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(54) **Title:** MODIFIED HUMAN IGM CONSTANT REGIONS FOR MODULATION OF COMPLEMENT-DEPENDENT CYTOLYSIS EFFECTOR FUNCTION

(57) **Abstract:**

MODIFIED HUMAN IGM CONSTANT REGIONS FOR MODULATION OF COMPLEMENT-DEPENDENT CYTOLYSIS EFFECTOR FUNCTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/483,087, filed April 7, 2017, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Complement dependent cytotoxicity is an effector function used by antibodies to engage components of the innate immune system to destroy invading microbes. Therapeutic antibodies can use the complement system to engage the complement system to attack cells targeted by the antibody, for example, tumor cells. The complement cascade is triggered by binding of the first component C1q to the Fc region of IgG's or to the Fc Mu region of IgM's. Hexamerization of antibodies results in formation of the hexameric C1q complex that further triggers downstream complement components and ends in formation of the membrane attack complex (MAC), which causes cell death by perforation of the cell membrane and resulting osmotic shock. Antibodies of the IgM isotype are multimeric (pentamers and hexamers) and are particularly well suited to binding and multimerization of C1q. Indeed, IgM antibodies can fix complement between 30 to 100 times better than corresponding IgG antibodies.

[0003] For those indications where an IgM antibody is used to multimerize a cell surface receptor to affect downstream signaling, however, it is not always desirable to retain this powerful complement fixation ability. For example, IgM antibodies could be used to activate T-cells by engaging TNF receptor superfamily members like CD40, OX40 or GITR. In each case, multimerization driven downstream signaling can be used to cause powerful agonist activity and proliferation of T-cells. However, if the complement fixation activity of IgM antibody is left intact, it could potentially counteract the agonist activity of IgM antibody on these targets. For this reason, there is a need in the art to identify mutations of the IgM heavy chain constant region that could reduce or eliminate complement fixation and resultant complement dependent cytotoxicity (CDC activity).

[0004] Mutations that enhance or reduce CDC activity in mouse IgM have been identified. See, e.g., Arya, S., et al., J. Immunol. 152: 1206-1212 (1994), Wright, J, F., *et al., J. Biol. Chem. 263*:11221-11226 (1988), and Wright, J.F., *et al., J. Biol. Chem.*

265:10506-10513 (1989). There remains a need in the art to identify and characterize modified human IgM antibodies with modified CDC activity.

SUMMARY

[0005] This disclosure provides a modified human IgM constant region that includes one or more amino acid substitutions relative to a wild-type human IgM constant region, where at least one amino acid substitution is at a position in the Cμ3 domain ranging from T302 of SEQ ID NO: 1 to K322 of SEQ ID NO: 1, and where a modified IgM antibody that includes the modified IgM constant region and a heavy chain variable region specific for a target antigen exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

[0006] In certain aspects, the modified human IgM constant region includes at least one amino acid substitution is at amino acid T302, C303, T304, V305, T306, H307, T308, D309, L310, P311, S312, P313, L314, K315, Q316, T317, I318, S319, R320, P321, and/or K322 of SEQ ID NO: 1. For example, the modified human IgM constant region can include at least one amino acid substitution is at position L310 of SEQ ID NO: 1, position P311 of SEQ ID NO: 1, position P313 of SEQ ID NO: 1, position K315 of SEQ ID NO: 1, or any combination thereof.

[0007] In certain aspects at least one amino acid substitution can be at position L310 of SEQ ID NO: 1, e.g., L310 of SEQ ID NO: 1 can be substituted with alanine (L310A), serine (L310S), aspartic acid (L310D) or glycine (L310G). In certain aspects L310 of SEQ ID NO: 1 can be substituted with alanine (L310A), for example, the modified IgM constant region can include SEQ ID NO: 15. In certain aspects L310 of SEQ ID NO: 1 can be substituted with aspartic acid (L310D), for example, the modified IgM constant region can include SEQ ID NO: 23.

[0008] In certain aspects at least one amino acid substitution can be at position P311 of SEQ ID NO: 1, e.g., P311 of SEQ ID NO: 1 can be substituted with alanine (P311A), serine (P311S), or glycine (P311G). In certain aspects P311 of SEQ ID NO: 1 can be substituted with alanine (P311A), for example, the modified IgM constant region can include SEQ ID NO: 2.

[0009] In certain aspects at least one amino acid substitution can be at position P313 of SEQ ID NO: 1, e.g., P313 of SEQ ID NO: 1 can be substituted with alanine (P313A), serine

(P313S), or glycine (P313G). In certain aspects P313 of SEQ ID NO: 1 can be substituted with serine (P313S), for example the modified IgM constant region can include SEQ ID NO: 3.

