

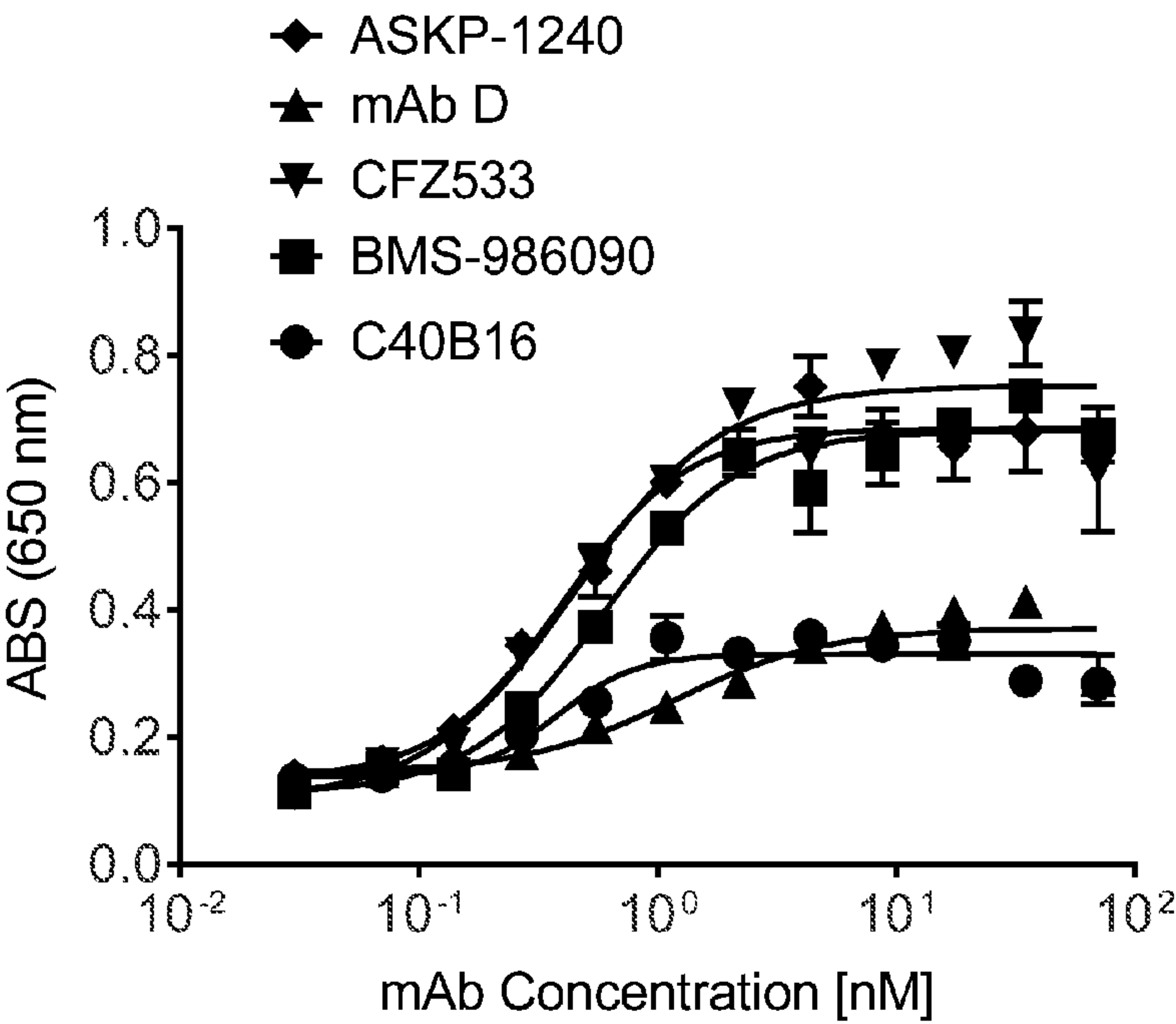


(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION
(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2016/09/30	(51) Cl.Int./Int.Cl. <i>C07K 16/28</i> (2006.01), <i>A61K 39/395</i> (2006.01), <i>A61P 1/00</i> (2006.01), <i>A61P 37/00</i> (2006.01)
(87) Date publication PCT/PCT Publication Date: 2017/04/06	(71) Demandeur/Applicant: JANSSEN BIOTECH, INC., US
(85) Entrée phase nationale/National Entry: 2018/03/28	(72) Inventeurs/Inventors: BABBE, HOLGER, US; FELIX, NATHAN, US; FRANSSON, JOHAN, CA; KIM, PAUL, US; SCULLY, MICHAEL, US; ZHOU, HONG, US
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(87) N° publication PCT/PCT Publication No.: 2017/059196	
(30) Priorité/Priority: 2015/09/30 (US62/234,812)	

(54) Titre : ANTICORPS ANTAGONISTES SE LIANT SPECIFIQUEMENT AU CD40 HUMAIN ET PROCEDES D'UTILISATION
(54) Title: ANTAGONISTIC ANTIBODIES SPECIFICALLY BINDING HUMAN CD40 AND METHODS OF USE

Figure 1.



(57) **Abrégé/Abstract:**
The present invention relates to antagonistic antibodies specifically binding human CD40, polynucleotides encoding the antibodies or antigen-binding fragments thereof, and methods of making and using the foregoing.

- (51) International Patent Classification:
A61K 39/395 (2006.01) A61P 1/00 (2006.01)
A61P 37/00 (2006.01)

(21) International Application Number:
PCT/US2016/054671

(22) International Filing Date:
30 September 2016 (30.09.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/234,812 30 September 2015 (30.09.2015) US

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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

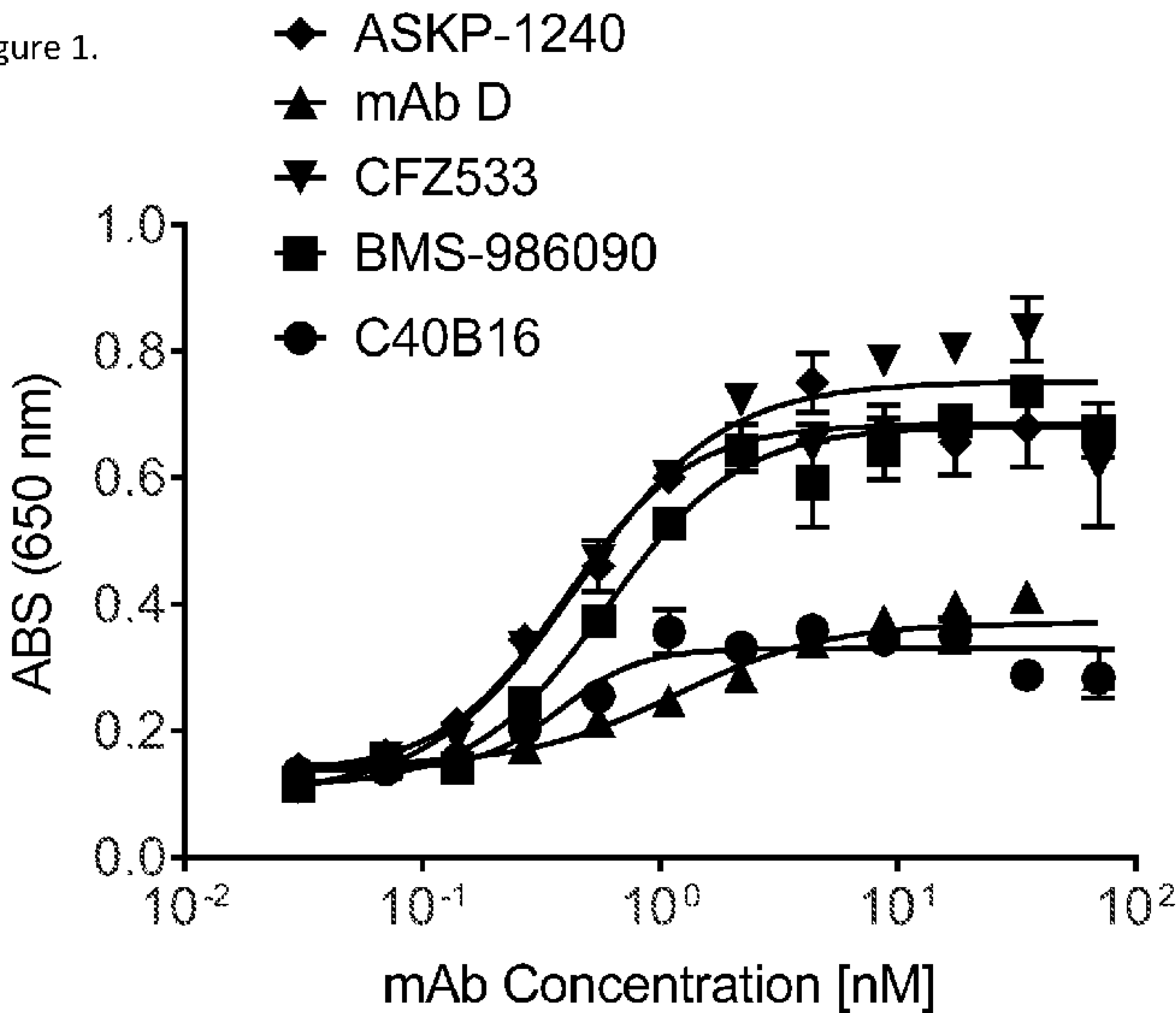
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
— with sequence listing part of description (Rule 5.2(a))

[Continued on next page]

(54) Title: ANTAGONISTIC ANTIBODIES SPECIFICALLY BINDING HUMAN CD40 AND METHODS OF USE

Figure 1.



(57) Abstract: The present invention relates to antagonistic antibodies specifically binding human CD40, polynucleotides encoding the antibodies or antigen-binding fragments thereof, and methods of making and using the foregoing.

WO 2017/059196 A3



(88) Date of publication of the international search report:
8 June 2017

Antagonistic Antibodies Specifically Binding Human CD40 and Methods of Use

Field of the Invention

The present invention relates to antagonistic antibodies specifically binding human CD40, polynucleotides encoding the antibodies or antigen-binding fragments thereof, and methods of making and using the foregoing.

Background of the Invention

The cell surface CD40 molecule is a member of the tumor necrosis factor receptor superfamily (TNFR) and a key regulator in both innate and adaptive immune responses. CD40 is constitutively expressed on antigen presenting cells, in particular B-cells, dendritic cells and macrophages, but can also be found on fibroblasts, synoviocytes, smooth muscle cells, endothelial cells and epithelial cells.

The natural ligand of CD40, designated CD154 or CD40L, is mainly expressed on activated T lymphocytes and platelets. The interaction of CD40 with CD40L on T cells induces both humoral and cell-mediated immune responses. CD40 regulates this ligand-receptor pair to activate B cells and other antigen-presenting cells (APC) including dendritic cells (DCs), driving T cell activation. For example, activation of CD40 on B cells induces B cell proliferation, somatic hypermutation, differentiation into antibody secreting cells and isotype switching in germinal centers of secondary lymphoid organs. *In vitro* studies have shown direct effects of CD40 activation on cytokine production (e.g. IL-6, IL-10, IL-12, TNF- α), expression of adhesion molecules and costimulatory receptors (e.g. ICAM, CD23, CD80 and CD86), and increased expression of MHC class I, MHC class II, and TAP transporter by B lymphocytes.

Antibodies that modulate the CD40/CD40L interaction are of interest in treating diseases such as inflammatory diseases, including autoimmune diseases.

Summary of the invention

The invention provides for an isolated antagonistic antibody or an antigen binding portion thereof specifically binding human CD40 of SEQ ID NO: 1, comprising a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 5, a HCDR2 of SEQ ID NO: 61, a HCDR3 of SEQ ID NO: 62, a light chain complementarity determining

region (LCDR) 1 of SEQ ID NO: 63, a LCDR2 of SEQ ID NO: 9 and a LCDR3 of SEQ ID NO: 10.

The invention also provides for an isolated antagonistic antibody or an antigen binding portion thereof specifically binding human CD40 of SEQ ID NO: 1, comprising certain HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3, VH, VL, HC and/or LC sequences.

The invention also provides for a pharmaceutical composition comprising the antibody of the invention and a pharmaceutically acceptable carrier.

The invention also provides for an immunoconjugate comprising the antibody of the invention linked to a therapeutic agent or an imaging agent.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 11, 21, 22, 23, 24, 25 or 26;
the VL of SEQ ID NOs: 12 or 27; or
the VH of SEQ ID NOs: 11, 21, 22, 23, 24, 25 or 26 and the VL of SEQ ID NOs: 12 or 27.

The invention also provides for an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NOs: 13, 14, 28, 29, 30, 31, 32, 33, or 34.

The invention also provides for an isolated polynucleotide encoding the heavy chain of SEQ ID NOs: 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46.

The invention also provides for an isolated polynucleotide encoding the light chain of SEQ ID NOs: 77 or 78.

The invention also provides for an isolated polynucleotide encoding the heavy chain of SEQ ID NOs: 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46 and a light chain of SEQ ID NOs: 77 or 78.

The invention also provides for an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NOs: 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 77 or 78.

The invention also provides for a vector comprising the polynucleotide of the invention.

The invention also provides for a host cell comprising the vector of the invention.

The invention also provides for a method of producing an antagonistic antibody or an antigen binding portion thereof specifically binding human CD40 of SEQ ID NO: 1,

comprising culturing the host cell of the invention in conditions wherein the antibody is expressed, and isolating the antibody.

The invention also provides for a method of treating a subject having an inflammatory disease, comprising administering to the subject in need thereof the isolated antibody of the invention for a time sufficient to treat the inflammatory disease.

The invention also provides for the antibody of the invention for use in therapy.

The invention also provides for an anti-idiotypic antibody binding to the antibody of the invention.

The invention also provides for a kit comprising the antibody of the invention.

Brief Description of the Drawings

Figure 1 shows that C40B16 as wild-type IgG1 demonstrated comparable minimal agonism when compared to Fc effector silent Antibody D. Fc effector silent ASKP-1240, CFZ533 and BMS-986090 mAbs demonstrated higher level of agonism when compared to C40B16. Agonism was assessed in HEK-Blue™ CD40L NF-κB activation assay.

Figure 2 shows that C40B16 does not induce agonism in an assay measuring antibody-mediated IL-12p40 production by human dendritic cells (DC), whereas ASKP-1240, CFZ533 and BMS-986090 induce IL-12p40 production. IL-12p40 production was evaluated at 6 different antibody concentrations, (350, 110, 35, 11, 3.5, and 1.1 nM) each represented by a separate column for each antibody in the Figure. DC+CD40L: positive control. DC only: negative control.

Figure 3A shows that 500 ng/ml concentrations of anti-CD40 antibodies C40B176, C40B179, C40B180 and C40B183 do not induce activation of dendritic cells (DC), whereas 350 nM ASKP-1240 induces IL-12p40 production. DC activation was assessed by IL-12p40 production by DCs in the presence of antibody. PP1B40: IgG1sigma isotype control; CNT09412: IgG4_PAA isotype control.

Figure 3B shows that high concentrations of anti-CD40 antibodies C40B176, C40B179, C40B180 and C40B183 do not induce proliferation of B cells, whereas 500 nM ASKP-1240 does. PP1B40: IgG1sigma isotype control; CNT09412: IgG4_PAA isotype control. Two separate dose response curves are shown for ASKP-1240.

Detailed Description of the Invention

Definitions

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as though fully set forth.

It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

As used herein, the singular forms “a,” “and,” and “the” include plural reference unless the context clearly dictates otherwise.

Although any methods and materials similar or equivalent to those described herein may be used in the practice for testing of the present invention, exemplary materials and methods are described herein.

“Specific binding” or “specifically binds” or “binds” refers to antibody binding to human CD40 with greater affinity than for non-related antigens. Typically, the antibody binds to human CD40 with a dissociation constant (K_D) of 1×10^{-8} M or less, for example 1×10^{-9} M or less, 1×10^{-10} M or less, 1×10^{-11} M or less, or 1×10^{-12} M or less, typically with a K_D that is at least one hundred fold less than its K_D for binding to a non-related antigen (for example, BSA, casein). The dissociation constant may be measured using standard procedures. Antibodies that specifically bind human CD40 may, however, have cross-reactivity to other related antigens, for example to the same antigen from other species (homologs), such as human or monkey, for example *Macaca fascicularis* (cynomolgus, cyno), *Pan troglodytes* (chimpanzee, chimp) or *Callithrix jacchus* (common marmoset, marmoset). While a monospecific antibody specifically binds one antigen or one epitope, a bispecific antibody specifically binds two distinct antigens or two distinct epitopes.

“Antibodies” is meant in a broad sense and includes immunoglobulin molecules including monoclonal antibodies including murine, human, humanized and chimeric monoclonal antibodies, antigen-binding fragments, bispecific or multispecific antibodies, dimeric, tetrameric or multimeric antibodies, single chain antibodies, domain antibodies and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding site of the required specificity. “Full length antibodies” are comprised of two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds as well as

multimers thereof (for example IgM). Each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (comprised of domains CH1, hinge CH2 and CH3). Each light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The VH and the VL regions may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each VH and VL is composed of three CDRs and four FR segments, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

“Complementarity determining regions (CDR)” are “antigen binding sites” in an antibody. CDRs may be defined using various terms: (i) Complementarity Determining Regions (CDRs), three in the VH (HCDR1, HCDR2, HCDR3) and three in the VL (LCDR1, LCDR2, LCDR3) are based on sequence variability (Wu and Kabat, J Exp Med 132:211-50, 1970; Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991). (ii) “Hypervariable regions”, “HVR”, or “HV”, three in the VH (H1, H2, H3) and three in the VL (L1, L2, L3) refer to the regions of an antibody variable domains which are hypervariable in structure as defined by Chothia and Lesk (Chothia and Lesk, Mol Biol 196:901-17, 1987). The International ImMunoGeneTics (IMGT) database (<http://www.imgt.org>) provides a standardized numbering and definition of antigen-binding sites. The correspondence between CDRs, HVs and IMGT delineations is described in Lefranc *et al.*, Dev Comparat Immunol 27:55-77, 2003. The term “CDR”, “HCDR1”, “HCDR2”, “HCDR3”, “LCDR1”, “LCDR2” and “LCDR3” as used herein includes CDRs defined by any of the methods described *supra*, Kabat, Chothia or IMGT, unless otherwise explicitly stated in the specification.

Immunoglobulins may be assigned to five major classes, IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA₁, IgA₂, IgG₁, IgG₂, IgG₃ and IgG₄. Antibody light chains of any vertebrate species can be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

“Antigen-binding fragment” refers to a portion of an immunoglobulin molecule that retains the antigen binding properties of the parental full length antibody. Exemplary

antigen-binding fragments are as heavy chain complementarity determining regions (HCDR) 1, 2 and/or 3, light chain complementarity determining regions (LCDR) 1, 2 and/or 3, a heavy chain variable region (VH), or a light chain variable region (VL), Fab, F(ab')₂, Fd and Fv fragments as well as domain antibodies (dAb) consisting of either one VH domain or one VL domain. VH and VL domains may be linked together via a synthetic linker to form various types of single chain antibody designs in which the VH/VL domains pair intramolecularly, or intermolecularly in those cases when the VH and VL domains are expressed by separate chains, to form a monovalent antigen binding site, such as single chain Fv (scFv) or diabody; described for example in Int. Pat. Publ. No. WO1998/44001, Int. Pat. Publ. No. WO1988/01649; Int. Pat. Publ. No. WO1994/13804; Int. Pat. Publ. No. WO1992/01047.

“Monoclonal antibody” refers to an antibody population with single amino acid composition in each heavy and each light chain, except for possible well known alterations such as removal of C-terminal lysine from the antibody heavy chain. Monoclonal antibodies typically bind one antigenic epitope, except that multispecific monoclonal antibodies bind two or more distinct antigens or epitopes. Bispecific monoclonal antibodies bind two distinct antigenic epitopes. Monoclonal antibodies may have heterogeneous glycosylation within the antibody population. Monoclonal antibody may be monospecific or multispecific, or monovalent, bivalent or multivalent. A multispecific antibody, such as a bispecific antibody or a trispecific antibody is included in the term monoclonal antibody.

“Isolated antibody” refers to an antibody or an antigen-binding fragment thereof that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody specifically binding human CD40 is substantially free of antibodies that specifically bind antigens other than human CD40). In case of a bispecific antibody, the bispecific antibody specifically binds two antigens of interest, and is substantially free of antibodies that specifically bind antigens other than the two antigens of interest. “Isolated antibody” encompasses antibodies that are isolated to a higher purity, such as antibodies that are 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% pure.

“Humanized antibodies” refers to antibodies in which the antigen binding sites are derived from non-human species and the variable region frameworks are derived from

human immunoglobulin sequences. Humanized antibodies may include intentionally introduced mutations in the framework regions so that the framework may not be an exact copy of expressed human immunoglobulin or germline gene sequences.

“Human antibodies” refers to antibodies having heavy and light chain variable regions in which both the framework and the antigen binding site are derived from sequences of human origin. If the antibody contains a constant region or a portion of the constant region, the constant region also is derived from sequences of human origin.

A human antibody comprises heavy or light chain variable regions that are derived from sequences of human origin if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin or rearranged immunoglobulin genes. Such exemplary systems are human immunoglobulin gene libraries displayed on phage, and transgenic non-human animals such as mice or rats carrying human immunoglobulin loci as described herein. A human antibody typically contain amino acid differences when compared to the human germline or rearranged immunoglobulin sequences due to, for example naturally occurring somatic mutations, intentional introduction of substitutions into the framework or antigen binding site and amino acid changes introduced during cloning and VDJ recombination in non-human animals. Typically, a human antibody is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical in amino acid sequence to an amino acid sequence encoded by a human germline or rearranged immunoglobulin gene. In some cases, a human antibody may contain consensus framework sequences derived from human framework sequence analyses, for example as described in Knappik *et al.*, J Mol Biol 296:57-86, 2000, or synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, for example as described in Shi *et al.*, J Mol Biol 397:385-96, 2010 and Int. Pat. Publ. No. WO2009/085462.

Antibodies in which antigen binding sites are derived from a non-human species are not included in the definition of human antibody.

“Recombinant” includes antibodies and other proteins that are prepared, expressed, created or isolated by recombinant means.

"Epitope" refers to a portion of an antigen to which an antibody specifically binds. Epitopes typically consist of chemically active (such as polar, non-polar or hydrophobic) surface groupings of moieties such as amino acids or polysaccharide side chains and may

have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be composed of contiguous and/or discontinuous amino acids that form a conformational spatial unit. For a discontinuous epitope, amino acids from differing portions of the linear sequence of the antigen come in close proximity in 3-dimensional space through the folding of the protein molecule.

“Multispecific” refers to an antibody that specifically binds at least two distinct antigens or two distinct epitopes within the antigens, for example three, four or five distinct antigens or epitopes.

“Bispecific” refers to an antibody that specifically binds two distinct antigens or two distinct epitopes within the same antigen. The bispecific antibody may have cross-reactivity to other related antigens or can bind an epitope that is shared between two or more distinct antigens.

“Variant” refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications for example, substitutions, insertions or deletions.

“Vector” refers to a polynucleotide capable of being duplicated within a biological system or that can be moved between such systems. Vector polynucleotides typically contain elements, such as origins of replication, polyadenylation signal or selection markers, that function to facilitate the duplication or maintenance of these polynucleotides in a biological system. Examples of such biological systems may include a cell, virus, animal, plant, and reconstituted biological systems utilizing biological components capable of duplicating a vector. The polynucleotide comprising a vector may be DNA or RNA molecules or a hybrid of these.

