ANTI-BCMA SINGLE DOMAIN ANTIBODIES AND APPLICATION THEREOF

TECHNICAL FIELD

[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

[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 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 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.

[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 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.

[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:

[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).

[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 comprising the amino acid sequences of CDR1, CDR2, and CDR3 as set forth in SEQ ID NO: 1.

[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).

[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 are genes of anti-BCMA single domain antibodies, said genes having 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.

[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 one or more genes selected from said group of genes of single domain antibodies 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.

[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.

[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.

[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.

[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.

[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.

[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)$_1$ 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. Accordingly, the light chain and the heavy chain can be divided to a variable region 00 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 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 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, 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, 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 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 terminor) 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 ºC 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 -80ºC 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 rmp 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 rmp 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 rmp. 0.5ml of buffer RPE was added, and the mixture was centrifuged at 14000 rmp 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 SfI, 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 32ºC, 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 32ºC. The culture was washed with 2YT culture medium, and 15% glycerol was added. The mixture was stored at -80 ºC.

[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 -80ºC for subsequent use.

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

[0084] Screening of BCMA-specific single domain antibody

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

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

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

[0088] Blocking: 1% CPBS, 300μl /well, 37ºC, 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	2.4×10^4/μl
Third round	5.32×10^{11}	150 μl/well x 5+ 350 μl	1/4=750 about 3000	3×10^4/μl

[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(lmol/1)

[0097] 3'primer 1μl(lmol/1)

[0098]

DEPC-treated ddH_2O	22μl

Total volume			50 μl	
PCR Reaction Conditions:				
94℃	3min			
94℃	30s	↕	30s	30 rounds
	55℃			
72℃	1min			

[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 ℃ overnight. A single colony was picked and inoculated in 15 ml LB medium solution containing ampicillin, and was cultured in a shaker at 37 ℃ overnight. 10 ml of culture was transferred to 1 L of 2YT culture solution containing ampicillin and cultured in a shaker at 37 ℃ 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-F_C antigen was diluted to 1 μg/ml with 0.05 M $NaHCO_3$ (pH 9.5). A 96-well place was coated with 100μl antigen overnight at 4 ℃. The 96-well plate was blocked with 300μl 0.5% BSA-PBS for 2 h at 37 ℃. The purified BCMA single domain antibodies with different dilution concentrations were added in 100μl/well at 37 ℃ 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 ℃ 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 lμg/ml, 100μl/well and incubated overnight at 4℃. 2% BSA was added for blocking, 300μl/well, incubated at 37 ℃ 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 ℃. TMB chromogenic solution was added, 100μl/well, and reacted in dark for 10 min. The reaction was quenched by adding 2M H_2SO_4 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

[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% CO_2 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 4ºC 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 ºC.

[00119] 3) Preparation of CART cells

[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 10^6 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

[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 10^4/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 ºC. 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:

Sequence	CDR1	CDR2	CDR3	Clone group
1	TYFMA	GGIRWSDGVPHYADSVKG	CASRGIADGSDFGS	G3
2	IKAMA	AYIRSGGTTNYADSVKG	CNADYSPPGSRFPDLGP	G1
3	ANTM	ARISTDGRTNYADSVKG	CNANWLSKFDY	NG7
4	VNAVA	AYIRRSGSTNYADSVKG	CNADFGSDYVVLGS	G5
5	IKALA	AYITSGGNTNYADSVRG	CNADFGEGTIISLGP	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	CFAWLGTDTY	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	AAITSGDSTNYADFVKG	CNALMVVRAGSNPEIGP	NG2
27	DYAIG	SCISSSDGSTYYADSVKG	CATPWVTYCPENLLFSY	G13-3#
28	LDAVG	ARIDRRGSTYYAVSVEG	CFAWQGAETH	G20
29	FNDMG	AAITSSRNTLYVDSVKG	CNPYPSPNNY	NG3
30	INAMG	AAITRSGKTNYADSVKG	CNGFYGSEFGP	NG4
31	RYAVG	ASITWSGDYTYYKDSVKG	CAADKSSFRLRGPGLYDY	NG5
32	YYAIG	SCISSRDGTTHYADSVKG	CATPWVTYCPENLLFSY	++
33	YYAIG	SAISNIDDDTYYEDSVKG	CAADKDVVVVRTGLSESDY	NG8
34	INAMA	AVITSGGRTMYAESVKG	CNGDWGSEGRVDLGP	NG9
35	IGDME	ASISAGPEMRSAGTPTYAKSVEG	CNADVLTYYNGRYSRDVY	NG10
36	INMS	ATITRHDSTHYSDSVKG	CSGFFLDGSTWRPY	G12-1
37	GYAVA	AAISSSDNSSPYYANVVKG	CAARYGTKRYVAREYDS	
38	INAMA	AYIRSSGTTMYADSVKG	CNGDYSPPGSTYPDLGP	NG11
39	DYAIG/YCPENLLFSY	SCITSSDGSTYYADSVKG/AAIRWSDGVPHYTDSVKG	CATPWVN/CASRGIADGSDFGSY	G15(bi)
40	ATTMA	ALITSDWHTKYADSVKD	CYARQAFSEPR	G11
41	IDAMG	ARLGSNGFTQYDISVEG	CFAWLGQDTV	NG12
42	NYAMG	ASVTRSGDNTYYKDSAKG	CAADKSSFRLRGPGVYDY	NG14
43	VMLMG	ASITSADYTTYAESVEG	CKVIAATVWGQETQVRQGLF	NG13
44	ARSMT	AVIMGGGSTMYADSVKG	CNADWGEVGFPNLGP	G21
45	TYAIG	AAISRRGNKTDYAESVKG	CAASARNFIGTQPLDYDY	NG23
46	NYALG	AAIDWRHSSYYADSVKG	CAASSLFPSSAPRQYDY	NG15
47	NYAMG	AAIVGSGDSTRYADSVKG	CASSSDPRVYIASTLDY	NG16
48	MFIMG	AAISRNSNLTYYFQSVKG	CNADYGPPVSIGP	G23
49	IKAMG	AGIVSSGNTNYADFVKG	CNALVVVTSASGPELAS	NG17
50	TYFMA	CNADYSPPGSRFPDLGP	AGIVSSGNTNYADFVKG	G1-3

51	NYAIA	SSTGSDGNLYTPSVRG	CVAGKRPVITTWIALDA	NG18
52	IDSMR	AHITSTGRTNYADAVKG	CNMVTTPYMH	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	CNGLCSDDRCYGDSLFAP	G16
58	LDAVG	ARIDSRGSAYYADSVEG	CFAYYGAQISFGP	G24
59	LDAMG	AHIDDDRGTAYYADFVKG	CFAWQGAETY	G19
60	VNAVA	AYIRRSGSTNYADSVKG	CNAGRTGDRFNLVAY	G5-1
61	TYFMA	GGIRWSDGVPHYADSVKG	CNADYSPPGSRFPDLGP	G26
62	IKAMA	AYIRSGGTNYADSVKG	CASRGIADGSDFGS	G27
63	LYAMG	AYIRSGGTTNYADSVKG	CNADYSPPGSRFPDLGP	G1-2
64	TYAMG	AAISRRGNKTDYAESVKG	CAASARNFIGTQPLDYDY	G28
65	GYFMA	GGIRWSDGVPHYADSK	CASRGIADGSDFGS	G29
66	INAMG	AAITKSNNINYADSBKG	CNGFFTLPGYSSEEFGP	G2-1