[0010] In certain aspects at least one amino acid substitution can be at position K315 of SEQ ID NO: 1, e.g., K315 of SEQ ID NO: 1 can be substituted with alanine (K315A), serine (K315S), aspartic acid (K315D), glutamine (K315Q), or glycine (P313G). In certain aspects K315 of SEQ ID NO: 1 can be substituted with alanine (K315A), for example the modified IgM constant region can include SEQ ID NO: 16. In certain aspects K315 of SEQ ID NO: 1 can be substituted with aspartic acid (K315D), for example the modified IgM constant region can include SEQ ID NO: 24. In certain aspects K315 of SEQ ID NO: 1 can be substituted with glutamine (K315Q), for example the modified IgM constant region can include SEQ ID NO: 25.

[0011] In certain aspects the modified human IgM constant region as provided herein can include two or more amino acid substitutions. For example, the modified human IgM constant region as provided herein can include amino acid substitutions at two or more of positions L310, P311, P313, or K315 of SEQ ID NO: 1.

[0012] In certain aspects, the modified human IgM constant region as provided herein can include amino acid substitutions at positions P311 and P313 of SEQ ID NO: 1. For example, P311 of SEQ ID NO: 1 can be substituted with alanine (P311A), serine (P311S), or glycine (P311G), and P313 of SEQ ID NO: 1 can be substituted with alanine (P313A), serine (P313S), or glycine (P313G). In certain aspects, P311 of SEQ ID NO: 1 can be substituted with alanine (P311A) and P313 of SEQ ID NO: 1 can be substituted with serine (P313S), for example the modified IgM constant region can include SEQ ID NO: 4.

[0013] In certain aspects, the modified human IgM constant region as provided herein can include amino acid substitutions at positions L310 and K315 of SEQ ID NO: 1. For example, L310 can be substituted with alanine (L310A) or serine (L310S) and K315 can be substituted with alanine (K315A) or serine (K315S), for example the modified IgM constant region can include SEQ ID NO: 17 or SEQ ID NO: 18.

[0014] In certain aspects, the modified human IgM constant region as provided herein can include amino acid substitutions at positions L310 and P311 of SEQ ID NO: 1. For example, L310 can be substituted with alanine (L310A) and P311 can be substituted with alanine (P311A), for example the modified IgM constant region can include SEQ ID NO: 19.

[0015] In certain aspects, the modified human IgM constant region as provided herein can include amino acid substitutions at positions L310 and P313 of SEQ ID NO: 1. For example, L310 can be substituted with alanine (L310A) and P313 can be substituted with serine (P313S), for example the modified IgM constant region can include SEQ ID NO: 20.

[0016] In certain aspects, the modified human IgM constant region as provided herein can include amino acid substitutions at positions P311 and K315 of SEQ ID NO: 1. For example, P311 can be substituted with alanine (P311A) and K315 can be substituted with alanine (K315A), for example the modified IgM constant region can include SEQ ID NO: 21.

[0017] In certain aspects, the modified human IgM constant region as provided herein can include amino acid substitutions at positions P313 and K315 of SEQ ID NO: 1. For example, P313 can be substituted with serine (P313S) and K315 can be substituted with alanine (K315A), for example the modified IgM constant region can include SEQ ID NO: 22.

[0018] In certain aspects, the maximum CDC activity achieved in a dose response assay by a target-specific IgM antibody that includes the modified human IgM constant region as provided herein is reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

[0019] In certain aspects, the antibody concentration effecting 50% CDC activity (EC_{50}) of a target-specific IgM antibody that includes the modified human IgM constant region as provided herein is increased by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, and least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

