

ANTI-BCMA SINGLE DOMAIN ANTIBODIES AND APPLICATION THEREOF

TECHNICAL FIELD

5 **[0001]** The present disclosure belongs to the field of biotechnologies. In particular, the present disclosure relates to a group of single domain antibodies against B cell maturation antigen (BCMA) and use thereof.

BACKGROUND

10 **[0002]** BCMA (B cell maturation antigen, BCMA) is a member of tumor necrosis factor receptor (TNFR) superfamily, which can bind to a B cell-activating factor (BAFF) or a B lymphocyte stimulator (BLyS) and a proliferation inducing ligand (APRIL). It is reported that in normal cells, BCMA is mainly expressed by plasmocytes and some mature B cells, but not expressed in most B cells or other organs. Multiple myeloma (MM) is a malignant tumor
15 characterized by massive proliferation of clonal plasmocytes. The RNA of BCMA is generally detected in MM cells, and the BCMA protein can be detected on the surfaces of plasmocytes of a patient with multiple myeloma. Accordingly, a candidate target antigen for immune treatment of MM is BCMA. At present, MM treatment can induce remission, but almost all the patients will eventually relapse and die. Some monoclonal antibody candidate drugs have shown a promise to
20 treat MM in pre-clinical studies and early clinical trials, but have not been universally approved by consensus, and no monoclonal antibody drug has been marketed. Clearly, there is an urgent need of new immunological therapy for MM, and an effective antigen-specific adoptive T cell therapy developed for this disease will be an important research progress.

25 **[0003]** Single domain antibody (sdAb), also known as nanobody, is a heavy chain antibody found in Alpaca blood in which a light chain is absent. By using the molecular biology technology in combination with nano-particle science, Belgian scientists have developed a novel, low molecular weight fragment of antibody which can bind to an antigen. It has a group of advantages, such as, simple structure, strong penetration, easy expression and purification, high affinity and stability, and no toxic and side reactions, or the like. Single domain antibodies for various target
30 antigens have been researched by use of a single domain antibody platform technology, and then used in the field of biomedicines.

[0004] The present disclosure aims to develop a group of promising anti-BCMA single domain antibodies for use in therapeutic antibody candidate drugs and chimeric antigen receptor T cells targeting BCMA.

SUMMARY OF THE INVENTION

[0005] The technical problem to be solved by the present disclosure is to provide a group of novel anti-BCMA single domain antibodies having good effects.

5 **[0006]** Another technical problem to be solved by the present disclosure is to develop various uses of anti-BCMA single domain antibodies.

[0007] In order to achieve the above objects, the present disclosure provides the following technical solutions:

10 **[0008]** The present disclosure provides a group of anti-BCMA single domain antibodies composed of a framework region and a complementary determining region, wherein the complementary determining region has an amino acid sequence selected from those of SEQ ID NOs: 1-66 (Annex 1: Amino Acid Sequences of Complementary Determining Regions of Screened BCMA-sdAbs).

15 **[0008a]** Disclosed herein is an anti-BCMA single domain antibody, said single domain antibody composed of framework regions and complementary determining regions, said complementary determining regions comprise the amino acid sequences of CDR1 consisting of TYFMA, CDR2 consisting of GGIRWSDGVOHYADSVKG, and CDR3 consisting of CASRGIADGSDFGS.

20 **[0009]** In some embodiments, the amino acid sequence of the complementary determining region has more than 80%, more than 85%, more than 90%, more than 95% or more than 99% identity to the amino acid sequence as set forth in SEQ ID NOs: 1-66.

[0010] Preferably, the difference between in amino acids is conservative substitution.

[0011] In some embodiments, the single domain antibody in the group has an amino acid sequence selected from SEQ ID NOs: 67-132 or is an amino acid sequence selected from SEQ ID NOs: 67-132 (Annex 2: Amino Acid Sequences of Screened BCMA-sdAbs).

25 **[0011a]** Disclosed herein is an anti-BCMA single domain antibody according to claim 1, wherein said single domain antibody comprises the amino acid sequences of CDR1, CDR2, and CDR3 as set forth in SEQ ID NO: 67

30 **[0012]** In some embodiments, the amino acid sequence of the single domain antibody has more than 80%, more than 85%, more than 90%, more than 95% or more than 99% identity to the amino acid sequence as set forth in SEQ ID NOs: 67-132.

[0013] Preferably, the difference between in amino acids is conservative substitution, more preferably, one or more conservative substitutions.

[0014] The present disclosure provides a group of genes of anti-BCMA single domain

antibodies having a nucleotide sequence selected from those of SEQ ID NOs: 133-198 (Annex 3: Nucleotide Sequences of Screened BCMA-sdAbs), or being a nucleotide sequence of SEQ ID NOs: 133-198, or being a nucleotide sequence encoding the above single domain antibodies.

[0014a] Disclosed herein is a gene of an anti-BCMA single domain antibody, wherein said gene of single domain antibody has a nucleotide sequence as set forth in SEQ ID NO: 133.

[0015] In some embodiments, the nucleotide sequence of the single domain antibody has more than 80%, more than 85%, more than 90%, more than 95% or more than 99% identity to the nucleotide sequence as set forth in SEQ ID NOs: 133-198.

[0016] Preferably, the difference between in bases is conservative substitution, more preferably, one or more conservative substitutions.

[0017] The present disclosure provides a polypeptide having one or more single domain antibodies selected from the group of single domain antibodies as described above.

[0017a] Disclosed herein is a polypeptide, having one or multiple single domain antibodies according to any one of claims 1-3.

[0018] Preferably, the plurality of single domain antibodies are the same or different.

[0019] The present disclosure provides an expression vector including one or more genes selected from the group of genes of single domain antibodies as described above.

[0019a] Disclosed herein is an expression vector, comprising the gene as defined herein, wherein said expression vector is preferably a prokaryotic cell expression vector, a eukaryotic cell expression vector or other cell expression vectors, more preferably, a lentiviral vector.

[0020] Preferably, the expression vector is a prokaryotic cell expression vector, a eukaryotic cell expression vector, or other cell expression vector(s).

[0021] The present disclosure provides a host cell including the above expression vector.

[0022] Preferably, the host cell is a prokaryotic expression cell, a eukaryotic expression cell, a fungus cell or a yeast cell, wherein the prokaryotic expression cell is preferably *Escherichia coli*.

[0022a] Disclosed herein is a host cell, comprising said expression vector as defined herein, wherein said host cell is preferably a prokaryotic expression cell, a eukaryotic expression cell, a fungus cell or a yeast cell, and said prokaryotic expression cell is preferably *Escherichia coli*.

[0023] The present disclosure provides a chimeric antigen receptor, having one or more single domain antibodies selected from the above group of single domain antibodies.

[0023a] Disclosed herein is a chimeric antigen receptor, having one or multiple single domain antibodies as defined herein.

[0024] Preferably, the more single domain antibodies are the same or different.

[0025] The present disclosure provides a T cell modified by a chimeric antigen receptor, which is modified by the above chimeric antigen receptor.

[0025a] Disclosed herein is a T cell, wherein said T cell expresses said chimeric antigen receptor as defined herein.

5 **[0026]** The present disclosure provides a pharmaceutical composition including one or more single domain antibodies selected from the above group of single domain antibodies as active ingredients.

[0026a] Disclosed herein is a pharmaceutical composition, comprising one or more single domain antibodies as defined herein as active ingredients.

10 **[0027]** The present disclosure provides a humanized anti-BCMA single domain antibody, which is obtained by humanizing the single domain antibody selected from the above group of single domain antibodies.

[0027a] Disclosed herein is the single domain antibody as defined herein wherein said single domain antibody is humanized.

15 **[0028]** The present disclosure provides use of the single domain antibody in the above group of single domain antibodies in detection of BCMA.

[0028a] Disclosed herein is an *in vitro* use of the single domain antibody as defined herein in detection of BCMA.

20 **[0029]** The present disclosure provides use of the single domain antibody in the above group of single domain antibodies for blocking an interaction between BAFF and BCMA.

[0029a] Disclosed herein is an *in vitro* use of the single domain antibody as defined herein for blocking an interaction between BAFF and BCMA.

[0030] In some embodiments, the single domain antibody is linked to one or more of a cytotoxic agent, an enzyme, a radioisotope, a fluorescent compound or a chemiluminescent compound.

25 **[0031]** The present disclosure provides use of the single domain antibody in the above group of single domain antibodies in preparation of a drug for treating a disease associated with abnormal BCMA expression.

[0031a] Disclosed herein is the use of the single domain antibody as defined herein in the manufacture of a medicament for treating a disease associated with high level of BCMA expression.

30 **[0032]** Preferably, the disease associated with abnormal BCMA expression is a multiple myeloma disease.

[0033] The present disclosure has the following beneficial technical effects:

[0034] The disclosure screens a group of anti-BCMA single domain antibodies. Compared with the existing antibodies, respective anti-BCMA single domain antibodies in the group have high activity and strong neutralization or binding capability. The group of single domain antibodies can specifically bind to human BCMA antigens or tumor cell strains expressing BCMA on the cell surfaces, effectively block the binding of BAFF antigen to BCMA, and generate a corresponding signal cascade effect. The group of single domain antibodies can be used for detecting and/or treating a plurality of diseases associated with abnormal BCMA expression.

[0035] Hereinafter the present disclosure will be described in details by reference to the accompanying drawings and examples, but the scope of the present disclosure is not limited thereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] Fig. 1 shows an amplification of common heavy chain antibody and genes of single chain antibodies via a first-round PCR. With reference to (Marker) (1500 BP, 1000 BP, 800 BP, 500 BP, 250 BP and 100 BP), PCR amplification products, there are common heavy chain gene amplification fragments having more than 800 BP and heavy chain antibody gene amplification fragments having less than 800 BP. 2 and 3 are PCR amplification products, which are heavy chain antibody gene amplification fragments having only about 500 BP.

[0037] Fig. 2 shows a VHH target gene fragment obtained in second-round PCR amplification. Marker (1500BP, 1200 BP, 1000 BP, 800 BP, 700 BP, 600 BP, 500 BP, 250 BP and 100 BP). 1 - 12 are PCR amplification products, which are heavy chain antibody VHH gene amplification fragments having about 500 BP.

[0038] Fig. 3 is an SDS-PAGE illustration of expressed BCMA-sdAbs before purification.

[0039] Fig. 4 shows an SDS-PAGE illustration of expressed BCMA-sdAbs after being purified by a nickel column.

[0040] Fig. 5 shows a concentration gradient of a purified BCMA single domain antibody binding to BCMA protein (ELISA).

[0041] Fig. 6 shows that a BCMA single domain antibody can competitively inhibit the binding of BAFF protein to BCMA protein.

[0042] Fig. 7 shows a killing efficiency of BCMA CART on tumor cells.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0043] The disclosure screens a group of anti-BCMA single domain antibodies by a group of

steps, which have potentials of high activity and high neutralization or binding capability. These single domain antibodies have similar structures (composed of a framework region and a complementary determining region), and similar functional effects. Thus, they can be considered as a group of anti-BCMA single domain antibodies having common structure and common property effects.

[0044] The term “BCMA”, as used herein, is a member of tumor necrosis factor receptor (TNFR) superfamily, which can bind to a B cell-activating factor or a B lymphocyte stimulator and a proliferation inducing ligand (APRIL)). Multiple myeloma (MM) is a malignant tumor characterized by massive proliferation of clonal plasmocytes. The RNA of BCMA is generally detected in MM cells, and the BCMA protein can be detected on the surfaces of plasmocytes in a patient with multiple myeloma.

[0045] The term “multiple myeloma (MM)” as used herein is a malignant tumor characterized by massive proliferation of clonal plasmocytes. At present, MM treatment can induce remission, but almost all the patients will eventually relapse and die. Some monoclonal antibodies have shown a promise to treat MM in pre-clinical studies and early clinical trials, but have not been universally approved. Clearly, there is an urgent need of new antibodies and new immunological therapy for MM.

[0046] New antibodies against BCMA are the development object, and finally the protective object of the present disclosure. The scope of the present disclosure encompasses the obtained anti-BCMA antibodies and various forms thereof (for example, single domain antibodies), as well as substances including the antibody as component (for example, pharmaceutical compositions, kits, vectors, chimeric antigen receptors, a chimeric antigen receptor modified T cells, or the like), uses (for example, uses for diagnosis, treatment or application, etc.). However, it should be understood by those skilled in the art that the protective objects of the present disclosure are not limited to these exemplified contents.