[00123] Annex 2

Sequence	Amino acid sequence	Clone group
67	EVQLQASGGGLAQAGGSLRLSCTASGRTFSTYFMAWFRQPPGKEREYVGGIRWSDGVPHYADS VKGRFTISRDNAKNTVYLQMNSLKSEDTAVYFCASRGIADGSDFGSYGQGTQVTVSS	G3
68	QVKLEESGGGLVQPGGSLRLSCAASGSIFSIKAMAWYRQAPGKQRELVAYIRSGGTTNYADSV KGRFTISRDIAKNTVYLQMNSLKPEDTAVYYCNADYSPPGSRFPDLGPWGQGTQVTVSS	G1
69	QVKLEESGGGLAQPGGSLRLSCAASGLVFSANTMAWYRRAPGKQRELVARISTDGRTNYADSV KGRFTISRDNREKTVFLQMNRLNPDDTAVYYCNANWLSKFDYWGQGTQVTVSS	NG7
70	DVQLQASGGGLVQAGGSLRLSCVASGSIFSVNAVAWYRQAPGKQRELVAYIRRSGSTNYADSV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNADFGSDYVVLGSWGQGTQVTVSS	G5
71	QVKLEESGGGLVQAGGSLRLSCAASGSIFSIKALAWYRQAPGKQRELVAYITSGGNTNYADSV RGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNADFGEGTIISLGPWGQGTQVTVSS	G9
72	EVQLVESGGGLVQPGGSLRLSCAASGSEFSINAMAWYRQAPGKQRELVAALTSGGNTHYADSV KGRFTISRDNAKNTWYLQMNSLKPEDTAVYYCNADFGTAGLVVLGPWGQGTQVTVSS	G7
73	EVQLQASGGGLVQPGGSLRLSCAASGSIFSINAMAWYRQAPGKQRELVAYIRSNGRTNYADSV KGRFTISRDNAKNTVYLQMNSLKLEDTAVYYCNADYGPPVSIGPWGQGTQVTVSS	G6-2
74	EVQLVESGGGLVQAGGSLRLSCVVSGSLLSIKAMAWFRQPPGKQRELVAAVTSGGSTHYLDSV KGRFTISRDNANTVHLQMNSLKPEDTAVYYCNADFGTDYVDLGPWGQGTQVTVSS	G10
75	DVQLQASGGGLVQPGGSLRLSCAVSGSIFSINAMGWYRQAPGKQRELVAAITKSNNINYADSV KGRFTISTDNAKNTVYLQMNSLKPEDTAVYYCNGFFALPGYSSEEFGPWGQGTQVTVSS	G2
76	EVQLVESGGGLVQPGGSLRLSCVASGNIFDMNRMGWYRQPPGKQRELVADIRDGGSTIYSDSV KGRFTISRDNAKNTLYLQMNSLKPDDTAVYYCNAGRTGDRFNLVAYWGQGTQVTVSS	G8
77	DVQLQASGGGLVQHGGSLRLSCEASGRTFSGYAMAWFRQAPGKEHEFVAAISSSSNSAPYYAN SVKGRFTISRDNAKNTVYLQMNNLQTEDTAVYYCAARYGTKRYVAREYDSWGQGTQVTVSS	G17
78	DVQLQASGGGVVQAGGSLRLSCTASGSIRSINGMGWSRVAPGKQRDFVARIDSRGSAYYADSV EGRFTISRDNAKNTVYLQVDTLKPEDTAVYYCFAWQGAETYWGLGTQVTVSS	G25
79	QVKLEESGGGLVQPGGSLRLSCAASGSIGDTYAMAWYRQAPGKQRDLVAYITNGGSTDYAASV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNGATRGAQLVFDWGQGTQVTVSS	NG1

80	QVKLEESGGGLVQHGGSLRLSCAASGGTFSNYAMAWFRQAPGKEREFVAAISVSANSAPYYAN SVKGRFTISRDNAKNTVYLQMNSLKTEDTAVYYCAARYGTKRYVAREYDSWGQGTQVTVSS	NG20
81	QVKLEESGGGLVQPGGSLRLSCAASGSSVSLNAMGWSRVQPGSTRDFVARIAADGSTHYADSV EGRFTISGDAARNTVYLQMDSLKPEDTAVYYCFAWLGTDTYWGQGTQVTVSS	NG21
82	DVQLQASGGGLVQAGGSLTLSCAASGSIGDNNAMGWSRTPPGKQREFVARIDSGGITRYADSL KGRFTVSRDTGKNTVSLQMNSLKAEDTGVYYCFAHVGGTIWGQGTQVTVSS	G14
83	QVQLVESGGGLVQPGGSLRLSCLPSGGIFTINSMGWYRQAPGKQRELVASITGGGSSRYADSV KGRFIMSRDNAKNMVYLQMNSLKPEDTAVYYCNTIPPARTQSDHGEWYDYWGQGTQVTVSS	NGS1
84	QVKLEESGGGLVQAGGSLRLSCAASSSIFSINMSWYRQAPGNERELVATITRHDSTHYSDSVK GRFTISRDDDKNTIYLQMNSLKPEDTAVYYCSGFFLDGSTWHPYWGQGTQVTVSS	G12
85	EVQLVESGGGLVQPGGSLRLSCAASGSIVSINAMAWYRQAPGKQRELVAYIRSNGSTNYADSV KGRFTISRDNAKNTVYLQMNSLKLEDTAVYYCNGFFTLPGYSSEEFGPWGQGTQVTVSS	G6
86	EVQLVESGGGLVQPGGSLRLSCAASESIFSINAMGWYRQAPGKQREYVAGITKGGRTNYADSV KGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCNGLCSGRECYGDSLFAAWGQGTQVTVSS	G22#
87	EVQLVESGGGLVQPGGSLRLSCAASGSIVSINAMAWYRQAPGKQRELVAYIRSNGRTNYADSV KGRFTISRDNAKNTVYLQMNSLKLEDTAVYYCSGFFLDGSTWHPYWGQGTQVTVSS	G6-1
88	EVQLVESGGGLAQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSCISSSDGSTHYADS VKGRFTISRDNARNTVTLQINSLKPEDTAVYYCATPWVTYCPENLLFSYWGQGTQVTVSS	G13#
89	QVKLEESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSCITSSDGSTYYADS VKGRFTISRDNANNTVHLQISNLKPEDTAVYYCATPWVTYCPENLLFSYWGQGTQVTVSS	G13-#
90	EVQLVESGGGLVQAGGSLTLSCAVSGSSFSIKAMGWYRLAPGKQRELVAAITSGGSTNYADSV KGRFTISRDSAKNTVYLQMNSLKPEDTAVYYCNGFFEYRGLEQLGPWGQGTQVTVSS	G31
91	DVQLQASGGGLVQPGGSLRLSCAASGSIVGIRAMTWYRQAPGKQRELVAVLTSAGKPMYADSV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNADFGTPGSVVLGPWGQGTQVTVSS	G4
92	QVKLEESGGGLVQPGGSLRLSCAASGSILSIEAMGWYRQTLGKQRELVAAITSGDSTNYADFV KGRFTISRDKAKNMVYLQMNSLKPEDTAVYFCNALMVVRAGSNPEIGPWGQGTQVTVSS	NG2
93	QVKLEESGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSCISSSDGSTYYADS VKGRFTISRDNANNTVHLQISNLKPEDTAVYYCATPWVTYCPENLLFSYWGQGTQVTVSS	G13-#
94	EVQLVESGGGLVQPGGSLRLSCVVSARGVSLDAVGWSRVAPGKQRDFVARIDRRGSTYYAVSV	G20