“Expression vector” refers to a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

“Polynucleotide” refers to a synthetic molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. cDNA is a typical example of a polynucleotide.

“Polypeptide” or “protein” refers to a molecule that comprises at least two amino acid residues linked by a peptide bond to form a polypeptide. Small polypeptides of less than 50 amino acids may be referred to as “peptides”.

“CD40” or “huCD40” refers to the human CD40 protein. CD40 is also known as Tumor necrosis factor receptor superfamily member 5 (TNFRSF5), CD40L receptor or CD154 receptor. The amino acid sequence of the full length human CD40 is shown in SEQ ID NO: 1. Human full length CD40 protein is a type I membrane protein with 277 amino acids. The signal sequence spans residues 1-20, the extracellular domain spans residues 21-193, the transmembrane domain spans residues 194-215, and the cytoplasmic domain spans residues 216-277 of SEQ ID NO: 1. Throughout the specification, the extracellular domain of CD40, “CD40-ECD”, refers to the CD40 fragment of residues 21-193 of SEQ ID NO: 1.

“Antagonist” or “antagonistic” refers to an antibody that specifically binds human CD40 and inhibits CD40 biological activity in the presence of CD40L in cellular assays such as CD40L-driven human B cell proliferation or CD40L-driven IL-12p40 production by human dendritic cells. The antagonist may inhibit CD40 biological activity in a statistically significant manner when compared to a control sample without the antibody. Alternatively, the antagonistic antibody specifically binding human CD40 may inhibit CD40 biological activity with an IC_{50} value of about 1 nM or less. CD40L in the assays may be provided as a soluble form or membrane-bound (*e.g.* as cells expressing CD40L, such as Jurkat cells).

“CD40 biological activity” refers to a measureable event in a cell occurring as a result of binding of CD40L to CD40 on human cells. CD40 biological activity may be for example proliferation of human B cells or production of IL-12p40 by human dendritic cells, or downstream activation of CD40 signaling pathways. CD40 biological activity may be measured using known methods and methods described herein.

“About” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. Unless explicitly stated otherwise within the Examples or elsewhere in the Specification in the context of a particular assay, result or embodiment, “about” means within one standard deviation per practice in the art, or a range of up to 5%, whichever is larger.

“In combination with” means that two or more therapeutics can be administered to a subject together in a mixture, concurrently as single agents or sequentially as single agents in any order.

“Cross-linking” refers higher order multimerization of CD40 on cells induced by an antibody specifically binding human CD40 binding to Fc γ RIIb *cis* or *trans*, resulting in induction of CD40 agonistic activity.

Conventional one and three-letter amino acid codes are used herein as shown in **Table 1.**

Table 1.

Amino acid	Three-letter code	One-letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Gln	E
Glutamine	Glu	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Antibodies of the invention

The invention provides antagonistic antibodies specifically binding human CD40, polynucleotides encoding the antibodies, vectors, host cells and methods of using the antibodies.

The antibodies of the invention are potent inhibitors of CD40 and have minimal agonistic activity. It is documented that antagonistic anti-CD40 antibodies, albeit being antagonists, can also have agonistic activity as a result of Fc-dependent cross-linking (for example see US7,537,763) and therefore pose a potential safety risk when administered to

subjects in which suppression of CD40 signaling is desired, such as patients with autoimmune disease. Preferable anti-CD40 antibodies for the treatment of conditions in which inhibition of CD40 biological function is desired are hence those that lack agonistic activity, or have minimal agonistic activity. Suitable therapeutic CD40 antibodies would thus be Fc-engineered to abolish Fc γ R binding, and as a result lack Fc-mediated cross-linking and potential for Fc-mediated agonism. Such effector silent Fc-engineered antibodies are for example ASKP-1240, CFZ533, BMS-986090 and Antibody D (“benchmark antibodies”). ASKP-1240 and CFZ533 are currently in clinical development for inflammatory or autoimmune diseases.

The antibodies of the invention demonstrate improved properties when compared to the benchmark antibodies. The antibody C40B16 of the invention has minimal agonistic activity and activates CD40 signaling to a lesser extent when compared to the benchmark antibodies ASKP-1240, CFZ533 and BMS-986090. As ASKP-1240, CFZ533 and BMS-986090 are Fc effector silent antibodies, the observed agonism of these antibodies may be epitope-dependent. C40B16 on the contrary is a wild-type IgG1 and therefore possesses neither epitope-dependent nor Fc-dependent agonism. Further, Fc engineered effector silent antibodies C40B176, C40B179, C40B180 and C40B183 of the invention demonstrate only minimal agonism but also up to 10-fold improved potency when compared to Antibody D.

The invention provides for an isolated antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 5, a HCDR2 of SEQ ID NO: 61, a HCDR3 of SEQ ID NO: 62, a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 63, a LCDR2 of SEQ ID NO: 9 and a LCDR3 of SEQ ID NO: 10.

SEQ ID NOs: 61, 62 and 63 represent the HCDR2, the HCDR3 and the LCDR1, genus sequences of antagonistic antibodies specifically binding CD40, the genera encompassing variants of a parental antibody C40B16 in which putative sites for post-translational modifications have been mutated. The antibodies within the genus are expected to display no shift in epitope, e.g. the antibodies comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 5, 61, 62, 63, 9 and 10 are expected to have similar characteristics when compared to the parental

C40B16 antibody. Exemplary such antibodies are antibodies comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 or the VH and the VL amino acid sequences of antibodies C40M141, C40M152, C40M142, C40M153, C40M144, C40M155, C40M148, C40M194, C40M198, C40M197, C40M201 or C40M126 as shown in **Table 2** and **Table 7**.

SEQ ID NO: 61 Genus HCDR2 sequence:

TIX₁X₂X₃GGGTYTADSVKG; wherein

X₁ is N, D or Q;

X₂ is N, Q or A; and

X₃ is S or A.

SEQ ID NO: 62 genus HCDR3 sequence

EGGKYYYYAX₁DV; wherein

X₁ is M or L

SEQ ID NO: 63 genus LCDR1 sequence

SGDKLGDKYAX₁; wherein

X₁ is C or A.

Table 2.

mAb	SEQ ID NO:					
	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
C40B16	5	6	7	8	9	10
C40B124	5	15	7	20	9	10
C40B135	5	15	7	20	9	10
C40B125	5	16	7	20	9	10
C40B136	5	16	7	20	9	10
C40B127	5	17	7	20	9	10
C40B138	5	17	7	20	9	10
C40B131	5	18	7	20	9	10
C40B176	5	18	19	20	9	10
C40B180	5	18	19	20	9	10
C40B179	5	17	19	20	9	10
C40B183	5	17	19	20	9	10

In some embodiments, the antibody competes for binding to human CD40 of SEQ ID NO: 1 with an antibody comprising

a heavy chain variable region (VH) of SEQ ID NO: 11 and a light chain variable region (VL) of SEQ ID NO: 12;

the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27, or

the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody binds to the same epitope on human CD40 of SEQ ID NO: 1 to which the antibody comprising

a heavy chain variable region (VH) of SEQ ID NO: 11 and a light chain variable region (VL) of SEQ ID NO: 12;

the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27, or

the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 binds to.

In some embodiments, the antibody binds human CD40 with a dissociation constant (K_D) of about 1.5×10^{-10} M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.01% polysorbate 20 (PS-20) and 100 µg/ml bovine serum albumin.

In some embodiments, the antibody inhibits soluble human CD40L-driven human tonsillar B cell proliferation with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody inhibits soluble human CD40L-driven production of IL-12p40 by human dendritic cells with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody binds human CD40 with a dissociation constant (K_D) of about 1.5×10^{-10} M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.01% PS-20 and 100 µg/ml bovine serum albumin, inhibits soluble human CD40L-driven human tonsillar B cell proliferation with an IC_{50} value of less than about 1×10^{-9} M and inhibits soluble human CD40L-driven production of IL-12p40 by human dendritic cells with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody is an IgG1, IgG2, IgG3 or IgG4 isotype.

In some embodiments, the antibody is an IgG1 isotype.

In some embodiments, the antibody is an IgG2 isotype.

In some embodiments, the antibody is an IgG3 isotype.

In some embodiments, the antibody is an IgG4 isotype.

In some embodiments, the antibody is an IgG1 isotype, optionally comprising a L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody is an IgG1/ λ isotype, optionally comprising the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody is an IgG1/ λ isotype comprising the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody is an IgG4 isotype, optionally comprising a S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4/ λ isotype, optionally comprising the S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4/ λ isotype comprising the S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4 isotype, optionally comprising a S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4/ λ isotype, optionally comprising the S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4/ λ isotype comprising the S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is a multispecific antibody, such as a bispecific antibody.

The antibody is suitable for use in therapy, for example in treating an inflammatory disease.

The antibody is suitable for use in therapy, for example in treating an autoimmune disease.

The antibody is suitable for use in therapy, for example in treating Addison's disease.

The antibody is suitable for use in therapy, for example in treating an ankylosing spondylitis.

The antibody is suitable for use in therapy, for example in treating an atherosclerosis.

The antibody is suitable for use in therapy, for example in treating an autoimmune hepatitis.

The antibody is suitable for use in therapy, for example in treating an autoimmune diabetes.

The antibody is suitable for use in therapy, for example in treating Graves' disease.

The antibody is suitable for use in therapy, for example in treating Guillain-Barre syndrome.

The antibody is suitable for use in therapy, for example in treating Hashimoto's disease.

The antibody is suitable for use in therapy, for example in treating, an idiopathic thrombocytopenia.

The antibody is suitable for use in therapy, for example in treating an inflammatory bowel disease (IBD).

The antibody is suitable for use in therapy, for example in treating a systemic lupus erythematosus.

The antibody is suitable for use in therapy, for example in treating a multiple sclerosis.

The antibody is suitable for use in therapy, for example in treating a myasthenia gravis.

The antibody is suitable for use in therapy, for example in treating a psoriasis.

The antibody is suitable for use in therapy, for example in treating an arthritis.

The antibody is suitable for use in therapy, for example in treating a scleroderma.

The antibody is suitable for use in therapy, for example in treating Sjogren's syndrome.

The antibody is suitable for use in therapy, for example in treating a systemic sclerosis.

The antibody is suitable for use in therapy, for example in treating a transplantation.

The antibody is suitable for use in therapy, for example in treating a kidney transplantation.

The antibody is suitable for use in therapy, for example in treating a skin transplantation.

The antibody is suitable for use in therapy, for example in treating a bone marrow transplantation.

The antibody is suitable for use in therapy, for example in treating a graft versus host disease (GVHD).

The antibody is suitable for use in therapy, for example in treating a type I diabetes.

The antibody is suitable for use in therapy, for example in treating a rheumatoid arthritis.

The antibody is suitable for use in therapy, for example in treating a juvenile arthritis.

The antibody is suitable for use in therapy, for example in treating a psoriatic arthritis.

The antibody is suitable for use in therapy, for example in treating Reiter's syndrome.

The antibody is suitable for use in therapy, for example in treating a gouty arthritis.

The antibody is suitable for use in therapy, for example in treating Crohn's disease.

The antibody is suitable for use in therapy, for example in treating an ulcerative colitis.

The antibody is suitable for use in therapy, for example in treating an inflammatory disease in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating an autoimmune disease in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating a rheumatoid arthritis in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating a systemic lupus erythematosus in combination with a second therapeutic agent.

The invention also provides for an isolated antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising

the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 5, 6, 7, 8, 9 and 10, respectively.

In some embodiments, the antibody comprises the VH and the VL of SEQ ID NOs: 11 and 12, respectively.

In some embodiments, the VH is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 13 and the VL is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 14.

In some embodiments, the antibody comprises a heavy chain of SEQ ID NO: 35 and a light chain of SEQ ID NO: 47.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 65 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 77.

The invention also provides for an isolated antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 5, 15, 7, 20, 9 and 10, respectively.

In some embodiments, the antibody comprises the VH and the VL of SEQ ID NOs: 21 and 27, respectively.

In some embodiments, the VH is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 28 and the VL is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 34.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 36 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 66 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 37 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 67 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

The invention also provides for an isolated antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 5, 16, 7, 20, 9 and 10, respectively.

In some embodiments, the antibody comprises the VH and the VL of SEQ ID NOs: 22 and 27, respectively.

In some embodiments, the VH is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 29 and the VL is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 34.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 38 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 68 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 39 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 69 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

The invention also provides for an isolated antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 5, 17, 7, 20, 9 and 10, respectively.

In some embodiments, the antibody comprises the VH and the VL of SEQ ID NOs: 23 and 27, respectively.

In some embodiments, the VH is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 30 and the VL is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 34.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 40 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 70 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 41 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 71 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

The invention also provides for an isolated antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 5, 18, 7, 20, 9 and 10, respectively.

In some embodiments, the antibody comprises the VH and the VL of SEQ ID NOs: 24 and 27, respectively.

In some embodiments, the VH is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 31 and the VL is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 34.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 42 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 72 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

The invention also provides for an isolated antagonistic or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 5, 18, 19, 20, 9 and 10, respectively.

In some embodiments, the antibody competes for binding to human CD40 of SEQ ID NO: 1 with an antibody comprising

a heavy chain variable region (VH) of SEQ ID NO: 11 and a light chain variable region (VL) of SEQ ID NO: 12;

the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27, or

the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody binds to the same epitope on human CD40 of SEQ ID NO: 1 to which the antibody comprising

a heavy chain variable region (VH) of SEQ ID NO: 11 and a light chain variable region (VL) of SEQ ID NO: 12;

the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27, or

the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 binds to.

In some embodiments, the antibody binds human CD40 with a dissociation constant (K_D) of about 1.5×10^{-10} M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.01% PS-20 and 100 µg/ml bovine serum albumin.

In some embodiments, the antibody inhibits soluble human CD40L-driven human tonsillar B cell proliferation with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody inhibits soluble human CD40L-driven production of IL-12p40 by human dendritic cells with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody binds human CD40 with a dissociation constant (K_D) of about 1.5×10^{-10} M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.01% PS-20 and 100 µg/ml bovine serum albumin, inhibits soluble human CD40L-driven human tonsillar B cell proliferation with an IC_{50} value of less than about 1×10^{-9} M and inhibits soluble human CD40L-driven production of IL-12p40 by human dendritic cells with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody comprises the VH and the VL of SEQ ID NOs: 25 and 27, respectively.

In some embodiments, the VH is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 32 and the VL is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 34.

In some embodiments, the antibody is an IgG1, IgG2, IgG3 or IgG4 isotype.

In some embodiments, the antibody is an IgG1 isotype.

In some embodiments, the antibody is an IgG2 isotype.

In some embodiments, the antibody is an IgG3 isotype.

In some embodiments, the antibody is an IgG4 isotype.

In some embodiments, the antibody is an IgG1 isotype, optionally comprising a L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody is an IgG1/ λ isotype, optionally comprising the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27 and is an IgG1/ λ isotype, optionally comprising the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27 and is an IgG1/ λ isotype comprising the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody is an IgG4 isotype, optionally comprising a S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4/ λ isotype, optionally comprising the S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27 and is an IgG4/ λ isotype, optionally comprising the S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27 and is an IgG4/ λ isotype comprising the S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4 isotype, optionally comprising a S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4/ λ isotype, optionally comprising the S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27 and is an IgG4/ λ isotype, optionally comprising the S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27 and is an IgG4/ λ isotype comprising the S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 43 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 73 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 44 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 74 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

In some embodiments, the antibody is a multispecific antibody, such as a bispecific antibody.

The antibody is suitable for use in therapy, for example in treating an inflammatory disease.

The antibody is suitable for use in therapy, for example in treating an autoimmune disease.

The antibody is suitable for use in therapy, for example in treating Addison's disease.

The antibody is suitable for use in therapy, for example in treating ankylosing spondylitis.

The antibody is suitable for use in therapy, for example in treating an atherosclerosis.

The antibody is suitable for use in therapy, for example in treating an autoimmune hepatitis.

The antibody is suitable for use in therapy, for example in treating an autoimmune diabetes.

The antibody is suitable for use in therapy, for example in treating Graves' disease.

The antibody is suitable for use in therapy, for example in treating Guillain-Barre syndrome.

The antibody is suitable for use in therapy, for example in treating Hashimoto's disease.

The antibody is suitable for use in therapy, for example in treating, an idiopathic thrombocytopenia.

The antibody is suitable for use in therapy, for example in treating an inflammatory bowel disease (IBD).

The antibody is suitable for use in therapy, for example in treating a systemic lupus erythematosus.

The antibody is suitable for use in therapy, for example in treating a multiple sclerosis.

The antibody is suitable for use in therapy, for example in treating a myasthenia gravis.

The antibody is suitable for use in therapy, for example in treating a psoriasis.

The antibody is suitable for use in therapy, for example in treating an arthritis.

The antibody is suitable for use in therapy, for example in treating a scleroderma.

The antibody is suitable for use in therapy, for example in treating Sjogren's syndrome.

The antibody is suitable for use in therapy, for example in treating a systemic sclerosis.

The antibody is suitable for use in therapy, for example in treating a transplantation.

The antibody is suitable for use in therapy, for example in treating a kidney transplantation.

The antibody is suitable for use in therapy, for example in treating a skin transplantation.

The antibody is suitable for use in therapy, for example in treating a bone marrow transplantation.

The antibody is suitable for use in therapy, for example in treating a graft versus host disease (GVHD).

The antibody is suitable for use in therapy, for example in treating a type I diabetes.

The antibody is suitable for use in therapy, for example in treating a rheumatoid arthritis.

The antibody is suitable for use in therapy, for example in treating a juvenile arthritis.

The antibody is suitable for use in therapy, for example in treating a psoriatic arthritis.

The antibody is suitable for use in therapy, for example in treating Reiter's syndrome.

The antibody is suitable for use in therapy, for example in treating a gouty arthritis.

The antibody is suitable for use in therapy, for example in treating Crohn's disease.

The antibody is suitable for use in therapy, for example in treating an ulcerative colitis.

The antibody is suitable for use in therapy, for example in treating an inflammatory disease, in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating an autoimmune disease, in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating a rheumatoid arthritis in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating a systemic lupus erythematosus in combination with a second therapeutic agent.

The invention also provides for an isolated antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 5, 17, 19, 20, 9 and 10, respectively.

In some embodiments, the antibody competes for binding to human CD40 of SEQ ID NO: 1 with an antibody comprising

a heavy chain variable region (VH) of SEQ ID NO: 11 and a light chain variable region (VL) of SEQ ID NO: 12;

the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27, or

the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody binds to the same epitope on human CD40 of SEQ ID NO: 1 to which the antibody comprising

a heavy chain variable region (VH) of SEQ ID NO: 11 and a light chain variable region (VL) of SEQ ID NO: 12;

the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27, or

the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 binds to.

In some embodiments, the antibody binds human CD40 with a dissociation constant (K_D) of about 1.5×10^{-10} M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.01% PS-20 and 100 µg/ml bovine serum albumin.

In some embodiments, the antibody inhibits soluble human CD40L-driven human tonsillar B cell proliferation with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody inhibits soluble human CD40L-driven production of IL-12p40 by human dendritic cells with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody binds human CD40 with a dissociation constant (K_D) of about 1.5×10^{-10} M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.01% PS-20 and 100 µg/ml bovine serum albumin, inhibits soluble human CD40L-driven human tonsillar B cell proliferation with an IC_{50} value of less than about 1×10^{-9} M and inhibits soluble human CD40L-driven production of IL-12p40 by human dendritic cells with an IC_{50} value of less than about 1×10^{-9} M.

In some embodiments, the antibody comprises the VH and the VL of SEQ ID NOs: 26 and 27, respectively.

In some embodiments, the VH is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 33 and the VL is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 34.

In some embodiments, the antibody is an IgG1, IgG2, IgG3 or IgG4 isotype.

In some embodiments, the antibody is an IgG1 isotype.

In some embodiments, the antibody is an IgG2 isotype.

In some embodiments, the antibody is an IgG3 isotype.

In some embodiments, the antibody is an IgG4 isotype.

In some embodiments, the antibody is an IgG1 isotype, optionally comprising a L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody is an IgG1/ λ isotype, optionally comprising the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 and is an IgG1/ λ isotype, optionally comprising the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 and is an IgG1/ λ isotype comprising the L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation when compared to the wild-type IgG1.

In some embodiments, the antibody is an IgG4 isotype, optionally comprising a S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4/ λ isotype, optionally comprising the S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 and is an IgG4/ λ isotype, optionally comprising the S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 and is an IgG4/ λ isotype comprising the S228P mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4 isotype, optionally comprising a S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody is an IgG4/ λ isotype, optionally comprising the S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 and is an IgG4/ λ isotype, optionally comprising the S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 and is an IgG4/ λ isotype comprising the S228P/F234A/L235A mutation when compared to the wild-type IgG4.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 45 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 75 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

In some embodiments, the antibody comprises the heavy chain of SEQ ID NO: 46 and the light chain of SEQ ID NO: 48.

In some embodiments, the heavy chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 76 and the light chain is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 78.

In some embodiments, the antibody is a multispecific antibody, such as a bispecific antibody.

The antibody is suitable for use in therapy, for example in treating an inflammatory disease.

The antibody is suitable for use in therapy, for example in treating an autoimmune disease.

The antibody is suitable for use in therapy, for example in treating Addison's disease.

The antibody is suitable for use in therapy, for example in treating ankylosing spondylitis.

The antibody is suitable for use in therapy, for example in treating an atherosclerosis.

The antibody is suitable for use in therapy, for example in treating an autoimmune hepatitis.

The antibody is suitable for use in therapy, for example in treating an autoimmune diabetes.

The antibody is suitable for use in therapy, for example in treating Graves' disease.

The antibody is suitable for use in therapy, for example in treating Guillain-Barre syndrome.

The antibody is suitable for use in therapy, for example in treating Hashimoto's disease.

The antibody is suitable for use in therapy, for example in treating, an idiopathic thrombocytopenia.

The antibody is suitable for use in therapy, for example in treating an inflammatory bowel disease (IBD).

The antibody is suitable for use in therapy, for example in treating a systemic lupus erythematosus.

The antibody is suitable for use in therapy, for example in treating a multiple sclerosis.

The antibody is suitable for use in therapy, for example in treating a myasthenia gravis.

The antibody is suitable for use in therapy, for example in treating a psoriasis.

The antibody is suitable for use in therapy, for example in treating an arthritis.

The antibody is suitable for use in therapy, for example in treating a scleroderma.

The antibody is suitable for use in therapy, for example in treating Sjogren's syndrome.

The antibody is suitable for use in therapy, for example in treating a systemic sclerosis.

The antibody is suitable for use in therapy, for example in treating a transplantation.

The antibody is suitable for use in therapy, for example in treating a kidney transplantation.

The antibody is suitable for use in therapy, for example in treating a skin transplantation.

The antibody is suitable for use in therapy, for example in treating a bone marrow transplantation.

The antibody is suitable for use in therapy, for example in treating a graft versus host disease (GVHD).

The antibody is suitable for use in therapy, for example in treating a type I diabetes.

The antibody is suitable for use in therapy, for example in treating a rheumatoid arthritis.

The antibody is suitable for use in therapy, for example in treating a juvenile arthritis.

The antibody is suitable for use in therapy, for example in treating a psoriatic arthritis.

The antibody is suitable for use in therapy, for example in treating Reiter's syndrome.

The antibody is suitable for use in therapy, for example in treating a gouty arthritis.

The antibody is suitable for use in therapy, for example in treating Crohn's disease.

The antibody is suitable for use in therapy, for example in treating an ulcerative colitis.