[0020] The disclosure further provides a modified human IgM antibody that includes the modified human IgM constant region as provided herein and further includes a heavy chain variable region (VH) situated amino terminal to the modified human IgM constant region, where the modified human IgM antibody specifically binds to a target antigen and exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody. In certain aspects, the modified human IgM antibody can be a pentameric or a hexameric antibody that includes five or six bivalent IgM binding units, respectively, where each binding unit includes two IgM heavy chains each including a VH situated amino terminal to the modified human IgM constant region, and two immunoglobulin light chains each including a light chain variable domain (VL) situated amino terminal to a human immunoglobulin light chain constant region. In certain aspects, the modified human IgM antibody as provided herein can be pentameric, further including a J-chain, or functional fragment thereof, or a functional variant thereof. In certain aspects the J-chain can include amino acids 23 to 158 of SEQ ID NO: 7 or functional fragment thereof, or a functional variant thereof. In certain aspects the J-chain or fragment or variant thereof can be a modified J-chain that further includes a heterologous polypeptide, where the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment or variant thereof. In certain aspects the heterologous polypeptide can be fused to the J-chain or fragment thereof via a peptide linker, e.g., a peptide linker includes at least 5 amino acids, but no more than 25 amino acids. In certain aspects the peptide linker can comprise, consist essentially of, or consist of GGGGSGGGGSGGGGS (SEQ ID NO: 12). In certain aspects, the heterologous polypeptide can be fused to the N-terminus of the J-chain or fragment or variant thereof, the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof. In certain aspects, the heterologous polypeptide can include a binding domain, e.g., an antibody or antigen-binding fragment thereof, e.g., an Fab fragment, an Fab' fragment, an F(ab')2 fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof. In certain aspects the modified J-chain can include a scFv fragment. In certain aspects, the heterologous polypeptide can specifically bind to CD3ε. In certain aspects, the modified J-chain can include the amino acid sequence SEQ ID NO: 9 (V15J) or SEQ ID NO: 11 (J15V). In certain aspects the modified J-chain can further include a signal peptide, e.g., the modified J-chain can include the amino acid sequence SEQ ID NO: 8 (V15J) or SEQ ID NO: 10 (J15V). In certain aspects the modified human IgM antibody as provided herein can direct T-cell-mediated killing of a cell expressing the target antigen at an activity level equivalent to that of a corresponding IgM antibody that is identical to the modified IgM antibody except for the modified IgM constant region. In certain aspects, the cell expressing the target antigen is a eukaryotic cell.

[0021] This disclosure further provides a polynucleotide that includes a nucleic acid sequence that encodes the modified human IgM constant region as provided herein, or a heavy chain polypeptide subunit of the modified human IgM antibody as provided herein.

Also provided is a composition that includes the provided polynucleotide. In certain aspects the composition further includes a nucleic acid sequence that encodes a light chain polypeptide subunit. In certain aspects, the light chain polypeptide subunit includes a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL. In certain aspects the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit can be on separate vectors. In certain aspects, the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit can be on a single vector. In certain aspects, the composition further includes a nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof. In certain aspects, the J-chain or fragment or variant thereof is a modified J-chain that further includes a heterologous polypeptide, where the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment thereof. In certain aspects, the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain can be on a single vector. In certain aspects, the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain can be each on separate vectors. All such vector or vectors are further provided by this disclosure. The disclosure further provides a host cell that includes the provided polynucleotides, compositions, vector, or vectors. In certain aspects, the host cell can express the modified human IgM constant region as provided herein, or the modified human IgM antibody as provided herein, or any functional fragment thereof. The disclosure further provides a method of producing the modified human IgM constant region as provided herein or the modified human IgM antibody as provided herein, where the method includes culturing the provided host cell, and recovering the constant region or antibody.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0022] **FIGURE 1** shows an SDS PAGE gel run under non-reducing conditions to show assembly of pentamers and hexamers. Gel patterns are depicted for the controls, IgM (pure), IgM + J (pure), and supernatant preparations (all without J chain) of IgM, and the IgM mutants, S283N, P313S, P311A, and D294G.

[0023] **FIGURE 2** shows percent complement dependent cytolysis (CDC) activity for 1) control anti-CD20 antibodies tested in the absence of complement (1.5.3 IgG and 1.5.3 IgM x V15J); 2) for control IgM (1.5.3 IgM, without J-chain) tested in the presence of complement; and 3) for corresponding 1.5.3 IgM mutants P311A, P313S, D294G, and S283N, all without J-chain, tested in the presence of complement.

[0024] **FIGURE 3** shows percent CDC activity for anti-CD20 x anti-CD3 bispecific IgM antibodies with an anti-CD3 modified J-chain including the control 1.5.3 IgM x V15J antibody, and corresponding 1.5.3 IgM x V15J mutants, P311A, P313S, D294G, and S283N.

[0025] **FIGURE 4** shows a T cell activation assay for a control anti-CD20 IgM lacking the anti-CD3 modified J-chain, and bispecific IgM antibodies that contain the anti-CD3 modified J-chain (V15J) including the control antibody, 1.5.3 IgM x V15J, and the corresponding 1.5.3 x V15J IgM mutants, D294G, S283N, P313S, and P311A.

[0026] **FIGURE 5** shows hybrid and reducing gels for 1.5.3 IgM x V15J, and the corresponding 1.5.3 x V15J IgM mutants, P311A, P313S, and the double mutant P311A/P313S. The double mutant is expressed and assembled as well as each of the single mutants.