	EGRSTISRDNAKNTVYLQLDTLKPEDTAVYYCFAWQGAETHWGLGTQVTVSS	
95	QVKLEESGGGLVQAGGSLTLSCVASGSHFSFNDMGWYRQDPWKGRDLVAAITSSRNTLYVDSV KGRFTISRDDAKNTVYLQMNNLKPEDTAVYYCNPYPSPNNYWGQGTQVTVSS	NG3
96	QVKLEESGGGLVQPGGSLRLSCAASGSPFTINAMGWYRQAPGKQRELVAAITRSGKTNYADSV KGRFTISGDNALTTVYLQMNNLQPEDTAVYYCNGFYGSEFGPWGQGTQVTVSS	NG4
97	QVKLEESGGGLVQAGGSATLSCSAPGDTLSRYAVGWFRQGPGQERDFVASITWSGDYTYYKDS VKGRFTISRDSVNNMVYLRMNSLKPEDTALYYCAADKSSFRLRGPGLYDYRGQGTQVTVSS	NG5
98	QVKLEESGGGLVQPGGSLRLSCAASGFTFDYYAIGWFRQAPGKEREGVSCISSRDGTTHYADS VKGRFTISRDNAKNTVYLQIDSLKPEDTAVYYCATPWVTYCPENLLFSYWGQGTQVTVSS	NG6#
99	QVKLEESGGGFVQPGGSLRLSCAASGFSLHYYAIGWFRQAPGKEREWVSAISNIDDDTYYEDS VKGRFTISRDNAKNTAYLQMNNLKPEDTAVYYCAADKDVVVVRTGLSESDYWGQGTQVTVSS	NG8
100	QVKLEESGGGLVQAGGSLRLSCAASGSIFGINAMAWYRQAPGKQRELVAVITSGGRTMYAESV KGRFAISRDVAKNTVYLQMNSLKPEDTAVYYCNGDWGSEGRVDLGPWGQGTQVTVSS	NG9
101	QVKLEESGGGLVQPGGTLRLSCAASGSIRSIGDMEWYRQAPGQQRELVASISAGPEMRSAGTP TYAKSVEGRFTISRDNIKNMMWLQMNSLRPEDTAVYSCNADVLTYYNGRYSRDVYWGQGTQVT VSS	NG10
102	QVKLEESGGGLVQAGGSLRLSCAASSSIFSINMSWYRQAPGNERELVATITRHDSTHYSDSVK GRFAISRDDDKNTIYLQMNSLKPEDTAVYYCSGFFLDGSTWRPYWGQGTQVTVSS	G12-1
103	DVQLQASGGGLVQPGGSLRLSCAASGRTLSGYAVAWFRQAPGKEREFVAAISSSDNSSPYYAN VVKGRFTISRDNAKNTVYLQMNSLQTEDTALYYCAARYGTKRYVAREYDSWGQGTQVTVSS	G17-1
104	QVKLEESGGGLVQPGGSLRLSCAASRSIFSINAMAWYRQAPGKQRELVAYIRSSGTTMYADSV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNGDYSPPGSTYPDLGPWGQGTQVTVSS	NG11
105	EVQLQASGGGLVQPGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSCITSSDGSTYYADS VKGRFTISRDNANNTVHLQISNLKPEDTAVYYCATPWVNYCPENLLFSYWGQGTQVTVSSQAQ VQLVESGGGLAQAGGSLRLSCTASGRTFSTYFMA WFRQPPGKEREYVGGIRWSDGVPHYTDSVKGRFTISRDNAKNTVYLQMNSLKSEDTAVYFCAS RGIADGSDFGSYGQGTQVTVSS	G15(bi)
106	QVKLEESGGGLVQAGGSLRLSCGASGIIFSATTMAWYRQAPGKQRELVALITSDWHTKYADSV KDRFSISRDNAKSTVHLQMNSLRSEDTAVYFCYARQAFSEPRWGQGTQVTVSS	G11

107	QVQLVDSGGGLVQPGGSLRLSCAASGSSGRIDAMGWSRVAPGKQRDFVARLGSNGFTQYDISV EGRFTISGDVAKNTIYLQMDTLKPEDTAVYYCFAWLGQDTVWGQGTQVTVSS	NG12
108	QVQLVDSGGGLVKAGASLRLSCAASGDALFNYAMGWFRQGPGKERDFVASVTRSGDNTYYKDS AKGRFTISRDDAKNTVYLQMNSLKPEDTAVYFCAADKSSFRLRGPGVYDYRGQGTQVTVSS	NG14
109	DVQLVDSGGGLVQAGGSLRLSCAVSGSDGRVMLMGWYRQAPGQQRDLVASITSADYTTYAESV EGRFTISTDNNKNTVYLQMNSLKPEDTAVYFCKVIAATVWGQETQVRQGLTFWGQGTQVTVSS	NG13
110	EVQLVESGGGLVQPGGSLRLSCVASGSISSARSMTWYRQALGKQRELVAVIMGGGSTMYADSV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNADWGGVGFPNLGPWGQGTQVTVSS	G21
111	DVQLQASGGGLVQIGDSVRLSCIASGGTFRTYAIGWFRQAPGAEREFVAAISRRGNKDYAESV KGRFTVSRDNAENTVYLQMNSLKPDDMGVYYCAASARNFIGTQPLDYDYWGQGTQVTVSS	NG23
112	QVKLEESGGGLVQAGGSLRLSCAASGWNLGNYALGWFRQAPGKEREFVAAIDWRHSSYYADSV KGRFTISRDNTKNMVYLQMSSLKLEDTRLYYCAASSLFPSSAPRQYDYWGQGTQVTVSS	NG15
113	DVQLVDSGGGLVQAGGSLRLSCVASGRTFSNYAMGWYRRRPGLEREFVAAIVGSGDSTRYADS VKGRFTISRDNAKNTVYLQMNTLKPEDTAVYYCASSSDPRVYIASTLDYWGQGTQVTVSS	NG16
114	QVQLVESGGGLVQAGGSLRLSCAASGRTFSMFIMGWFRQAPGKERELVAAISRNSNLTYYFQS VKGRFTISRDNAKNTVYLQMNSLKLEDTAVYYCNADYGPPVSIGPWGQGTQVTVSS	G23
115	QVKLEESGGGWVQPGGSLRLSCVVSGRILSIKAMGWYRQAPGKQREYVAGIVSSGNTNYADFV KGRFTISGDNAKNTVFLQMNSLKPEDTAVYYCNALVVVTSASGPELASWGQGTQVTVSS	NG17
116	DVQLVDSGGGLAQAGGSLRLSCTASGRTFSTYFMAWFRQPPGKQRELVAYIRSGGTTNYADSV KGRFTISRDIAKNTVYLQMNSLKPEDTAVYYCNADYSPPGSRFPDLGPWGQGTQVTVSS	G1-3
117	QVKLEESGGGLVQPGGSLTLSCAASGFTLDNYAIAWFRQAPGREREWVSSTGSDGNLYTPSVR GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCVAGKRPVITTWIALDAWGQGTQVTVSS	NG18
118	DVQLVDSGGGLVQAGGSLRLSCAASGTFSSIDSMRWFRRAPGKEREFVAHITSTGRTNYADAV KGRFTISRDNAKNTMWLQMDNLKPDDTAVYYCNMVTTPYMHWGQGTQVTVSS	NG24
119	QVKLEESGGGLVQAGGSLKLSCVASGSRFSENAMGWYHQAPDKQRTLVAAITSSRSTLYIDSV KGRFTISRDNAKNTVYLQMSNLKPEDTGVYYCNPYPSPNSYWGQGTQVTVSS	NG25
120	QVKLEESGGGLVQPGGSLRLSCAASGLVFSANKMGWYRQAPGKQRELVARISTDGRTNYADSV KGRFTISRDNAEKTVFLQMNSLNPDDTAVYYCNANWLDKYDYWGQGTQVTVSS	NG19
121	QVKLEESGGGLVEPGGSLRLSCVASGSISSARSMTWYRQAHGKQRELVAVITSGGSTMYADSV	NG26(