The antibody is suitable for use in therapy, for example in treating an inflammatory disease in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating an autoimmune disease in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating a rheumatoid arthritis in combination with a second therapeutic agent.

The antibody is suitable for use in therapy, for example in treating a systemic lupus erythematosus in combination with a second therapeutic agent.

Competition between binding to human CD40 with antibodies of the invention comprising certain VH and VL sequences may be assayed *in vitro* using following protocol: His-tagged recombinant soluble human CD40 (CD40-ECD-his) is used in the assay. 5 μ L of anti-his mAb (10 μ g/mL, R&D Systems, MAB050) is directly coated on MSD HighBind plates for 2 hours at room temperature and then blocked with 5% MSD Blocker A buffer for an additional 2 hours at room temperature. 25 μ L of 10 μ g/mL CD40-ECD-his protein is added to be captured by anti-his mAb. After incubation with gentle shaking at room temperature 2 hours, plates are washed 3x with 0.1 M HEPES buffer, pH 7.4, followed by the addition of the mixture of 10 nM Ruthenium (Ru)-labeled reference anti-CD40 mAb or a Fab portion thereof which is pre-incubated at room temperature for 30 minutes with different concentrations, from 2 μ M to 1 nM, of a test anti-CD40 antibody. After incubation with gentle shaking at room temperature 1 hours, plates are washed 3x

with 0.1M HEPES buffer (pH 7.4). MSD Read Buffer T is diluted with distilled water (4-fold) and dispensed into each well then analyzed with a SECTOR Imager 6000 (Meso Scale Discovery, Gaithersburg, MD). The test antibody competes for binding to human CD40 with a reference antibody (e.g. an antibody comprising the VH of SEQ ID NO: 11 and the VL of SEQ ID NO: 12, the antibody comprising the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27 or the antibody comprising the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27) when the test antibody reduces the MDS signal obtained in the above assay using the Ru-labeled reference antibody or a Fab portion thereof by more than 90%. Antibodies that compete for binding to CD40 with an antibody comprising the VH of SEQ ID NO: 11 and the VL of SEQ ID NO: 12, the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27 or the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27 may be generated by isolating antibodies specifically binding human CD40 using phage display libraries, and screening the generated antibodies for their ability to compete for binding to CD40 with the aforementioned antibodies.

The CD40 epitope the antibody of the invention binds to may be resolved for example using hydrogen/deuterium exchange (H/D exchange) or by analyzing a crystal structure of the antibody in complex with CD40. Two CD40 antibodies “bind the same epitope on CD40” when 80% or more CD40 amino acid residues protected by the antibody by at least 5% difference in deuteration levels through H/D exchange are identical between the two antibodies, or when 80% or more CD40 surface exposed amino acid residues determined to bind the antibody in a crystal structure of a complex of the antibody and CD40 are identical between the two antibodies. In the crystal structure of a complex of the antibody and CD40, the epitope residues are those CD40 residues that reside within 4 Å distance or less from any of the antibody CDR residues.

In an H/D exchange assay, CD40 protein is incubated in the presence or absence of the antibody in deuterated water for predetermined times resulting in deuterium incorporation at exchangeable hydrogen atoms which are unprotected by the antibody, followed by protease digestion of the protein and analyses of the peptide fragments using LC-MS. In an exemplary assay, 5 µL of the test antibody (10 µg) or 5 µL of the complex of CD40 and the test antibody (10 & 7.35 µg, respectively) is incubated with 120 µL deuterium oxide labeling buffer (50mM phosphate, 100mM sodium chloride at pH 7.4) for 0 sec, 60 sec, 300 sec, 1800 sec, 7200 sec, and 14400 sec. Deuterium exchange is

quenched by adding 63 μ L of 5 M guanidine hydrochloride and final pH is 2.5. The quenched sample is subjected to on-column pepsin/protease type XIII digestion and LC-MS analysis. For pepsin/protease type XIII digestion, 5 μ g of the samples in 125 μ L control buffer (50mM phosphate, 100mM sodium chloride at pH 7.4) are denatured by adding 63 μ L of 5 M guanidine hydrochloride (final pH is 2.5) and incubating the mixture for 3 min. Then, the mixture is subjected to on-column pepsin/protease type XIII digestion and the resultant peptides analyzed using an UPLC-MS system comprised of a Waters Acquity UPLC coupled to a Q ExactiveTM Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo). Raw MS data is processed using HDX WorkBench, software for the analysis of H/D exchange MS data. The deuterium levels are calculated using the average mass difference between the deuteriated peptide and its native form (t_0). Peptide identification is done through searching MS/MS data against the CD40 sequence with Mascot. The mass tolerance for the precursor and product ions is 20 ppm and 0.05 Da, respectively.

For X-ray crystallography, CD40 and the test antibody are expressed and purified using standard protocols. The CD40/test antibody complex is incubated overnight at 4°C, concentrated, and separated from the uncomplexed species using size-exclusion chromatography. The complex is crystallized by the vapor-diffusion method from various known test solutions for example solutions containing PEG3350, ammonium citrate and 2-(N-morpholino)ethanesulfonic acid (MES).

Antibodies binding the same epitope on CD40 as a reference antibody may be generated by isolating antibodies binding CD40 using phage display libraries, selecting those antibodies that compete with the reference antibody for binding to CD40 by 100%, and identifying the antibody epitope by H/D exchange or by X-ray crystallography.

Alternatively, mice or rabbits may be immunized using peptides encompassing the epitope residues, and the generated antibodies may be evaluated for their binding within the recited region.

The affinity of an antibody to human or cyno CD40 may be determined experimentally using any suitable method. An exemplary method utilizes ProteOn XPR36, Biacore 3000 or KinExA instrumentation, ELISA or competitive binding assays known to those skilled in the art. The measured affinity of a particular antibody to CD40 may vary if measured under different conditions (e.g., osmolarity, pH). Thus, measurements of affinity and other binding parameters (e.g., K_D , K_{on} , and K_{off}) are typically made with standardized

conditions and a standardized buffer, such as the buffer described herein. Skilled in the art will appreciate that the internal error for affinity measurements for example using Biacore 3000 or ProteOn (measured as standard deviation, SD) can typically be within 5-33% for measurements within the typical limits of detection. Therefore the term “about” reflects the typical standard deviation in the assay. For example, the typical SD for a K_D of 1×10^{-9} M is up to $\pm 0.33 \times 10^{-9}$ M.

In the B cell proliferation assay, 1×10^5 human tonsil B cells may be cultured in RPMI medium containing glutamax, 10% FBS and 1% Pen/Strep, titrations of each of the antibodies specifically binding human CD40 may be added to the cells, followed by addition of 0.5 $\mu\text{g/ml}$ soluble human CD154. Cells may be cultured for 48 hours at 37°C , pulsed with 3H-thymidine (1 $\mu\text{Ci/well}$) in 50 μl medium and cultured for 16-18 hours before harvest and counting.

In the DC IL-12p40 production assay, human DCs may be generated by culturing purified human monocytes ($2.5 \times 10^6/\text{well}$ 6-well plates) for 6 days in 3ml RPMI medium containing glutamax, 25mM HEPES, 10% FBS, 1% Pen/Strep and 50ng each GM-CSF and IL-4. On day 3, 1ml of the medium may be removed and replaced with 2ml fresh medium containing 50 ng/ml each of GM-CSF and IL-4. On day 6, DCs may be plated into 96-well plates (100,000 cells/well) followed by titrations of each of the antibodies specifically binding human CD40 and addition of 1 $\mu\text{g/ml}$ of soluble human CD154 to the cultures. Cells may be cultured for 48 hours before collecting and analyzing supernatants for IL-12p40 by MSD.

Immune effector properties of the antibodies of the invention may be enhanced or silenced through Fc modifications. For example, Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. may be provided and/or controlled by modifying residues in the Fc responsible for these activities.

In some embodiments, the antibody of the invention has reduced binding to Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIIa or Fc γ RIIIb.

“Reduced binding” refers to reduced binding of the antibody of the invention having at least one mutation in the Fc region to an Fc γ receptor (Fc γ R) when compared to the binding of the parental antibody without the mutation to the same Fc γ R. “Reduced

binding” may be at least about 100-fold, at least about 500-fold, at least about 1000-fold, at least about 5000-fold, at least about 10,000-fold, or at least about 20,000-fold reduced binding. In practice, antibodies exhibiting “reduced binding” to a particular FcγR refer to antibodies that have statistically insignificant effector function mediated by the particular FcγR.

In some embodiments, the antibody of the invention comprises at least one mutation in the Fc region that reduces binding of the antibody to an FcγR.

In some embodiments, the FcγR is FcγRI, FcγRIIa or FcγRIIIa or FcγRIIIb.

In some embodiments, the at least one mutation in the Fc region is a L234A mutation, a L235A mutation, a G237A mutation, a P238S mutation, a M252Y mutation, a S254T mutation, a T256E mutation, a H268A mutation, a A330S mutation or a P331S mutation, wherein residue numbering is according to the EU Index.

In some embodiments, the antibody of the invention comprises a L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation in the Fc region, wherein residue numbering is according to the EU Index.

In some embodiments, the at least one mutation in the Fc region is a V234A mutation, a G237A mutation, a P238S mutation, a M252Y mutation, a S254T mutation, a T256E mutation, a H268A mutation, a V309L mutation, an A330S mutation or a P331S mutation, wherein residue numbering is according to the EU Index.

In some embodiments, the antibody of the invention comprises a V234A/G237A/P238S/H268A/V309L/A330S/P331S mutation in the Fc region, wherein residue numbering is according to the EU Index.

In some embodiments, the antibody of the invention comprises a S228P mutation in the Fc region, wherein residue numbering is according to the EU Index.

In some embodiments, the antibody of the invention comprises a F234A mutation in the Fc region, wherein residue numbering is according to the EU Index.

In some embodiments, the antibody of the invention comprises a L235A mutation in the Fc region, wherein residue numbering is according to the EU Index.

In some embodiments, the antibody of the invention comprises a S228P/F234A/L235A mutation, wherein residue numbering is according to the EU Index.

Binding of the antibodies of the invention to FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa and FcγRIIIb may be evaluated using recombinant soluble forms or cell-associated forms

of the Fc γ receptors. For example, direct or indirect, e.g., competitive binding, measurements may be applied for assessing relative affinities and avidities of the antibodies of the invention to various Fc γ R. In an exemplary assay, a test antibody binding to soluble Fc γ R captured on a plate is evaluated using competitive binding between 1 μ g/ml biotinylated human IgG1 and serial dilutions of the test antibody pre-complexed with antigen.

In some embodiments, the antibody of the invention comprising at least one mutation in the Fc region has reduced antibody dependent cellular cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis, ("ADCP") and/or reduced complement dependent cytotoxicity (CDC).

"Antibody-dependent cellular cytotoxicity", "antibody-dependent cell-mediated cytotoxicity" or "ADCC" is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells with effector cells possessing lytic activity, such as natural killer cells, monocytes, macrophages and neutrophils via Fc gamma receptors (Fc γ R) expressed on effector cells. For example, NK cells express Fc γ RIIIa, whereas monocytes express Fc γ RI, Fc γ RII and Fc γ RIIIa. To assess ADCC activity of the antibodies of the invention, the antibody may be added to target cells in combination with immune effector cells, which may be activated by the antigen antibody complexes resulting in cytolysis of the target cell. Cytolysis is generally detected by the release of label (e.g. radioactive substrates, fluorescent dyes or natural intracellular proteins) from the lysed cells. Exemplary effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Exemplary target cells include cells expressing CD40.

"Antibody-dependent cellular phagocytosis" ("ADCP") refers to a mechanism of elimination of antibody-coated target cells by internalization by phagocytic cells, such as macrophages or dendritic cells. ADCP may be evaluated using monocyte-derived macrophages as effector cells and cells expressing CD40 engineered to express GFP or other labeled molecule as target cells. Effector:target cell ratio may be for example 4:1. Effector cells may be incubated with target cells for 4 hours with or without the test CD40 antibody. After incubation, cells may be detached using accutase. Macrophages may be identified with anti-CD11b and anti-CD14 antibodies coupled to a fluorescent label, and percent phagocytosis may be determined based on % GFP fluorescent in the CD11⁺CD14⁺ macrophages using standard methods.

“Complement-dependent cytotoxicity”, or “CDC”, refers to a mechanism for inducing cell death in which an Fc effector domain of a target-bound antibody binds and activates complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement components on the target cell surface that facilitate ADCC by binding complement receptors (e.g., CR3) on leukocytes. CDC of CD40-expressing cells may be measured for example by plating cells expressing CD40 in an appropriate medium, adding anti-CD40 antibodies into the mixture, followed by addition of pooled human serum. After incubation period, percentage (%) lysed cells may be detected as % propidium iodide stained cells in FACS assay using standard methods.

“Reduced ADCC”, “reduced CDC” and “reduced ADCP” refers to a statistically significant reduction in ADCC, CDC and/or ADCP mediated by the antibody of the invention comprising at least one mutation in the Fc region when compared to the same antibody without the mutation. ADCC, CDC and/or ADCP, such as assays described herein and in assays described in U.S. Pat. No. 8,871,204.

Variants of the antibodies of the invention comprising the VH or the VL amino acid sequences shown in **Table 7** are within the scope of the invention. For example, variants may comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen amino acid substitutions in the VH and/or the VL that do not adversely affect the characteristics of the antibodies. In some embodiments, the sequence identity may be about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to the antibody VH or the VL amino acid sequence of the invention.

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions × 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The percent identity between two amino acid sequences may be determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences may be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the

GAP program in the GCG software package (available at http://_www_gcg_com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

In some embodiments, the antibody of the invention comprises the VH that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH of SEQ ID NOs: 11, 21, 22, 23, 24, 25 or 26 and the VL that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VL of SEQ ID NOs: 12 or 27, wherein the antibody exhibits one or more of the following properties:

inhibits 0.5 µg/ml human soluble CD40L-driven human tonsillar B cell proliferation with an IC₅₀ value of less than about 1x10⁻⁹ M;

inhibits 1 µg/ml human soluble CD40L-driven production of IL-12p40 by human dendritic cells with an IC₅₀ value of less than about 1x10⁻⁹ M; or

binds to human CD40 with a dissociation constant (K_D) of about 5x10⁻⁹ M or less, when the K_D is measured using ProteOn XPR36 system using experimental design described in Example 4.

In some embodiments, the antibody of the invention comprises the VH and the VL which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH of SEQ ID NO: 11 and the VL of SEQ ID NO: 12.

In some embodiments, the antibody of the invention comprises the VH and the VL which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody of the invention comprises the VH and the VL which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH of SEQ ID NO: 22 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody of the invention comprises the VH and the VL which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody of the invention comprises the VH and the VL which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH of SEQ ID NO: 24 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody of the invention comprises the VH and the VL which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody of the invention comprises the VH and the VL which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27.

In some embodiments, the antibody of the invention comprises the VH of SEQ ID NO: 11 and the VL of SEQ ID NO: 12, wherein the VH, the VL or both the VH and the VL comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen conservative amino acid substitutions and the antibody exhibits one or more of the following properties:

inhibits 0.5 µg/ml human soluble CD40L-driven human tonsillar B cell proliferation with an IC_{50} value of less than about 1×10^{-9} M;

inhibits 1 µg/ml human soluble CD40L-driven production of IL-12p40 by human dendritic cells with an IC_{50} value of less than about 1×10^{-9} M; or

binds to human CD40 with a dissociation constant (K_D) of about 5×10^{-9} M or less, when the K_D is measured using ProteOn XPR36 system using experimental design described in Example 4.

In some embodiments, the antibody of the invention comprises the VH of SEQ ID NO: 11 and the VL of SEQ ID NO: 12, wherein the VH, the VL or both the VH and the VL comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen conservative amino acid substitutions.

In some embodiments, the antibody of the invention comprises the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 27, wherein the VH, the VL or both the VH and the VL comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen conservative amino acid substitutions.

In some embodiments, the antibody of the invention comprises the VH of SEQ ID NO: 22 and the VL of SEQ ID NO: 27, wherein the VH, the VL or both the VH and the VL comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen conservative amino acid substitutions.

In some embodiments, the antibody of the invention comprises the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 27, wherein the VH, the VL or both the VH and the

VL comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen conservative amino acid substitutions.

In some embodiments, the antibody of the invention comprises the VH of SEQ ID NO: 24 and the VL of SEQ ID NO: 27, wherein the VH, the VL or both the VH and the VL comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen conservative amino acid substitutions.

In some embodiments, the antibody of the invention comprises the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27, wherein the VH, the VL or both the VH and the VL comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen conservative amino acid substitutions.

In some embodiments, the antibody of the invention comprises the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27, wherein the VH, the VL or both the VH and the VL comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen conservative amino acid substitutions.

“Conservative modification” refers to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequences. Conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are those in which the amino acid is replaced with an amino acid residue having a similar side chain. The families of amino acid residues having similar side chains are well defined and include amino acids with acidic side chains (e.g., aspartic acid, glutamic acid), basic side chains (e.g., lysine, arginine, histidine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), uncharged polar side chains (e.g., glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine, tryptophan), aromatic side chains (e.g., phenylalanine, tryptophan, histidine, tyrosine), aliphatic side chains (e.g., glycine, alanine, valine, leucine, isoleucine, serine, threonine), amide (e.g., asparagine, glutamine), beta-branched side chains (e.g., threonine, valine, isoleucine) and sulfur-containing side chains (cysteine, methionine). Furthermore, any native residue in the polypeptide may be substituted with alanine, as has been previously described for alanine scanning mutagenesis (MacLennan *et al.*, *Acta Physiol. Scand. Suppl.* 643:55-67, 1998; Sasaki *et al.*, *Adv. Biophys.* 35:1-24, 1998). Amino acid substitutions to the antibodies of the invention may be made by well-known methods for example by PCR mutagenesis (US Pat.

No. 4,683,195). Alternatively, libraries of variants may be generated using known methods, for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp). The resulting antibody variants may be tested for their characteristics using assays described herein.

Although the embodiments illustrated in the Examples comprise pairs of variable regions, one from a heavy chain and one from a light chain, a skilled artisan will recognize that alternative embodiments may comprise single heavy or light chain variable regions. The single variable region may be used to screen for variable domains capable of forming a two-domain specific antigen-binding fragment capable of for example specifically binding to human CD40. The screening may be accomplished by phage display screening methods using for example hierarchical dual combinatorial approach disclosed in Int. Pat. Publ. No. WO1992/01047.

Antibodies of the invention may be generated using various technologies. For example, the hybridoma method of Kohler and Milstein, *Nature* 256:495, 1975 may be used to generate monoclonal antibodies. In the hybridoma method, a mouse or other host animal, such as a hamster, rat, rabbit or monkey, is immunized with human, marmoset or cyno CD40 or fragments of CD40, such as soluble form of CD40, followed by fusion of spleen cells from immunized animals with myeloma cells using standard methods to form hybridoma cells (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Colonies arising from single immortalized hybridoma cells are screened for production of antibodies with desired properties, such as specificity of binding, cross-reactivity or lack thereof, and affinity for the antigen.

Various host animals may be used to produce the antibodies of the invention. For example, Balb/c mice may be used to generate antibodies. The antibodies made in Balb/c mice and other non-human animals may be humanized using various technologies to generate more human-like sequences. Exemplary humanization techniques including selection of human acceptor frameworks are known and include CDR grafting (U.S. Pat. No. 5,225,539), SDR grafting (U.S. Pat. No. 6,818,749), Resurfacing (Padlan, *Mol Immunol* 28:489-499, 1991), Specificity Determining Residues Resurfacing (U.S. Pat. Publ. No. 20100261620), human-adaptation (or human framework adaptation) (U.S. Pat. Publ. No. US2009/0118127), Superhumanization (U.S. Pat. No. 7,709, 226) and guided

selection (Osbourn *et al* (2005) *Methods* 36:61-68, 2005; U.S. Pat. No. 5,565,332). In these methods, CDRs of parental antibodies are transferred onto human frameworks that may be selected based on their overall homology to the parental frameworks, based on framework CDR length, homology or canonical structure information, or a combination thereof.

Humanized antibodies may be further optimized to improve their selectivity or affinity to a desired antigen by incorporating altered framework support residues to preserve binding affinity (backmutations) by techniques such as those disclosed as described in Int. Pat. Publ. No. WO90/007861 and in Int. Pat. Publ. No. WO92/22653, or by introducing variation to any of the CDRs to improve for example affinity of the antibody.

Transgenic mice carrying human immunoglobulin (Ig) loci in their genome may be used to generate human antibodies against a target protein, and are described in for example Int. Pat. Publ. No. WO90/04036, U.S. Pat. No. 6150584, Int. Pat. Publ. No. WO99/45962, Int. Pat. Publ. No. WO02/066630, Int. Pat. Publ. No. WO02/43478, Lonberg *et al* (1994) *Nature* 368:856-9; Green *et al* (1994) *Nature Genet.* 7:13-21; Green & Jakobovits (1998) *Exp. Med.* 188:483-95; Lonberg and Huszar (1995) *Int. Rev. Immunol.* 13:65-93; Bruggemann *et al* (1991) *Eur. J. Immunol.* 21:1323-1326; Fishwild *et al* (1996) *Nat. Biotechnol.* 14:845-851; Mendez *et al* (1997) *Nat. Genet.* 15:146-156; Green (1999) *J. Immunol. Methods* 231:11-23; Yang *et al* (1999) *Cancer Res.* 59:1236-1243; Bruggemann and Taussig (1997) *Curr. Opin. Biotechnol.* 8:455-458; Int. Pat. Publ. No. WO02/043478). The endogenous immunoglobulin loci in such mice may be disrupted or deleted, and at least one complete or partial human immunoglobulin locus may be inserted into the mouse genome using homologous or non-homologous recombination, using transchromosomes, or using minigenes. Companies such as Regeneron (http://_www_regeneron_com), Harbour Antibodies (http://_www_harbourantibodies_com), Open Monoclonal Technology, Inc. (OMT) (http://_www_omtinc_net), KyMab (http://_www_kymab_com), Trianni (http://_www.trianni_com) and Ablexis (http://_www_ablexis_com) may be engaged to provide human antibodies directed against a selected antigen using technology as described above.

Human antibodies may be selected from a phage display library, where the phage is engineered to express human immunoglobulins or portions thereof such as Fabs, single

chain antibodies (scFv), or unpaired or paired antibody variable regions (Knappik *et al.*, J.Mol Biol 296:57-86, 2000; Krebs *et al.*, J Immunol Meth 254:67-84,2001; Vaughan *et al.*, Nature Biotechnology 14:309-314, 1996; Sheets *et al.*, PITAS (USA) 95:6157-6162, 1998; Hoogenboom and Winter, J Mol Biol 227:381,1991; Marks *et al.*, J Mol Biol 222:581, 1991). The antibodies of the invention may be isolated for example from phage display libraries expressing antibody heavy and light chain variable regions as fusion proteins with bacteriophage pIX coat protein as described in Shi *et al.*, J Mol Biol 397:385-96, 2010 and Int. Pat. Publ. No. WO09/085462). The libraries may be screened for phage binding to human and/or cyno CD40 and the obtained positive clones may be further characterized, the Fabs isolated from the clone lysates, and expressed as full length IgGs. Such phage display methods for isolating human antibodies are described in for example: U.S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698 to Ladner *et al.*; U.S. Patent Nos. 5,427,908 and 5,580,717 to Dower *et al.*; U.S. Patent Nos. 5,969,108 and 6,172,197 to McCafferty *et al.*; and U.S. Patent Nos. 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081 to Griffiths *et al.*

Preparation of immunogenic antigens and monoclonal antibody production may be performed using any suitable technique, such as recombinant protein production. The immunogenic antigens may be administered to an animal in the form of purified protein, or protein mixtures including whole cells or cell or tissue extracts, or the antigen may be formed *de novo* in the animal's body from nucleic acids encoding said antigen or a portion thereof.