[0047] The term “single domain antibody (sdAb)” as used herein refers to a fragment containing a single variable domain in an antibody, and is also known as nanobody. Like a complete antibody, it can selectively bind to a specific antigen. Compared with the mass of the complete antibody (150-160kDa), the single domain antibody (only about 12-15kDa) is much smaller. The first single domain antibody was made from alpaca heavy chain antibodies by artificial engineering, and known as “VHH segment”. In a preferred embodiment, the present disclosure uses the single domain antibody of the alpaca, whereas those skilled in the art should understand that the present disclosure can also encompass single domain antibodies derived from other species. Without limitation, the single domain antibody of the present disclosure is an anti-

BCMA single domain antibody.

[0048] The term “framework region” is also known as a skeleton region. The sequences of about 110 amino acids near the N-terminals of the H chain and the L chain of immunoglobulin vary greatly, while the amino acid sequences in other positions are relatively constant.

5 Accordingly, the light chain and the heavy chain can be divided to a variable region (V) and a constant region (C). The variable region contains an HVR (hypervariable region), also known as complementary-determining region (CDR) and a frame region (FR). The variability of FR is less than that of CDR. There are four FR molecules in total, that is, FR1, FR2, FR3 and FR4, respectively. During the recognition of antibody, four FR molecules crimp so that CDR molecules
10 are close to each other. It should be understood that, the present disclosure is not limited to specific framework region(s), and those skilled in the art can select or obtain appropriate framework region(s) according to practical requirements without departing from the protective scope of the present disclosure.

[0049] The term “complementary determining region (CDR)”, the whole antibody molecule
15 can be divided into a constant region and a variable region. In the variable region, some amino acid residues are highly variable, and the regions in which the compositions and arrangement orders of these amino acid residues are more prone to vary are called hypervariable regions. There are three hyper-variable regions (HVR) in the V regions of the L chain and the H chain, which can form a precise complementation with the antigen determinants in terms of spatial structure,
20 and thus the hyper-variable regions are also called complementary determining regions.

[0050] The term “identity” of sequence as used herein is interchangeably used with “homology”, and refers to a similarity degree between sequences as measured by sequence alignment softwares, such as BLAST. The sequence alignment methods and softwares are well-known by those skilled in the art. Modified nucleotide sequences can be obtained by substitution,
25 deletion and/or addition of one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or more) amino acids or bases in a known sequence. For example, by modifying the amino acid sequence or nucleotide sequence as set forth in one or more of sequence SEQ ID NOs: 1-198 of the present disclosure via conventional means (for example, by conservative substitution), it is feasible to obtain sequences having more than 80%, more than 85%, more than
30 90%, more than 95% or more than 99% sequence identity to these sequences, and having substantially the same properties, which are encompassed within the protective scope of the present disclosure. Preferably, the present disclosure obtains sequence identity by conservative substitution, but is not limited thereto.

[0051] The term “amino acid sequence” refers to an arrangement in which amino acids are

linked to each other to form a peptide chain (or polypeptide), wherein the amino acid sequence can only be read in one direction. There are more than 100 types of different amino acids, twenty of which are commonly used. The present disclosure does not exclude the case that other substances (e.g., saccharides, lipids, and other modifications) are attached to the amino acid chains, and is not limited to the 20 amino acids that are commonly used, either.

[0052] The term “nucleotide sequence” refers to an arrangement of bases in DNAs or RNAs, namely, an arrangement of A, T, G and C in DNA, or an arrangement of A, U, G and C in mRNA. It also includes arrangements of bases in rRNA, tRNA and mRNA. It should be understood that the antibody gene of the present disclosure also encompasses, in addition to DNA sequences, RNA (rRNA, tRNA and mRNA) and their complementary sequences. It will also be understood that genes encoding the antibodies of the present disclosure are not equivalent to the sequences as set forth in SEQ ID NOs: 133-198 of the present disclosure, and the genes which encode the antibodies of the present disclosure but are different from the nucleotide sequences as set forth in SEQ ID NOs: 133-198 are also within the protective scope of the present disclosure.

[0053] In some embodiments, the polypeptide, the pharmaceutical composition, the chimeric antibody receptor or the CART of the present disclosure comprises one single domain antibody, it should be understood that the present disclosure is not limited thereto. The above substances of the present disclosure can contain two, three, or more single domain antibodies, wherein the more single domain antibodies are the same or different. Moreover, in addition to the single domain antibodies of the present disclosure, other antibodies or single domain antibodies that are not contained in the present disclosure can also be included without going beyond the scope of the present disclosure.

[0054] The term “expression vectors” refers to a vector that incorporates expression elements (such as, promoter, RBS, or terminator) on the basis of the basic backbone of a cloning vector so that a target gene can be expressed. The expression vector comprises four parts: a target gene, a promoter, a terminator and a marker gene. The present disclosure includes, but is not limited to, a prokaryotic cell expression vector, a eukaryotic cell expression vector or other cell expression vectors.

[0055] “Chimeric antigen receptor (CAR)” is a core component of “chimeric antigen receptor T cell (CART)”, which imparts a T cell with an ability to recognize tumor antigens in an independent manner, so that the T cell modified by CAR is capable of recognizing a broader range of targets as compared with a natural T cell surface receptor. The basic design of CAR comprises a tumor-associated antigen binding region, an extracellular hinge region, a transmembrane region, and an intracellular signal region.

[0056] In an embodiment of the present disclosure, the chimeric antigen receptor or chimeric antigen receptor T cell of the present disclosure can contain one, two or more single domain antibodies of the present disclosure, which can be the same or different.

[0057] In an embodiment of the present disclosure, the “pharmaceutical composition” of the present disclosure can contain one, two or more single domain antibodies of the present disclosure, which can be the same or different.

[0058] The term “humanized” antibody refers to an antibody in which the constant regions (namely CH and CL regions) of the antibodies or the whole antibodies are encoded by human antibody genes. The humanized antibody can greatly reduce the immune side reaction of a heterologous antibody in a human organism. The humanized antibody includes several types including chimeric antibodies, modified antibodies and full human antibodies. It will be appreciated that those skilled in the art can prepare suitable humanized forms of the single domain antibodies of the present disclosure according to the practical requirements, which are within the scope of the present disclosure.

[0059] The term “lentivirus” as used herein is one genus of Retroviridae including eight viruses that can infect humans and vertebrates, wherein the primary infection cells are mainly lymphocytes and macrophages, and the infected individuals will eventually develop the diseases. The types of lentiviruses include, for example, human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), equine infectious anemia (EIA), and feline immunodeficiency virus (FIV). The progress of lentiviral vector research is rapid and intensive. This vector can effectively integrate foreign genes into host chromosomes, so as to achieve persistent expression. In terms of infectability, it can effectively infect neurons, hepatocytes, cardiomyocytes, tumor cells, endothelial cells, stem cells and other types of cells, so as to achieve good gene therapeutic effect. In addition, those skilled in the art can also select other suitable vectors other than lentivirus, which are within the protective scope of the present disclosure.

[0060] Hereinafter the present disclosure will be described in details by reference to the following examples. However, the present disclosure is not limited to the specific details of these examples, because for persons skilled in the art, other variations are well established, or obvious according to the direct disclosure and the appended claims. Therefore, all the technologies achieved based on the above description of the present disclosure shall fall within the scope of the present disclosure.

[0061] Unless otherwise specified, all the experimental methods described in the following examples are conventional methods; and all the reagents and biomaterials are commercially available, unless otherwise specified.

[0062] Example 1: Construction of anti-BCMA antigen-specific single domain antibody library

[0063] 1) Immunization of Alpaca with BCMA antigen

[0064] It is performed according to the conventional immunization method. Briefly, adult healthy alpacas were subject to multipoint subcutaneous injections at their necks and backs with BCMA antigen (Human TNFRSF17/BCMA/CD269 Protein, purchased from Beijing Yiqiao Shenzhou, Product No. 10620-H15H) with a total weight of about 2 mg, in which the antigen and an equal volume of Freund's adjuvant were added. Immunizations were carried out for 4-6 times. The absorption of mass at the injection sites was followed to confirm correct immunization. The immunization interval time was 7-15 days. After the fourth immunization, serum was collected to determine the immune titer of the antigen. When the titer reached 10,000 times or more (ELISA method), about 100 ml of whole blood was collected, and lymphocytes were separated and stored at -80 °C for subsequent use.

[0065] 2) Separation and RNA extraction of peripheral blood lymphocytes of alpaca

[0066] Peripheral blood lymphocytes of alpacas were separated by using a QIAGEN kit (QIAamp RNA Blood Mini Kit (50), Product No. 52304) following the instructions. Briefly, to 1 ml of full blood was added 5-10 ml of red blood cell lysate. The mixture was uniformly mixed, and placed in an ice bath for 30 min. It was centrifuged for 10 min at 2000 rpm after red blood cells were lysed. The supernatant was discarded, and an additional 1-2 ml of red blood cell lysate was added and uniformly mixed. The mixture was placed in an ice bath for 10 min to lyse residual red blood cells, and then centrifuged at 2000 rpm for 10 min. The supernatant was discarded, and 0.3 ml of lysate was added to mix with leukocytes uniformly. The resultant mixture was stored at -80°C for subsequent use.

[0067] RNA purification was carried out by using a QIAGEN kit (QIAamp RNA Blood Mini Kit (50), Product No. 52304) following the instructions. Briefly, to 0.3 ml of the separated alpaca lymphocytes was added 0.3 ml of buffer RLT, and the mixture was mixed well with shaking. The mixed liquid from the last step was transferred to a collection tube equipped with an adsorption column (QIAshredderSpinColumn), and centrifuged at 14,000 rpm for 2 min. The filtrate in the collection tube was transferred to a new centrifuge tube. 0.5 ml of 70% ethanol was added into the filtrate, and uniformly mixed upside down. The mixture was centrifuged at 10000 rpm for 15s, the waste liquid in the collection tube was discarded, and the adsorption column was re-placed into the collection tube. The adsorption column was transferred to a new 2 ml collection tube, 0.7 ml of buffer RW1 was added, and the mixture was centrifuged at 10000 rpm for 15s. The adsorption column was transferred to a new 2 ml collection tube, 0.5 ml of buffer RPE was added, and the mixture was centrifuged for 15s at 10000 rpm. 0.5ml of buffer RPE was added, and the

mixture was centrifuged at 14000 rpm for 3 min. The adsorption column was transferred to a new 1.5 ml centrifuge tube, and 30-50 µl RNase-free water was added dropwise into the middle of an adsorption membrane in the air. The mixture was placed at room temperature for 2-5 min, and centrifuged at 12,000 rpm for 1 min. The plasmid solution was collected into the centrifuge tube, and measured for the RNA concentration.

[0068] 3) Variable region-VHH of heavy chain antibody

[0069] Synthesis of a first chain of cDNA: A cDNA synthesis kit (MiniBESTAgarose Gel DNA Extraction Kit ver.4.0, TAKARA Company) was used following the instructions. With this template, two sets of primers were used to perform PCR amplification of the VHH gene fragment of the heavy chain antibody. By using a Nested PCR method, the fragments of greater than 800bp in the first PCR amplification are common heavy chain gene fragments, and the fragments between 800bp and 500bp are heavy chain antibody gene fragments with deletion of light chains (see Fig. 1). The gene fragments of heavy chain antibodies with deletion of light chain were recovered by gel cutting, and used as the template to obtain the VHH target gene (~500bp) by

PCR amplification with VHH specific primers (see Fig. 2).