	KGRFTISRDSAKNTVYLQMNSLKPEDTAVYYCNADWGEVGFVNLGPWGQGTQVTVSS	G21-1)
122	EVQLVESGGGLVQPGGSLRLSCAASGSIFGFNGVAWFRQAPGKGRELVAVIRSGGNTYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNVDYSPPGSLVPDLGPWGQGTQVTVSS	G18
123	EVQLEESGGGLVQPGGSLRLSCAASGSIASINAMGWYRQAPGKQRELVAAITRGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYSCNGLCSDDRCYGDSLFAPWGPGTQVTVSS	G16#
124	EVQLVESGGGLVQPGGSLRLSCLVSGRGVSLDAVGWSRVAPGKQRDFVARIDSRGSAYYADSVEGRFTISRDNAKNTVYLQVDTLKPEDTAVYYCFAYYGAQISFGPWGQGTQVTVSS	G24
125	DVQLQASGGGLVQPGGSLRLSCVVSGRGVNLDAMGWSRVAPGKQRDFVAHIDDRGTAYYADFVKGRSTISRDNAKNTVYLQVDTLKPEDTAVYYCFAWQGAETYWGLGTRVTVSS	G19
126	EVQLVESGGGLVQAGGSLRLSCVASGSIFSVNAVAWYRQAPGKQRELVAYIRRSGSTNYADSVKGRFTISRDNAKNTLYLQMNSLKPDDTAVYYCNAGRTGDRFNLVAYWGQGTQVTVSS	G5-1
127	EVQLVESGGGLAQAGGSLRLSCTASGRTFSTYFMAWFRQPPGKEREYVGGIRWSDGVPHYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNADYSPPGSRFPDLGPWGQGTQVTVSS	G26
128	EVQLQASGGGLVQPGGSLRLSCVASGSIFSIKAMAWYRQAPGKQRELVAYIRSGGTTNYADSVKGRFTISRDIAKNTVYLQMNSLKSEDTAVYFCASRGIADGSDFGSYGQGTQVTVSS	G27
129	EVQLVESGGGLVQAGASVRLSCAASGRANSLYAMGWFRQAPGKQRELVAYIRSGGTTNYADSVKGRFTISRDIAKNTVYLQMNSLKPEDTAVYYCNADYSPPGSRFPDLGPWGQGTQVTVSS	G1-2
130	EVQLVESGGGLVQIGDSVRLSCIASGGTFRTYAMGWFRQAPGAEREFVAAISRRGNKTDYAESVKGRFTVSRDNAENTVYLQMNSLKPDDMGVYYCAASARNFIGTQPLDYDYWGQGTQVTVSS	G28
131	QVKLEESGGGMVQAGGSLRLSCVASGRSFVGYFMAWFRQPPGKEREYVGGIRWSDGVPHYADSVKGRFTISRDNAKNTVYLQMNSLKSEDTAVYFCASRGIADGSDFGSYGQGTQVTVSS	G29
132	QVKLVESGGGLVQPGGSLRLSCAASGSIFSINAMGWYRQAPGKQRELVAAITKSNNINYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNGFFTLPGYSSEEFGPWGQGTQVTVSS	G2-1

[00124] Annex 3

Sequence	Nucleotide acid sequence	Clone group
133	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTACAGCCTCTGGACGCACCTTCAGTACCTATTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAAGAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTTCCACACTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAATCTGAGGACACGGCCGTTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCT GACTTTGGTTCCTACGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G3
134	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTACAGCCTCTGGACGCACCTTCAGTACCTATTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAAGAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTTCCACACTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAATCTGAGGACACGGCCGTTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCT GACTTTGGTTCCTACGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G1
135	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTAAGACTCTCC TGTGCAGCCTCTGGACTCGTCTTCAGTGCCAATACCATGGCCTGGTACCGCCGGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCACGTATTAGCACTGACGGACGTACAAACTACGCGGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACCGCGAGAAGACGGTGTTTCTGCAAATGAACAGG CTGAACCCTGACGACACGGCCGTCTATTACTGTAATGCAAACTGGCTCAGTAAATTTGACTAC TGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG7
136	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAGCATCTTCAGTGTCAATGCCGTGGCCTGGTACCGCCAGGCTCCAGGG AAACAGCGCGAGTTGGTCGCATATATACGTCGTAGTGGTAGCACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACAGCCGTCTATTACTGTAATGCAGATTTCGGTAGCGACTATGTCGTC CTCGGTTCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G5

137	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCTTCAGTATCAAAGCCTTGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTACTAGTGGTGGTAACACAAACTATGCAGACTCCGTG AGGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAACAGC CTGAAACCTGAGGACACAGCCGTCTATTACTGTAATGCAGATTTCGGAGAAGGGACTATCATA TCCCTTGGACCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G9
138	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCGAATTCAGTATCAATGCCATGGCGTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCACTTACTAGTGGTGGTAACACTCACTATGCGGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGTGGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAATGCAGATTTCGGAACTGCGGGTTTGGTA GTGCTGGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G7
139	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGTCAGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTCGTAGTAATGGCCGCACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATTTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACTTGAGGACACGGCCGTCTATTACTGTAATGCAGACTACGGGCCTCCAGTATCCATT GGTCCTTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G6-2
140	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGTAGTCTCTGGAAGTCTCCTCAGTATCAAAGCCATGGCCTGGTTCCGCCAGCCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCTGTTACTAGTGGTGGAAGCACACACTATTTAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAACACGGTGCATCTGCAAATGAACAGCCTG AAACCTGAGGACACAGCTGTCTATTACTGTAATGCAGATTTCGGTACTGACTATGTCGACTTA GGGCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G10
141	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGTCTCTGGAAGCATCTTCAGTATCAATGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAACAGCGCGAGTTGGTCGCAGCTATTACTAAAAGTAATAACATAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCACAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAATGGATTCTTCGCTTTGCCTGGGTACAGT	G2

	AGTGAAGAATTTGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
142	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAACATCTTCGATATGAATCGGATGGGCTGGTACCGCCAGCCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGATATTCGTGATGGCGGTTCTACAATTTATTCAGATTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAAATGAACAGC CTGAAACCTGACGACACAGCCGTGTATTATTGTAATGCGGGGCGGACAGGGGATCGTTTTAAT TTGGTGGCGTATTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G8
143	GATGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCACGGGGGCTCTCTGAGACTCTCC TGTGAAGCCTCTGGACGCACCTTCAGTGGCTATGCCATGGCCTGGTTCCGCCAGGCTCCAGGA AAGGAACATGAATTTGTAGCAGCTATTAGCTCAAGTAGTAATAGTGCCCCATACTATGCAAAT TCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTTTATCTACAAATG AACAACCTACAAACTGAGGACACGGCCGTTTATTACTGTGCAGCCCGGTACGGTACGAAACGG TACGTCGCCCGGGAGTATGACTCGTGGGGCCAAGGGACCCAGGTCACCGTCTCCTCA	G17
144	GATGTGCAGCTGCAGGCGTCTGGGGGAGGCGTCGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTACAGCCTCTGGAAGCATCCGCAGTATCAATGGCATGGGCTGGTCGCGCGTGGCTCCAGGG AAGCAGCGCGACTTCGTCGCACGTATTGATAGTAGGGGTAGCGCATACTATGCAGACTCCGTA GAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAGTGGACACG CTGAAACCTGAGGACACGGCCGTCTATTATTGCTTTGCGTGGCAGGGTGCGGAAACATATTGG GGCCTGGGGACCCAGGTCACCGTCTCCTCA	G25
145	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGCGACTCTCC TGTGCAGCCTCTGGAAGCATCGGCGATACCTATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGACTTGGTCGCATATATTACTAATGGTGGTAGCACGGACTACGCAGCCTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTCTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTACTACTGTAATGGAGCTACCCGTGGTGCACAGTTAGTC TTCGACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG1

146	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTGCAGCACGGGGGCTCTCTGAGACTCTCC TGTGCAGCCTCTGGAGGCACGTTCAGTAACTATGCCATGGCCTGGTTCCGCCAGGCTCCAGGA AAGGAGCGTGAATTTGTAGCAGCTATTAGCGTGAGTGCTAATAGTGCCCCATACTATGCAAAT TCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTTTATCTGCAAATG AACAGCCTAAAAACTGAGGACACGGCCGTTTATTACTGTGCAGCCCGGTACGGTACGAAACGA TACGTCGCCCGGGAGTATGACTCGTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG20
147	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGCGACTCTCC TGCGCAGCCTCTGGAAGTAGCGTCAGTCTCAATGCCATGGGCTGGTCGCGCGTGCAACCAGGA AGTACGCGCGACTTCGTCGCACGGATTGCTGCCGATGGTAGCACTCACTATGCAGACTCCGTG AGGGCCGGTTCACCATCTCCGGGGACGCCGCCAGGAACACGGTGTATCTACAAATGGATTCGC TGAAACCCGAAGACACGGCCGTCTATTACTGTTTTGCGTGGCTGGGTACGGACACGTACTGGG GCCAGGGGACCCAGGTCACCGTCTCCTCA	NG21
148	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGACACTCTCC TGTGCAGCCTCTGGAAGCATCGGCGATAACAATGCCATGGGCTGGTCCCGCACGCCTCCAGGG AAGCAGCGCGAGTTCGTCGCACGTATAGATAGTGGGGGGATCACACGCTATGCAGACTCCCTG AAGGGCCGATTCACTGTCTCCAGAGACACCGGCAAGAACACGGTGTCTCTGCAAATGAACAGC CTGAAAGCTGAGGACACAGGCGTCTATTACTGTTTTGCACATGTCGGTGGTACTATCTGGGGC CAGGGGACCCAGGTCACCGTCTCCTCA	G14
149	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTTTACCCTCTGGAGGCATCTTCACTATCAATAGCATGGGCTGGTATCGGCAGGCTCCAGGG AAACAGCGCGAGTTGGTCGCAAGTATCACTGGTGGTGGTAGTTCACGTTATGCAGACTCCGTG AAGGGCCGATTCATCATGTCCAGAGACAACGCCAAGAACATGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAATACAATCCCCCCGGCCCGGACCCAAAGC GATCATGGGGAGTGGTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NGS1
150	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGAGGGTCTCTGAGACTCTCC TGCGCAGCCTCTAGCAGCATCTTCAGTATCAATATGAGCTGGTACCGCCAGGCTCCAGGGAAC GAGCGCGAGTTGGTCGCAACTATTACACGGCATGATAGCACACACTATTCAGACTCCGTGAAG GGCCGATTCACCATCTCCAGAGACGACGACAAGAACACGATATATCTGCAAATGAACAGCCTG AAACCTGAGGACACGGCCGTCTATTACTGTTCTGGGTTTTTTCTGGACGGTAGTACCTGGCAC	G12

	CCATATTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
151	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGTCAGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTCGTAGTAATGGCAGCACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATTTCCAGAGACAACGCCAAGAACACGGTCTACCTGCAAATGAACAGC CTGAAACTTGAGGACACGGCCGTCTATTATTGTAATGGATTCTTCACTTTGCCTGGGTACAGT AGTGAAGAATTTGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G6
152	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGCCTGGAGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGAGAGCATCTTCAGTATCAACGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTATGTCGCAGGCATTACTAAGGGTGGGCGTACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACGACGCCAAGAATACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAAGACACGGCCGTCTATTACTGTAATGGTTTGTGCTCAGGCAGAGAGTGTTAT GGGGACTCCCTTTTTGCCGCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCAGGATCCGAA CAAAAACTGATCAGCGAAGAAGATCTGAACCATCACCATCACCATTAGTGA	G22#
153	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGTCAGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTCGTAGTAATGGCCGCACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATTTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACTTGAGGACACGGCCGTCTATTACTGTTCTGGGTTTTTTCTGGACGGTAGTACCTGG CACCCATATTGGGGCCAGGGCACCCAGGTCACCGTCTCCTCA	G6-1
154	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTCGATGATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGTGAGGGGGTCTCATGTATTAGTAGTAGTGATGGTAGCACACACTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAGGAACACGGTGACTCTGCAAATAAAC AGCCTGAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCCTGGGTGACCTATTGCCCC GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G13#

155	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTCGATGATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGCGAGGGGGTCTCATGTATTACGAGTAGTGATGGTAGCACATACTATGCAGACTCT GTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAACAACACGGTGCATCTGCAAATAAGC AACCTAAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCCTGGGTGACCTACTGCCCC GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G13-2#
156	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGACACTCTCC TGTGCAGTCTCTGGAAGCAGCTTCAGTATCAAGGCCATGGGCTGGTACCGCCTGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCAATTACTAGTGGTGGTAGCACGAACTATGCGGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAGCGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACAGCCGTCTATTACTGTAATGGTTTTTTCGAGTATAGGGGTCTTGAA CAATTGGGCCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G31
157	GATGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGTCGGTATCCGTGCCATGACGTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGTTCTTACTAGTGCTGGTAAACCTATGTATGCCGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAACGCAGATTTCGGGACTCCGGGTTCAGTA GTACTGGGTCCTTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G4
158	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCCTCAGTATCGAGGCCATGGGCTGGTACCGCCAGACTCTTGGG AAGCAGCGCGAATTGGTCGCAGCTATTACTAGTGGTGATAGCACAAACTATGCAGACTTCGTG AAGGGCCGATTCACCATCTCCAGAGACAAGGCCAAGAACATGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTTCTGTAATGCCCTAATGGTAGTTAGGGCTGGCTCG AATCCCGAAATTGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG2
159	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTCGATGATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGTGAGGGGGTCTCATGTATTAGTAGTAGTGATGGTAGCACATACTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACACGGTGTATCTGCAAATAAAC AGCCTGAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCCTGGGTGACCTACTGCCCC	G13-3#

	GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
160	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGTGCAGCCTGGGGGGTCTCTGAGACTGTCC TGTGTAGTCTCTGCAAGGGGCGTCAGTCTCGATGCCGTGGGCTGGTCGCGCGTGGCTCCAGGG AAGCAGCGCGACTTCGTCGCACGTATTGATCGAAGGGGTAGTACATACTATGCAGTGTCCGTA GAGGGCCGATCCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAACTGGACACG CTGAAACCTGAGGACACGGCCGTCTATTATTGTTTTGCATGGCAGGGTGCGGAAACACATTGG GGCCTGGGGACCCAGGTCACCGTCTCCTCA	G20
161	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGACCCTCTCC TGTGTAGCCTCTGGAAGCCACTTCAGTTTCAATGACATGGGCTGGTATCGCCAGGATCCGTGG AAGGGGCGCGACTTGGTCGCGGCTATTACTAGTAGTCGTAACACACTTTATGTAGACTCCGTG AAGGGCCGGTTCACCATCTCCAGAGACGACGCCAAGAACACGGTGTATCTACAAATGAACAAC CTGAAACCTGAGGACACAGCCGTCTATTACTGTAACCCGTACCCTTCCCCAAATAACTACTGG GGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG3
162	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCCCCTTCACGATCAATGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCAATTACTCGTAGTGGTAAGACGAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCGGAGACAACGCCCTGACCACGGTGTATCTGCAAATGAACAAC CTGCAACCTGAAGACACGGCCGTCTATTACTGTAATGGGTTCTACGGGTCTGAATTTGGGCCC TGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG4
163	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTCCAGGCTGGGGGCTCTGCGACGCTCTCC TGTTCAGCCCCTGGAGACACCTTAAGTAGATACGCCGTGGGCTGGTTCCGCCAGGGGCCAGGG CAGGAGCGTGATTTTGTAGCATCCATTACCTGGAGTGGTGATTACACATACTATAAAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAGTGTCAACAACATGGTGTATCTGCGAATGAAC AGCCTGAAACCTGAGGACACGGCCCTGTATTACTGTGCAGCCGATAAGAGTTCCTTTAGACTC CGAGGCCCTGGATTATATGACTACAGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG5

164	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTCGATTATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGCGAGGGGGTCTCATGTATTAGTAGTAGGGATGGTACCACCCACTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACACGGTGTATCTGCAAATAGAC AGCCTGAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCCTGGGTGACCTACTGCCCC GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG6#
165	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTCGTACAGCCTGGGGGGTCACTGAGACTCTCC TGTGCAGCCTCGGGATTCAGTTTGCATTATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGCGAGTGGGTCTCTGCCATTAGTAATATTGATGATGACACATACTATGAAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGCGTATCTGCAAATGAAC AACCTGAAACCTGAGGACACGGCCGTTTATTACTGTGCAGCAGATAAGGATGTAGTGGTAGTG CGTACGGGTCTCAGCGAGTCTGACTATTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG8
166	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCTTCGGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAACTGGTCGCAGTTATTACCAGTGGTGGACGCACAATGTATGCAGAGTCCGTG AAGGGCCGATTCGCCATCTCCAGAGACGTCGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAAGACACAGCCGTCTATTACTGTAATGGAGACTGGGGGTCGGAGGGTAGGGTG GACCTTGGACCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG9
167	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGACGCTGAGACTCTCC TGTGCCGCCTCGGGAAGCATTCGCAGTATCGGCGACATGGAGTGGTACCGCCAGGCTCCAGGA CAGCAGCGCGAGTTGGTCGCAAGTATTAGTGCTGGCCCTGAGATGCGTAGTGCTGGTACCCCA ACTTATGCAAAGTCCGTGGAGGGCCGATTCACCATCTCCAGAGACAACATCAAGAACATGATG TGGCTGCAAATGAACAGCCTGAGACCTGAAGACACGGCCGTCTATTCCTGTAATGCCGACGTT CTGACGTACTATAATGGTAGATACTCCCGAGATGTCTACTGGGGCCAGGGGACCCAGGTCACC GTCTCCTCA	NG10
168	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGCGCAGCCTCTAGCAGCATCTTCAGTATCAATATGAGCTGGTACCGCCAGGCTCCAGGGAAC GAGCGCGAGTTGGTCGCAACTATTACACGACATGATAGTACACACTATTCAGACTCCGTGAAG GGCCGATTCGCCATCTCCAGAGACGACGACAAGAACACGATATATCTGCAAATGAACAGCCTG	G12-1

	AAACCTGAGGACACGGCCGTCTATTACTGTTCTGGATTTTTTCTGGACGGTAGTACCTGGCGG CCATATTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
169	GATGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGACGCACCCTCAGTGGCTATGCCGTGGCCTGGTTCCGCCAGGCTCCAGGA AAGGAGCGTGAGTTTGTAGCAGCCATTAGCTCGAGTGATAATAGTAGCCCATATTATGCAAAT GTCGTGAAGGGTCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTTTATCTGCAAATG AACAGCCTGCAAACTGAGGACACGGCCCTTTATTACTGTGCAGCCCGGTACGGTACGAAACGG TACGTCGCCCGGGAGTATGACTCGTGGGGTCAGGGGACCCAGGTCACCGTCTCCTCA	G17-1
170	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTAGAAGCATCTTCAGTATCAATGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATATATTCGTAGTAGTGGTACCACAATGTATGCGGATTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTATTGTAACGGAGATTACTCCCCGCCCGGCAGCACG TACCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG11
171	GAGGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGATTCACTTTCGATGATTATGCCATAGGCTGGTTCCGCCAGGCCCCAGGG AAGGAGCGCGAGGGGGTCTCATGTATTACGAGTAGTGATGGTAGCACATACTATGCAGACTCT GTGAAGGGCCGATTCACCATCTCTAGAGACAATGCCAACAACACGGTGCATCTGCAAATAAGC AACCTAAAACCTGAGGATACGGCCGTTTATTACTGTGCGACCCCCTGGGTGAACTACTGCCCC GAGAACCTTCTGTTTAGTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCACAGGCCCAG GTACAGCTGGTGGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCCTGT ACAGCCTCTGGACGCACCTTCAGTACCTATTTCATGGCCTGGTTCCGCCAGCCTCCAGGGAAA GAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTTCCACACTATACAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAACAGC CTGAAATCTGAGGACACGGCCGTTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCTGAC TTTGGTTCCTACGGCCAGGGGACCCAGGTCACCGTCTCCTCAGGATCCGAACAAAAACTGATC AGCGAAGAAGATCTGAACCATCACCATCACCATTAGTGA	G15(bi)