The antibodies of the invention may be human or humanized.

In some embodiments, the antibody of the invention comprises a VH framework derived from human germline gene VH3_3-23 (SEQ ID NO: 49).

In some embodiments, the antibody of the invention comprises a VL framework derived from human germline gene VL3_3R (IGLV3-1) (SEQ ID NO: 50).

The antibodies of the invention may be of IgA, IgD, IgE, IgG or IgM type. The antibodies of the invention may be of IgG1, IgG2, IgG3, IgG4 type.

The antibodies of the invention may further be engineered to generate modified antibodies with similar or altered properties when compared to the parental antibodies. The VH, the VL, the VH and the VL, the constant regions, VH framework, VL framework, or any or all of the six CDRs may be engineered in the antibodies of the invention.

The antibodies of the invention may be engineered by CDR grafting. One or more CDR sequences of the antibodies of the invention may be grafted to a different framework sequence. CDR grafting may be done using methods described herein. In some embodiments, the antibodies of the invention comprise a VH that comprises the HCDR1 of SEQ ID NO: 5, the HCDR2 of SEQ ID NO: 6, 15, 16, 17 or 18, the HCDR3 of SEQ ID NOs: 7 or 19, and the VL that comprises the LCDR1 of SEQ ID NOs: 8 or 20, the LCDR2 of SEQ ID NO: 9 and/or the LCDR3 of SEQ ID NO: 10, wherein the VH framework is not derived from VH3_3-23 (SEQ ID NO: 49) and the VL framework is not derived from VL3_3R (IGLV3-1) (SEQ ID NO: 50). The framework sequences to be used may be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA and the encoded protein sequences for human heavy and light chain variable region genes can be found at IMGT®, the international ImMunoGeneTics information system® http://_www-imgt_org. Framework sequences that may be used to replace the existing framework sequences in the antibodies of the invention are those that show the highest percent identity to C40B16, C40B124, C40B135, C40B125, C40B136, C40B127, C40B138, C40B131, C40B176, C40B180, C40B179 or C40B183 VH or VL at amino acid level.

The framework sequences of the parental and engineered antibodies may further be modified, for example by backmutations to restore and/or improve binding of the resulting antibody to the antigen as described for example in U.S. Pat No. 6,180,370. The framework sequences of the parental and engineered antibodies may further be modified by mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T-cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as “deimmunization” and described in further detail in U.S. Pat. Publ. No. 20030153043.

The CDR residues of the antibodies of the invention may be mutated to improve one or more binding properties of the antibody of interest. Site-directed mutagenesis or PCR-mediated mutagenesis may be performed to introduce the mutation(s) and the effect on antibody binding, or other functional property of interest, may be evaluated in *in vitro* or *in vivo* assays as described herein and provided in the Examples. Exemplary substitutions that may be introduced are conservative modifications as discussed *supra*. Moreover,

typically no more than one, two, three, four or five residues within a CDR region are altered.

Antibodies of the invention may be post-translationally modified by processes such as glycosylation, isomerization, deglycosylation or non-naturally occurring covalent modification such as the addition of polyethylene glycol moieties (pegylation) and lipidation. Such modifications may occur *in vivo* or *in vitro*. For example, the antibodies of the invention may be conjugated to polyethylene glycol (PEGylated) to improve their pharmacokinetic profiles. Conjugation may be carried out by techniques known to those skilled in the art. Conjugation of therapeutic antibodies with PEG has been shown to enhance pharmacodynamics while not interfering with function (Knigh *et al.*, Platelets 15:409-18, 2004; Leong *et al.*, Cytokine 16:106-19, 2001; Yang *et al.*, Protein Eng. 16:761-70, 2003).

Antibodies or antigen-binding fragments thereof of the invention may be modified to improve stability, selectivity, cross-reactivity, affinity, immunogenicity or other desirable biological or biophysical property are within the scope of the invention. Stability of an antibody is influenced by a number of factors, including core packing of individual domains that affects their intrinsic stability, protein/protein interface interactions that have impact upon the HC and LC pairing, burial of polar and charged residues, (4) H-bonding network for polar and charged residues; and surface charge and polar residue distribution among other intra- and inter-molecular forces (Worn *et al.*, *J Mol Biol* 305:989-1010, 2001). Potential structure destabilizing residues may be identified based upon the crystal structure of the antibody or by molecular modeling in certain cases, and the effect of the residues on antibody stability may be tested by generating and evaluating variants harboring mutations in the identified residues. One of the ways to increase antibody stability is to raise the thermal transition midpoint (T_m) as measured by differential scanning calorimetry (DSC). In general, the protein T_m is correlated with its stability and inversely correlated with its susceptibility to unfolding and denaturation in solution and the degradation processes that depend on the tendency of the protein to unfold (Remmele *et al.*, Biopharm 13:36-46, 2000). A number of studies have found correlation between the ranking of the physical stability of formulations measured as thermal stability by DSC and physical stability measured by other methods (Gupta *et al.*, AAPS PharmSci 5E8, 2003; Zhang *et al.*, J Pharm Sci 93:3076-89, 2004; Maa *et al.*, Int J Pharm 140:155-68, 1996;

Bedu-Addo *et al.*, *Pharm Res* 21:1353-61, 2004; Remmele *et al.*, *Pharm Res* 15:200-8, 1997). Formulation studies suggest that a Fab T_m has implication for long-term physical stability of a corresponding mAb.

In some embodiments, the antibody of the invention is a multispecific antibody.

In some embodiments, the antibody of the invention is a bispecific antibody.

The monospecific antagonistic antibodies specifically binding CD40 of the invention may be engineered into bispecific antibodies which are also encompassed within the scope of the invention.

Full length bispecific antibodies may be generated for example using Fab arm exchange (e.g., half-molecule exchange, exchanging one heavy chain-light chain pair) between two monospecific bivalent antibodies by introducing substitutions at the heavy chain CH3 interface in each half molecule to favor heterodimer formation of two antibody half molecules having distinct specificity either *in vitro* in cell-free environment or using co-expression. The Fab arm exchange reaction is the result of a disulfide-bond isomerization reaction and dissociation-association of CH3 domains. The heavy chain disulfide bonds in the hinge regions of the parental monospecific antibodies are reduced. The resulting free cysteines of one of the parental monospecific antibodies form an inter heavy-chain disulfide bond with cysteine residues of a second parental monospecific antibody molecule and simultaneously CH3 domains of the parental antibodies release and reform by dissociation-association. The CH3 domains of the Fab arms may be engineered to favor heterodimerization over homodimerization. The resulting product is a bispecific antibody having two Fab arms or half molecules which each bind a distinct epitope.

Bispecific antibodies may also be generated using designs such as the Triomab/Quadroma (Trion Pharma/Fresenius Biotech), Knob-in-Hole (Genentech), CrossMAbs (Roche) and the electrostatically-induced CH3 interaction (Chugai, Amgen, NovoNordisk, Oncomed), the LUZ-Y (Genentech), the Strand Exchange Engineered Domain body (SEEDbody)(EMD Serono), the Biclonic (Merus) and as DuoBody® Products (Genmab A/S).

The Triomab quadroma technology may be used to generate full length bispecific antibodies of the invention. Triomab technology promotes Fab arm exchange between two parental chimeric antibodies, one parental mAb having IgG2a and the second parental mAb having rat IgG2b constant regions, yielding chimeric bispecific antibodies.

The “knob-in-hole” strategy (see, e.g., Intl. Publ. No. WO 2006/028936) may be used to generate full length bispecific antibodies. Briefly, selected amino acids forming the interface of the CH3 domains in human IgG can be mutated at positions affecting CH3 domain interactions to promote heterodimer formation. An amino acid with a small side chain (hole) is introduced into a heavy chain of an antibody specifically binding a first antigen and an amino acid with a large side chain (knob) is introduced into a heavy chain of an antibody specifically binding a second antigen. After co-expression of the two antibodies, a heterodimer is formed as a result of the preferential interaction of the heavy chain with a “hole” with the heavy chain with a “knob”. Exemplary CH3 substitution pairs forming a knob and a hole are (expressed as modified position in the first CH3 domain of the first heavy chain/ modified position in the second CH3 domain of the second heavy chain): T366Y/F405A, T366W/F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S_L368A_Y407V.

The CrossMAb technology may be used to generate full length bispecific antibodies of the invention. CrossMAbs, in addition to utilizing the “knob-in-hole” strategy to promote Fab arm exchange, have in one of the half arms the CH1 and the CL domains exchanged to ensure correct light chain pairing of the resulting bispecific antibody (see e.g. U.S. Patent No. 8,242,247).

Other cross-over strategies may be used to generate full length bispecific antibodies by exchanging variable or constant, or both domains between the heavy chain and the light chain or within the heavy chain in the bispecific antibodies, either in one or both arms. These exchanges include for example VH-CH1 with VL-CL, VH with VL, CH3 with CL and CH3 with CH1 as described in Int. Patent Publ. Nos. WO2009/080254, WO2009/080251, WO2009/018386 and WO2009/080252.

Other strategies such as promoting heavy chain heterodimerization using electrostatic interactions by substituting positively charged residues at one CH3 surface and negatively charged residues at a second CH3 surface may be used, as described in US Patent Publ. No. US2010/0015133; US Patent Publ. No. US2009/0182127; US Patent Publ. No. US2010/028637 or US Patent Publ. No. US2011/0123532. In other strategies, heterodimerization may be promoted by following substitutions (expressed as modified position in the first CH3 domain of the first heavy chain/ modified position in the second CH3 domain of the second heavy chain): L351Y_F405A_Y407V/T394W,

T366I_K392M_T394W/F405A_Y407V, T366L_K392M_T394W/F405A_Y407V, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, Y407A/T366A_K409F, or T350V_L351Y_F405A_Y407V/T350V_T366L_K392L_T394W as described in U.S. Patent Publ. No. US2012/0149876 or U.S. Patent Publ. No. US2013/0195849.

LUZ-Y technology may be utilized to generate bispecific antibodies. In this technology, a leucine zipper is added into the C terminus of the CH3 domains to drive the heterodimer assembly from parental mAbs that is removed post-purification as described in Wranik *et al.*, (2012) *J Biol Chem* 287(52): 42221-9.

SEEDbody technology may be utilized to generate bispecific antibodies. SEEDbodies have, in their constant domains, select IgG residues substituted with IgA residues to promote heterodimerization as described in U.S. Patent No. US20070287170.

Bispecific antibodies may be generated *in vitro* in a cell-free environment by introducing asymmetrical mutations in the CH3 regions of two monospecific homodimeric antibodies and forming the bispecific heterodimeric antibody from two parent monospecific homodimeric antibodies in reducing conditions to allow disulfide bond isomerization according to methods described in Int. Patent Publ. No. WO2011/131746. In the methods, the first monospecific bivalent antibody and the second monospecific bivalent antibody are engineered to have certain substitutions at the CH3 domain that promoter heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. Substitutions that may be used are F405L in one heavy chain and K409R in the other heavy chain. The incubation conditions may optimally be restored to non-reducing. Exemplary reducing agents that may be used are 2- mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl)phosphine (TCEP), L-cysteine and beta-mercaptoethanol. For example, incubation for at least 90 min at a temperature of at least 20°C in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

Substitutions are typically made at the DNA level to a molecule such as the constant domain of the antibody using standard methods.

The antibodies of the invention may be engineered into various well known antibody forms.

In some embodiments, the bispecific antibodies include recombinant IgG-like dual targeting molecules, wherein the two sides of the molecule each contain the Fab fragment or part of the Fab fragment of at least two different antibodies; IgG fusion molecules, wherein full length IgG antibodies are fused to an extra Fab fragment or parts of Fab fragment; Fc fusion molecules, wherein single chain Fv molecules or stabilized diabodies are fused to heavy-chain constant-domains, Fc-regions or parts thereof; Fab fusion molecules, wherein different Fab-fragments are fused together; ScFv- and diabody-based and heavy chain antibodies (e.g., domain antibodies, nanobodies) wherein different single chain Fv molecules or different diabodies or different heavy-chain antibodies (e.g. domain antibodies, nanobodies) are fused to each other or to another protein or carrier molecule.

Polynucleotides, vectors, host cells

The invention also provides for an isolated polynucleotide encoding any of the antibody heavy chain variable regions, any of the antibody light chain variable regions, or any of the antibody heavy chains and/or the antibody light chains of the invention.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 11, 21, 22, 23, 24, 25 or 26.

The invention also provides for an isolated polynucleotide encoding the VL of SEQ ID NOs: 12 or 27.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 11, 21, 22, 23, 24, 25 or 26 and the VL of SEQ ID NOs: 12 or 27.

The invention also provides for an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NOs: 13, 14, 28, 29, 30, 31, 32, 33, or 34.

The invention also provides for an isolated polynucleotide encoding the heavy chain of SEQ ID NOs: 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46.

The invention also provides for an isolated polynucleotide encoding the light chain of SEQ ID NOs: 77 or 78.

The invention also provides for an isolated polynucleotide encoding the heavy chain of SEQ ID NOs: 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46 and a light chain of SEQ ID NOs: 77 or 78.

The invention also provides for an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NOs: 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 77 or 78.

The polynucleotide sequences encoding the VH or the VL or a fragment thereof of the antibodies of the invention or the heavy chain and the light chain of the antibodies of the invention may be operably linked to one or more regulatory elements, such as a promoter or enhancer, that allow expression of the nucleotide sequence in the intended host cell. The polynucleotide may be a cDNA.

The invention also provides for a vector comprising the polynucleotide of the invention. Such vectors may be plasmid vectors, viral vectors, vectors for baculovirus expression, transposon based vectors or any other vector suitable for introduction of the synthetic polynucleotide of the invention into a given organism or genetic background by any means. For example, polynucleotides encoding light and/or heavy chain variable regions of the antibodies of the invention, optionally linked to constant regions, are inserted into expression vectors. The light and/or heavy chains may be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains may be operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Such control sequences include signal sequences, promoters (e.g. naturally associated or heterologous promoters), enhancer elements, and transcription termination sequences, and are chosen to be compatible with the host cell chosen to express the antibody. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the proteins encoded by the incorporated polynucleotides.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 13 and the polynucleotide of SEQ ID NO: 14.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 28 and the polynucleotide of SEQ ID NO: 34.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 29 and the polynucleotide of SEQ ID NO: 34.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 30 and the polynucleotide of SEQ ID NO: 34.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 31 and the polynucleotide of SEQ ID NO: 34.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 32 and the polynucleotide of SEQ ID NO: 34.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 33 and the polynucleotide of SEQ ID NO: 34.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 65 and the polynucleotide of SEQ ID NO: 77.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 66 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 67 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 68 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 69 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 70 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 71 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 72 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 73 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 74 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 75 and the polynucleotide of SEQ ID NO: 78.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 76 and the polynucleotide of SEQ ID NO: 78.

Suitable expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression

vectors contain selection markers such as ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance or neomycin resistance to permit detection of those cells transformed with the desired DNA sequences.

Suitable promoter and enhancer elements are known in the art. For expression in a eukaryotic cell, exemplary promoters include light and/or heavy chain immunoglobulin gene promoter and enhancer elements; cytomegalovirus immediate early promoter; herpes simplex virus thymidine kinase promoter; early and late SV40 promoters; promoter present in long terminal repeats from a retrovirus; mouse metallothionein-I promoter; and various art-known tissue specific promoters. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

Large numbers of suitable vectors and promoters are known to those of skill in the art; many are commercially available for generating a subject recombinant constructs. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia).

The invention also provides for a host cell comprising one or more vectors of the invention. "Host cell" refers to a cell into which a vector has been introduced. It is understood that the term host cell is intended to refer not only to the particular subject cell but to the progeny of such a cell, and also to a stable cell line generated from the particular subject cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Such host cells may be eukaryotic cells, prokaryotic cells, plant cells or archeal cells.

Escherichia coli, bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species are examples of prokaryotic host cells. Other microbes, such as yeast, are also useful for expression. *Saccharomyces* (e.g., *S. cerevisiae*) and *Pichia* are examples of suitable yeast host cells. Exemplary eukaryotic cells may be of mammalian, insect, avian or other animal origins. Mammalian eukaryotic cells include immortalized cell lines such as hybridomas or myeloma cell lines such as SP2/0 (American Type Culture Collection (ATCC), Manassas,

VA, CRL-1581), NS0 (European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646) and Ag653 (ATCC CRL-1580) murine cell lines. An exemplary human myeloma cell line is U266 (ATCC CRL-TIB-196). Other useful cell lines include those derived from Chinese Hamster Ovary (CHO) cells such as CHO-K1SV (Lonza Biologics, Walkersville, MD), CHO-K1 (ATCC CRL-61) or DG44.

The invention also provides for a method of producing the antibody of the invention comprising culturing the host cell of the invention in conditions that the antibody is expressed, and recovering the antibody produced by the host cell. Methods of making antibodies and purifying them are known. Once synthesized (either chemically or recombinantly), the whole antibodies, their dimers, individual light and/or heavy chains, or other antibody fragments such as VH and/ or VL, may be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, high performance liquid chromatography (HPLC) purification, gel electrophoresis, and the like (see generally Scopes, Protein Purification (Springer- Verlag, N.Y., (1982))). A subject antibody may be substantially pure, e.g., at least about 80% to 85% pure, at least about 85% to 90% pure, at least about 90% to 95% pure, or at least about 98% to 99%, or more, pure, e.g., free from contaminants such as cell debris, macromolecules, etc. other than the subject antibody.

The invention also provides for a method for producing an antagonistic antibody specifically binding CD40 of SEQ ID NO: 1, comprising:

- incorporating the first polynucleotide encoding the VH of the antibody and the second polynucleotide encoding the VL of the antibody into an expression vector;
- transforming a host cell with the expression vector;
- culturing the host cell in culture medium under conditions wherein the VL and the VH are expressed and form the antibody; and
- recovering the antibody from the host cell or culture medium.

The polynucleotides encoding certain VH or VL sequences of the invention may be incorporated into vectors using standard molecular biology methods. Host cell transformation, culture, antibody expression and purification are done using well known methods.

Methods of Treatment

Antagonistic antibodies specifically binding CD40 of the invention, for example antibodies C40B16, C40B124, C40B135, C40B125, C40B136, C40B127, C40B138, C40B131, C40B176, C40B180, C40B179 or C40B183, may be used for the treatment and/or prevention of any condition or disease wherein antagonizing the effects of CD40 may be therapeutically effective and may reduce the symptoms of the disease. Examples thereof include the treatment of inflammatory diseases such as autoimmune diseases wherein the induction of tolerance and/or the suppression of humoral immunity are therapeutically desirable. Diseases that may be treated with the antibodies of the invention are autoimmune diseases, Addison's disease, an ankylosing spondylitis, an atherosclerosis, an autoimmune hepatitis, an autoimmune diabetes, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, an idiopathic thrombocytopenia, an inflammatory bowel disease (IBD), a systemic lupus erythematosus, a multiple sclerosis, a myasthenia gravis, a psoriasis, an arthritis, a scleroderma, Sjogren's syndrome, a systemic sclerosis, a transplantation, a kidney transplantation, a skin transplantation, a bone marrow transplantation, a graft versus host disease (GVHD), a type I diabetes, a rheumatoid arthritis, a juvenile arthritis, a psoriatic arthritis, Reiter's syndrome, an ankylosing spondylitis, or a gouty arthritis, Crohn's disease or an ulcerative colitis.

The invention also provides for a method of treating an arthritis, comprising administering a therapeutically effective amount of the antibody of the invention to a subject in need thereof for a time sufficient to treat the arthritis.

In some embodiments, the arthritis is a juvenile arthritis, a rheumatoid arthritis, a psoriatic arthritis, Reiter's syndrome, an ankylosing spondylitis, or a gouty arthritis.

The invention also provides for a method of treating a lupus, comprising administering a therapeutically effective amount of the antibody of the invention to a subject in need thereof for a time sufficient to treat the lupus.

In some embodiments, the lupus is a systemic lupus erythematosus (SLE) or a cutaneous lupus erythematosus (CLE).

In some embodiments, the subject has lupus nephritis.

In some embodiments, the subject has a cutaneous lupus erythematosus.

The invention also provides for a method of treating an inflammatory bowel disease, comprising administering a therapeutically effective amount of the antibody of the

invention to a subject in need thereof for a time sufficient to treat the inflammatory bowel disease.

In some embodiments, the inflammatory bowel disease is Crohn's disease.

In some embodiments, the inflammatory bowel disease is an ulcerative colitis.

"Treatment" or "treat" refers to therapeutic treatment. Subjects in need of treatment include those subjects diagnosed with the disorder or experiencing at least one of the symptoms of the disease. Subjects that may be treated also include those prone to or susceptible to have the disorder, or those in which the disorder is to be prevented.

Beneficial or desired clinical results include alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. Beneficial treatment result include, in a subject who has received treatment, reduction in the levels of inflammatory cytokines, adhesion molecules, proteases, immunoglobulins, combinations thereof, increased production of anti-inflammatory proteins, a reduction in the number of autoreactive cells, an increase in immune tolerance, inhibition of autoreactive cell survival, and/or a decrease in one or more symptoms mediated by stimulation of CD40-expressing cells by CD154.

Clinical response may be assessed using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, flow cytometry or fluorescence-activated cell sorter (FACS) analysis, histology, gross pathology, and blood chemistry, including but not limited to changes detectable by ELISA, RIA, chromatography, and the like.

The methods of the invention may be used to treat a subject belonging to any animal classification. Examples of subjects that may be treated include mammals such as humans, rodents, dogs, cats and farm animals.

The antibodies of the invention may be useful in the preparation of a medicament for such treatment, wherein the medicament is prepared for administration in dosages defined herein.

The antibodies of the invention may be administered in combination with a second therapeutic agent.

The second therapeutic agent may be any known therapy for inflammatory diseases, such as autoimmune diseases, including any agent or combination of agents that

are known to be useful, or which have been used or are currently in use for treatment. Such therapies and therapeutic agents include surgery or surgical procedures (e.g. splenectomy, lymphadenectomy, thyroidectomy, plasmapheresis, leukopheresis, cell, tissue, or organ transplantation, intestinal procedures, organ perfusion, and the like), therapy such as steroid therapy and non-steroidal therapy, hormone therapy, cytokine therapy, therapy with dermatological agents (for example, topical agents used to treat skin conditions such as allergies, contact dermatitis, and psoriasis), immunosuppressive therapy, and other anti-inflammatory drugs including monoclonal antibodies.

The second therapeutic agent may be a corticosteroid, an immunosuppressant, a cytotoxic drug, or a B-cell modulator.

In some embodiments, the antibodies of the invention are administered in combination with a second therapeutic agent. Exemplary second therapeutic agents are corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), salicylates, hydroxychloroquine, sulfasalazine, cytotoxic drugs, immunosuppressive drugs, immunomodulatory antibodies, methotrexate, cyclophosphamide, mizoribine, chlorambucil, cyclosporine, tacrolimus (FK506 ; ProGrafrM), mycophenolate mofetil, and azathioprine (6-mercaptopurine), sirolimus (rapamycin), deoxyspergualin, leflunomide and its malononitriloamide analogs; anti-CTLA4 antibodies and Ig fusions, anti-B lymphocyte stimulator antibodies (e.g., LYMPHOSTAT-BTM) and CTLA4-Ig fusions, anti-CD80 antibodies, anti-T cell antibodies such as anti-CD3 (OKT3), anti-CD4, corticosteroids such as, for example, clobetasol, halobetasol, hydrocortisone, triamcinolone, betamethasone, fluocinole, fluocinonide, prednisone, prednisolone, methylprednisolone; non-steroidal anti-inflammatory drugs (NSAIDs) such as, for example, sulfasalazine, medications containing mesalamine (known as 5-ASA agents), celecoxib, diclofenac, etodolac, fenpropfen, flurbiprofen, ibuprofen, ketoprofen, meclofamate, meloxicam, nabumetone, naproxen, oxaprozin, piroxicam, rofecoxib, salicylates, sulindac, and tolmetin; phosphodiesterase-4 inhibitors, anti-TNF α antibodies REMICADE® (infliximab), SIMPONI® (golimumab) and HUMIRA® (adalimumab), thalidomide or its analogs such as lenalidomide.