[0070] Synthesis of primers:

[0071] First-round PCRFd5' primer:

[0072] YF-1: CGC CAT CAA GGT ACC AGT TGA (SEQ ID NO:199)

[0073] YF-2: GGG GTA CCT GTC ATC CAC GGA CCA GCT GA (SEQ ID NO:200)

[0074] First-round PCR Bd3' primer:

[0075] YBN:CAG CCG GCC ATG GCC SMK GTR CAG CTG GTG GAK TCT GGG GGA G (SEQ ID NO:201)

[0076] Second-round PCR primer:

[0077] YV-BACK: CAT GTG CATGGCCTA GAC TCG CGG CCCAGC CGG CCA TGG CC (SEQ ID NO:202)

[0078] YV-FOR: CAT GTG TAG ATT CCT GGC CGG CCT GGC CTG AGG AGA CGG TGA CCT GG (SEQ ID NO:203)

[0079] 4) Ligation of VHH fragment and phage display vector and electric transformation of TG1 competent cells

[0080] After the VHH fragment and the pHEN6 vector plasmid were subjected to single digestion with SfiI, the VHH fragment and the pHEN6 vector (Conrath, KEM other. Antimicrob Agents Chemother (Antimicrobial Chemotherapy) 2001,45: (10) 2807-12, Chinese patent

ZL20111028003.1)) were ligated by a ligase, and then electrically transformed into TG1 competent cells, which were used to coat a plate, and detected by colony PCR for verification of the antibody insertion rate. Detection of recombinant gene cloning efficiency: an LB/Amp plate was coated with an electrically transformed bacterial solution, cultured overnight at 32°C, and detected by colony PCR for verification of the ligation efficiency of the antibodies on the next day. The ligation efficiency of the phage-antibody library was more than 90%. The LB/Amp plate was coated with the electrically transformed bacterial solution, and cultured overnight at 32°C. The culture was washed with 2YT culture medium, and 15% glycerol was added. The mixture was stored at -80 °C.

10 **[0081]** 5) Preparation of VHH phage antibody library

[0082] Helper phage M13K07 (Invitrogen) was added into the antibody library for rescue: the phage antibody library was prepared according to a conventional method and stored at -80°C for subsequent use.

[0083] Example 2: Preparation of single domain antibody of BCMA

15 **[0084]** Screening of BCMA-specific single domain antibody

[0085] First-round: BCMA protein concentration 150µg/ml, 150µl/well, 1 micropore, incubate overnight at 4°C.

[0086] Second-round: BCMA protein concentration 10-100µg/ml, 150µl/well, 5 micropores, incubate overnight at 4°C.

20 **[0087]** Third-round: BCMA protein concentration 10-50µg/ml, 150µl/well, 5 micropores, incubate overnight at 4°C.

[0088] Blocking: 1% CPBS, 300µl /well, 37°C, incubate for 2 h.

Screening Round No.	Total amount of added phage antibody library	Elution solution + Tris-HCl	Number of single colony	Elution titer
First round	5.6×10^{11}	300 µl+200 µl	10	50/µl
Second round	5.25×10^{11}	150 µl/well x 5+ 350 µl	1/4=600 about 2400	$2.4 \times 10^4/\mu\text{l}$
Third round	5.32×10^{11}	150 µl/well x 5+ 350 µl	1/4=750 about 3000	$3 \times 10^4/\mu\text{l}$

[0089] 2. Picking of positive clones via phage ELISA

[0090] A single colony was randomly picked from an agar plate screened for grown colonies in the third round, inoculated and cultured in a 96-well culture plate containing an Amp 2YT liquid culture medium, and subject to superinfection of helper phages to induce the expression of the phage antibody. The expression supernatant was harvested, and then an ELISA assay was carried out with BCMA as an antigen. BCMA-positive wells were selected, and subject to DNA sequencing to identify the gene sequence of the anti-BCMA single domain antibody clones. A series of single domain antibody gene sequences including those in Annex 3 were obtained and used for further expression and screening of the single domain antibodies with high specificity and high activity.

[0091] Example 3: Construction of expression plasmid of specific BCMA single domain antibody

[0092] The specific BCMA single domain antibody gene obtained in Example 3 was amplified by PCR to obtain PCR products with restriction enzymes BbsI and BamHI sites. The PCR products and vectors (pSJF2 vector) (kim ls. Biosic Biochem. 2002, 66(5): 1148-51, Chinese patent ZL 201110280031) were treated with restriction enzymes BbsI and BamHI respectively, and recombined by ligation with T4 ligase to obtain the plasmid sdAb-pSJF2 that can be efficiently expressed in Escherichia coli, which was subject to gene sequencing to determine the correctness of its sequence.

[0093] 1) PCR amplification conditions required for obtaining VHH target genes, and compositions of 50μl PCR system:

- [0094]** MIX 25μl
- [0095]** Positive colony clone 1μl
- [0096]** 5'primer 1μl(1mol/1)
- [0097]** 3'primer 1μl(1mol/1)

[0098]

DEPC-treated ddH ₂ O		22μl	
Total volume		50 μl	
PCR Reaction Conditions:			
94°C	3min		30s
94°C	30s		
	55°C		
72°C	1min		
			30 rounds

[0099] 5' primer—GAA GAAGAA GAC AA CAG GCC SVK GTG MAG CTG GWG GAK TCT (SEQ ID NO:204)

[00100] 3' primer ——gaagatctccggatccTGAGGAGACGGTGACCTGGGT(SEQ ID NO:205)

[00101] 2) The target gene and the vector were digested, ligated, and transformed into TG1 cells. The products were subject to PCR for identifying the clones containing the target fragment, which were subject to gene sequencing so as to obtain the BCMA single domain antibody expression plasmid with a correct gene sequence.

[00102] Example 4: Expression and purification of anti-BCMA single domain antibody

[00103] The strains containing the plasmid BCMAsdAb-pSJF2 in example 3 were inoculated on an LB culture plate containing ampicillin at 37 °C overnight. A single colony was picked and inoculated in 15 ml LB medium solution containing ampicillin, and was cultured in a shaker at 37 °C overnight. 10 ml of culture was transferred to 1 L of 2YT culture solution containing ampicillin and cultured in a shaker at 37 °C at 240 rpm/min. After OD value reached 0.4-0.6, 0.5-1.0 mM IPTG was added and additionally incubated overnight. The above solution was centrifuged for collecting bacteria. The bacteria were lysed by adding lysozym and centrifuged, and the soluble single domain antibody protein in the supernatant was collected. A protein with the purity of more than 95% was obtained by Ni⁺ ion affinity chromatography. Fig. 3 shows the expressed anti-BCMA single domain antibody protein, and Fig. 4 shows SDS-PAGE electrophoresis results of expressed BCMA-sdAbs purified by a nickel column.

[00104] Example 5: Affinity assay test of BCMA single domain antibody

[00105] 1) Preparation of sample

[00106] Antigen: Bio-BCMA was diluted to 10μg/ml with 1X dynamic buffer (1X PBS, containing 0.05%Tween 20, 0.1% BSA, pH7.2);

[00107] Single domain antibody was gradually diluted into 400 nM, 200 nM, 100 nM, 50 nM, 25 nM, 12.5 nM and 6.25 nM with 1X kinetic buffer;

[00108] 2) Sample test

[00109] The antigen to be tested was loaded through an SA sensor. The antigen was diluted by 5 gradients, and all the BCMA single domain antibodies had an affinity of 50nm, 20nm, 10nm, 1nm, 0.1nm and 0.01nm.

[00110] Example 6: Binding test of purified BCMA single domain antibody and BCMA antigen(ELISA)

[00111] The BCMA-Fc antigen was diluted to 1 µg/ml with 0.05 M NaHCO₃ (pH 9.5). A 96-well plate was coated with 100µl antigen overnight at 4 °C. The 96-well plate was blocked with 300µl 0.5% BSA-PBS for 2 h at 37 °C. The purified BCMA single domain antibodies with different dilution concentrations were added in 100µl/well at 37 °C for 1 h. The plate was washed three times with 0.05% PBST. Mouse anti-His-HRP diluted in 1:5000 fold was added in 100 µl/well at 37 °C for 1 hour. The plate was washed three times with 0.05% PBST. 100 µl of TMB was added and kept in dark place at room temperature for 20 min. 100 µl of 1 mol/L HCl was added to quench the reaction. The OD value of the sample at 450 nm was measured by a microplate reader. Fig. 5 shows the concentration gradient of purified BCMA single domain antibody binding to BCMA protein (ELISA). Except that the binding ability of the two antibodies of B35 (13) and B92 (6-1) to the BCMA antigen was relatively low, the binding ability of the rest 11 antibodies to BCMA antigen was very high.

[00112] Example 7: Binding competitive inhibition test of BCMA single domain antibody on BAFF and BCMA

[00113] Because BCMA can bind to BAFF, the functional BCMA single domain antibody should be able to competitively inhibit the binding of BAFF to BCMA. BAFF protein coated a detachable ELISA plate according to 1µg/ml, 100µl/well and incubated overnight at 4°C. 2% BSA was added for blocking, 300µl/well, incubated at 37 °C for 2 hours. The BCMA single domain antibody was diluted to a final concentration of 10µg/ml. 100µl BCMA(10µg/ml) single domain antibody was added, 2µl of BAFF (5µg/ml) protein was added in each well to be uniformly mixed. Goat anti-rabbit IgG HRP (1:5000) was diluted, 100µl /well, incubated for 1h at 37 °C. TMB chromogenic solution was added, 100µl/well, and reacted in dark for 10 min. The reaction was quenched by adding 2M H₂SO₄ at 50µl/well. The OD value was measured at 450 nm. Fig. 6 shows that the BCMA single domain antibody can competitively inhibit the binding of BAFF protein to BCMA protein. Different BCMA single domain antibodies could competitively inhibit the binding of BAFF protein to BCMA protein, and the inhibition rate ranged from 34% to 92%.

[00114] Example 8: The study on BCMA single domain antibody as recognition antibody targeting specific BCMA antigen on CART cell

[00115] 1) Construction of vector

[00116] A BCMA single domain antibody gene and a second-generation CAR structure gene were synthesized. The two genes were spliced by overlapping PCR to obtain a BCMA CAR gene. After the synthetic gene was obtained, molecular cloning was carried out. First, PCR products of two gene fragments were obtained. Then, overlapping PCR was carried out to obtain BCMA CAR gene with the second-generation CAR structure in which two fragments are linked. Through

enzyme digestion of Pre vector and BCMA CAR gene, ligation, transformation, cloning, plasmid upgrading and sequencing, the BCMA CAR-expressed lentiviral vector Pre-Lenti-EF1 BCMA with a correct sequence was obtained.

[00117] 2) Packaging of lentivirus

5 **[00118]** On the day before virus packaging, 293T cells were digested by trypsin and spread in 150 cm culture dish. The cells were incubated in 5% CO₂ culture box for 8-24 h. When the adherent cells reached 80% of the total culture dish area, the 293T cells were transfected. Pre-Lenti-EF1 BCMA CAR: psPAX2: pMD2G = 4:3:1 was co-transfected with lipofectamine 2000. The virus supernatant was collected after 48 hours, and centrifuged at 4°C at 1250 rpm for 5 min to remove the dead 293T cells and cell debris. Then, the virus supernatant was filtered, concentrated, sub-packaged, and stored in a refrigerator at -80 °C.

[00119] 3) Preparation of CART cells

15 **[00120]** 10 ml of fresh blood was taken from healthy volunteers. Peripheral blood mononuclear cells (PBMC) were isolated with lymphocyte isolation solution, and then T cells were isolated and purified by magnetic beads. 2 x 10⁶ T cells/well were seeded into a 6-well plate, cultured in an x-vivo 15 culture medium containing IL-2 (1000 U/ml) and stimulated with anti-CD3 for 24 h. After 24 hours of stimulation, a BCMA virus solution was added and infected overnight. 2 ml of culture medium was added on the second day. After 6-7 days of infection, the expression of CAR was evaluated by flow cytometry. The positive rate of expressing anti-BCMA-CAR by transfected T cells was analyzed using biotinylated BCMA via flow cytometry.

[00121] 4) Determination of killing vitality

25 **[00122]** In a cell killing test, an LDH detection kit (Promega) was used for detection. CART cells/T cells: target cells were set with four gradients, which were 0.5:1, 1:1, 2:1 and 4:1, respectively. Daudi cells 3 x 10⁴/well, and the rest wells were supplemented to 200 µL with an X-VIVO-containing culture medium/1640 culture medium. The 96-well plate was cultured in a 5% CO₂ incubator at 37 °C. After 17 h, 20 µl of lysate was added into the maximum release well, and the cells were uniformly mixed to be completely ruptured. The 96-well plate was incubated in the CO₂ incubator for 2 h. Two hours later, the maximum release well was observed. After target cells were completely lysed, 50µL of supernatant was sucked from each well to the 96-well plate with a flat bottom, and then 50µL of substrate solution was added to each well, development was carried out for 30 min in the dark. After 30 min, the mixture was observed for the color change, wherein the colors of the maximum release MM.1S well and the CART cell well should be darker. A microplate reader was used for measurement at a wavelength of 490 nm. The killing results were seen in Fig. 7. BCMA chimeric antigen receptor modified T cells can specifically kill

BCMA-positive cells with a very high killing activity of more than 20%, and has no killing effect on BCMA-negative cells.