172	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGGAGCATCTGGAATTATTTTTAGTGCCACTACCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCACTGATTACTAGTGATTGGCACACAAAGTATGCAGACTCCGTG AAGGACCGATTCTCCATTTCCAGAGACAACGCCAAGAGCACGGTGCACCTGCAAATGAACAGC CTGAGATCTGAAGACACAGCAGTCTATTTTTGTTATGCCCGCCAAGCCTTCAGTGAGCCTCGT TGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G11
173	CAGGTACAGCTGGTGGATTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGATTGTCC TGTGCAGCCTCTGGAAGCAGCGGCAGAATCGATGCCATGGGCTGGTCGCGCGTGGCTCCAGGG AAGCAGCGCGACTTCGTCGCACGTCTTGGCAGTAATGGATTCACACAGTATGACATCTCCGTG GAGGGCCGATTCACCATCTCCGGGGACGTCGCCAAGAATACGATATATCTGCAAATGGACACG CTGAAACCTGAGGACACGGCCGTCTATTACTGTTTTGCGTGGCTGGGGCAAGATACCGTGTGG GGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG12
174	CAGGTACAGCTGGTGGATTCTGGGGGAGGATTGGTAAAGGCTGGGGCATCTCTGAGACTCTCC TGTGCAGCCTCTGGAGACGCCTTATTTAACTACGCCATGGGCTGGTTTCGCCAGGGGCCAGGG AAGGAGCGTGACTTTGTAGCATCTGTTACCAGGAGTGGTGATAATACATACTATAAAGACTCC GCGAAGGGCCGATTCACCATCTCCAGAGACGACGCCAAGAACACGGTATATCTGCAAATGAAC AGCCTGAAACCTGAGGACACGGCCGTTTATTTCTGTGCAGCAGATAAGAGTTCCTTTAGGCTC CGAGGCCCTGGAGTATATGACTACAGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG14
175	CAGGTACAGCTGGTGGATTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGTCTCTGGAAGCGACGGCCGAGTCATGCTCATGGGCTGGTACCGCCAGGCTCCAGGG CAGCAGCGCGACCTGGTCGCATCTATTACTAGTGCAGATTACACAACCTATGCAGAATCCGTC GAGGGCCGATTCACCATCTCCACAGACAACAACAAGAACACAGTGTATCTACAAATGAACAGC CTGAAGCCTGAAGACACAGCCGTCTATTTTTGTAAAGTAATTGCGGCGACGGTCTGGGGCCAG GAGACCCAGGTCAGGCAGGGTTTGACATTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG13
176	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAGCATCTCCAGTGCCAGATCCATGACCTGGTACCGCCAGGCTCTAGGG AAGCAGCGCGAGTTGGTCGCAGTGATTATGGGTGGCGGTAGCACGATGTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTACAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTATTGTAATGCAGACTGGGGGGGAGTCGGGTTTCCG	G21

	AACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
177	GATGTGCAGCTGCAGGCGTCTGGGGGAGGATTGGTGCAAATTGGGGACTCTGTGAGACTCTCC TGTATAGCCTCTGGAGGCACCTTCAGAACTTATGCTATCGGTTGGTTCCGCCAGGCTCCAGGG GCTGAGCGTGAATTTGTAGCTGCCATTAGCCGGCGCGGTAATAAGACAGATTATGCAGAGTCC GTGAAGGGCCGATTCACAGTCTCCAGAGACAACGCCGAGAATACGGTGTATTTGCAAATGAAC AGCCTGAAACCTGATGACATGGGCGTTTATTACTGTGCAGCGTCGGCGCGTAATTTCATCGGC ACCCAGCCACTTGATTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG23
178	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTACAGGCTGGGGGCTCTCTGAGACTCTCC TGTGCAGCCTCTGGATGGAACCTTGGTAATTATGCCTTGGGCTGGTTCCGCCAGGCTCCAGGG AAGGAGCGTGAGTTTGTAGCAGCTATCGACTGGCGTCATAGTTCATACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACACCAAGAACATGGTGTATCTGCAAATGAGCAGC CTGAAACTTGAGGACACGCGCCTTTATTACTGTGCAGCATCAAGCCTATTCCCTAGTAGTGCT CCCCGTCAGTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG15
179	CAGGTACAGCTGGTGGATTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTGTAGCCTCTGGACGCACCTTCAGTAATTATGCCATGGGCTGGTACCGCCGACGTCCAGGG CTGGAGCGTGAATTTGTAGCAGCTATTGTTGGGAGTGGTGATAGCACAAGGTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAAC ACGCTGAAACCTGAGGACACGGCCGTTTATTACTGTGCGTCATCCTCCGACCCGCGGGTTTAT ATAGCAAGTACTCTCGATTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG16
180	CAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTGCAGCCTCTGGACGCACCTTCAGTATGTTTATCATGGGCTGGTTCCGCCAGGCTCCAGGG AAGGAGCGTGAATTAGTAGCAGCTATTAGCCGGAATAGTAATCTCACATACTATTTTCAGTCC GTGAAAGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAACTTGAGGACACGGCCGTCTATTACTGTAATGCAGACTACGGGCCTCCAGTATCC ATTGGTCCTTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G23

181	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTGGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTAGTCTCTGGAAGGATCCTCAGTATCAAGGCCATGGGCTGGTACCGCCAGGCTCCTGGG AAGCAGCGCGAGTACGTCGCAGGTATTGTTAGCAGTGGTAATACAAACTATGCAGACTTCGTG AAGGGCCGATTCACCATCTCCGGAGACAACGCCAAGAACACGGTGTTTCTGCAAATGAACAGC CTGAAACCTGAAGACACGGCCGTCTATTACTGTAATGCCCTAGTGGTCGTTACTAGTGCCTCG GGTCCCGAGTTGGCTTCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG17
182	GATGTACAGCTGGTGGATTCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTACAGCCTCTGGACGCACCTTCAGTACCTATTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAGCAGCGCGAGTTGGTCGCATACATTCGTAGTGGTGGTACGACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACATCGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGCAATGCAGATTACTCCCCGCCCGGCAGCCGG TTCCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G1-3
183	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGACACTCTCC TGCGCAGCCTCTGGATTCACCTTGGATAATTATGCCATAGCGTGGTTCCGCCAGGCCCCAGGG AGGGAGCGCGAGTGGGTCTCATCAACTGGTAGTGATGGTAACTTATATACACCGTCCGTGAGG GGCCGATTCACCATTTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGCCTG AAACCTGAGGACACGGCCGTTTATTATTGTGTAGCAGGGAAGAGACCGGTAATTACTACATGG ATTGCTTTGGACGCATGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG18
184	GATGTACAGCTGGTGGATTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAACATTCTCCAGTATCGATTCCATGCGCTGGTTCCGGCGGGCTCCAGGA AAGGAGCGCGAATTTGTCGCACATATTACTAGCACGGGTAGGACAAACTATGCAGACGCCGTG AAGGGCCGATTTACCATCTCTAGAGACAACGCCAAGAACACGATGTGGCTGCAAATGGACAAC CTGAAACCTGACGACACGGCCGTCTATTATTGCAATATGGTGACGACTCCTTATATGCACTGG GGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG24
185	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAAACTCTCC TGTGTAGCCTCTGGAAGCCGCTTCAGTGAAAATGCCATGGGCTGGTATCACCAGGCTCCAGAC AAACAGCGCACCTTGGTCGCAGCTATTACTAGTAGTCGTAGCACTCTTTATATAGACTCCGTG AAGGGCCGCTTCACCATCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAGCAAC CTGAAACCTGAGGACACCGGCGTCTATTACTGTAACCCGTACCCTTCCCCAAATTCCTACTGG	NG25

	GGCCAGGGGACCCAGGTCACCGTCTCCTCA	
186	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTAAGACTCTCC TGTGCAGCCTCTGGACTCGTCTTCAGTGCCAATAAGATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCACGTATTAGCACTGACGGACGTACAAACTATGCGGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCGAGAAGACGGTGTTTCTGCAAATGAACAGC CTGAATCCTGACGACACGGCCGTCTATTACTGTAATGCAAACTGGCTCGATAAATATGACTAC TGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG19
187	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGGAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTGGCCTCTGGAAGCATCTCCAGTGCCAGATCCATGACCTGGTACCGCCAGGCTCACGGG AAGCAGCGCGAGTTGGTCGCAGTTATTACTAGTGGCGGTAGCACAATGTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAGCGCCAAGAACACGGTGTATCTACAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTATTGTAATGCAGACTGGGGGGAAGTCGGGTTTGTG AACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	NG26(G 21-1)
188	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCTTCGGTTTCAATGGCGTGGCCTGGTTCCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGTTATTCGTAGTGGTGGTAACACGCTCTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGTAATGTAGATTACTCCCCGCCCGGTAGTCTG GTTCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G18
189	GAGGTACAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCGCCAGTATCAATGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCAGCTATTACTAGAGGTGGTAGCACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTATATCTGCAAATGAACAGC CTGAAACCGGAGGACACGGCCGTCTATTCATGTAATGGTTTGTGCTCAGACGATCGGTGTTAT GGGGACTCCCTTTTTGCCCCCTGGGGCCCGGGGACCCAGGTCACCGTCTCCTCA	G16#