The antibodies of the invention may be administered in combination with a second therapeutic agent simultaneously, sequentially or separately.

Treatment effectiveness or RA may be assessed using effectiveness as measured by clinical responses defined by the American College of Rheumatology criteria, the European

League of Rheumatism criteria, or any other criteria. See for example, Felson et al. (1995) Arthritis Rheum. 38 : 727-35 and van Gestel et al. (1996) Arthritis Rheum. 39: 34-40.

Administration/Pharmaceutical Compositions

The invention also provides for pharmaceutical compositions of the antagonistic antibodies specifically binding CD40 of the invention and a pharmaceutically acceptable carrier. For therapeutic use, the antibodies the invention may be prepared as pharmaceutical compositions containing an effective amount of the antibody as an active ingredient in a pharmaceutically acceptable carrier. "Carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the active compound is administered. Such vehicles may be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine may be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the antibodies of the invention in such pharmaceutical compositions may vary widely, *i.e.*, from less than about 0.5%, usually to at least about 1% to as much as 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% by weight and will be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected. Suitable vehicles and formulations, including other human proteins, *e.g.*, human serum albumin, are described, for example, in *e.g.* Remington: The Science and Practice of Pharmacy, 21st Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5, Pharmaceutical Manufacturing pp 691-1092, See especially pp. 958-989.

The mode of administration of the antibodies of the invention in the methods of the invention may be any suitable route such as parenteral administration, *e.g.*, intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, transmucosal (oral, intranasal, intravaginal, and rectal) or other means appreciated by the skilled artisan, as well known in the art.

The antibodies of the invention may be administered to the subject by any suitable route, for example parentally by intravenous (*i.v.*) infusion or bolus injection, intramuscularly or subcutaneously or intraperitoneally. *i.v.* infusion may be given over for, example, 15, 30, 60, 90, 120, 180, or 240 minutes, or over 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours.

The dose given to the subject is sufficient to alleviate or at least partially arrest the disease being treated (“therapeutically effective amount”) and may be from about 0.005 mg/kg to about 100 mg/kg, for example about 0.05 mg/kg to about 20 mg/kg, about 0.1 mg/kg to about 20 mg/kg, about 1 mg to about 20 mg/kg, about 4 mg/kg, about 8 mg/kg, about 16 mg/kg or about 24 mg/kg, or, e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg, but may even higher, for example about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90 or 100 mg/kg.

A fixed unit dose may also be given, for example, 50, 100, 200, 500 or 1000 mg, or the dose may be based on the patient's surface area, e.g., 500, 400, 300, 250, 200, or 100 mg/m². Usually between 1 and 8 doses, (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) may be administered to treat the inflammatory disease, such as an autoimmune disease such or a rheumatoid arthritis, but 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more doses may be given.

The administration of the antibodies of the invention may be repeated after one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, two months, three months, four months, five months, six months or longer. Repeated courses of treatment are also possible, as is chronic administration. The repeated administration may be at the same dose or at a different dose. For example, the antibodies of the invention may be administered at 0.1 mg/kg, at 1 mg/kg, at 5 mg/kg, at 8 mg/kg or at 16 mg/kg at weekly interval for 8 weeks, followed by administration at 8 mg/kg or at 16 mg/kg every two weeks for an additional 16 weeks, followed by administration at 8 mg/kg or at 16 mg/kg every four weeks by intravenous infusion.

The antibodies of the invention may be provided by maintenance therapy, such as, e.g., once a week, once a month, once in two months, once in three months, once in four months, once in five months, or once in six months over one or more years.

For example, the antibodies of the invention may be provided as a daily dosage in an amount of about 0.1-100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

The antibodies of the invention may also be administered prophylactically in order to reduce the risk of developing a disease and/or delay the onset of the disease to be treated.

An exemplary pharmaceutical composition of the invention for intramuscular injection may be prepared to contain 1 ml sterile buffered water, and between about 1 ng to about 100 mg/kg, *e.g.* about 50 ng to about 30 mg/kg or about 5 mg to about 25 mg/kg, of the antibody of the invention.

An exemplary pharmaceutical composition of the invention for intravenous infusion may be made up to contain about 200 ml of sterile Ringer's solution, and about 8 mg to about 2400 mg, about 400 mg to about 1600 mg, or about 400 mg to about 800 mg of the antibodies of the invention for administration to a 80 kg patient. Methods for preparing parenterally administrable compositions are well known and are described in more detail in, for example, "Remington's Pharmaceutical Science", 15th ed., Mack Publishing Company, Easton, PA.

"Therapeutically effective amount" of the antibodies of the invention effective in the treatment of a disease may be determined by standard research techniques. For example, *in vitro* assays may be employed to help identify optimal dosage ranges. Optionally, the dosage of the antibodies of the invention that may be effective in the treatment of a disease such as the inflammatory disease may be determined by administering the antibodies to relevant animal models well known in the art. Selection of a particular effective dose may be determined (*e.g.*, via clinical trials) by those skilled in the art based upon the consideration of several factors. Such factors include the disease to be treated or prevented, the symptoms involved, the patient's body mass, the patient's immune status and other factors known by the skilled artisan. The precise dose will also depend on the route of administration, and the severity of disease, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model

test systems. The antibodies of the invention may be tested for their efficacy and effective dosage using any of the models described herein.

The antibodies of the invention may be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional protein preparations and well known lyophilization and reconstitution techniques can be employed.

Anti-idiotypic antibodies

The present invention provides for an anti-idiotypic antibody binding to the antibody of the invention.

The invention also provides for an anti-idiotypic antibody specifically binding the antibody comprising the VH of SEQ ID NO: 11 and the VL or SEQ ID NO: 12.

The invention also provides for an anti-idiotypic antibody specifically binding the antibody comprising the VH of SEQ ID NO: 21 and the VL or SEQ ID NO: 27.

The invention also provides for an anti-idiotypic antibody specifically binding the antibody comprising the VH of SEQ ID NO: 22 and the VL or SEQ ID NO: 27.

The invention also provides for an anti-idiotypic antibody specifically binding the antibody comprising the VH of SEQ ID NO: 23 and the VL or SEQ ID NO: 27.

The invention also provides for an anti-idiotypic antibody specifically binding the antibody comprising the VH of SEQ ID NO: 24 and the VL or SEQ ID NO: 27.

The invention also provides for an anti-idiotypic antibody specifically binding the antibody comprising the VH of SEQ ID NO: 25 and the VL or SEQ ID NO: 27.

The invention also provides for an anti-idiotypic antibody specifically binding the antibody comprising the VH of SEQ ID NO: 26 and the VL or SEQ ID NO: 27.

An anti-idiotypic (Id) antibody is an antibody which recognizes the antigenic determinants (*e.g.* the paratope or CDRs) of the antibody. The Id antibody may be antigen-blocking or non-blocking. The antigen-blocking Id may be used to detect the free antibody in a sample (*e.g.* CD40 antibody of the invention described herein). The non-blocking Id may be used to detect the total antibody (free, partially bond to antigen, or fully bound to antigen) in a sample. An Id antibody may be prepared by immunizing an animal with the antibody to which an anti-Id is being prepared.

An anti-Id antibody may also be used as an immunogen to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. An anti-anti-Id may be epitopically identical to the original mAb, which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity. Anti-Id antibodies may be varied (thereby producing anti-Id antibody variants) and/or derivatized by any suitable technique.

Immunoconjugates

An "immunoconjugate" refers to the antibody of the invention conjugated to one or more heterologous molecule(s).

In some embodiments, the antibody of the invention is conjugated to one or more cytotoxic agents or an imaging agent.

Exemplary cytotoxic agents include chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), and radionuclides.

The cytotoxic agent may be one or more drugs, such as to a maytansinoid (see, e.g., U.S. Patent No. 5,208,020, 5,416,06), an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see, e.g., U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298), a dolastatin, a calicheamicin or derivative thereof (see, e.g., U.S. Patent Nos. 5,712,374, 5,714,586, 5,739, 116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman *et al.*, (1993) *Cancer Res* 53:3336-3342; and Lode *et al.*, (1998) *Cancer Res* 58:2925-2928); an anthracycline such as daunomycin or doxorubicin (see, e.g., Kratz *et al.*, (2006) *Current Med. Chem* 13:477-523; Jeffrey *et al.*, (2006) *Bioorganic & Med Chem Letters* 16:358-362; Torgov *et al.*, (2005) *Bioconj Chem* 16:717-721; Nagy *et al.*, (2000) *Proc Natl Acad Sci USA* 97:829-834; Dubowchik *et al.*, *Bioorg. & Med. Chem. Letters* 12: 1529-1532 (2002); King *et al.*, (2002) *J Med Chem* 45:4336-4343; and U.S. Patent No. 6,630,579), methotrexate, vindesine, a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel.

The cytotoxic agent may also be an enzymatically active toxin or fragment thereof, such as diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-

S), momordica charantia inhibitor, curcin, crocin, saponaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

The cytotoxic agent or an imaging agent may also be a radionuclide. Exemplary radionuclides include Ac-225, At-211, I-131, I-125, Y-90, Re-186, Re-188, Sm-153, Bi-212, P-32, Pb-212 and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc-99m or I-123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as I-123, I-131, In-111, F-19, C-13, N-15 or O-17.

Conjugates of the antibodies of the invention and the heterologous molecule may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HQ), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin may be prepared as described in Vitetta *et al.*, (1987) *Science* 238: 1098. Carbon- 14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX- DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, e.g., W094/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari *et al.*, (1992) *Cancer Res* 52: 127-131; U.S. Patent No. 5,208,020) may be used.

Conjugates of the antibodies of the invention and the heterologous molecule may be prepared with cross-linker reagents such as BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo- SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

The invention also provides for an immunoconjugate comprising the antibody specifically binding CD40 of SEQ ID NO: 1 of the invention linked to a therapeutic agent or an imaging agent.

Diagnostic uses and kits

Kits

The invention also provides for a kit comprising the antagonistic antibody specifically binding human CD40 of the invention.

The kit may be used for therapeutic uses and as diagnostic kits.

The kit may be used to detect the presence of CD40 in a biological sample.

In some embodiments, the kit comprises the antagonistic antibody specifically binding human CD40 of the invention and reagents for detecting the antibody. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

In some embodiments, the kit comprises the antibody of the invention in a container and instructions for use of the kit.

In some embodiments, the antibody in the kit is labeled.

In some embodiments, the kit comprises the antibody C40B16, C40B124, C40B135, C40B125, C40B136, C40B127, C40B138, C40B131, C40B176, C40B180, C40B179 or C40B183.

Methods of detecting CD40

The invention also provides for a method of detecting CD40 in a sample, comprising obtaining the sample, contacting the sample with the antibody of the invention, and detecting the antibody bound to CD40 in the sample.

In some embodiments, the sample may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, circulating tumor cells, cells that are not tissue associated (*i.e.*, free cells), tissues (*e.g.*, surgically resected tumor tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

The antibodies of the invention may be detected using known methods. Exemplary methods include direct labeling of the antibodies using fluorescent or chemiluminescent labels, or radiolabels, or attaching to the antibodies of the invention a moiety which is readily detectable, such as biotin, enzymes or epitope tags. Exemplary labels and moieties are ruthenium, ^{111}In -DOTA, ^{111}In - diethylenetriaminepentaacetic acid (DTPA), horseradish peroxidase, alkaline phosphatase and beta-galactosidase, poly-histidine (HIS tag), acridine dyes, cyanine dyes, fluorone dyes, oxazin dyes, phenanthridine dyes, rhodamine dyes and Alexafluor® dyes.

The antibodies of the invention may be used in a variety of assays to detect CD40 in the sample. Exemplary assays are western blot analysis, radioimmunoassay, surface plasmon resonance, immunoprecipitation, equilibrium dialysis, immunodiffusion, electrochemiluminescence (ECL) immunoassay, and immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

The present invention will now be described with reference to the following specific, non-limiting examples.

General Materials and Methods

Generation of antigens used in the studies

Cloning, expression and purification of the antigens was done using standard methods. The amino acid sequences of the proteins used are shown below.

Full length human CD40 (huCD40); SEQ ID NO: 1

MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLCQPGQKLVS DCTEFTE
TECLPCGESEFLDTWNRETHCHQH KYCDPNLGLRVQQKGTSETDTICTCEEGWHC
TSEACESCVLHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCE
TKDLVVQQAGTNKTDVVCGPQDRLRALVVIPIIFGILFAILLVLVFIKKVAKKPTNK
APHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ

Human CD40 extracellular domain (huCD40-ECD); SEQ ID NO: 2

EPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETETECCLPCGESEFLDTWNRETHC
 HQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHRSCSPGFGVK
 QIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTNKTDVVCGP
 QDRLR

***Macaca fascicularis* (cynomolgous, herein referred to as cyno) CD40 (cCD40); SEQ ID NO: 3**

MVRLPLQCVLWGCLLTAVYPEPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTE
 TECLPCSESEFLDTWNRETRCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGLHCM
 SESCOESCVPHRSCCLPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCRPWTSCETK
 DLVVQQAGTNKTDVVCGPQDRQRALVVIPICLGILFVILLVLVFIKKVAKKPNDK
 APHPKQEPQEINFLDDLPGSNPAAPVQETLHGCQPVTQEDGKESRISVQERQ

Cyno CD40 extracellular domain (cCD40-ECD); SEQ ID NO: 4

EPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECCL
 PCSESEFLDTWNRETRCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGLHCMSESC
 ESCVPHRSCCLPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCRPWTSCETKDLV
 VQQAGTNKTDVVCGPQDRQR

Soluble human CD154; SEQ ID NO: 60

MQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVTLENGKQLTVKRQ
 GLYYIYAQVTFCNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIH
 LGGVFELQPGASVFNVTDPVSQVSHGTGFTSFGLLKL

Full length human CD154; SEQ ID NO: 64

MIETYNQTSRSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDER
 NLHEDFVFMKTIQRCNTGERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEM
 QKGDQNPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVTLENGKQLTVKRQG
 LYYIYAQVTFCNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHL
 GGVFELQPGASVFNVTDPVSQVSHGTGFTSFGLL

Binding assay in primary human and cyno Dendritic Cells (DCs)

Human monocytes were isolated from either frozen/fresh PBMC using CD14 negative isolation kit per manufacturer's protocol (MACS Miltenyi). Cyno monocytes were isolated from fresh PBMC using CD14 positive isolation kit per manufacturer's protocol (MACS Miltenyi). To generate DCs, monocytes were cultured for 5 days in complete RPMI medium (Invitrogen) in the presence of 100ng/ml human GM-CSF and human IL-4 (Peprotech) and medium was replenished every 2 days. On day 5, DCs were stimulated with 100ng/ml LPS (Sigma) for 24 hours. Cells were then stained with each of the tested CD40 antibodies at different concentration in flow cytometry buffer (PBS + 1% FBS; BD Bioscience) in 100 µl volume for 30 minutes on ice followed with two washes with flow buffer. Cells were then stained for an additional 30 minutes on ice with APC-conjugated anti human IgG (Jackson ImmunoResearch) at the recommended dilution (1:100) and washed twice with flow buffer. Cells were analyzed for percent positive and Mean Fluorescence Intensity (MFI) to determine the antibody binding using Fortessa (BD Bioscience).

Binding assay in Raji (B cells lymphoma cell line) and HEK-Blue™ CD40L NF-κB cell lines

Raji cells were obtained from ATCC and HEK-Blue™ CD40L NF-κB cell line was obtained from Invivogen. Cells were cultured in complete RPMI medium per company's recommendation. Staining was done as described above for binding assay in primary human and cyno DCs.

Human DC IL-12p40 production assay

Human DCs were generated by culturing purified human monocytes (2.5x10⁶/well 6-well plates) for 6 days in 3ml RPMI medium containing glutamax, 25mM HEPES, 10% FBS, 1% Pen/Strep and 50ng each GM-CSF and IL-4. On day 3, 1ml of the medium was removed and replaced with 2ml fresh medium containing 50ng/ml each of GM-CSF and IL-4. For agonist assays, day 6 DCs were plated into 96-well plates (100,000 cells/well) followed by titrations of each of the CD40 antibodies. For antagonist assays, day 6 DCs were plated into 96-well plates (100,000 cells/well) followed by titrations of each of the CD40 antibodies and addition of 1µg/ml of soluble human CD154 (R&D Systems) to the

cultures. For both assays, cells were cultured for 48 hours before collecting and analyzing supernatants for IL-12p40 by MSD (according to manufacturer's directions). For Jurkat D1.1 (ATCC) antagonist assays, day 6 DCs were plated into 96-well plates (10,000 cells/well) followed by titrations of each of the CD40 antibodies, after which irradiated Jurkat D1.1 cells (1,000 rads; 100,000/well) were added to the cultures. Cells were cultured for 24 hours before collecting and analyzing supernatants for IL-12p40 by MSD.

Human B cell proliferation assay

Human tonsillar B cells were plated into 96-well plates (100,000 cells/well) in RPMI medium containing glutamax, 10% FBS and 1% Pen/Strep. For agonist assays, titrations of each of the CD40 antibodies were added to the cells and cultured for 48 hours. For antagonist assays, titrations of each of the CD40 antibodies were added to the cells, followed by addition of 0.5 µg/ml soluble human CD154 (R&D Systems). Cells were cultured for 48 hours at 37C. For Jurkat D1.1 antagonist assays, titrations of each of the CD40 antibodies were added to the cells along with IL-21 (100ng/ml final, Thermo Fisher Scientific), after which irradiated Jurkat D1.1. cells (5,000 rads; 100,000/well) were added to the cultures and incubated at 37C for 48 hours. For all assays, after 48 hours cells were pulsed with 3H-thymidine (1uCi/well) in 50µl medium and cultured for 16-18 hours before harvest and counting.

Cyno B cell proliferation assay

Cyno spleen cells were plated into 96-well plates (100,000 cells/well) in RPMI medium containing glutamax, 10% FBS and 1% Pen/Strep. Titrations of each of the CD40 antibodies were added to the cells, followed by addition of 0.5 µg/ml soluble human CD154 (R&D Systems). Cells were cultured for 48 hours at 37C. After 48 hours, cells were pulsed with 3H-thymidine (1uCi/well) in 50µl medium and cultured for 16-18 hours before harvest and counting.

HEK-Blue™ CD40L NF-κB activation assay

HEK-Blue™ CD40L cell lines stably express human CD40 and NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP). Activation of CD40 on HEK-Blue™ CD40L cells induce downstream signaling events leading to activation of NF-κB and

secretion of SEAP, which can be measured by QUANTI-Blue™ substrate conversion. These cells were used to assess the ability of the antibodies to either block (antagonize) CD40-CD154 interaction or activate (agonize) CD40.

Inhibition of CD40 Dependent NF-kB Activation in HEK-Blue CD40L Cell Line

HEK-Blue™ CD40L cells (Invivogen), which were maintained according to the vendor's protocol, were seeded into 96 well tissue culture plates in 100 µl volume (2.5×10^4 cells/well). The assay plates were covered and incubated overnight (37°C, 5% CO₂) to allow the cells to recover. On the following day, a 4X solution of rhCD154-ECD-His (80 ng/ml final concentration) and 4X solution of CD40 antibodies, or Fabs, (1 – 25 µg/ml final concentration) were pre-mixed and the resulting 2X solution added to the 96 well assay plates containing the cells (200 µl/well final volumes). After 16-24 h incubation (37°C, 5% CO₂), 40 µl aliquots of the supernatants were mixed with 160 µl of pre-warmed QUANTI-Blue™ (Invivogen) solution and incubated for 30-60 minutes prior to obtaining absorbance readings at 650 nm.

CD40 Dependent NF-kB Activation in HEK-Blue CD40L NFkB-SEAP Cell Line

HEK-Blue™ CD40L cells were seeded as described above and recovered overnight. On the following day, 2X CD40 antibody solutions (1 – 25 µg/ml final concentration) were added to the plate at 100 µl/well and incubated overnight (37°C, 5% CO₂). After 16-24 h incubation (37°C, 5% CO₂), 40 µl aliquots of the supernatants were analyzed as described above.

Example 1. Isolation of antibodies specifically binding human CD40 using rats expressing human immunoglobulin loci

Antibodies were generated using transgenic rats expressing human immunoglobulin loci, the OmniRat®; OMT, Inc. The OmniRat® endogenous immunoglobulin loci are replaced by human Igκ and Igλ loci and a chimeric human/rat IgH locus with V, D and J segments of human origin linked to the rat C_H locus. The IgH locus contains 22 human V_{HS}, all human D and J_H segments in natural configuration linked to the rat C_H locus. Generation and characterization of the OmniRat® is described in Osborn, *et al.* J Immunol 190: 1481-1490, 2013; and Int. Pat. Publ. No. WO 14/093908.

Separate cohorts of five rats were immunized with recombinant human and cyno CD40 ECD-His or human and cyno CD40 ECD-Fc proteins. Following a 31-34 day immunization regimen, lymph nodes were harvested from two rats and used to generate hybridomas. The generated hybridomas were screened for binding to both human and cyno CD40-ECD. Hybridomas exhibiting statistically significant binding to both human and cyno CD40-ECD following one-way ANOVA with a Dunnett's mean comparison post-test were cloned and their V regions sequenced using standard procedures

Example 2. Engineering of C40B16

C40B16 was generated by using the OmniRat® and identified having antagonist activity in the HEK-Blue™ CD40L NF-κB activation assay based on the criteria that an antagonist had a signal that is lower than the 3 x standard deviation of the mean signal of HEK-Blue™ CD40L cells treated with rhCD154-ECD-his alone.

C40B16 VH and VL were sequenced using standard methods and the framework sequences compared to the closest germline gene sequences in order to identify potential immunogenicity risks. C40B16 VH amino acid sequence was most homologous to IGHV3-23 (**SEQ ID NO: 49**) with 2 amino acid changes in the framework. C40B16 VL framework was identical to that of IGLV3-1 (**SEQ ID NO: 50**).

The variable regions of C40B16 were engineered to reduce possible immunogenicity and/or developability risk(s) by generating mutations at positions R43, H82, N52, S54 and/or M108 in the VH (residue numbering according to **SEQ ID NO: 11**) and at position C33 in the VL (residue numbering according to **SEQ ID NO: 12**) to eliminate potential heterogeneity caused by unpaired cysteines. The generated VH/VL domains were cloned as effector silent Fc isoforms IgG1sigma or IgG4PAA. IgG1sigma contains mutations L234A, L235A, G237A, P238S, H268A, A330S, and P331S when compared to the wild-type IgG1. IgG4PAA contains mutations S228P, F234A and L235A when compared to the wild-type IgG4. Residue numbering is according to the EU Index. **Table 3** shows the generated antibodies and introduced mutations when compared to the parental C40B16 mAb. A C33A mutation was engineered in the VL of all generated antibodies when compared to the parental C40B16 VL.

Table 4 shows the HCDR1 and the HCDR2 amino acid sequences of the antibodies.