Annex 1:

Seque nce	CDR1	CDR2	CDR3	Clone group
1	TYFMA	GGIRWSDGVPHYADSVKG	CASRGIADGSDFGS	G3
2	IKAMA	AYIRSGGTTNYADSVKG	CNADYSPPGSRFPDLGP	G1
3	ANTM	ARISTDGRNYADSVKG	CNANWLSKFDY	NG7
4	VNAVA	AYIRRSGSTNYADSVKG	CNADFGSDYVVLGS	G5
5	IKALA	AYITSGGNTNYADSVRG	CNADFGEGTIIISLGP	G9
6	INAMA	AALTSGGNTHYADSVKG	CNADFGTAGLVVLGP	G7
7	INAMA	AYIRSNGRTNYADSVKG	CNADYGPPVSIGP	G6-2
8	IKAMA	AAVTSGGSTHYLDSVKG	CNADFGTDYVDLGP	G10
9	INAMG	AAITKSNNINYADSVKG-	CNGFFALPGYSSEEFGP	G2
10	MNRMG	ADIRDGGSTIYSDSVKG	CNAGRTGDRFNLVAY	G8
11	GYAMA	AAISSSSNSAPYYANSVKG	CAARYGTKRYVAREYDS	G17
12	INGMG	ARIDSRGSAYYADFVEG	CFAWQGAETY	G25
13	TYAMA	AYITNGGSTDYAASVKG	CNGATRGAQLVFD	NG1
14	NYAMA	AAISVSANSAPYYANSVKG	CAARYGTKRYVAREYDS	NG20
15	LNAMG	ARIAADGSTHYADSVEG	CFAWLGTDY	NG21
16	NNAMG	ARIDSGGITRYADSLKG	CFAHVGGTI	G14
17	INSMG	ASITGGGSSRYADSVKG	CNTIPPARTQSDHGEWYDY	NGS1
18	IN-MS	ATTRHDSTHYSDSVKG	CSGFFLDGSTWHPY	G12
19	INAMA	AYIRSNGSTNYADSVKG	CNGFFTLPGYSSEEFGP	G6
20	INAMG	AGITKGGRTNYADSVKG	CNGLCSGRECYGDSLFAA	G22#
21	INAMA	AYIRSNGRTNYADSVKG	CSGFFLDGSTWHPY	G6-1
22	DYAIG	SCISSSDGSTHYADSVKG	CATPWVTYCPENLLFSY	G13#
23	DYAIG	SCITSSDGSTYYADSVKG	CATPWVTYCPENLLFSY	G13-2#

24	IKAMG	AAITSGGSTNYADSVKG	CNGFFEYRGLEQLGP	G31
25	IRAMT	AVLTSAGKPMYADSVKG	CNADFGTPGSVVLGP	G4
26	IEAMG	AAITSGDSTNYADSVKG	CNALMVVRAGSNPEIGP	NG2
27	DYAIG	SCISSSDGSTYYADSVKG	CATPWVTYCPENLLFSY	G13-3#
28	LDAVG	ARIDRRGSTYYAVSVEG	CFAWQGAETH	G20
29	FNDMG	AAITSSRNTLYVDSVKG	CNPYPSPNNY	NG3
30	INAMG	AAITRSGKTNADSVKG	CNGFYGSEFGP	NG4
31	RYAVG	ASITWSGDYTTYKDSVKG	CAADKSSFRLRGPGLYDY	NG5
32	YYAIG	SCISSRDGTTHYADSVKG	CATPWVTYCPENLLFSY	++
33	YYAIG	SAISNIDDDTYEDSVKG	CAADKDVVVVRTGLSESDY	NG8
34	INAMA	AVITSGGRTMYAESVKG	CNGDWGSEGRVDLGP	NG9
35	IGDME	ASISAGPEMRSAGTPTYAKSV EG	CNADVLTYNGRYSRDVY	NG10
36	INMS	ATITRHDSTHYSDSVKG	CSGFFLDGSTWRPY	G12-1
37	GYAVA	AAISSSDNSSPYANVVKG	CAARYGTKRYVAREYDS	
38	INAMA	AYIRSSGTMYADSVKG	CNGDYSPPGSTYPLGP	NG11
39	DYAIG/YCPENLLFS Y	SCITSSDGSTYYADSVKG/ AAIRWSDGVPHYTDSVKG	CATPWVN/CASRGADGSDFG SY	G15 (bi)
40	ATTMA	ALITSDWHTKYADSVKD	CYARQAFSEPR	G11
41	IDAMG	ARLGSNGFTQYDISVEG	CFAWLGGDTV	NG12
42	NYAMG	ASVTRSGDNTYYKDSAKG	CAADKSSFRLRGPVYDY	NG14
43	VMLMG	ASITSADYTTAESVEG	CKVIAATVWGQETQVRQGLF	NG13
44	ARSMT	AVIMGGGSTMYADSVKG	CNADWGEVGFNLGP	G21
45	TYAIG	AAISRRGNKTDYAESVKG	CAASARNFIGTQPLDYDY	NG23
46	NYALG	AAIDWRHSSYYADSVKG	CAASSLPSSAPRQYDY	NG15
47	NYAMG	AAIVGSGDSTRYADSVKG	CASSSDPRVYIASTLDY	NG16
48	MFIMG	AAISRNSNLTYFYQSVKG	CNADYGPPVSIGP	G23
49	IKAMG	AGIVSSGNTNYADSVKG	CNALVVVTSASGPGLAS	NG17
50	TYFMA	CNADYSPPGSRFPDLGP	AGIVSSGNTNYADSVKG	G1-3

51	NYAIA	SSTGSDGNLYTPSVRG	CVAGKRPVITTWIALDA	NG18
52	IDSMR	AHITSTGRTNYADAVKG	CNMVTTTPYMH	NG24
53	ENAMG	AAITSSRSTLYIDSVKG	CNPYPSPNSY	NG25
54	ANKMG	ARISTDGRTNYADSVKG	CNANWLDKYDY	NG19
55	ARSMT	AVITSGGSTMYADSVKG	CNADWGEVGFVNLGP	NG26 (G21-1)
56	FNGVA	AVIRSGGNTLYADSVKG	CNVDYSPPGSLVPDLGP	G18
57	INAMG	AAITRGGSTNYADSVKG	CNGLCSDDRCYGDSLFP	G16
58	LDAVG	ARIDSRGSAYYADSVKG	CFAYYGAQISFGP	G24
59	LDAMG	AHIDDDRGRTAYYADFVKG	CFAWQGAETY	G19
60	VNAVA	AYIRRS GSTNYADSVKG	CNAGRTGDRFNLVAY	G5-1
61	TYFMA	GGIRWSDGVPHYADSVKG	CNADYSPPGSRFPDLGP	G26
62	IKAMA	AYIRSGGTNYADSVKG	CASRG IADGSDFGS	G27
63	LYAMG	AYIRSGGTTNYADSVKG	CNADYSPPGSRFPDLGP	G1-2
64	TYAMG	AAISRRGNKTDYAESVKG	CAASARNFIGTQPLDYDY	G28
65	GYFMA	GGIRWSDGVPHYADSK	CASRG IADGSDFGS	G29
66	INAMG	AAITKSNININADSBKG	CNGFFTLPGYSSEFGP	G2-1

[00123] Annex 2

Sequence	Amino acid sequence	Clone group
67	EVQLQASGGGLAQAGGSLRLSCTASGRTFSTYFMAWFRQPPGKEREYVGGIRWSDGVPHYADS VKGRFTISRDNAKNTVYLLQMNSLKSEDTAVYFCASRG IADGSDFGSYGQGTQVTVSS	G3
68	QVKLEESGGGLVQPGGSLRLSCAASGSIFS IKAMAWYRQAPGKQREL VAYIRSGGTTNYADSV KGRFTISRDI AKNTVYLLQMNSLKPEDTAVYYCNADYSPPGSRFPDLGPWGQGTQVTVSS	G1
69	QVKLEESGGGLAQPGGSLRLSCAASGLVFSANTMAWYRRAPGKQRELVARISTDGRNTNYADSV KGRFTISRDNREKTVFLQMNRNLNPD TAVYYCNANWLSKFDYWGQGTQVTVSS	NG7
70	DVQLQASGGGLVQAGGSLRLSCVASGSIFSVNAVAWYRQAPGKQREL VAYIRRSGSTNYADSV KGRFTISRDNAKNTVYLLQMNSLKPEDTAVYYCNADFGSDYVVLGSGWGQGTQVTVSS	G5
71	QVKLEESGGGLVQAGGSLRLSCAASGSIFS IKALAWYRQAPGKQREL VAYITSGGNTNYADSV RGRFTISRDNAKNTVYLLQMNSLKPEDTAVYYCNADFGEGTI ISLGPWGQGTQVTVSS	G9
72	EVQLVESGGGLVQPGGSLRLSCAASGSEFSINAMAWYRQAPGKQRELVAALTSGGNTHYADSV KGRFTISRDNAKNTWYLLQMNSLKPEDTAVYYCNADFGTAGLVVLGPWGQGTQVTVSS	G7
73	EVQLQASGGGLVQPGGSLRLSCAASGSIFSINAMAWYRQAPGKQREL VAYIRSNGRNTNYADSV KGRFTISRDNAKNTVYLLQMNSLKLEDTAVYYCNADYGPPVSI GPWGQGTQVTVSS	G6-2
74	EVQLVESGGGLVQAGGSLRLSCVVSGSLLSIKAMAWFRQPPGKQRELVA AVTSGGSTHYLDSV KGRFTISRDNANTVHLQMNSLKPEDTAVYYCNADFGTDYVDLGPWGQGTQVTVSS	G10
75	DVQLQASGGGLVQPGGSLRLSCAVSGSIFSINAMGWYRQAPGKQRELVA AITKSNINNYADSV KGRFTISTDNAKNTVYLLQMNSLKPEDTAVYYCNGFFALPGYSSEEF GPWGQGTQVTVSS	G2
76	EVQLVESGGGLVQPGGSLRLSCVASGNIFDMNRMGWYRQPPGKQREL VADIRDGGSTIYSDSV KGRFTISRDNAKNTLYLLQMNSLKPD TAVYYCNAGRTGDRFNLVAYWGQGTQVTVSS	G8
77	DVQLQASGGGLVQHGGSLRLSCEASGRTFSGYAMAWFRQAPGKEHEFVAAISSSSNSAPYYAN SVKGRFTISRDNAKNTVYLLQMNNLQTEDTAVYYCAARYG TKRYVAREYDSWGQGTQVTVSS	G17
78	DVQLQASGGGVVQAGGSLRLSCTASGSIRSINGMWSRVAPGKQRDFVARIDSRGSAYYADSV EGRFTISRDNAKNTVYLLQVDTLKPEDTAVYYCFAWQGAETYWGLGTQVTVSS	G25
79	QVKLEESGGGLVQPGGSLRLSCAASGSIGDTYAMAWYRQAPGKQRDLVAYITNGGSTDYAASV KGRFTISRDNAKNTVYLLQMNSLKPEDTAVYYCNGATRGAQLVFDWGQGTQVTVSS	NG1

