC**ACRY---DRSYFDY**WGQGTTLTVSS	118
60	1L5VH001-6-5_0907	RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYC**ACRY---DRSYFDY**WGQGTTLTVSS	118
61	1L5VH001-6-11_0907	RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYC**ACRY---DRSYFDY**WGQGTTLTVSS	118

FIG. 10B

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62  1L5VH003-6-2_0910  RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYCACRY---DRSYFDYWGQGTTLTVSS  118
63  1L5VH001-6-12_0907 RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYCACRY---DRSYFDYWGQGTTLTVSS  118
64  1L5VH003-3-4_0907  RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYCACRY---DRSYFDYWGQGTTLTVSS  118
65  1L5VH003-3-8_0907  RLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYCACRY---DRSYFDYWGQGTTLTVSS  118
66  CTC2_VH            RFNEKFKGKATLSVDTSSTTAYMHLFSLTSDDSAVYYCVRS---------GDFWGQGTTLTVSS  113
67  CTD2_VH            RFNEKFKGKATLSVDTSSTTAYMHLFSLTSDDSAVYYCVRS---------GDFWGQGTTLTVSS  113
68  CTA5_VH            YYADSVKDRFTISRDDSQSMLYLQMFNLKTEDTAMYYCVRD---------GWWWGQGTSVTVSS  115
69  CTB11_VH           YYADSVKDRFTISRDDSQSMLYLQMFNLKTEDTAMYYCVRD---------GWWWGQGTSVTVSS  115
70  1N23VH3-5          YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
71  1N23VH3-7          YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
72  1N23VH2-1          YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
73  1K23VH2-1_0910     YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
74  1K23VH1-4_0907     YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
75  1K23VH1-10_0907    YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
76  1N23VH1-5          YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
77  1K23VH8-4_0907     YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
78  1K23VH8-5_0907     YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
79  1K23VH8-9_0907     YYNPSLKSRISITRDTSKNQYYLQLNSVTTEDTATYYCARYNSLRLGAMDYWGQGTSVTVSS  120
80  1E9_VH1E3          YYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGGNS-----YYGWGQGTLVTVSS  116
81  1E9_VH1D5          YYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGGNS-----FRDWGQGTLVTVSS  116
82  1A12_VH            YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDRED-----GMDVWGQGTTVTVSA  117
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FIG. 10B Cont.

Unique Light Chain CDR Sequence Alignments

SEQ ID NO.	CDR1	SEQ ID NO.	CDR2	SEQ ID NO.	CDR3
83	QSLVHSNGNTY	89	KVS	96	SQSAHVP-WT
84	QNIVHSNGNTY	90	KIS	97	SQSTHVP-PT
85	KSLLHSNGNTY	91	RMS	98	FQGSHV--LT
86	QNI-----NSF	92	HMS	99	MQHLEYP-LT
87	SNI----GNNY	93	RTN	100	LQQLEYP-FT
88	KL-----GNKY	94	DNN	101	MQGLEYP-LT
		95	QDK	102	LQYDDFP-LT
				103	SSYTSSSSWV
				104	QAWDSRTVVI

FIG. 11

Unique Heavy Chain CDR Sequence Alignments

SEQ ID NO.	CDR1	SEQ ID NO.	CDR2	SEQ ID NO.	CDR3
105	GYTFTSYW	113	IYPG--NGDT	122	ARG-----GIAWFAY
106	GFTFTNYW	114	IYPG--NGET	123	ASGYP------YFAY
107	GYSFTSYW	115	IYPG--DGET	124	AKG--D--GNFWFAY
108	GYTFTTFW	116	IHPS--DSET	125	ACRY-D---RSYFDY
109	GFTFNANA	117	IYPG--DAAT	126	VRS---------GDF
110	GDSVTSGY	118	IRSKTRNYAI	127	VR---D------GWW
111	GFTFSSYW	119	ISYS---GNT	128	ARYNS-LLRLGAMDY
112	GFTFSNYG	120	IKRD--GSEK	129	ARGGN-S-----YYG
		121	ISYD--GINK	130	ARGGN-S-----FRD
				131	AKDRED----G-MDV

FIG. 12