Table 5 shows the HCDR3 and the LCDR1 amino acid sequences of the antibodies.

Table 6 shows the LCDR2 and the LCDR3 amino acid sequences of the antibodies.

Table 7 shows the SEQ ID NOs: of the amino acid and cDNA sequences of the VH and the VL regions of the antibodies.

Table 8 shows the SEQ ID NOs: of the amino acid sequences of the heavy chains and the light chains of the antibodies.

Table 9 shows the amino acid or polynucleotide sequences corresponding to SEQ ID NOs: 11-14, 21-52 and 65-78.

Table 3.

mAb	VH Mutation compared to C40B16	Isotype
C40B16	parental	IgG1
C40B124	R43K,H82Q,N52D,S54A	IgG1Sigma
C40B135	R43K,H82Q,N52D,S54A	IgG4PAA
C40B125	R43K,H82Q,N52Q,S54A	IgG1Sigma
C40B136	R43K,H82Q,N52Q,S54A	IgG4PAA
C40B127	R43K,H82Q,N53Q,S54A	IgG1Sigma
C40B138	R43K,H82Q,N53Q,S54A	IgG4PAA
C40B131	R43K,H82Q,N53A,S54A	IgG4PAA
C40B176	R43K,H82Q,N53A,S54A, M108L	IgG1Sigma
C40B180	R43K,H82Q,N53A,S54A, M108L	IgG4PAA
C40B179	R43K,H82Q,N53Q,S54A, M108L	IgG1Sigma
C40B183	R43K,H82Q,N53Q,S54A, M108L	IgG4PAA

Table 4.

mAb	HCDR1		HCDR2	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
C40B16	SYAMS	5	TINNSGGGTYTADSVKG	6
C40B124	SYAMS	5	TIDNAGGGTYTADSVKG	15
C40B135	SYAMS	5	TIDNAGGGTYTADSVKG	15
C40B125	SYAMS	5	TIQNAGGGTYTADSVKG	16

C40B136	SYAMS	5	TIQNAGGGTTYADSVKG	16
C40B127	SYAMS	5	TINQAGGGTTYADSVKG	17
C40B138	SYAMS	5	TINQAGGGTTYADSVKG	17
C40B131	SYAMS	5	TINAAGGGTTYADSVKG	18
C40B176	SYAMS	5	TINAAGGGTTYADSVKG	18
C40B180	SYAMS	5	TINAAGGGTTYADSVKG	18
C40B179	SYAMS	5	TINQAGGGTTYADSVKG	17
C40B183	SYAMS	5	TINQAGGGTTYADSVKG	17

Table 5.

mAb	HCDR3		LCDR1	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
C40B16	EGGKYYYYAMDV	7	SGDKLGDKYAC	8
C40B124	EGGKYYYYAMDV	7	SGDKLGDKYAA	20
C40B135	EGGKYYYYAMDV	7	SGDKLGDKYAA	20
C40B125	EGGKYYYYAMDV	7	SGDKLGDKYAA	20
C40B136	EGGKYYYYAMDV	7	SGDKLGDKYAA	20
C40B127	EGGKYYYYAMDV	7	SGDKLGDKYAA	20
C40B138	EGGKYYYYAMDV	7	SGDKLGDKYAA	20
C40B131	EGGKYYYYAMDV	7	SGDKLGDKYAA	20
C40B176	EGGKYYYYALDV	19	SGDKLGDKYAA	20
C40B180	EGGKYYYYALDV	19	SGDKLGDKYAA	20
C40B179	EGGKYYYYALDV	19	SGDKLGDKYAA	20
C40B183	EGGKYYYYALDV	19	SGDKLGDKYAA	20

Table 6.

mAb	LCDR2		LCDR3	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
C40B16	QDSRRPS	9	QAWASSTVV	10
C40B124	QDSRRPS	9	QAWASSTVV	10
C40B135	QDSRRPS	9	QAWASSTVV	10
C40B125	QDSRRPS	9	QAWASSTVV	10
C40B136	QDSRRPS	9	QAWASSTVV	10

C40B127	QDSRRPS	9	QAWASSTVV	10
C40B138	QDSRRPS	9	QAWASSTVV	10
C40B131	QDSRRPS	9	QAWASSTVV	10
C40B176	QDSRRPS	9	QAWASSTVV	10
C40B180	QDSRRPS	9	QAWASSTVV	10
C40B179	QDSRRPS	9	QAWASSTVV	10
C40B183	QDSRRPS	9	QAWASSTVV	10

Table 7.

mAb	VH name	VH amino acid SEQ ID NO:	VH DNA SEQ ID NO:	VL name	VL amino acid SEQ ID NO:	VL DNA SEQ ID NO:
C40B16		11	13		12	14
C40B124	C40H60	21	28	C40L71	27	34
C40B135	C40H60	21	28	C40L71	27	34
C40B125	C40H61	22	29	C40L71	27	34
C40B136	C40H61	22	29	C40L71	27	34
C40B127	C40H63	23	30	C40L71	27	34
C40B138	C40H63	23	30	C40L71	27	34
C40B131	C40H56	24	31	C40L71	27	34
C40B176	C40H83	25	32	C40L71	27	34
C40B180	C40H83	25	32	C40L71	27	34
C40B179	C40H86	26	33	C40L71	27	34
C40B183	C40H86	26	33	C40L71	27	34

Table 8.

mAb	HC protein SEQ ID NO:	HC DNA SEQ ID NO:	LC protein SEQ ID NO:	LC DNA SEQ ID NO:
C40B16	35	65	47	77
C40B124	36	66	48	78
C40B135	37	67	48	78
C40B125	38	68	48	78
C40B136	39	69	48	78

C40B127	40	70	48	78
C40B138	41	71	48	78
C40B131	42	72	48	78
C40B176	43	73	48	78
C40B180	44	74	48	78
C40B179	45	75	48	78
C40B183	46	76	48	78

Table 9.

Protein	Sequence	SEQ ID NO:
C40B16 VH protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGRGLEWVSTINNSGGGTYAD SVKGRFTISRDN SKNTLYLHMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSS	11
C40B16 VL protein	SYELTQPPSVSVSPGQTASITCSGDKLGDKYA CWYQQKPGQSPVLVIYQDSRRPSGIPERFSGS NSGNTATLTISGTQAMDEADYYCQAWASSTV VFGGGTKLTVL	12
C40B16 VH DNA	GAGGTGCAGCTGGTGAATCTGGCGGAGGA CTGGTGCAGCCTGGCGGCAGCCTGAGACTG TCTTGTGCCGCCAGCGGCTTCACCTTCAGCA GCTACGCTATGAGCTGGGTCCGACAGGCCC CTGGCAGAGGACTCGAGTGGGTGTCCACCA TCAACAACAGCGGCGGAGGCACCTACTACG CCGACAGCGTGAAGGGCAGATTCACCATCA GCCGGGACAACAGCAAGAACACCCTGTACC TGCACATGAACAGCCTGCGGGCCGAGGACA CCGCCGTGTACTATTGTGCCAAAGAGGGCG GCAAGTACTACTACTATGCCATGGACGTGT GGGGCCAGGGCACCAACCGTGACAGTGTCAT CT	13
C40B16 VL DNA	TCCTACGAGCTGACCCAGCCTCCCTCCGTGT CTGTGTCTCCTGGCCAGACCGCCAGCATCAC CTGTAGCGGCGACAAGCTGGGCGATAAGTA	14

	CGCCTGCTGGTATCAGCAGAAGCCCGGCCA GAGCCCCGTGCTGGTCATCTACCAGGACAG CAGAAGGCCCAGCGGCATCCCCGAGAGATT CAGCGGCAGCAACAGCGGCAATACCGCCAC CCTGACCATCAGCGGCACCCAGGCCATGGA CGAGGCCGATTACTATTGTCAGGCCTGGGC CAGCAGCACCGTGGTGTGTTGGCGGCGGAAC AAAGCTGACCGTGCTG	
C40H60 protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTIDNAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSS	21
C40H61 protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTIQNAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSS	22
C40H63 protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINQAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSS	23
C40H56 protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINAAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSS	24
C40H83 protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINAAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYALDVWGQGTTVTVSS	25
C40H86 protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINQAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYALDVWGQGTTVTVSS	26
C40L71 protein	SYELTQPPSVSVSPGQTASITCSGDKLGDKYA AWYQQKPGQSPVLVIYQDSRRPSGIPERFSGS NSGNTATLTISGTQAMDEADYYCQAWASSTV VFGGGTKLTVL	27
C40H60 DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCGACAACGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC	28

	CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGC	
C40H61 DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCCAGAACGCGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGC	29
C40H63 DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACCAGGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGC	30
C40H56 DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACGCCGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGC	31
C40H83 DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC	32

	CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACGCCCGCCGGCGGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCCTGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGC	
C40H86 DNA	GAGGTGCAGCTGGTGGAGAGCGGGCGGCGGC CTGGTGCAGCCCCGGCGGCAGCCTGCGGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACCAGGCCGGCGGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCCTGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGC	33
C40L71 DNA	AGCTACGAGCTGACCCAGCCCCCAGCGTG AGCGTGAGCCCCGGCCAGACCGCCAGCATC ACCTGCAGCGGCGACAAGCTGGGCGACAAG TACGCCGCCTGGTACCAGCAGAAGCCCGGC CAGAGCCCCGTGCTGGTGATCTACCAGGAC AGCCGGCGGCCCAGCGGCATCCCCGAGCGG TTCAGCGGCAGCAACAGCGGCAACACCGCC ACCCTGACCATCAGCGGCACCCAGGCCATG GACGAGGCCGACTACTACTGCCAGGCCTGG GCCAGCAGCACCGTGGTGTTCTGGCGGCGGC ACCAAGCTGACCGTGCTG	34
C40B16 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGRGLEWVSTINNSGGGTYAD SVKGRFTISRDN SKNTLYLHMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLH	35

	QDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	
C40B124 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTIDNAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPEAAGASSVFLFPPKPK DTLMISRTPEVTCVVVDVSAEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	36
C40B135 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTIDNAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSSAS TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLS VVTVPSSSLGKTYTCNV DHKPSNTKVDKRV ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI	37

	AVEWESNGQPENNYKTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLGK	
C40B125 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTIQNAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPEAAGASSVFLFPPKPK DTLMISRTPEVTCVVVDVSAEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	38
C40B136 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTIQNAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSSAS TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLS VVTVPSSSLGKTYTCNV DHKPSNTKVDKRV ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMHEALHNHYTQK	39

	SLSLSLGK	
C40B127 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINQAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPEAAGASSVFLFPPKPK DTL MISRTPEVTCVVVDVSAEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDS DGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	40
C40B138 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINQAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSSAS TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGKTYTCNV D HKPSNTKVDKRV ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTTPVLDS DGSFFLYSR LTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLGK	41
C40B131 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY	42

	AMSWVRQAPGKGLEWVSTINAAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYAMDVWGQGTTVTVSSAS TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGKTYTCNV D HKPSNTKVDKRV ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLGK	
C40B176 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINAAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYYALDVWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPEAAGASSVFLFPPKPK DTL MISRTPEVTCVVVDVSAEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	43
C40B180 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINAAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV	44

	YYCAKEGGKYYYYALDVWGQGTTVTVSSAS TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTTPVLDS DGSFFLYSR LTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLGK	
C40B179 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINQAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYALDVWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPEAAGASSVFLFPPKPK DTLMISRTPEVTCVVVDVSAEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDS DGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	45
C40B183 heavy chain protein	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSTINQAGGGTTYAD SVKGRFTISRDN SKNTLYLQMNSLRAEDTAV YYCAKEGGKYYYYALDVWGQGTTVTVSSAS TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE	46

	PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTKTYTCNVDPHKPSNTKVDKRV ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLGK	
C40B16 light chain protein	SYELTQPPSVSVSPGQTASITCSGDKLGDKYA CWYQQKPGQSPVLVIYQDSRRPSGIPERFSGS NSGNTATLTISGTQAMDEADYYCQAWASSTV VFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAG VETTTPSKQSNNKYAASSYLSLTPEQWKSHRS YSCQVTHEGSTVEKTVAPTECS	47
C40B124, C40B135, C40B125, C40B136, C40B127, C40B138, C40B131, C40B176, C40B180, C40B179, C40B183 Light chain protein	SYELTQPPSVSVSPGQTASITCSGDKLGDKYA AWYQQKPGQSPVLVIYQDSRRPSGIPERFSGS NSGNTATLTISGTQAMDEADYYCQAWASSTV VFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAG VETTTPSKQSNNKYAASSYLSLTPEQWKSHRS YSCQVTHEGSTVEKTVAPTECS	48
IGHV3-23	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADS VKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCAK	49
IGLV3-1	SYELTQPPSVSVSPGQTASITCSGDKLGDKYA CWYQQKPGQSPVLVIYQDSKRPSGIPERFSGS NSGNTATLTISGTQAMDEADYYCQAWDSSTA	50
IgG1sigma Fc	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS	51

	LSSVVTVPSSSLGTQTYICNVN HKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGASSVFLFPP KPKDTLMISRTPEVTCVVVDVSAEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPSSIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNV FSCSVMHEAL HNHYTQKSLSLSPGK	
IgG4PAA	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYTCNV D HKPSNTKVDKR VESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVVDV SQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNV FSCSVMHEALHNHYTQ KSLSLSLGK	52
C40B16 heavy chain DNA	GAGGTGCAGCTGGTGG AATCTGGCGGAGGA CTGGTGCAGCCTGGCGGCAGCCTGAGACTG TCTTGTGCCGCCAGCGGCTTCACCTTCAGCA GCTACGCTATGAGCTGGGTCCGACAGGCC CTGGCAGAGGACTCGAGTGGGTGTCCACCA TCAACAACAGCGGCGGAGGCACCTACTACG CCGACAGCGTGAAGGGCAGATTCACCATCA GCCGGGACAACAGCAAGAACACCCTGTACC TGCACATGAACAGCCTGCGGGCCGAGGACA CCGCCGTGTACTATTGTGCCAAAGAGGGCG GCAAGTACTACTACTATGCCATGGACGTGT GGGGCCAGGGCACCAACCGTGACAGTGTCAT CTGCCTCCACCAAGGGGCCATCGGTCTTCCC CCTGGCACCCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCGT GGAACTCAGGCGCCCTGACCAGCGGCGTGC ACACCTTCCCGGCTGTCCTACAGTCCTCAGG ACTCTACTCCCTCAGCAGCGTGGTGACCGTG CCCTCCAGCAGCTTGGGCACCCAGACCTAC ATCTGCAACGTGAATCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAAGTTGAGCCCAA TCTTGTGACAAAAC T CACACATGCCCACCGT GCCCAGCACCTGAACTCCTGGGGGGACCGT CAGTCTTCCTCTTCCCCCAAAACCCAAGGA CACCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTG	65

	GACGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTACAACAGCAC GTACCGTGTGGTCAGCGTCCTCACCGTCCTG CACCAGGACTGGCTGAATGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGCCCTCCCA GCCCCCATCGAGAAAACCATCTCCAAAGCC AAAGGGCAGCCCCGAGAACCACAGGTGTAC ACCCTGCCCCCATCCCGGGAGGAGATGACC AAGAACCAGGTCAGCCTGACCTGCCTGGTC AAAGGCTTCTATCCCAGCGACATCGCCGTG GAGTGGGAGAGCAATGGGCAGCCGGAGAA CAACTACAAGACCACGCCTCCCGTGCTGGA CTCCGACGGCTCCTTCTTCTCTACAGCAAG CTCACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGTCTTCTCATGCTCCGTGATGCATG AGGCTCTGCACAACCACTACACGCAGAAGA GCCTCTCCCTGTCTCCGGGTAAA	
C40B124 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCGACAACGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCCTCCTCCAAGAGCACCTCTGG GGGCACAGCGGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTC GTGGAACCTCAGGCGCCCTGACCAGCGGCGT GCACACCTTCCCGGCTGTCCTACAGTCCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACC TACATCTGCAACGTGAATCACAAGCCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCC AAATCTTG TGACAAA ACTCACACATGCCCA CCGTGCCCAGCACCTGAAGCAGCAGGGGCA TCTTCAGTCTTCTCTTCCCCC AAAACCCA AGGACACCCTCATGATCTCCCGGACCCCTG AGGTCACATGCGTGGTGGTGGACGTGAGCG CCGAAGACCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGGAGGTGCATAATGCCA AGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTCAGCGTCCTCACC	66

	GTCCTGCACCAGGACTGGCTGAATGGCAAG GAGTACAAGTGCAAGGTCTCCAACAAAGCC CTCCCATCCTCCATCGAGAAAACCATCTCCA AAGCCAAAGGGCAGCCCCGAGAACCACAG GTGTACACCCTGCCCCCATCCCGGGAGGAG ATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACA ACTACAAGACCACGCCTCCCGTGC TGGACTCCGACGGCTCCTTCTTCCTCTACAG CAAGCTCACC GTGGACAAGAGCAGGTGGCA GCAGGGGAACGTCTTCTCATGCTCCGTGATG CATGAGGCTCTGCACAACCACTACACGCAG AAGAGCCTCTCCCTGTCTCCGGGTAAA	
C40B135 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCGACAACGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCAACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCCGTCTTCC CCCTGGCGCCCTGCTCCAGGAGCACCTCCG AGAGCACAGCCGCCCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGA ACTCAGGCGCCCTGACCAGCGGCG TGCACACCTTCCCGGCTGTCTTACAGTCCTC AGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACGAAAAC CTACACCTGCAACGTAGATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGTC CAAATATGGTCCCCCATGCCCAACCATGCCCA GCACCTGAGGCCGCCGGGGGACCATCAGTC TTCCTGTTCCCCC AAAACCCAAGGACACTC TCATGATCTCCCGGACCCCTGAGGTCACGTG CGTGGTGGTGGACGTGAGCCAGGAAGACCC CGAGGTCCAGTTCAACTGGTACGTGGATGG CGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTTCAACAGCACGTACCG TGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGGCCTCCCGTCCTCC ATCGAGAAAACCATCTCCAAAGCCAAAGGG	67

	CAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCCAGGAGGAGATGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTACCCCAGCGACATCGCCGTGGAGTGG GAGAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGAC GGCTCCTTCTTCCTCTACAGCAGGCTAACCG TGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGTAAA	
C40B125 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCCAGAACGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCAACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCCTCCTCCAAGAGCACCTCTGG GGGCACAGCGGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTC GTGGAACTCAGGCGCCCTGACCAGCGGCGT GCACACCTTCCCGGCTGTCCTACAGTCCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACC TACATCTGCAACGTGAATCACAAGCCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCC AAATCTTGTGACAAAACCTCACACATGCCCA CCGTGCCCAGCACCTGAAGCAGCAGGGGCA TCTTCAGTCTTCCTCTTCCCCC AAAACCCA AGGACACCCTCATGATCTCCCGGACCCCTG AGGTCACATGCGTGGTGGTGGACGTGAGCG CCGAAGACCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGAGGTGCATAATGCCA AGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTCAGCGTCCTCACC GTCCTGCACCAGGACTGGCTGAATGGCAAG GAGTACAAGTGCAAGGTCTCCAACAAAGCC CTCCCATCCTCCATCGAGAAAACCATCTCCA AAGCCAAAGGGCAGCCCCGAGAACCACAG GTGTACACCCTGCCCCCATCCCGGGAGGAG ATGACCAAGAACCAGGTCAGCCTGACCTGC	68

	CTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAACACTACAAGACCACGCCTCCCGTGC TGGACTCCGACGGCTCCTTCTTCCTCTACAG CAAGCTCACCGTGGACAAGAGCAGGTGGCA GCAGGGGAACGTCTTCTCATGCTCCGTGATG CATGAGGCTCTGCACAACCACTACACGCAG AAGAGCCTCTCCCTGTCTCCGGGTAAA	
C40B136 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCCAGAACGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCAACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCCGTCTTCC CCCTGGCGCCCTGCTCCAGGAGCACCTCCG AGAGCACAGCCGCCCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGAACCTCAGGCGCCCTGACCAGCGGCG TGCACACCTTCCCGGCTGTCCTACAGTCCTC AGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACGAAAAC CTACACCTGCAACGTAGATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGTC CAAATATGGTCCCCCATGCCCACCATGCCCA GCACCTGAGGGCCGCCGGGGGACCATCAGTC TTCCTGTTCCCCCCTAAAACCCAAGGACACTC TCATGATCTCCCGGACCCCTGAGGTCACGTG CGTGGTGGTGGACGTGAGCCAGGAAGACCC CGAGGTCCAGTTCAACTGGTACGTGGATGG CGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTTCAACAGCACGTACCG TGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGGCCTCCCGTCCTCC ATCGAGAAAACCATCTCCAAAGCCAAAGGG CAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCAGGAGGAGATGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTACCCAGCGACATCGCCGTGGAGTGG GAGAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGAC	69

	GGCTCCTTCTTCTCTACAGCAGGCTAACCG TGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGTAAA	
C40B127 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACCAGGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTTACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCAACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCCTCCTCCAAGAGCACCTCTGG GGGCACAGCGGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTC GTGGAACCTCAGGCGCCCTGACCAGCGGCGT GCACACCTTCCCGGCTGTCCTACAGTCCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACC TACATCTGCAACGTGAATCACAAGCCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCC AAATCTTGTGACAAAACCTCACACATGCCCA CCGTGCCCAGCACCTGAAGCAGCAGGGGCA TCTTCAGTCTTCTCTTCCCCCCTTCCCA AGGACACCCTCATGATCTCCCGGACCCCTG AGGTACATGCGTGGTGGTGGACGTGAGCG CCGAAGACCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGGAGGTGCATAATGCCA AGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTCAGCGTCCTCACC GTCCTGCACCAGGACTGGCTGAATGGCAAG GAGTACAAGTGCAAGGTCTCCAACAAAGCC CTCCCATCCTCCATCGAGAAAACCATCTCCA AAGCCAAAGGGCAGCCCCGAGAACCACAG GTGTACACCCTGCCCCCATCCCGGGAGGAG ATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAACCTACAAGACCACGCCTCCCGTGC TGGACTCCGACGGCTCCTTCTTCTCTACAG CAAGCTCACCGTGGACAAGAGCAGGTGGCA GCAGGGGAACGTCTTCTCATGCTCCGTGATG	70

	CATGAGGCTCTGCACAACCACTACACGCAG AAGAGCCTCTCCCTGTCTCCGGGTAAA	
C40B138 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACCAGGCCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCAACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCCGTCTTCC CCCTGGCGCCCTGCTCCAGGAGCACCTCCG AGAGCACAGCCGCCCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGAACCTCAGGCGCCCTGACCAGCGGCG TGCACACCTTCCCGGCTGTCCTACAGTCCTC AGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACGAAAAC CTACACCTGCAACGTAAGATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGTC CAAATATGGTCCCCCATGCCCACCATGCCCA GCACCTGAGGCGCCGGGGGACCATCAGTC TTCCTGTTCCCCCCTAAAACCAAGGACACTC TCATGATCTCCCGGACCCCTGAGGTCACGTG CGTGGTGGTGGACGTGAGCCAGGAAGACCC CGAGGTCCAGTTCAACTGGTACGTGGATGG CGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTTCAACAGCACGTACCG TGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGGCCTCCCGTCCTCC ATCGAGAAAACCATCTCCAAAGCCAAAGGG CAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCCAGGAGGAGATGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTACCCCAGCGACATCGCCGTGGAGTGG GAGAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGAC GGCTCCTTCTTCTCTACAGCAGGCTAACCG TGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGTAAA	71
C40B131 heavy chain	GAGGTGCAGCTGGTGGAGAGCGGCGGC	72