80	QVKLEESGGGLVQHGGSLRLSCAASGGTFSNYAMAWFRQAPGKEREVAAISVSANSAPYYAN SVKGRFTISRDNANTVYLMNSLKTEDTAVYYCAARYGKRYVAREYDSWGQGTQVTVSS	NG20
81	QVKLEESGGGLVQPGGSLRLSCAASGSSVSLNAMGWSRVQPGSTRDFVARIAADGSTHYADSV EGRFTISGDAARNTVYLMQDSLKPEDTAVYYCFAWLGTDTYWGQGTQVTVSS	NG21
82	DVQLQASGGGLVQAGGSLTLSCAASGSIGDNNAMGWSRTPPGKQREFVARIDSGGITRYADSL KGRFTVSRDTGKNTVSLQMNSLKAEDTGYYCFAHVGGTIWGQGTQVTVSS	G14
83	QVQLVESGGGLVQPGGSLRLSCLPSGGIFTINSMGWYRQAPGKQRELVASITGGSSRYADSV KGRFIMSRDNAKNMVYLMNSLKPEDTAVYYCNTIPPARTQSDHGEWYDYWGQGTQVTVSS	NGS1
84	QVKLEESGGGLVQAGGSLRLSCAASSSIFSNMSWYRQAPGNERELVATITRHDSTHYSDSVK GRFTISRDDDKNTIYLMNSLKPEDTAVYYCSGFFLDGSTWHPYWGQGTQVTVSS	G12
85	EVQLVESGGGLVQPGGSLRLSCAASGSIVSINAMAWYRQAPGKQRELVAIIRSNGSTNYADSV KGRFTISRDNANTVYLMNSLKLDTAVYYCNGFFTLPGYSSEEFGPWGQGTQVTVSS	G6
86	EVQLVESGGGLVQPGGSLRLSCAASESIFSNAMGWYRQAPGKQREYVAGITKGGRTNYADSV KGRFTISRDDAKNTVYLMNSLKPEDTAVYYCNGLCSGRECYGDSLFAAWGQGTQVTVSS	G22#
87	EVQLVESGGGLVQPGGSLRLSCAASGSIVSINAMAWYRQAPGKQRELVAIIRSNGRNYADSV KGRFTISRDNANTVYLMNSLKLDTAVYYCNGFFLDGSTWHPYWGQGTQVTVSS	G6-1
88	EVQLVESGGGLAQAGGSLRLSCAASGFTDDYAIGWFRQAPGKEREGVSCISSSDGSTHYADS VKGRFTISRDNARNTVTLQINSLKPEDTAVYYCATPWVITYCPENLLFSYWGQGTQVTVSS	G13#
89	QVKLEESGGGLVQPGGSLRLSCAASGFTDDYAIGWFRQAPGKEREGVSCITSSDGSTYYADS VKGRFTISRDNANNTVHLQISNLKPEDTAVYYCATPWVITYCPENLLFSYWGQGTQVTVSS	G13-#
90	EVQLVESGGGLVQAGGSLTLSCAVSGSSFSIKAMGWYRLAPGKQRELVAAITSGGSTNYADSV KGRFTISRDSAKNTVYLMNSLKPEDTAVYYCNGFFEYRGLEQLGPWGQGTQVTVSS	G31
91	DVQLQASGGGLVQPGGSLRLSCAASGSIVGIRAMTWYRQAPGKQRELVAVLTSAGKPMYADSV KGRFTISRDNANTVYLMNSLKPEDTAVYYCNADFGTPGSVVLGPWGQGTQVTVSS	G4
92	QVKLEESGGGLVQPGGSLRLSCAASGSILSIEAMGWYRQTLGKQRELVAAITSGDSTNYADFV KGRFTISRDKAKNMVYLMNSLKPEDTAVYFCNALMVVRAGSNPEIGPWGQGTQVTVSS	NG2
93	QVKLEESGGGLVQPGGSLRLSCAASGFTDDYAIGWFRQAPGKEREGVSCISSSDGSTYYADS VKGRFTISRDNANNTVHLQISNLKPEDTAVYYCATPWVITYCPENLLFSYWGQGTQVTVSS	G13-#
94	EVQLVESGGGLVQPGGSLRLSCVVSARGVSLDAVGWSRVAPGKQRDFVARIDRRGSTYYAVSV G20	G20

	EGRSTISRDNAKNTVYLQLDTLKPEDTAVYYCFAWQGAETHWGLGTQVTVSS	
95	QVKLEESGGGLVQAGGSLTLSCVASGSHFSFNDMGWYRQDPWKGRDLVAAITSSRNTLYVDSV KGRFTISRDDAKNTVYLMNNLKPEDTAVYYCNPYPSPNNYWGQGTQVTVSS	NG3
96	QVKLEESGGGLVQPGGSLRLSCAASGSPFTINAMGWYRQAPGKQRELVAAITRSGKTNYADSV KGRFTISGDNALTTVYLMNNLQPEDTAVYYCNGFYGSEFGPWGQGTQVTVSS	NG4
97	QVKLEESGGGLVQAGGSATLSCSAPGDTLSRYAVGWFRQGPQGERDFVASITWSGDYTYKDS VKGRFTISRDSVNNMVYLRMNSLKPEDTALYYCAADKSSFRLRGPGLYDYRGQGTQVTVSS	NG5
98	QVKLEESGGGLVQPGGSLRLSCAASGFTFDYYAIGWFRQAPGKEREGVSCISSRDGTHYADS VKGRFTISRDNAKNTVYLQIDSLKPEDTAVYYCATPWVITYCPENLLFSYWGQGTQVTVSS	NG6#
99	QVKLEESGGGFVQPGGSLRLSCAASGFSLHYAIGWFRQAPGKEREWVSAISNIDDDTYEDS VKGRFTISRDNAKNTAYLQMNLSKPEDTAVYYCAADKDVVVVRTGLSESDYWGQGTQVTVSS	NG8
100	QVKLEESGGGLVQAGGSLRLSCAASGSIFGINAMAWYRQAPGKQRELVAVITSGGRTMYAESV KGRFAISRDAKNTVYLMNSLKPEDTAVYYCNGDWGSEGRVDLGPWGQGTQVTVSS	NG9
101	QVKLEESGGGLVQPGGTLRLSCAASGSIRSIGMEWYRQAPGQRELVASISAGPEMRSAGTP TYAKSVEGRFTISRDNINKMMWLQMNLSRPEDTAVYSCNADVLTYYNGRYSRDVYWGQGTQVT VSS	NG10
102	QVKLEESGGGLVQAGGSLRLSCAASSSIFSINMSWYRQAPGNERELVATITRHDSTHYSDSVK GRFAISRDDDKNTIYLMNSLKPEDTAVYYCSGFFLDGSTWRPYWGQGTQVTVSS	G12-1
103	DVQLQASGGGLVQPGGSLRLSCAASGRTLSGYAVAWFRQAPGKEREFVAAISSDNSSPYAN VVKGRFTISRDNAKNTVYLMNSLQTEDTALYYCAARYGTKRYVAREYDSWGQGTQVTVSS	G17-1
104	QVKLEESGGGLVQPGGSLRLSCAASRSIFSINAMAWYRQAPGKQRELVAIYIRSSGTTMYADSV KGRFTISRDNAKNTVYLMNSLKPEDTAVYYCNGDYSPPGSTYPLGPWGQGTQVTVSS	NG11
105	EVQLQASGGGLVQPGGSLRLSCAASGFTFDYYAIGWFRQAPGKEREGVSCITSSDGSTYYADS VKGRFTISRDNANNTVHLQISNLKPEDTAVYYCATPWVNYCPENLLFSYWGQGTQVTVSSQAQ VQLVESGGGLAQAGGSLRLSCTASGRTFSTYFMA WFRQPPGKEREYVGGIRWSDGVPHYTDSVKGRFTISRDNAKNTVYLMNSLKSEDTAVYFCAS RGIADGSDFGSYGQGTQVTVSS	G15 (b i)
106	QVKLEESGGGLVQAGGSLRLSCGASGIIFSATTMAWYRQAPGKQRELVALITSDWHTKYADSV KDRFSISRDNASTVHLQMNLSRSEDVAVYFCYARQAFSEPRWGQGTQVTVSS	G11

107	QVQLVDSGGGLVQPGGSLRLSCAASGSSGRIDAMGWSRVAPGKQRDFVARLGSNFTQYDISV EGRFTISGDVAKNTIYLMQMDTLKPEDTAVYYCFAWLQDQTVWGQGTQVTVSS	NG12
108	QVQLVDSGGGLVKAGASLRLSCAASGDALFNYAMGWFRQGPGERDFVASVTRSGDNTYYKDS AKGRFTISRDDAKNTVYLMQNSLKPEDTAVYFCAADKSSFRLRGPVYDYRGQGTQVTVSS	NG14
109	DVQLVDSGGGLVQAGGSLRLSCAVSGSDGRVMLMGWYRQAPGQQRDLVASITSADYTTAIESV EGRFTISTDNNKNTVYLMQNSLKPEDTAVYFCKVIAATVWGQETQVRQGLTFWGQGTQVTVSS	NG13
110	EVQLVESGGGLVQPGGSLRLSCVASGSISSARSMTWYRQALGKQRELVAIVIMGGGSTMYADSV KGRFTISRDNKNTVYLMQNSLKPEDTAVYYCNADWGGVGFNLPWPWGQGTQVTVSS	G21
111	DVQLQASGGGLVQIGDSVRLSCIASGGTFRTYAIGWFRQAPGAEREFVAAISRRGNKDYAESV KGRFTVSRDNAENTVYLMQNSLKPDDMGVYYCAASARNFIGTQPLDYDYWGQGTQVTVSS	NG23
112	QVKLEESGGGLVQAGGSLRLSCAASGWNLGNYALGWFRQAPGKEREFVAAIDWRHSSYYADSV KGRFTISRDNNTKNMVYLMQSSLKLEDTRLYYCAASSLPSSAPRQYDYWGQGTQVTVSS	NG15
113	DVQLVDSGGGLVQAGGSLRLSCVASGRTFSNYAMGWYRRRPGLEREFVAAIVSGSDSTRYADS VKGRFTISRDNKNTVYLMQNTLKPEDTAVYYCASSSDPRVYIASTLDYWGQGTQVTVSS	NG16
114	QVQLVESGGGLVQAGGSLRLSCAASGRTFSMFIMGWFRQAPGKERELVAAISRNSNLTYFQS VKGRFTISRDNKNTVYLMQNSLKLEDTAVYYCNADYGPPVSIQWPWGQGTQVTVSS	G23
115	QVKLEESGGGWVQPGGSLRLSCVVSGRILSIKAMGWYRQAPGKQREYVAGIVSSGNTNYADSV KGRFTISGDNAKNTVFLQMNSLKPEDTAVYYCNALVVVTSASGPELASWGQGTQVTVSS	NG17
116	DVQLVDSGGGLAQAGGSLRLSCTASGRTFSTYFMAWFRQPPGKQRELVAIIRSGGTTNYADSV KGRFTISRDIKNTVYLMQNSLKPEDTAVYYCNADYSPPGSRFPDLGPWGQGTQVTVSS	G1-3
117	QVKLEESGGGLVQPGGSLTLSCAASGFTLDNYAIAWFRQAPGREREWVSSTGSDGNLYTPSVR GRFTISRDNKNTVYLMQNSLKPEDTAVYYCVAGKRPVITTWIALDAWGQGTQVTVSS	NG18
118	DVQLVDSGGGLVQAGGSLRLSCAASGTFSSIDSMRWFRAPGKEREFVAHITSTGRNTYADAV KGRFTISRDNKNTMWLQMDNLKPDDTAVYYCNMVTTPYMHWGQGTQVTVSS	NG24
119	QVKLEESGGGLVQAGGSLKLSCVASGSRFSENAMGWYHQAPDKQRTLVAAITSSRSTLYIDSV KGRFTISRDNKNTVYLMQNSLKPEDTGYYCNPYPSPNSYWGQGTQVTVSS	NG25
120	QVKLEESGGGLVQPGGSLRLSCAASGLVFSANKMGWYRQAPGKQRELVARISTDGRNTYADSV KGRFTISRDNKNTVFLQMNSLNPDDTAVYYCNANWLDKYDYWGQGTQVTVSS	NG19
121	QVKLEESGGGLVEPGGSLRLSCVASGSISSARSMTWYRQAHGKQRELVAIVITSGGSTMYADSV	NG26 (