190	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGTGCAGCCTGGGGGGTCTCTGAGACTGTCC TGTCTAGTCTCTGGAAGGGGCGTCAGTCTCGATGCCGTGGGCTGGTCGCGCGTGGCTCCAGGG AAGCAGCGCGACTTCGTCGCACGTATTGATAGTAGGGGTAGCGCATACTATGCAGACTCCGTA GAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAGTGGACACG CTGAAACCTGAGGACACGGCCGTCTATTATTGTTTTGCGTACTACGGGGCTCAAATATCTTTT GGTCCGTGGGGCCAGGGGACCCAGGTCACCGTCTCTTCA	G24
191	GATGTGCAGCTGCAGGCGTCTGGGGGAGGATTGGTGCAGCCTGGGGGGTCTCTGAGACTGTCC TGTGTAGTCTCTGGAAGGGGCGTCAATCTCGATGCCATGGGCTGGTCGCGCGTGGCTCCAGGG AAGCAGCGCGACTTCGTCGCACATATTGATGATAGGGGTACCGCATACTATGCAGACTTCGTA AAGGGCCGATCCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAGTGGACACG CTGAAACCTGAGGACACGGCCGTCTATTATTGCTTTGCGTGGCAGGGTGCGGAAACATATTGG GGCCTGGGGACCCGGGTCACCGTCTCCTCAGGATCCGAACCAAAACTGATCAACGAAGAACAT CTGAACCATCACCATCACCATTATTGA	G19
192	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAGCATCTTCAGTGTCAATGCCGTGGCCTGGTACCGCCAGGCTCCAGGG AAACAGCGCGAGTTGGTCGCATATATACGTCGTAGTGGTAGCACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATCTGCAAATGAACAGC CTGAAACCTGACGACACAGCCGTGTATTATTGTAATGCGGGGCGGACAGGGGATCGTTTTAAT TTGGTGGCGTATTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G5-1
193	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGCGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTACAGCCTCTGGACGCACCTTCAGTACCTATTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAAGAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTTCCACACTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAACCTGAGGACACGGCCGTCTATTACTGCAATGCAGATTACTCCCCGCCCGGCAGC CGGTTCCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G26
194	GAGGTGCAGCTGCAGGCGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGTAGCCTCTGGAAGCATCTTCAGTATCAAAGCCATGGCCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATACATTCGTAGTGGTGGTACGACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACATCGCCAAGAACACGGTGTATCTGCAAATGAACAGC	G27

	CTGAAATCTGAGGACACGGCCGTTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCTGCT TTGGTTCCTACGGCCAGGGGACCCAGGTCACCGTCTCCTCA	
195	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGGCTGGGGCCTCCGTGAGACTCTCC TGTGCAGCCTCTGGACGCGCCAACAGTTTGTATGCCATGGGCTGGTTCCGCCAGGCTCCAGGG AAGCAGCGCGAGTTGGTCGCATACATTCGTAGTGGTGGTACGACAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACATCGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTACTGCAATGCAGATTACTCCCCGCCCGGCAGCCGG TTCCCTGACTTAGGTCCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G1-2
196	GAGGTACAGCTGGTGGAATCTGGGGGAGGATTGGTGCAAATTGGGGACTCTGTGAGACTCTCC TGTATAGCCTCTGGAGGCACCTTCAGAACTTATGCTATGGGTTGGTTCCGCCAGGCTCCAGGG GCTGAGCGTGAATTTGTAGCTGCCATTAGCCGGCGCGGTAATAAGACAGATTATGCAGAGTCC GTGAAGGGCCGATTCACAGTCTCCAGAGACAACGCCGAGAATACGGTGTATTTGCAAATGAAC AGCCTGAAACCTGATGACATGGGCGTTTATTACTGTGCAGCGTCGGCGCGTAATTTCATCGGC ACCCAGCCACTTGATTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G28
197	CAGGTAAAGCTGGAGGAGTCTGGGGGAGGAATGGTGCAGGCTGGGGGCTCTCTGAGACTCTCC TGTGTAGCCTCTGGACGCTCCTTCGTTGGCTATTTCATGGCCTGGTTCCGCCAGCCTCCAGGG AAAGAGCGTGAATACGTAGGCGGTATTAGGTGGAGTGATGGTGTTCCACACTATGCAGACTCC GTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATTTGCAAATGAAC AGCCTGAAATCTGAGGACACGGCCGTTTATTTTTGTGCATCACGGGGTATTGCGGATGGATCT GACTTTGGTTCCTACGGCCAGGGGACCCAGGTCACCGTCTCCTCA	G29
198	GAGGTACAGCTGGTGGAATCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCC TGTGCAGCCTCTGGAAGCATCTTCAGTATCAATGCCATGGGCTGGTACCGCCAGGCTCCAGGG AAGCAGCGCGAATTGGTCGCAGCTATTACTAAAAGTAATAACATAAACTATGCAGACTCCGTG AAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGC CTGAAACCTGAGGACACGGCCGTCTATTATTGTAATGGATTCTTCACTTTGCCTGGGTACAGT AGTGAAGAATTTGGTCCCTGGGGCCTGGGGACCCAGGTCACCGTCTCCTCA	G2-1

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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, CDR2, and CDR3 as set forth in SEQ ID NO: 1.

2. The anti-BCMA single domain antibody according to claim 1, wherein said 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 sequences of CDR1, CDR2, and CDR3 as set forth in SEQ ID NO: 1, preferably, the difference in amino acids is conservative substitution.

3. The 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.

4. 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.

5. The anti-BCMA single domain antibody according to claim 4, wherein 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, preferably, the difference in amino acids is conservative substitution.

6. Genes of anti-BCMA single domain antibodies, said genes of single domain antibody have a nucleotide sequence as set forth in SEQ ID NO: 133.

7. The genes of anti-BCMA single domain antibodies according to claim 6, having a nucleotide sequence with 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 NO: 133, wherein the difference in bases is preferably a conservative substitution.

8. A polypeptide, having one or multiple single domain antibodies according to any one of claims 1-5.

9. An expression vector, comprising one or more genes selected from said genes of single domain antibodies according to claim 6 or 7, 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.

10. A host cell, comprising said expression vector according to claim 9, 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*.

11. A chimeric antigen receptor, having one or multiple single domain antibodies according to any one of claims 1-5.

12. A T cell , wherein said T cell expresses said chimeric antigen receptor according to claim 11.

13. A pharmaceutical composition, comprising one or more single domain antibodies according to any one of claims 1-5 as active ingredients.

14. The single domain antibody according to any one of claims 1-5, wherein said single domain antibody is humanized.

15. An *in vitro* use of said single domain antibody according to any one of claims 1-5 in detection of BCMA.

16. An *in vitro* use of said single domain antibody according to any one of claims 1-5 for blocking an interaction between BAFF and BCMA.

17. The use according to claim 16, 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.

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

19. The use according to claim 18, wherein said disease associated with abnormal BCMA expression is a multiple myeloma.