DNA	CTGGTGCAGCCCCGGCGGCAGCCTGCGGGCTG AGCTGCGCCGCCAGCGGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACGCCGCCGGCGGCAGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCATGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGCGCCTCCACCAAGGGGCCATCCGTCTTCC CCCTGGCGCCCTGCTCCAGGAGCACCTCCG AGAGCACAGCCGCCCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGA ACTCAGGCGCCCTGACCAGCGGCG TGCACACCTTCCCGGCTGTCCTACAGTCCTC AGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACGAAAAC CTACACCTGCAACGTAGATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGTC CAAATATGGTCCCCCATGCCCAACCATGCCCA GCACCTGAGGCCGCCGGGGGACCATCAGTC TTCCTGTTCCCCC AAAACCCAAGGACACTC TCATGATCTCCCGGACCCCTGAGGTCACGTG CGTGGTGGTGGACGTGAGCCAGGAAGACCC CGAGGTCCAGTTCAACTGGTACGTGGATGG CGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTTCAACAGCACGTACCG TGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGGCCTCCCGTCCTCC ATCGAGAAAACCATCTCCAAAGCCAAAGGG CAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCCAGGAGGAGATGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTACCCCAGCGACATCGCCGTGGAGTGG GAGAGCAATGGGCAGCCGGAGAACA ACTAC AAGACCACGCCTCCCGTGCTGGACTCCGAC GGCTCCTTCTTCCTCTACAGCAGGCTAACCG TGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGTAAA	
C40B176 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCCGGCGGCAGCCTGCGGGCTG AGCTGCGCCGCCAGCGGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC	73

	CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACGCCGCGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCCTGGACGTG TGGGGCCAGGGCACCAACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCCTCCTCCAAGAGCACCTCTGG GGGCACAGCGGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTC GTGGAACCTCAGGCGCCCTGACCAGCGGCGT GCACACCTTCCCGGCTGTCCTACAGTCCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACC TACATCTGCAACGTGAATCACAAGCCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCC AAATCTTGTGACAAAACCTCACACATGCCCA CCGTGCCCAGCACCTGAAGCAGCAGGGGCA TCTTCAGTCTTCTCTTCCCCCCTTCTTCA AGGACACCCTCATGATCTCCCGGACCCCTG AGGTCACATGCGTGGTGGTGGACGTGAGCG CCGAAGACCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGGAGGTGCATAATGCCA AGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTCAGCGTCCTCACC GTCCTGCACCAGGACTGGCTGAATGGCAAG GAGTACAAGTGCAAGGTCTCCAACAAGCC CTCCCATCCTCCATCGAGAAAACCATCTCCA AAGCCAAAGGGCAGCCCCGAGAACCACAG GTGTACACCCTGCCCCCATCCCGGGAGGAG ATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAACCTACAAGACCACGCCTCCCGTGC TGGACTCCGACGGCTCCTTCTTCTCTACAG CAAGCTCACCGTGGACAAGAGCAGGTGGCA GCAGGGGAACGTCTTCTCATGCTCCGTGATG CATGAGGCTCTGCACAACCACTACACGCAG AAGAGCCTCTCCCTGTCTCCGGGTAAA	
C40B180 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACGCCGCGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTCACCATC	74

	AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCCTGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGCGCCTCCACCAAGGGGCCATCCGTCTTCC CCCTGGCGCCCTGCTCCAGGAGCACCTCCG AGAGCACAGCCGCCCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGAATCAGGCGCCCTGACCAGCGGCG TGCACACCTTCCCGGCTGTCCTACAGTCCTC AGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACGAAAAC CTACACCTGCAACGTAAGATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGTC CAAATATGGTCCCCCATGCCCACCATGCCCA GCACCTGAGGGCCGCCGGGGGACCATCAGTC TTCCTGTTCCCCCCTAAAACCCAAGGACACTC TCATGATCTCCCGGACCCCTGAGGTCACGTG CGTGGTGGTGGACGTGAGCCAGGAAGACCC CGAGGTCCAGTTCAACTGGTACGTGGATGG CGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTTCAACAGCACGTACCG TGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGGCCTCCCGTCCTCC ATCGAGAAAACCATCTCCAAAGCCAAAGGG CAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCAGGAGGAGATGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTACCCAGCGACATCGCCGTGGAGTGG GAGAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGAC GGCTCCTTCTTCTCTACAGCAGGCTAACCG TGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGTAAA	
C40B179 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACCAGGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC	75

	GGCAAGTACTACTACTACGCCCTGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCTCCTCCAAGAGCACCTCTGG GGGCACAGCGGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTC GTGGAACCTCAGGCGCCCTGACCAGCGGCGT GCACACCTTCCCGGCTGTCCTACAGTCCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACC TACATCTGCAACGTGAATCACAAGCCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCC AAATCTTGTGACAAAACCTCACACATGCCCA CCGTGCCCAGCACCTGAAGCAGCAGGGGCA TCTTCAGTCTTCTCTTCCCCCCTTCAACCA AGGACACCCTCATGATCTCCCGGACCCCTG AGGTCACATGCGTGCTGGTGGACGTGAGCG CCGAAGACCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGAGGTGCATAATGCCA AGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTCAGCGTCCTCACC GTCCTGCACCAGGACTGGCTGAATGGCAAG GAGTACAAGTGCAAGGTCTCCAACAAGCC CTCCCATCCTCCATCGAGAAAACCATCTCCA AAGCCAAAGGGCAGCCCCGAGAACCACAG GTGTACACCCTGCCCCCATCCCGGGAGGAG ATGACCAAGAACCAGGTGAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAACCTACAAGACCACGCCTCCCGTGC TGGACTCCGACGGCTCCTTCTTCTCTACAG CAAGCTCACCGTGGACAAGAGCAGGTGGCA GCAGGGGAACGTCTTCTCATGCTCCGTGATG CATGAGGCTCTGCACAACCACTACACGCAG AAGAGCCTCTCCCTGTCTCCGGGTAAA	
C40B183 heavy chain DNA	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGC CTGGTGCAGCCCGGCGGCAGCCTGCGGCTG AGCTGCGCCGCCAGCGGCTTCACCTTCAGC AGCTACGCCATGAGCTGGGTGCGGCAGGCC CCCGGCAAGGGCCTGGAGTGGGTGAGCACC ATCAACCAGGCCGGCGGCGGCACCTACTAC GCCGACAGCGTGAAGGGCCGGTTACCATC AGCCGGGACAACAGCAAGAACACCCTGTAC CTGCAGATGAACAGCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGCGCCAAGGAGGGC GGCAAGTACTACTACTACGCCCTGGACGTG TGGGGCCAGGGCACCACCGTGACCGTGAGC AGCGCCTCCACCAAGGGCCCATCCGTCTTCC	76

	CCCTGGCGCCCTGCTCCAGGAGCACCTCCG AGAGCACAGCCGCCCTGGGCTGCCTGGTCA AGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGAACCTCAGGCGCCCTGACCAGCGGCG TGCACACCTTCCCGGCTGTCCTACAGTCCTC AGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACGAAAAC CTACACCTGCAACGTAGATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGTC CAAATATGGTCCCCCATGCCCAACCATGCCCA GCACCTGAGGCCGCCGGGGGACCATCAGTC TTCCTGTTCCCCCCTAAAACCCAAGGACACTC TCATGATCTCCCGGACCCCTGAGGTCACGTG CGTGGTGGTGGACGTGAGCCAGGAAGACCC CGAGGTCCAGTTCAACTGGTACGTGGATGG CGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTTCAACAGCACGTACCG TGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAACGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGGCCTCCCGTCCTCC ATCGAGAAAACCATCTCCAAAGCCAAAGGG CAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCCAGGAGGAGATGACCAAGAAC CAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTACCCCAGCGACATCGCCGTGGAGTGG GAGAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGAC GGCTCCTTCTTCTCTACAGCAGGCTAACCG TGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGTAAA	
C40B16 light chain DNA	TCCTACGAGCTGACCCAGCCTCCCTCCGTGT CTGTGTCTCCTGGCCAGACCGCCAGCATCAC CTGTAGCGGCGACAAGCTGGGCGATAAGTA CGCCTGCTGGTATCAGCAGAAGCCCGGCCA GAGCCCCGTGCTGGTCATCTACCAGGACAG CAGAAGGCCAGCGGCATCCCCGAGAGATT CAGCGGCAGCAACAGCGGCAATACCGCCAC CCTGACCATCAGCGGCACCCAGGCCATGGA CGAGGCCGATTACTATTGTCAGGCCTGGGC CAGCAGCACCGTGGTGTTTGGCGGCGGAAC AAAGCTGACCGTGCTGGGTCAGCCCAAGGC TGCACCCAGTGTCCTCTGTTCCTCCCGCCCTCC TCTGAGGAGCTTCAAGCCAACAAGGCCACA CTGGTGTGTCTCATAAGTGACTTCTACCCGG GAGCCGTGACAGTGGCCTGGAAGGCCGATA GCAGCCCCGTCAAGGCGGGAGTGGAGACCA	77

	CCACACCCTCCAAACAAAGCAACAACAAGT ACGCGGCCAGCAGCTATCTGAGCCTGACGC CTGAGCAGTGGAAGTCCCACAGAAGCTACA GCTGCCAGGTCACGCATGAAGGGAGCACCG TGGAGAAGACAGTGGCCCCTACAGAATGTT CA	
C40B124, C40B135, C40B125, C40B136, C40B127, C40B138, C40B131, C40B176, C40B180, C40B179, C40B183 Light chain DNA	AGCTACGAGCTGACCCAGCCCCCAGCGTG AGCGTGAGCCCCGGCCAGACCGCCAGCATC ACCTGCAGCGGCGACAAGCTGGGCGACAAG TACGCCGCCTGGTACCAGCAGAAGCCCCGGC CAGAGCCCCGTGCTGGTGATCTACCAGGAC AGCCGGCGGCCAGCGGCATCCCCGAGCGG TTCAGCGGCAGCAACAGCGGCAACACCGCC ACCCTGACCATCAGCGGCACCCAGGCCATG GACGAGGCCGACTACTACTGCCAGGCCTGG GCCAGCAGCACCGTGGTGTTCGGCGGCGGC ACCAAGCTGACCGTGCTGGGTCAGCCCAAG GCTGCACCCAGTGTCACTCTGTTCCCGCCCT CCTCTGAGGAGCTTCAAGCCAACAAGGCCA CACTGGTGTGTCTCATAAGTGACTTCTACCC GGGAGCCGTGACAGTGGCCTGGAAGGCCGA TAGCAGCCCCGTCAAGGCGGGAGTGGAGAC CACCACACCCTCCAAACAAAGCAACAACAA GTACGCGGCCAGCAGCTATCTGAGCCTGAC GCCTGAGCAGTGGAAGTCCCACAGAAGCTA CAGCTGCCAGGTCACGCATGAAGGGAGCAC CGTGGAGAAGACAGTGGCCCCTACAGAATG TTCA	78

Example 3. Characterization of antagonistic anti-CD40 antibodies

The parental C40B16 and the engineered variants were tested for their antagonistic activity in a spectrum of assays including ability of the antibodies to inhibit human soluble CD40L (sCD40L) or membrane-bound CD40L (mCD40L)-mediated proliferation of human or cyno B cells, and inhibition of IL-12p40 production by human DCs. The experiments were conducted according to protocols described in Example 1. Membrane-bound CD40L was provided on Jurkat cells in the assays.

All antibodies demonstrated antagonism in the assays performed.

Table 10 shows the IC₅₀ values for inhibition of soluble or membrane-bound CD40L-driven B cell proliferation. **Table 11** shows the IC₅₀ values for inhibition of soluble or membrane-bound CD40L-driven IL-12p40 production by human dendritic cells.

Table 10.

	B-cell proliferation IC ₅₀ nM +/- SD		
mAb	sCD40L-driven human B-cell proliferation	mCD40L-driven human B-cell proliferation	sCD40L-driven cyno B-cell proliferation
C40B16			
C40B124		0.118 +/-0.003 (4)	
C40B135	0.056 +/-0.040 (3)	0.131 +/-0.012 (4)	0.077 +/-0.024 (2)
C40B125	0.088 +/-0.025 (6)	0.128 +/-0.019 (6)	0.083 +/-0.029 (2)
C40B136	0.087 +/-0.056 (6)	0.122 +/-0.013 (4)	0.065 +/-0.038 (2)
C40B127	0.073 +/-0.010 (2)	0.111 +/-0.009 (4)	0.066 +/-0.002 (2)
C40B138	0.048 +/-0.035 (2)	0.106 +/-0.000 (2)	0.075 +/-0.050 (2)
C40B131		0.099 +/-0.003 (4)	
C40B176	0.132 +/-0.105 (4)	0.181 +/-0.054 (4)	0.108 +/-0.029 (4)
C40B180	0.092 +/-0.047 (4)	0.136 +/-0.071 (4)	0.109 +/-0.047 (4)
C40B179	0.132 +/-0.030 (4)	0.178 +/-0.053 (4)	0.119 +/-0.026 (4)
C40B183	0.103 +/-0.053 (4)	0.140 +/-0.018 (4)	0.110 +/-0.029 (4)
sCD40L: soluble CD40L			
mCD40L: membrane-bound CD40L			
The number in parenthesis indicates the number of times the assay was repeated			

Table 11.

mAb	DC IL-12p40 production IC ₅₀ nM +/- SD	
	sCD40L-driven	mCD40L-driven
C40B16		
C40B124	0.387 +/-0.09 (3)	0.507 +/-0.07 (5)
C40B135	0.329 +/-0.02 (3)	0.430 +/-0.10 (5)
C40B125	0.244 +/-0.04 (5)	0.393 +/-0.09 (5)
C40B136	0.246 +/-0.05 (5)	0.379 +/-0.08 (5)
C40B127	0.270 +/-0.07 (3)	0.383 +/-0.08 (5)
C40B138	0.262 +/-0.06 (3)	0.341 +/-0.10 (5)
C40B131	0.330 +/-0.18 (3)	0.323 +/-0.12 (5)
C40B176	0.220 +/-0.11 (3)	0.245 +/-0.06 (2)
C40B180	0.225 +/-0.06 (3)	0.222 +/-0.07 (2)
C40B179	0.184 +/-0.07 (3)	0.297 +/-0.06 (2)
C40B183	0.208 +/-0.09 (3)	0.291 +/-0.07 (2)
sCD40L: soluble CD40L		
mCD40L: membrane-bound CD40L		
The number in parenthesis indicates the number of times the assay was repeated		

Affinity of the antibodies to human CD40 was assessed using Surface Plasmon Resonance (SPR) using a ProteOn XPR36 system (BioRad). A biosensor surface was prepared by coupling anti-Human IgG Fc (Jackson cat#109-005-098) to the modified alginate polymer layer surface of a GLC chip (BioRad, Cat#176-5011) using the manufacturer instructions for amine-coupling chemistry. Approximately 5000 RU (response units) of mAbs were immobilized. The kinetic experiments were performed at 25°C in running buffer (DPBS+0.01%P20+100µg/ml BSA). To perform kinetic experiments, 200 RU of mAbs were captured followed by injections of analytes (human or cyno CD40) at 5 concentrations (in a 4-fold serial dilution). The association phase was

monitored for 3 minutes at 50 $\mu\text{L}/\text{min}$, then followed by 15 minutes of buffer flow (dissociation phase). The chip surface was regenerated with two 18 second pulses of 100 mM H_3PO_4 (Sigma, Cat#7961) at 100 $\mu\text{L}/\text{min}$.

The collected data were processed using ProteOn Manager software. First, the data was corrected for background using inter-spots. Then, double reference subtraction of the data was performed by using the buffer injection for analyte injections. The kinetic analysis of the data was performed using a Langmuir 1:1 binding model. The results were reported in the format of K_a (On-rate), K_d (Off-rate) and K_D (equilibrium dissociation constant).

Summary of kinetics affinity for the mAbs for binding to human CD40 is shown in **Table 12**. The parameters reported in this table were obtained from a 1:1 Langmuir binding model for all samples. Affinity, $K_D = k_d/k_a$.

Table 12.

mAb	k_a (1/Ms)	k_d (1/s)	K_D (M)
C40B16	4.27E+06	1.57E-04	3.68E-11
C40B124	2.30E+06	9.56E-05	4.16E-11
C40B135	2.93E+06	1.45E-04	4.96E-11
C40B125	3.89E+06	1.36E-04	3.49E-11
C40B136	3.30E+06	1.13E-04	3.43E-11
C40B127	3.79E+06	1.15E-04	3.03E-11
C40B138	3.58E+06	2.15E-04	5.99E-11
C40B131	3.52E+06	2.86E-04	8.12E-11
C40B176	2.86E+06	3.07E-04	1.07E-10
C40B180	2.67E+06	3.66E-04	1.37E-10
C40B179	2.86E+06	2.67E-04	9.33E-11
C40B183	2.91E+06	3.50E-04	1.21E-10

Example 4. Antagonistic antibodies specifically binding human CD40 are potent inhibitors of CD40/CD40L pathway and display minimal agonistic activity

Four antagonistic antibodies (C40B176, C40B179, C40B180 and C40B183) were benchmarked against anti-CD40 antibodies currently in clinical developments. The antibodies used in comparisons were: Astellas/Kirin ASKP-1240 (HC SEQ ID NO: 53, LC SEQ ID NO: 54, Fc silent IgG4 with S228P and L235E mutations), Antibody D (HC SEQ ID NO: 55, LC SEQ ID NO: 56, Fc silent IgG1 with L234A and L235A mutations; described in US8,591,900 as antibody B IgG1KOb), Novartis CFZ533 (HC SEQ ID NO: 57, LC SEQ ID NO: 58, Fc silent IgG1 with N297A mutation), and BMS-986090 (HC SEQ ID NO: 59, IgG4 with S228P mutation).

SEQ ID NO: 53 (ASKP-1240 HC)

QLQLQESGPGLLKPSSETLSLTCTVSGGSISSPGYYGGWIRQPPGKGLEWIGSIYKSGS
TYHNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCTRPVVRYFGWFDPWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPP
CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG
VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS
KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLK

SEQ ID NO: 54 (ASKP-1240 LC)

AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKAPKLLIYDASNLESG
VPSRFSGSGSGTDFTLTISLQPEDFATYYCQQFNSTYPTFGQGKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 55 (Antibody D HC)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSDYGMHWVRQAPGKGLEWVAYISSG
NRIYYADTVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARQDGYRYAMDY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA

LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKS
 CDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
 SLSPGK

SEQ ID NO: 56 (Antibody D LC)

DIVMTQSPDSLAVSLGEKVTINCKSSQSLLNSGNQKNYLTWHQQKPGQPPKLLIYW
 TSTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYTYPLTFGGGKVEIK
 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV
 TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 57 (CFZ533 HC)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYE
 ESNRYHADSVKGRFTISRDNISKITLYLQMNSLRTEDTAVYYCARDGGIAAPGPDY
 WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
 LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKS
 CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
 PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
 SLSPGK

SEQ ID NO: 58 (CFZ533 LC)

DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLISLGS
 NRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFGPGTKVDIRR
 TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
 EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 59 (BMS-986090 HC)

EVQLLESGGGLVQPGGSLRLSCAASGFTFRDYEMWWVRQAPGKGLERSAINPQG
 TRTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKLPFRFSDRGQGT
 LVTVSSASTESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
 QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK
 CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDI
 AVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
 HNHYTQKSLSLSLGK

Table 13 shows the IC₅₀ values for inhibition of soluble or membrane-bound CD40L-driven human or cyno B cell proliferation for the tested antibodies. **Table 14** shows the IC₅₀ values for inhibition of soluble or membrane-bound CD40L-driven IL-12p40 production by human dendritic cells (DC) and the affinity kinetics for each mAb. mAbs C40B176, C40B179, C40B180 and C40B183 exhibited comparable potency when compared to the best performing benchmark mAb ASKP-1240.

Table 13.

mAb	B cell proliferation; IC ₅₀ nM +/- SD		
	Soluble CD40L human B-cells	Membrane CD40L human B-cells	Soluble CD40L cyno B-cells
C40B176	0.132 +/-0.105 (4)	0.181 +/-0.054 (4)	0.108 +/-0.029 (4)
C40B180	0.092 +/-0.047 (4)	0.136 +/-0.071 (4)	0.109 +/-0.047 (4)
C40B179	0.132 +/-0.030 (4)	0.178 +/-0.053 (4)	0.119 +/-0.026 (4)
C40B183	0.103 +/-0.053 (4)	0.140 +/-0.018 (4)	0.110 +/-0.029 (4)
ASKP-1240	0.079 +/-0.017 (12)	0.132 +/-0.042 (10)	0.146 +/- 0.063 (8)
Antibody D	0.680 +/-0.323 (4)	1.926 +/-0.368 (2)	
CFZ533	0.223 +/-0.117 (4)	0.279 +/-0.062 (2)	
BMS-986090	0.288 +/-0.136 (4)	0.393 +/-0.002 (2)	

Table 14.

mAb	Affinity K _D (M)	Human DC IL-12p40 production; IC ₅₀ nM +/- SD	
		Soluble CD40L	Membrane CD40L
C40B176	4.96E-11	0.220 +/-0.11 (3)	0.245 +/-0.06 (2)
C40B180	5.99E-11	0.225 +/-0.06 (3)	0.222 +/-0.07 (2)
C40B179	3.43E-11	0.184 +/-0.07 (3)	0.297 +/-0.06 (2)
C40B183	1.07E-10	0.208 +/-0.09 (3)	0.291 +/-0.07 (2)

ASKP-1240	1.81E-10	0.119 +/-0.05 (6)	0.280 +/-0.11 (7)
Antibody D	5.8E-10	0.781 +/-0.30 (3)	3.630 +/-1.19 (5)
CFZ533	9.36E-12	0.320 +/-0.12 (3)	0.948 +/-0.29 (5)
BMS-986090	8.7E-10	0.285 +/-0.17 (2)	0.822 +/-0.39 (5)

Possible undesired agonistic activity of the mAbs was assessed using HEK-Blue™ CD40L NF-κB activation assay and IL12p40 production by dendritic cells as readouts without cross-linking using experimental protocols as described in Example 1. In addition to effector silent ASKP-1240, CFZ533, BMS-986090 and Antibody D, the parental antibody C40B16 on wild-type IgG1 was evaluated.

C40B16 as wild-type IgG1 and Antibody D on silent Fc exhibited comparable non-existing or minimal agonism in the assay. ASKP-1240, CFZ533 and BMS-986090 exhibited some agonism as anti-CD40 antibody concentration increased. **Figure 1** shows the dose response curves of the NF-κB reporter assay. **Figure 2** shows the antibody-mediated IL12p40 production by DCs using antibody concentrations between 350 nM and 1.1 nM.

The results suggest that ASKP-1240, CFZ533 and BMS-986090 may exhibit V-region driven agonism as the agonism was demonstrated in effector silent Fc.

Agonism was also assessed at 500 ng/ml concentration of antibodies in DC or B-cell agonistic assays, comparing ASKP-1240 and C40B176, C40B179, C40B180 and C40B183. At higher antibody concentrations, ASKP-1240 induced IL-12p40 production by DCs (**Figure 3A**) and proliferation of B cells (**Figure 3B**), whereas C40B176, C40B179, C40B180 and C40B183 did not.

Overall, the results suggest that C40B16 and its variants C40B176, C40B179, C40B180 and C40B183, as well as Antibody D have reduced agonist potential compared to ASKP-1240, CFZ533 and BMS-986090. Notably, the results also suggest that the potency of C40B176, C40B179, C40B180 and C40B183 in cell based assays is as much as 10-fold better than the Antibody D molecule, and comparable to the most potent benchmark molecule (ASKP-1240).