	KGRFTISRDSAKNTVYLMQNSLKPEDTAVYYCNADWGEVGFVNLGPWGQGTQVTVSS	G21-1)
122	EVQLVESGGGLVQPGGSLRLSCAASGSIFGFNGVAWFRQAPGKGRELVAIVIRSGGNTYADSVK GRFTISRDNAKNTVYLMQNSLKPEDTAVYYCNVDYSPPGSLVPDLGPWGQGTQVTVSS	G18
123	EVQLEESGGGLVQPGGSLRLSCAASGSIASINAMGWYRQAPGKQRELVAAITRGGSTNYADSV KGRFTISRDNAKNTVYLMQNSLKPEDTAVYSCNGLCSDDRCYGDSL FAPWGP GTQVTVSS	G16#
124	EVQLVESGGGLVQPGGSLRLSCLVSGRGVSLDAVGWSRVAPGKQRDFVARIDS RGSAYYADSV EGRFTISRDNAKNTVYLVQVDTLKPEDTAVYYCFAYYGAQISFGPWGQGTQVTVSS	G24
125	DVQLQASGGGLVQPGGSLRLSCVVSGRGVNLDAMGWSRVAPGKQRDFVAHIDDRGTAYYADFV KGRSTISRDNAKNTVYLVQVDTLKPEDTAVYYCFAWQGAETYWGLGTRVTVSS	G19
126	EVQLVESGGGLVQAGGSLRLSCVASGSIFSVNAVAVYRQAPGKQRELVAIIRRS GSTNYADSV KGRFTISRDNAKNTLYLMQNSLKPDDTAVYYCNAGRTGDRFNLVAYWGQGTQVTVSS	G5-1
127	EVQLVESGGGLAQAGGSLRLSCTASGRTFSTYFMAWFRQPPGKEREYVGGIRWSDGVPHYADS VKGRFTISRDNAKNTVYLMQNSLKPEDTAVYYCNADYSPPGSRFPDLGPWGQGTQVTVSS	G26
128	EVQLQASGGGLVQPGGSLRLSCVASGSIFSIKAMAWYRQAPGKQRELVAIIRSGGTTNYADSV KGRFTISRDI AKNTVYLMQNSLKSEDTAVYFCASRG IADGSDFGSYGQGTQVTVSS	G27
129	EVQLVESGGGLVQAGASVRLSCAASGRANS LYAMGWFRQAPGKQRELVAIIRSGGTTNYADSV KGRFTISRDI AKNTVYLMQNSLKPEDTAVYYCNADYSPPGSRFPDLGPWGQGTQVTVSS	G1-2
130	EVQLVESGGGLVQIGDSVRLSCIASGGTFRTYAMGWFRQAPGAEREFVAAISRRGNKTDYAES VKGRFTVSRDNAENTVYLMQNSLKPDDMGVYYCAASARNF IGTQPLDYDYWGQGTQVTVSS	G28
131	QVKLEESGGGMVQAGGSLRLSCVASGRSFVGYFMAWFRQPPGKEREYVGGIRWSDGVPHYADS VKGRFTISRDNAKNTVYLMQNSLKSEDTAVYFCASRG IADGSDFGSYGQGTQVTVSS	G29
132	QVKLVESGGGLVQPGGSLRLSCAASGSIFSINAMGWYRQAPGKQRELVAAITKSNNINYADSV KGRFTISRDNAKNTVYLMQNSLKPEDTAVYYCNGFFTLPGYSSEEFGPWGQGTQVTVSS	G2-1

[00124] Annex 3

Seq uenc e	Nucleotide acid sequence	Clone group
133	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTACAGCCTCTGGACGCACCTTCAGTACCTATTTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAAGAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTTCCACACTATGCAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAATCTGAGGACACGGCCGTTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCT GACTTTGGTTCCTACGGCCAGGGGACCCAGGTACCGTCTCCTCA	G3
134	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTACAGCCTCTGGACGCACCTTCAGTACCTATTTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAAGAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTTCCACACTATGCAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAATCTGAGGACACGGCCGTTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCT GACTTTGGTTCCTACGGCCAGGGGACCCAGGTACCGTCTCCTCA	G1
135	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTAAGACTCTCC TGTGCAGCCTCTGGACTCGTCTTCAGTGCCAATACCATGGCCTGGTACCGCCGGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCACGTATTAGCACTGACGGACGTACAAACTACGCGGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACCGCGAGAAGACGGTGTCTGCAAATGAACAGG CTGAAACCCTGACGACACGGCCGTCATTACTGTAATGCAAACTGGCTCAGTAAATTTGACTAC TGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	NG7
136	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAGCATCTTCAGTGTCAATGCCGTGGCCTGGTACCGCCAGGCTCCAGGG AAACAGCGCGAGTTGGTCGCATATATACGTCTAGTGGTAGCACAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACAGCCGTCATTACTGTAATGCAGATTTCCGTAGCGACTATGTCGTC CTCGGTTCTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	G5

137	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCTTCAGTATCAAAGCCTTGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTACTAGTGGTGGTAACACAACTATGCAGACTCCGTG AGGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAACAGC CTGAAACCTGAGGACACAGCCGTCTATTACTGTAATGCAGATTTGCGAGAAGGGACTATCATA TCCCTTGGACCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G9
138	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCGAATTCAGTATCAATGCCATGGCGTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCACTTACTAGTGGTGGTAACACTCACTATGCGGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGTGGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAATGCAGATTTGGAAGTGCAGGTTTGGTA GTGCTGGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G7
139	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGTCAGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTCTAGTAATGGCCGCACAACTATGCAGACTCCGTG AAGGGCCGATTACCATTTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACTTGAGGACACGGCCGTCTATTACTGTAATGCAGACTACGGGCCTCCAGTATCCATT GGTCCTTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G6-2
140	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGTCTCTGAGACTCTCC TGTGTAGTCTCTGGAAGTCTCCTCAGTATCAAAGCCATGGCCTGGTTCCGCCAGCCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCTGTTACTAGTGGTGAAGCACACACTATTTAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAACACGGTGCATCTGCAAATGAACAGCCTG AAACCTGAGGACACAGCTGTCTATTACTGTAATGCAGATTTCCGTACTGACTATGTCGACTTA GGGCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G10
141	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTCTCTGAGACTCTCC TGTGCAGTCTCTGGAAGCATCTTCAGTATCAATGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAACAGCGCGAGTTGGTCGCAGCTATTACTAAAAGTAATAACATAAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAATGGATTCTTCGCTTTGCCTGGGTACAGT	G2

	AGTGAAGAATTTGGTCCCTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	
142	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAACATCTTCGATATGAATCGGATGGGCTGGTACCGCCAGCCTCCAGGG AAGCAGCGGAGTTGGTCGCAGATATTCTGTATGGCGTTCTACAATTTATTCAGATTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAAAATGAACAGC CTGAAACCTGACGACACAGCCGTGTATTATTGTAATGCGGGGCGGACAGGGGATCGTTTTAAT TTGGTGGCGTATTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	G8
143	GATGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCACGGGGGCTCTCTGAGACTCTCC TGTGAAGCCTCTGGACGCACCTTCAGTGGCTATGCCATGGCCTGGTTCCGCCAGGCTCCAGGA AAGGAACATGAATTTGTAGCAGCTATTAGCTCAAGTAGTAATAGTGCCCCATACTATGCAAAT TCCGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTTTATCTACAAATG AACAACTACAACTGAGGACACGGCCGTTTATTACTGTGCAGCCCGGTACGGTACGAAACGG TACGTGCCCCGGGAGTATGACTCGTGGGGCCAAGGGACCCAGGTACCGTCTCCTCA	G17
144	GATGTGCAGCTGCAGGCGTCTGGGGGAGGCGTCGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTACAGCCTCTGGAAGCATCCGCAGTATCAATGGCATGGGCTGGTCGCGCGTGGCTCCAGGG AAGCAGCGGACTTCGTTCGCAGTATTGATAGTAGGGGTAGCGCATACTATGCAGACTCCGTA GAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAGTGACACG CTGAAACCTGAGGACACGGCCGTCTATTATTGCTTTGCGTGGCAGGGTGCGGAAACATATTGG GGCCTGGGGACCCAGGTACCGTCTCCTCA	G25
145	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGCGACTCTCC TGTGCAGCCTCTGGAAGCATCGGCGATACCTATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGGACTTGGTCGCATATATTACTAATGGTGGTAGCACGGACTACGCAGCCTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTCTATCTGCAAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTACTACTGTAATGGAGCTACCCGTGGTGACAGTTAGTC TTCGACTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	NG1

146	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTGCAGCACGGGGGCTCTCTGAGACTCTCC TGTGCAGCCTCTGGAGGCACGTTCACTAATATGCCATGGCCTGGTTCCGCCAGGCTCCAGGA AAGGAGCGTGAATTTGTAGCAGCTATTAGCGTGAGTGCTAATAGTGCCCCATACTATGCAAAT TCCGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTTTATCTGCAAATG AACAGCCTAAAACTGAGGACACGGCCGTTTATTACTGTGCAGCCCGGTACGGTACGAAACGA TACGTCGCCCCGGGAGTATGACTCGTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG20
147	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGCGACTCTCC TGCGCAGCCTCTGGAAGTAGCGTCAGTCTCAATGCCATGGGCTGGTCGCGCGTGCAACCAGGA AGTACGCGGACTTCGTGCGACGGATTGCTGCCGATGGTAGCACTCACTATGCAGACTCCGTG AGGGCCGGTTACCATCTCCGGGGACGCCGCCAGGAACACGGTGTATCTACAAATGGATTTCGC TGAAACCCGAAGACACGGCCGTCTATTACTGTTTTGCGTGGCTGGGTACGGACACGTAAGTGGG GCCAGGGGACCCAGGTCACCGTCTCCTCA	NG21
148	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGACACTCTCC TGTGCAGCCTCTGGAAGCATCGGCGATAACAATGCCATGGGCTGGTCCCGCACGCCTCCAGGG AAGCAGCGGAGTTGTCGCGACGTATAGATAGTGGGGGGATCACACGCTATGCAGACTCCCTG AAGGGCCGATTCACTGTCTCCAGAGACACCGGCAAGAACACGGTGTCTCTGCAAATGAACAGC CTGAAAGCTGAGGACACAGGCGTCTATTACTGTTTTGCACATGTCGGTGGTACTATCTGGGGC CAGGGGACCCAGGTCACCGTCTCCTCA	G14
149	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTTTACCCTCTGGAGGCATCTTCACTATCAATAGCATGGGCTGGTATCGGCAGGCTCCAGGG AAACAGCGGAGTTGGTCGCAAGTATCACTGGTGGTGGTAGTTCACGTTATGCAGACTCCGTG AAGGGCCGATTCATCATGTCCAGAGACAACGCCAAGAACATGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAATACAATCCCCCGGCCCGGACCCAAAGC GATCATGGGGAGTGGTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NGS1
150	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGAGGGTCTCTGAGACTCTCC TGCGCAGCCTCTAGCAGCATCTTCACTATCAATATGAGCTGGTACCGCCAGGCTCCAGGGAAC GAGCGCGAGTTGGTCGCAACTATTACACGGCATGATAGCACACACTATTCACTCCGTGAAG GGCCGATTACCATCTCCAGAGACGACGACAAGAACACGATATATCTGCAAATGAACAGCCTG AAACCTGAGGACACGGCCGTCTATTACTGTTCTGGGTTTTTTCTGGACGGTAGTACCTGGCAC	G12

	CCATATTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
151	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGTCAGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTCGTAGTAATGGCAGCACAACTATGCAGACTCCGTG AAGGGCCGATTACCATTTCCAGAGACAACGCCAAGAACACGGTCTACCTGCAAATGAACAGC CTGAAACTTGAGGACACGGCCGTCTATTATTGTAATGGATTCTTCACTTTGCCTGGGTACAGT AGTGAAGAATTTGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G6
152	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGCCTGGAGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGAGAGCATCTTCAGTATCAACGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTATGTGCGAGGCATTACTAAGGGTGGGCGTACAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACGACGCCAAGAATACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAAGACACGGCCGTCTATTACTGTAATGGTTTGTGCTCAGGCAGAGAGTGTTAT GGGGACTCCCTTTTTGCCGCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCAGGATCCGAA CAAAACTGATCAGCGAAGAAGATCTGAACCATCACCATCACCATTAGTGA	G22#
153	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGTCAGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTCGTAGTAATGGCCGCACAACTATGCAGACTCCGTG AAGGGCCGATTACCATTTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACTTGAGGACACGGCCGTCTATTACTGTTCTGGGTTTTTTCTGGACGGTAGTACCTGG CACCCATATTGGGGCCAGGGCACCCAGGTCACCGTCTCCTCA	G6-1
154	GAGGTACAGCTGGTGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTTCGATGATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGTGAGGGGGTCTCATGTATTAGTAGTAGTGATGGTAGCACACACTATGCAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAATGCCAGGAACACGGTGA CTCTGCAAATAAAC AGCCTGAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCCTGGGTGACCTATTGCCCC GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G13#