We claim:

- 1) An isolated antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 5, a HCDR2 of SEQ ID NO: 61, a HCDR3 of SEQ ID NO: 62, a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 63, a LCDR2 of SEQ ID NO: 9 and a LCDR3 of SEQ ID NO: 10.
- 2) The isolated antibody or the antigen-binding fragment thereof of claim 1, wherein the antibody competes for binding to human CD40 of SEQ ID NO: 1 with an antibody comprising
 - a) a heavy chain variable region (VH) of SEQ ID NO: 11 and a light chain variable region (VL) of SEQ ID NO: 12;
 - b) the VH of SEQ ID NO: 25 and the VL of SEQ ID NO: 27; or
 - c) the VH of SEQ ID NO: 26 and the VL of SEQ ID NO: 27.
- 3) The isolated antibody or the antigen-binding fragment thereof of claim 1 or 2, wherein the antibody comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
 - a) SEQ ID NOs: 5, 6, 7, 8, 9 and 10, respectively.
 - b) SEQ ID NOs: 5, 15, 7, 20, 9 and 10, respectively;
 - c) SEQ ID NOs: 5, 16, 7, 20, 9 and 10, respectively;
 - d) SEQ ID NOs: 5, 17, 7, 20, 9 and 10, respectively;
 - e) SEQ ID NOs: 5, 18, 7, 20, 9 and 10, respectively;
 - f) SEQ ID NOs: 5, 18, 19, 20, 9 and 10, respectively; or
 - g) SEQ ID NOs: 5, 17, 19, 20, 9 and 10, respectively.
- 4) The antibody or the antigen-binding fragment thereof of any of the claims 1-3, wherein the antibody binds human CD40 with a dissociation constant (K_D) of about 1.5×10^{-10} M or less, when the K_D is measured using ProteOn XPR36 system at 25°C in Dulbecco's phosphate buffered saline containing 0.01% polysorbate 20 (PS-20) and 100 µg/ml bovine serum albumin.
- 5) The isolated antibody or the antigen-binding fragment thereof of any of the claims 1-4, wherein the antibody inhibits soluble human CD40L-driven human tonsillar B cell proliferation with an IC_{50} value of less than about 1×10^{-9} M.

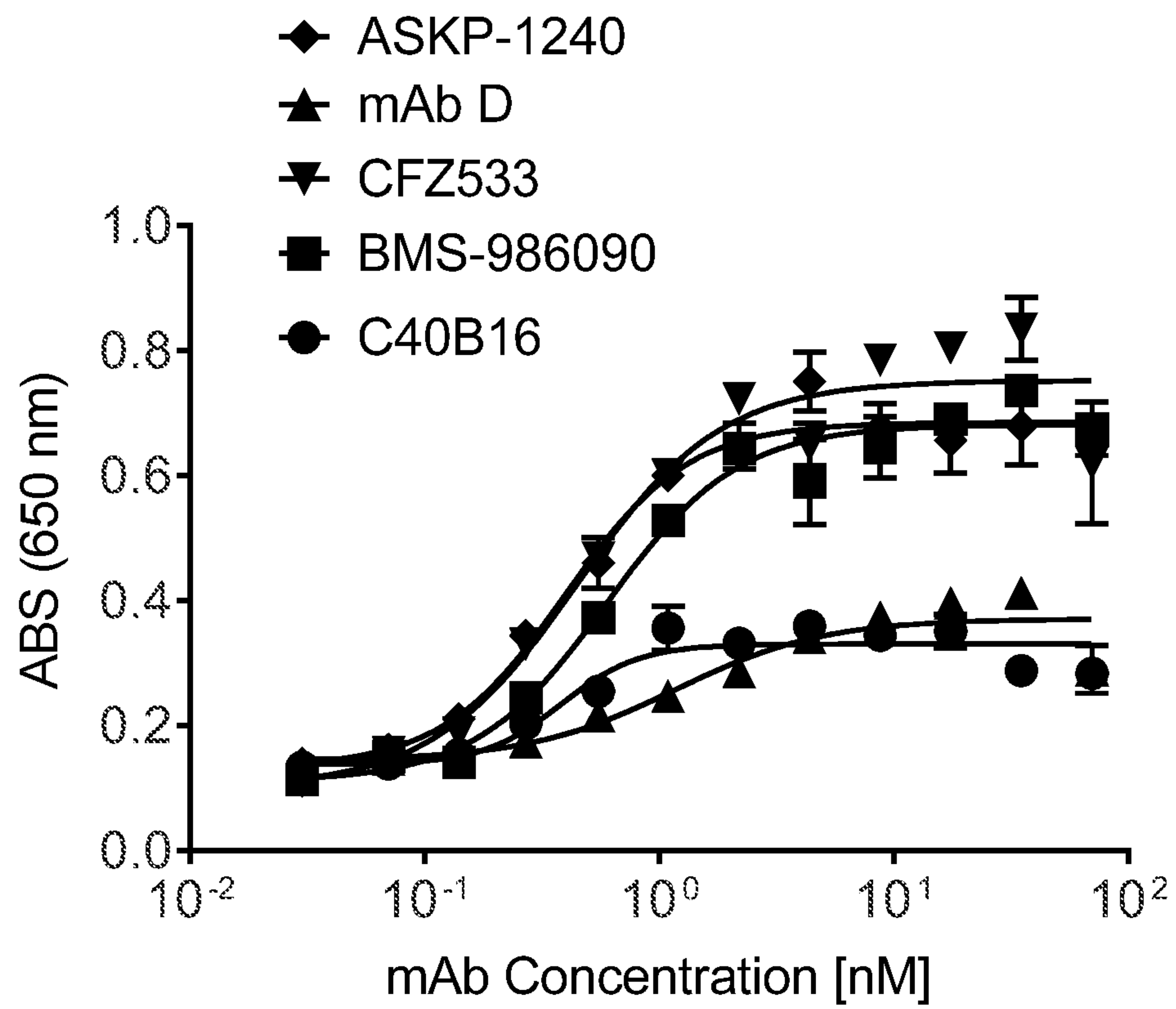
- 6) The isolated antibody or the antigen-binding fragment thereof of any of the claims 1-5, wherein the antibody inhibits soluble human CD40L-driven production of IL-12p40 by human dendritic cells with an IC_{50} value of less than about 1×10^{-9} M.
- 7) The isolated antibody or the antigen-binding fragment thereof of any of the claims 1-6, comprising the VH and the VL of
 - a) SEQ ID NOs: 11 and 12, respectively;
 - b) SEQ ID NOs: 21 and 27, respectively;
 - c) SEQ ID NOs: 22 and 27, respectively;
 - d) SEQ ID NOs: 23 and 27, respectively;
 - e) SEQ ID NOs: 24 and 27, respectively;
 - f) SEQ ID NOs: 25 and 27, respectively; or
 - g) SEQ ID NOs: 26 and 27, respectively.
- 8) The isolated antibody or the antigen-binding fragment thereof of any of the claims 1-7, which is an IgG1, IgG2, IgG3 or IgG4 isotype.
- 9) The isolated antibody or the antigen-binding fragment thereof of claim 8, wherein the antibody comprises at least one mutation in an Fc region that reduces binding of the antibody to a Fcγ receptor when compared to a wild-type Fc.
- 10) The isolated antibody or the antigen-binding fragment thereof of claim 9, wherein the at least one mutation in the Fc region is a S228P mutation, a F234A mutation, a L234A mutation, a L235A mutation, a G237A mutation, a P238S mutation, a H268A mutation, a A330S mutation or a P331S mutation, wherein residue numbering is according to the EU Index.
- 11) The isolated antibody or the antigen-binding fragment thereof of claim 9, wherein the antibody is an IgG1 isotype and comprises a L234A/L235A/G237A/P238S/H268A/A330S/P331S mutation.
- 12) The isolated antibody or the antigen-binding fragment thereof of claim 9, wherein the antibody is an IgG4 isotype and comprises a S228P/F234A/L235A mutation.
- 13) The isolated antibody or the antigen-binding fragment thereof of any of the claims 1-7, comprising a heavy chain and a light chain of
 - a) SEQ ID NOs: 35 and 47, respectively;
 - b) SEQ ID NOs: 36 and 48, respectively;
 - c) SEQ ID NOs: 37 and 48, respectively;

- d) SEQ ID NOs: 38 and 48, respectively;
 - e) SEQ ID NOs: 39 and 48, respectively;
 - f) SEQ ID NOs: 40 and 48, respectively;
 - g) SEQ ID NOs: 41 and 48, respectively;
 - h) SEQ ID NOs: 42 and 48, respectively;
 - i) SEQ ID NOs: 43 and 48, respectively;
 - j) SEQ ID NOs: 44 and 48, respectively;
 - k) SEQ ID NOs: 45 and 48, respectively; or
 - l) SEQ ID NOs: 46 and 48, respectively.
- 14) The antibody of any or the antigen-binding fragment thereof of the claims 1-13, wherein the antibody is a bispecific or a multispecific antibody.
- 15) A pharmaceutical composition comprising the isolated antibody or the antigen-binding fragment thereof of any of the claims 1-14 and a pharmaceutically acceptable carrier.
- 16) An immunoconjugate comprising the isolated antibody or the antigen-binding fragment thereof of any of the claims 1-14 linked to a cytotoxic agent or an imaging agent.
- 17) An isolated polynucleotide encoding
- a) the VH of SEQ ID NOs: 11, 21, 22, 23, 24, 25 or 26;
 - b) the VL of SEQ ID NOs: 12 or 27; or
 - c) the VH of SEQ ID NOs: 11, 21, 22, 23, 24, 25 or 26 and the VL of SEQ ID NOs: 12 or 27.
- 18) An isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NOs: 13, 14, 28, 29, 30, 31, 32, 33, or 34.
- 19) A vector comprising the polynucleotide of claim 17.
- 20) A vector comprising the polynucleotide of claim 18.
- 21) A host cell comprising the vector of claim 19.
- 22) A host cell comprising the vector of claim 20.
- 23) A method of producing an antagonistic antibody or an antigen-binding fragment thereof specifically binding human CD40 of SEQ ID NO: 1, comprising culturing the host cell of claim 21 or 22 in conditions wherein the antibody is expressed, and isolating the antibody.

- 24) A method of treating a subject having an inflammatory disease, comprising administering to the subject in need thereof the isolated antibody or the antigen-binding fragment thereof of any of the claims 1-14 for a time sufficient to treat the inflammatory disease.
- 25) The method of claim 24, wherein the inflammatory disease is an autoimmune disease.
- 26) The method of claim 24, wherein the inflammatory disease is Addison's disease, an ankylosing spondylitis, an atherosclerosis, an autoimmune hepatitis, an autoimmune diabetes, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, an idiopathic thrombocytopenia, an inflammatory bowel disease (IBD), a systemic lupus erythematosus, lupus nephritis, cutaneous lupus erythematosus, a multiple sclerosis, a myasthenia gravis, a psoriasis, an arthritis, a scleroderma, Sjogren's syndrome, a systemic sclerosis, a transplantation, a kidney transplantation, a skin transplantation, a bone marrow transplantation, a graft versus host disease (GVHD) or a type I diabetes.
- 27) The method of claim 26, wherein the arthritis is a rheumatoid arthritis, a juvenile arthritis, a psoriatic arthritis, Reiter's syndrome, an ankylosing spondylitis, or a gouty arthritis.
- 28) The method of claim 26, wherein the IBD is Crohn's disease or an ulcerative colitis.
- 29) The method of claim 24 further administering a second therapeutic agent.
- 30) The method of claim 29, wherein the second therapeutic agent is nonsteroidal anti-inflammatory drugs (NSAIDs), salicylates, hydroxychloroquine, sulfasalazine, corticosteroids, cytotoxic drugs, immunosuppressive drugs and/or antibodies.
- 31) The antibody or the antigen-binding fragment thereof of any of the claims 1-14 of the pharmaceutical composition of claim 15 for use in therapy.
- 32) An anti-idiotypic antibody binding to the antibody or the antigen-binding fragment thereof of claim 7.
- 33) A kit comprising the antibody or the antigen-binding fragment thereof of claim 7.
- 34) The kit of claim 33, further comprising reagents for detecting the antibody and instructions of use.

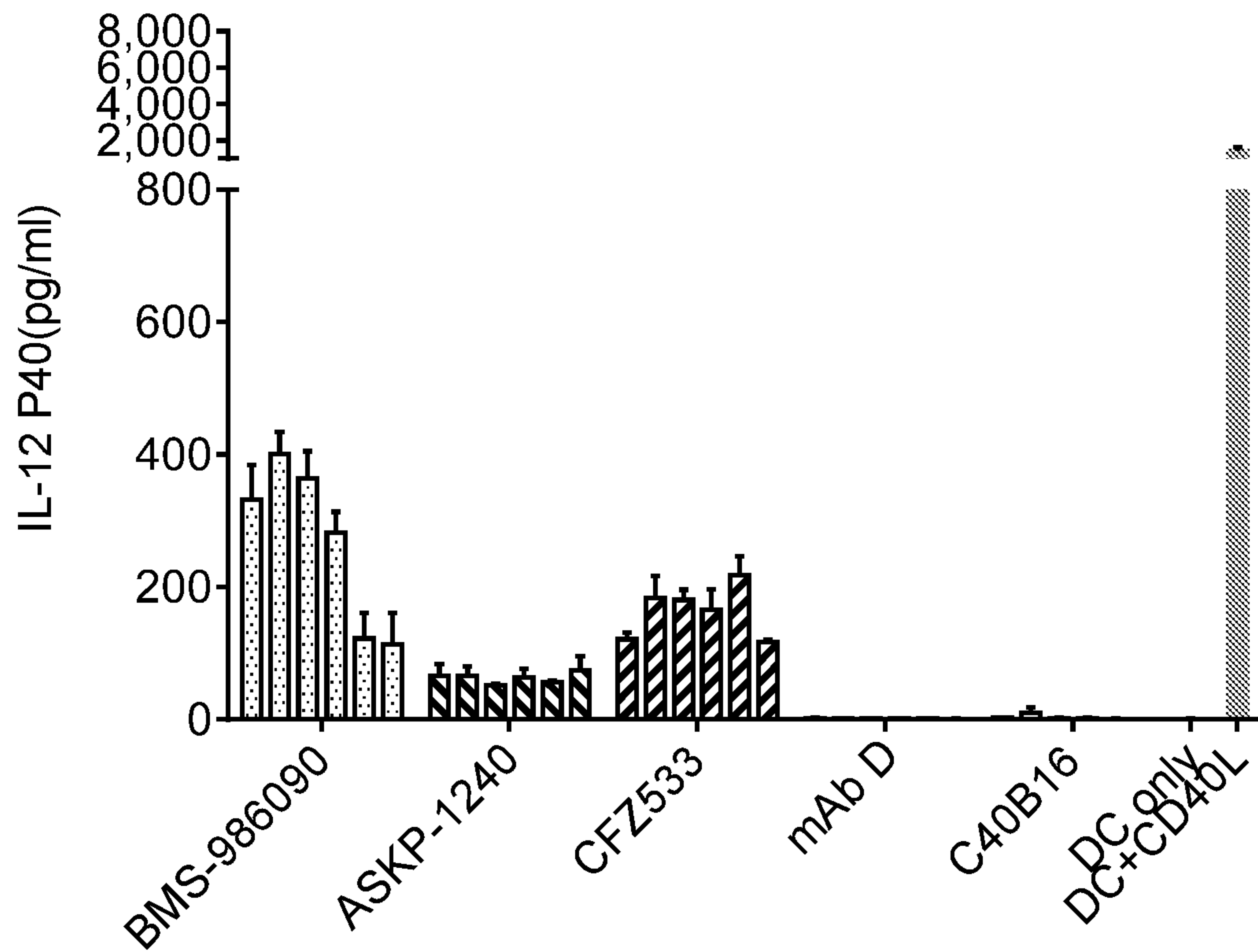
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Figure 1.



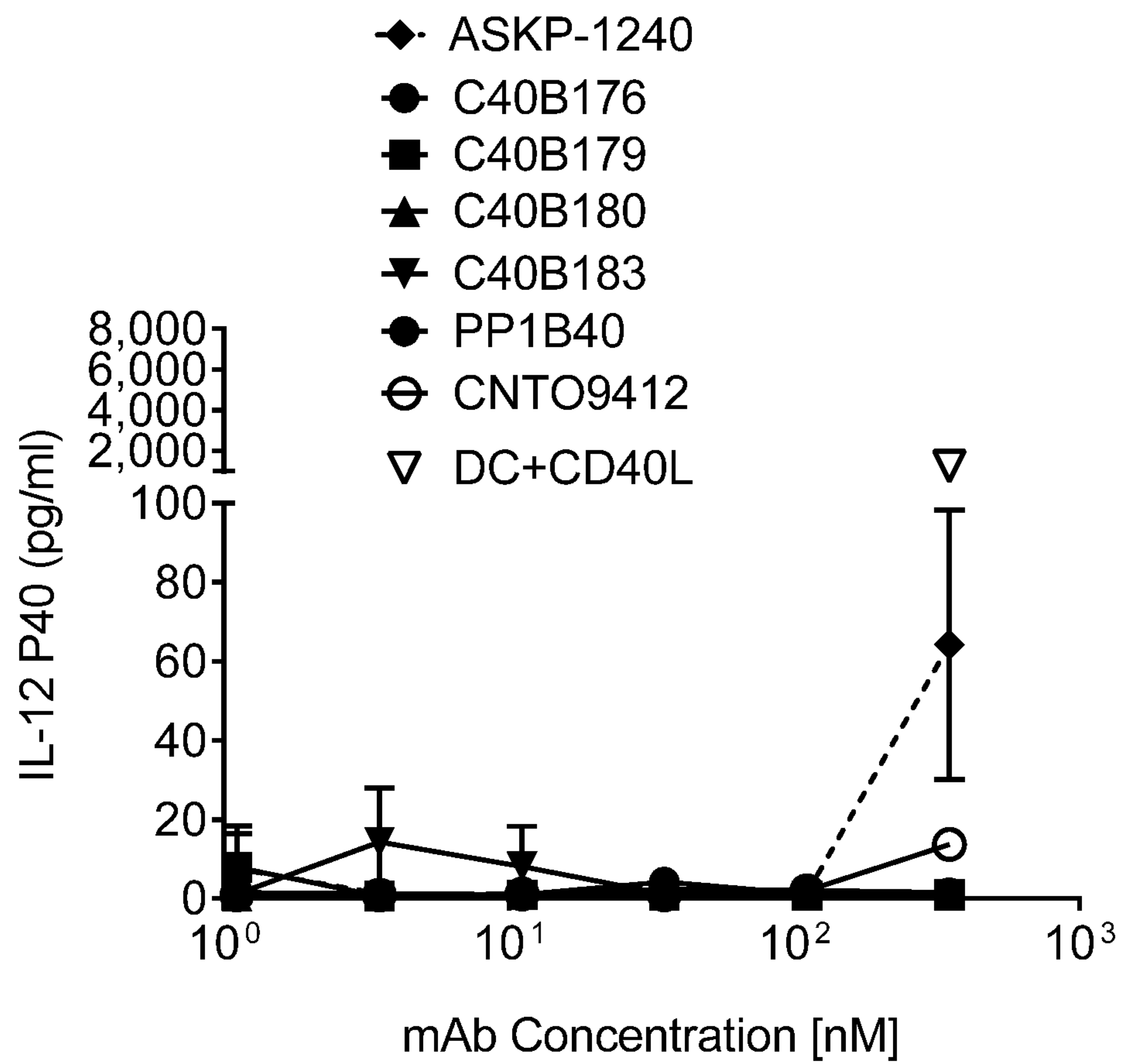
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Figure 2.



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Figure 3A.



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Figure 3B.

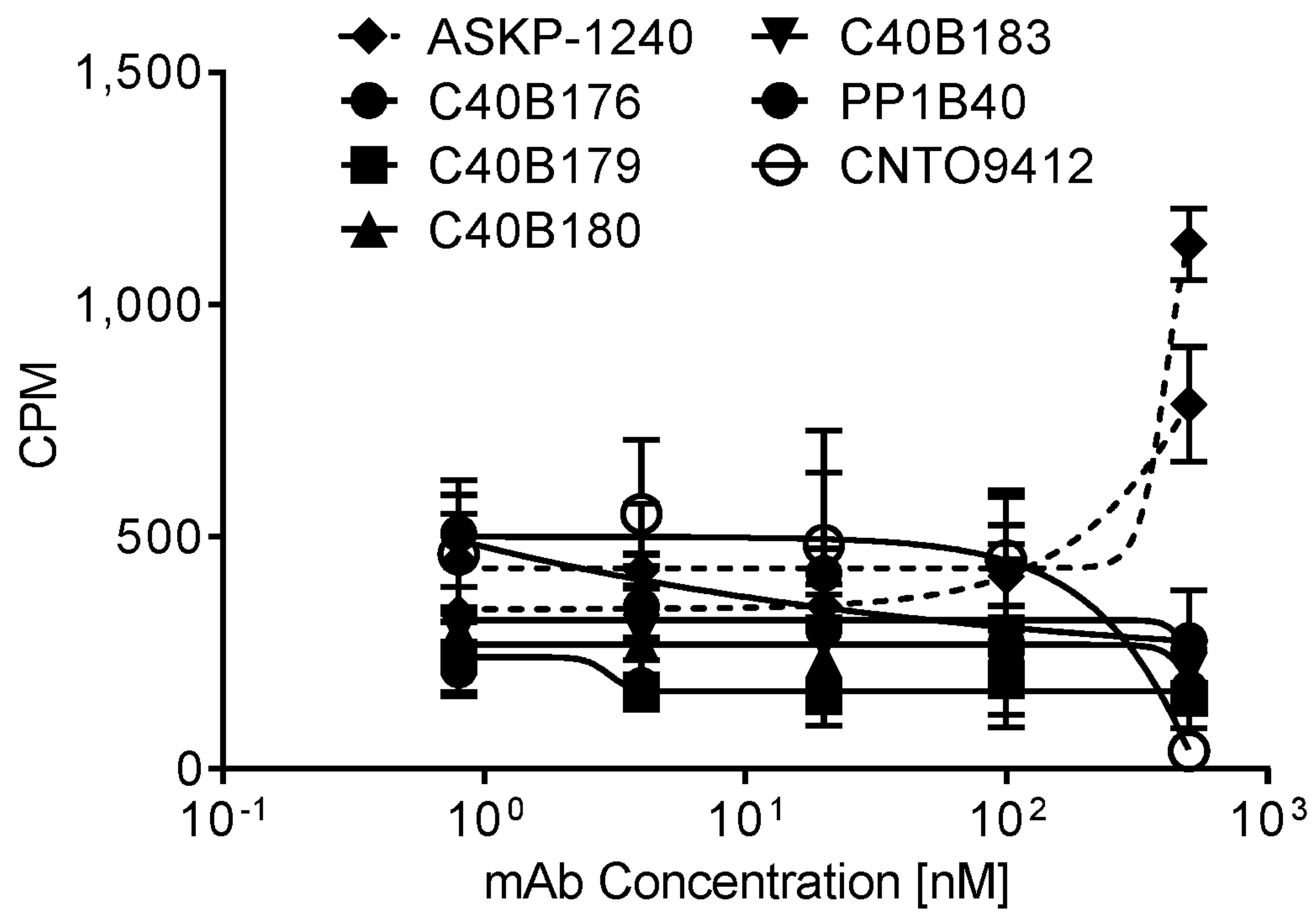


Figure 1.