155	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTTCGATGATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGCGAGGGGGTCTCATGTATTACGAGTAGTGATGGTAGCACATACTATGCAGACTCT GTGAAGGGCCGATTACCATCTCCAGAGACAATGCCAACAACACGGTGCATCTGCAAATAAGC AACCTAAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCCTGGGTGACCTACTGCCCC GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G13-2#
156	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGACACTCTCC TGTGCAGTCTCTGGAAGCAGCTTCAGTATCAAGGCCATGGGCTGGTACCGCCTGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCAATTACTAGTGGTGGTAGCACGAACTATGCGGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAGCGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACAGCCGTCTATTACTGTAATGGTTTTTTTCGAGTATAGGGGTCTTGAA CAATTGGGCCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G31
157	GATGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGTCGGTATCCGTGCCATGACGTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGTTCTTACTAGTGCTGGTAAACCTATGTATGCCGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAACGCAGATTTCCGGGACTCCGGGTTCAGTA GTACTGGGTCCCTTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G4
158	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCCTCAGTATCGAGGCCATGGGCTGGTACCGCCAGACTCTTGGG AAGCAGCGGAATTGGTCGCAGCTATTACTAGTGGTGATAGCACAACTATGCAGACTTCGTG AAGGGCCGATTACCATCTCCAGAGACAAGGCCAAGAACATGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTTCTGTAATGCCCTAATGGTAGTTAGGGCTGGCTCG AATCCCGAAATTGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG2
159	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTTCGATGATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGTGAGGGGGTCTCATGTATTAGTAGTAGTGATGGTAGCACATACTATGCAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAATGCCAAGAACACGGTGTATCTGCAAATAAAC AGCCTGAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCCTGGGTGACCTACTGCCCC	G13-3#

	GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
160	GAGGTACAGCTGGTGAATCTGGGGGAGGATTGGTGCAGCCTGGGGGTCTCTGAGACTGTCC TGTGTAGTCTCTGCAAGGGGCGTCAGTCTCGATGCCGTGGGCTGGTCGCGGTGGCTCCAGGG AAGCAGCGCGACTTCGTGCGACGTATTGATCGAAGGGGTAGTACATACTATGCAGTGTCCGTA GAGGGCCGATCCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAACTGGACACG CTGAAACCTGAGGACACGGCCGTCTATTATTGTTTTGCATGGCAGGGTGCGGAAACACATTGG GGCCTGGGGACCCAGGTCACCGTCTCCTCA	G20
161	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGTCTCTGACCCTCTCC TGTGTAGCCTCTGGAAGCCACTTCAGTTTCAATGACATGGGCTGGTATCGCCAGGATCCGTGG AAGGGGCGCGACTTGGTCGCGGTATTACTAGTAGTCGTAACACACTTTATGTAGACTCCGTG AAGGGCCGTTTACCATCTCCAGAGACGACGCCAAGAACACGGTGTATCTACAAATGAACAAC CTGAAACCTGAGGACACAGCCGTCTATTACTGTAACCCGTACCCTTCCCCAAATAACTACTGG GGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG3
162	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCCCCTTCACGATCAATGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGAGCAATTACTCGTAGTGGTAAGACGAACATATGCAGACTCCGTG AAGGGCCGATTACCATCTCCGAGACAACGCCCTGACCACGGTGTATCTGCAAATGAACAAC CTGCAACCTGAAGACACGGCCGTCTATTACTGTAATGGGTTCTACGGGTCTGAATTTGGGCCC TGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG4
163	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTCCAGGCTGGGGGTCTGCGACGCTCTCC TGTTACAGCCCCTGGAGACACCTTAAGTAGATACGCCGTGGGCTGGTTCCGCCAGGGGCCAGGG CAGGAGCGTGATTTTGTAGCATCCATTACCTGGAGTGGTGATTACACATACTATAAAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAGTGTCAACAACATGGTGTATCTGCGAATGAAC AGCCTGAAACCTGAGGACACGGCCCTGTATTACTGTGCAGCCGATAAGAGTTCCTTTAGACTC CGAGGCCCTGGATTATATGACTACAGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG5

164	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTCGATTATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGCGAGGGGGTCTCATGTATTAGTAGTAGGGATGGTACCACCCACTATGCAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAATGCCAAGAACACGGTGTATCTGCAAATAGAC AGCCTGAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCTGGGTGACCTACTGCCCC GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG6#
165	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTCGTACAGCCTGGGGGGTCACTGAGACTCTCC TGTGCAGCCTCGGGATTCACTTTCGATTATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGCGAGTGGGTCTCTGCCATTAGTAATATTGATGATGACACATACTATGAAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGCGTATCTGCAAATGAAC AACCTGAAACCTGAGGACACGGCCGTTTATTACTGTGCAGCAGATAAGGATGTAGTGGTAGTG CGTACGGGTCTCAGCGAGTCTGACTATTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG8
166	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCTTCGGTATCAATGCCATGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAACTGGTCGCAGTTATTACCAGTGGTGGACGCACAATGTATGCAGAGTCCGTG AAGGGCCGATTGCGCATCTCCAGAGACGTCGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAAGACACAGCCGTCTATTACTGTAATGGAGACTGGGGGTCTGGAGGGTAGGGTG GACCTTGGAACCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG9
167	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGACGCTGAGACTCTCC TGTGCCGCCTCGGAAGCATTCGCAGTATCGGCGACATGGAGTGGTACCGCCAGGCTCCAGGA CAGCAGCGCGAGTTGGTCGCAAGTATTAGTGCTGGCCCTGAGATGCGTAGTGCTGGTACCCCA ACTTATGCAAAGTCCGTGGAGGGCCGATTACCATCTCCAGAGACAACATCAAGAACATGATG TGGCTGCAAATGAACAGCCTGAGACCTGAAGACACGGCCGTCTATTCTGTAATGCCGACGTT CTGACGTACTATAATGGTAGATACTCCCGAGATGTCTACTGGGGCCAGGGGACCCAGGTCACC GTCTCCTCA	NG10
168	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGCGCAGCCTCTAGCAGCATCTTCAGTATCAATATGAGCTGGTACCGCCAGGCTCCAGGGAAC GAGCGCGAGTTGGTCGCAACTATTACACGACATGATAGTACACACTATTCAGACTCCGTGAAG GGCCGATTGCGCATCTCCAGAGACGACGACAAGAACACGATATATCTGCAAATGAACAGCCTG	G12-1

	AAACCTGAGGACACGGCCGTCTATTACTGTTCTGGATTTTTCTGGACGGTAGTACCTGGCGG CCATATTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
169	GATGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGACGCACCCTCAGTGGCTATGCCGTGGCCTGGTTCCGCCAGGCTCCAGGA AAGGAGCGTGAGTTTGTAGCAGCCATTAGCTCGAGTGATAATAGTAGCCCATATTATGCAAAT GTCGTGAAGGGTCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTTTATCTGCAAATG AACAGCCTGCAAACCTGAGGACACGGCCCTTTATTACTGTGCAGCCCGGTACGGTACGAAACGG TACGTCGCCCCGGGAGTATGACTCGTGGGGTCAGGGGACCCAGGTCACCGTCTCCTCA	G17-1
170	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTAGAAGCATCTTCAGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTCGTAGTAGTGGTACCACAATGTATGCGGATTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTATTGTAACGAGATTACTCCCCGCCGGCAGCACG TACCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG11
171	GAGGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTCGATGATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGCGAGGGGGTCTCATGTATTACGAGTAGTGATGGTAGCACATACTATGCAGACTCT GTGAAGGGCCGATTACCATCTCTAGAGACAATGCCAACAACACGGTGCATCTGCAAATAAGC AACCTAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCTGGGTGAACTACTGCCCC GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCACAGGCCCAG GTACAGCTGGTGGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCCTGT ACAGCCTCTGGACGCACCTTCAGTACCTATTTTCATGGCCTGGTTCCGCCAGCCTCCAGGGAAA GAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTCCACACTATACAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAACAGC CTGAAATCTGAGGACACGGCCGTTATTTTTGTGCATCACGGGTATTGCGGATGGATCTGAC TTTGGTTCTACGGCCAGGGGACCCAGGTCACCGTCTCCTCAGGATCCGAACAAAACTGATC AGCGAAGAAGATCTGAACCATCACCATCACCATTAGTGA	G15 (bi)

172	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGGAGCATCTGGAATTATTTTATGTGCCACTACCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCACTGATTACTAGTGATTGGCACACAAAGTATGCAGACTCCGTG AAGGACCGATTCTCCATTTCCAGAGACAACGCCAAGAGCACGGTGCACCTGCAAATGAACAGC CTGAGATCTGAAGACACAGCAGTCTATTTTGTATGCCCCGCAAGCCTTCAGTGAGCCTCGT TGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G11
173	CAGGTACAGCTGGTGGATTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGATTGTCC TGTGCAGCCTCTGGAAGCAGCGGCAGAATCGATGCCATGGGCTGGTCGCGGTGGCTCCAGGG AAGCAGCGCGACTTCGTGCGACGTCTTGGCAGTAATGGATTACACAGTATGACATCTCCGTG GAGGGCCGATTACCATCTCCGGGGACGTGCGCAAGAATACGATATATCTGCAAATGGACACG CTGAAACCTGAGGACACGGCCGTCTATTACTGTTTTGCGTGGCTGGGGCAAGATACCGTGTGG GGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG12
174	CAGGTACAGCTGGTGGATTCTGGGGGAGGATTGGTAAAGGCTGGGGCATCTCTGAGACTCTCC TGTGCAGCCTCTGGAGACGCCTTATTTAACTACGCCATGGGCTGGTTTCGCCAGGGGCCAGGG AAGGAGCGTGACTTTGTAGCATCTGTTACCAGGAGTGGTGATAATACATACTATAAAGACTCC GCGAAGGGCCGATTACCATCTCCAGAGACGACGCCAAGAACACGGTATATCTGCAAATGAAC AGCCTGAAACCTGAGGACACGGCCGTTTATTTCTGTGCAGCAGATAAGAGTTCCTTTAGGCTC CGAGGCCCTGGAGTATATGACTACAGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG14
175	CAGGTACAGCTGGTGGATTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGTCTCTGGAAGCGACGGCCGAGTCATGCTCATGGGCTGGTACCGCCAGGCTCCAGGG CAGCAGCGCGACCTGGTCGCATCTATTACTAGTGCAGATTACACAACCTATGCAGAATCCGTC GAGGGCCGATTACCATCTCCACAGACAACAACAAGAACACAGTGTATCTACAAATGAACAGC CTGAAGCCTGAAGACACAGCCGTCTATTTTGTAAAGTAATTGCGGCGACGGTCTGGGGCCAG GAGACCCAGGTCAGGCAGGGTTTGACATTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG13
176	GAGGTACAGCTGGTGGAACTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAGCATCTCCAGTGCCAGATCCATGACCTGGTACCGCCAGGCTCTAGGG AAGCAGCGCGAGTTGGTCGCACTGATTATGGGTGGCGGTAGCACGATGTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTACAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTATTGTAATGCAGACTGGGGGGGAGTCGGGTTTCCG	G21

	AACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
177	GATGTGCAGCTGCAGGCGTCTGGGGGAGGATTGGTGCAAATTGGGGACTCTGTGAGACTCTCC TGTATAGCCTCTGGAGGCACCTTCAGAACTTATGCTATCGGTTGGTTCCGCCAGGCTCCAGGG GCTGAGCGTGAATTTGTAGCTGCCATTAGCCGGCGCGTAATAAGACAGATTATGCAGAGTCC GTGAAGGGCCGATTCACAGTCTCCAGAGACAACGCCGAGAATACGGTGTATTTGCAAATGAAC AGCCTGAAACCTGATGACATGGGCGTTTATTACTGTGCAGCGTCGGCGCGTAATTTTCATCGGC ACCCAGCCACTTGATTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG23
178	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTACAGGCTGGGGGCTCTCTGAGACTCTCC TGTGCAGCCTCTGGATGGAACCTTGGTAATTATGCCTTGGGCTGGTTCCGCCAGGCTCCAGGG AAGGAGCGTGAGTTTGTAGCAGCTATCGACTGGCGTCATAGTTCATACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACACCAAGAACATGGTGTATCTGCAAATGAGCAGC CTGAAACTTGAGGACACGCGCCTTTATTACTGTGCAGCATCAAGCCTATTCCTAGTAGTGCT CCCCGTCAGTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG15
179	CAGGTACAGCTGGTGGATTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTGTAGCCTCTGGACGCACCTTCAGTAATTATGCCATGGGCTGGTACCGCCGACGTCCAGGG CTGGAGCGTGAATTTGTAGCAGCTATTGTTGGGAGTGGTGATAGCACAAGGTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAAC ACGCTGAAACCTGAGGACACGGCCGTTTATTACTGTGCGTCATCCTCCGACCCGCGGGTTTAT ATAGCAAGTACTCTCGATTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG16
180	CAGGTACAGCTGGTGAATCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTGCAGCCTCTGGACGCACCTTCAGTATGTTTATCATGGGCTGGTTCCGCCAGGCTCCAGGG AAGGAGCGTGAATTAGTAGCAGCTATTAGCCGGAATAGTAATCTCACATACTATTTTCAGTCC GTGAAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAACTTGAGGACACGGCCGCTATTACTGTAATGCAGACTACGGGCCTCCAGTATCC ATTGGTCCTTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G23

181	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTGGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTAGTCTCTGGAAGGATCCTCAGTATCAAGGCCATGGGCTGGTACCGCCAGGCTCCTGGG AAGCAGCGCGAGTACGTGCGAGGTATTGTTAGCAGTGGTAATACAACTATGCAGACTTCGTG AAGGGCCGATTACCATCTCCGAGACAACGCCAAGAACACGGTGTTTCTGCAAATGAACAGC CTGAAACCTGAAGACACGGCCGTCTATTACTGTAATGCCCTAGTGGTCGTTACTAGTGCCTCG GGTCCCGAGTTGGCTTCTCTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	NG17
182	GATGTACAGCTGGTGGATTCTGGGGGAGGATTGGCGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTACAGCCTCTGGACGCACCTTCAGTACCTATTTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAGCAGCGCGAGTTGGTGCATACATTTCGTAGTGGTGGTACGACAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACATCGCCAAGAACACGGTGATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGCAATGCAGATTACTCCCCGCCCGGCAGCCGG TTCCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	G1-3
183	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGCGCAGCCTCTGGATTACCTTGGATAATTATGCCATAGCGTGGTTCCGCCAGGCCCCAGGG AGGGAGCGCGAGTGGGTCTCATCACTGGTAGTGATGGTAACCTATATACACCGTCCGTGAGG GGCCGATTACCATTTCCAGAGACAACGCCAAGAACACGGTGATCTGCAAATGAACAGCCTG AAACCTGAGGACACGGCCGTTTATTATTGTGTAGCAGGGAAGAGACCGGTAATTACTACATGG ATTGCTTTGGACGCATGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	NG18
184	GATGTACAGCTGGTGGATTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAACATTCTCCAGTATCGATTCCATGCGCTGGTTCCGGCGGGCTCCAGGA AAGGAGCGGAATTTGTGCGACATATTACTAGCACGGGTAGGACAACTATGCAGACGCCGTG AAGGGCCGATTTACCATCTCTAGAGACAACGCCAAGAACACGATGTGGCTGCAAATGGACAAC CTGAAACCTGACGACACGGCCGTCTATTATTGCAATATGGTGACGACTCCTTATATGCACTGG GGCCAGGGGACCCAGGTACCGTCTCCTCA	NG24
185	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAAACTCTCC TGTGTAGCCTCTGGAAGCCGCTTCAGTGAAAATGCCATGGGCTGGTATCACCAGGCTCCAGAC AAACAGCGCACCTTGGTGCAGCTATTACTAGTAGTCGTAGCACTCTTTATATAGACTCCGTG AAGGGCCGCTTACCATCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAGCAAC CTGAAACCTGAGGACACGGCGTCTATTACTGTAACCCGTACCCCTCCCAAATTCCTACTGG	NG25

	GGCCAGGGGACCCAGGTCACCGTCTCCTCA	
186	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTAAGACTCTCC TGTGCAGCCTCTGGACTCGTCTTCAGTGCCAATAAGATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCACGTATTAGCACTGACGGACGTACAACTATGCGGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCGAGAAGACGGTGTTTCTGCAAATGAACAGC CTGAATCCTGACGACACGGCCGTCTATTACTGTAATGCAAACCTGGCTCGATAAATATGACTAC TGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG19
187	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGGAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTGGCCTCTGGAAGCATCTCCAGTGCCAGATCCATGACCTGGTACCGCCAGGCTCACGGG AAGCAGCGCGAGTTGGTCGCAGTTATTACTAGTGGCGGTAGCACAATGTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAGCGCCAAGAACACGGTGTATCTACAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTATTGTAATGCAGACTGGGGGGAAGTCGGGTTTGTG AACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG26 (G 21-1)
188	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCTTCGGTTTCAATGGCGTGGCTGGTTCCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGTTATTCGTAGTGGTGGTAACACGCTCTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAATGTAGATTACTCCCCGCCCGGTAGTCTG GTTCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G18
189	GAGGTACAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGCCAGTATCAATGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCTATTACTAGAGGTGGTAGCACAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAACAGC CTGAAACCGGAGGACACGGCCGTCTATTCATGTAATGGTTTGTGCTCAGACGATCGGTGTTAT GGGGACTCCCTTTTGGCCCCCTGGGGCCCGGGGACCCAGGTCACCGTCTCCTCA	G16#

190	GAGGTACAGCTGGTGAATCTGGGGGAGGATTGGTGCAGCCTGGGGGGTCTCTGAGACTGTCC TGTCTAGTCTCTGGAAGGGGCGTCAGTCTCGATGCCGTGGGCTGGTCGCGGTGGCTCCAGGG AAGCAGCGCGACTTCGTGCGACGTATTGATAGTAGGGGTAGCGCATACTATGCAGACTCCGTA GAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAGTGGACACG CTGAAACCTGAGGACACGGCCGTCTATTATTGTTTTGCGTACTACGGGGCTCAAATATCTTTT GGTCCGTGGGGCCAGGGGACCCAGGTACCGTCTCTTCA	G24
191	GATGTGCAGCTGCAGGCGTCTGGGGGAGGATTGGTGCAGCCTGGGGGGTCTCTGAGACTGTCC TGTGTAGTCTCTGGAAGGGGCGTCAATCTCGATGCCATGGGCTGGTCGCGGTGGCTCCAGGG AAGCAGCGCGACTTCGTGCGACATATTGATGATAGGGGTACCGCATACTATGCAGACTTCGTA AAGGGCCGATCCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAGTGGACACG CTGAAACCTGAGGACACGGCCGTCTATTATTGCTTTGCGTGGCAGGGTGCGGAAACATATTGG GGCCTGGGGACCCGGGTACCGTCTCCTCAGGATCCGAACCAAACTGATCAACGAAGAACAT CTGAACCATCACCATCACCATTATTGA	G19
192	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAGCATCTTCAGTGTCAATGCCGTGGCCTGGTACCGCCAGGCTCCAGGG AAACAGCGCGAGTTGGTCGCATATATACGTGCTAGTGGTAGCACAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAATGCCAAGAACACGCTGTATCTGCAAATGAACAGC CTGAAACCTGACGACACAGCCGTGTATTATTGTAATGCGGGGCGGACAGGGGATCGTTTTAAT TTGGTGGCGTATTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	G5-1
193	GAGGTACAGCTGGTGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTACAGCCTCTGGACGCACCTTCAGTACCTATTTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAAGAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTCCACACTATGCAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAACCTGAGGACACGGCCGTCTATTACTGCAATGCAGATTACTCCCCGCCCGGCAGC CGGTTCCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA	G26
194	GAGGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAGCATCTTCAGTATCAAAGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATACATTGCTAGTGGTGGTACGACAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACATCGCCAAGAACACGGTGTATCTGCAAATGAACAGC	G27

	CTGAAATCTGAGGACACGGCCGTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCTGCT TTGGTTCCTACGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
195	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGGCTGGGGCTCCGTGAGACTCTCC TGTGCAGCCTCTGGACGCGCCAACAGTTTGTATGCCATGGGCTGGTTCCGCCAGGCTCCAGGG AAGCAGCGCAGTTGGTCGCATACATTTCGTAGTGGTGGTACGACAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACATCGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGCAATGCAGATTACTCCCCGCCCGGCAGCCGG TTCCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G1-2
196	GAGGTACAGCTGGTGAATCTGGGGGAGGATTGGTGCAAATTGGGGACTCTGTGAGACTCTCC TGTATAGCCTCTGGAGGCACCTTCAGAACTTATGCTATGGGTTGGTTCCGCCAGGCTCCAGGG GCTGAGCGTGAATTTGTAGCTGCCATTAGCCGGCGCGTAATAAGACAGATTATGCAGAGTCC GTGAAGGGCCGATTACAGTCTCCAGAGACAACGCCGAGAATACGGTGTATTTGCAAATGAAC AGCCTGAAACCTGATGACATGGGCGTTTATTACTGTGCAGCGTCGGCGCGTAATTCATCGGC ACCCAGCCACTTGATTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G28
197	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGAATGGTGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTGTAGCCTCTGGACGCTCCTTCGTTGGCTATTTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAAGAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTTCACACTATGCAGACTCC GTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAATCTGAGGACACGGCCGTTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCT GACTTTGGTTCCTACGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G29
198	GAGGTACAGCTGGTGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCTTCAGTATCAATGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCAATTGGTCGCAGCTATTACTAAAAGTAATAACATAAACTATGCAGACTCCGTG AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTATTGTAATGGATTCTTCACTTTGCCTGGGTACAGT AGTGAAGAATTTGGTCCCTGGGGCTGGGGACCCAGGTCACCGTCTCCTCA	G2-1

CLAIMS

1. An anti-BCMA single domain antibody, said single domain antibody is composed of framework regions and complementary determining regions, said complementary determining regions comprise the amino acid sequences of CDR1 consisting of TYFMA, CDR2 consisting of
5 GGIRWSDGVOHYADSVKG, and CDR3 consisting of CASRGIADGSDFGS.
2. An anti-BCMA single domain antibody, wherein said single domain antibody comprises the amino acid sequences of CDR1, CDR2, and CDR3 as set forth in SEQ ID NO: 67.
3. The anti-BCMA single domain antibody according to claim 1, wherein said single domain antibody has an amino acid sequence as set forth in SEQ ID NO: 67.
- 10 4. A gene of an anti-BCMA single domain antibody, wherein said gene of single domain antibody has a nucleotide sequence as set forth in SEQ ID NO: 133.
5. A polypeptide, having one or multiple single domain antibodies according to any one of claims 1-3.
6. An expression vector, comprising the gene in claim 4, wherein said expression vector is
15 preferably a prokaryotic cell expression vector, a eukaryotic cell expression vector or other cell expression vectors, more preferably, a lentiviral vector.
7. A host cell, comprising said expression vector according to claim 6, wherein said host cell is preferably a prokaryotic expression cell, a eukaryotic expression cell, a fungus cell or a yeast cell, and said prokaryotic expression cell is preferably *Escherichia coli*.
- 20 8. A chimeric antigen receptor, having one or multiple single domain antibodies according to any one of claims 1-3.
9. A T cell, wherein said T cell expresses said chimeric antigen receptor according to claim 8.
10. A pharmaceutical composition, comprising one or more single domain antibodies
25 according to any one of claims 1-3 as active ingredients.
11. The single domain antibody according to any one of claims 1-3, wherein said single domain antibody is humanized.
12. An *in vitro* use of said single domain antibody according to any one of claims 1-3 in detection of BCMA.
- 30 13. An *in vitro* use of said single domain antibody according to any one of claims 1-3 for blocking an interaction between BAFF and BCMA.

14. The use according to claim 13, wherein said single domain antibody is linked to one or more of a cytotoxicity agent, an enzyme phase, a radioisotope, a fluorescent compound or a chemiluminescent compound.

5 15. Use of said single domain antibody according to any one of claims 1-3 in the manufacture of a medicament for treating a disease associated with high level of BCMA expression.

16. The use according to claim 15, wherein said disease associated with high level of BCMA expression is a multiple myeloma.