[0027] **FIGURE 6** shows CDC activity for 1.5.3 IgM x V15J, and the corresponding 1.5.3 x V15J IgM mutants, P311A, P313S, and the double mutant P311A/P313S. The double mutant shows complete elimination of CDC activity.

[0028] **FIGURE 7** shows a T cell activation assay for 1.5.3 IgM x V15J, and the corresponding 1.5.3 x V15J IgM mutants, P311A, P313S, and the double mutant P311A/P313S. The double mutant is as effective at activating T-cells as each of the single mutants.

[0029] **FIGURE 8** shows CDC activity for 1.5.3 IgM without J-chain, 1.5.3 IgM x V15J, the corresponding 1.5.3 x V15J IgM mutants, P311A, P313S, L310A, K315A, and the double mutant P311A/P313S. The double mutant shows complete elimination of CDC activity.

[0030] **FIGURE 9A** shows CDC activity for 1.5.3 IgM without J-chain, 1.5.3 IgM x V15J, the 1.5.3 x V15J IgM mutants L310D and L310A, and the double mutant P311A/P313S.

[0031] **FIGURE 9B** shows CDC activity for 1.5.3 IgM without J-chain, 1.5.3 IgM x V15J, and the 1.5.3 x V15J IgM mutants K315D, K315Q, and K315A.

DETAILED DESCRIPTION

Definitions

[0032] The term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule," is understood to represent one or more binding molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0033] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0034] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary of Biochemistry and Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0035] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0036] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, and derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a biological source or produced by recombinant technology but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0037] A polypeptide as disclosed herein can be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides can have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt many different conformations, are referred to as unfolded. As used herein, the term glycoprotein refers to a protein coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid, *e.g.*, a serine or an asparagine.

[0038] By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated as disclosed herein, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0039] As used herein, the term "a non-naturally occurring polypeptide" or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the polypeptide that are, or could be, determined or interpreted by a judge or an administrative or judicial body, to be "naturally-occurring."

[0040] Other polypeptides disclosed herein are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" as disclosed herein include any polypeptides which retain at least some of the properties of the corresponding native antibody or polypeptide, for example, specifically binding to an antigen. Fragments of polypeptides include, for example, proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of, *e.g.*, a polypeptide include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. In certain aspects, variants can be non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives are polypeptides that have been altered to exhibit additional features not found on the original polypeptide. Examples include fusion proteins. Variant polypeptides can also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a polypeptide can also refer to a subject polypeptide having one or more amino acids chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides that contain one or more derivatives of the twenty standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

[0041] A "conservative amino acid substitution" is one in which one amino acid is replaced with another amino acid having a similar side chain. Families of amino acids having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. In certain embodiments, conservative substitutions in the sequences of the polypeptides and antibodies of the present disclosure do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen to which the binding molecule binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen-binding are well-known in the art (see, *e.g.*, Brummell *et al.*, *Biochem.* 32: 1180-1 187 (1993); Kobayashi *et al.*, *Protein Eng.* 12(10):879-884 (1999); and Burks *et al.*, *Proc. Natl. Acad. Sci. USA* 94:.412-417 (1997)).

[0042] The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA), cDNA, or plasmid DNA (pDNA). A polynucleotide can comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The terms "nucleic acid" or "nucleic acid sequence" refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide.

[0043] By an "isolated" nucleic acid or polynucleotide is intended any form of the nucleic acid or polynucleotide that is separated from its native environment. For example, gel-purified polynucleotide, or a recombinant polynucleotide encoding a polypeptide contained in a vector would be considered to be "isolated." Also, a polynucleotide segment, *e.g.*, a PCR product, which has been engineered to have restriction sites for cloning is considered to be "isolated." Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in a non-native solution such as a buffer or saline. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides, where the transcript is not one that would be found in nature. Isolated polynucleotides or nucleic acids further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid can be or can include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

[0044] As used herein, the term "a non-naturally occurring polynucleotide" or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the nucleic acid or polynucleotide that are, or could be, determined or interpreted by a judge, or an administrative or judicial body, to be "naturally-occurring."

[0045] As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector can contain a single coding region, or can comprise two or more coding regions, *e.g.*, a single vector can separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid can include heterologous coding regions, either fused or unfused to another coding region. Heterologous coding regions include without limitation, those encoding specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0046] In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter can be a cell-specific promoter that directs substantial transcription of the DNA in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription.

[0047] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0048] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0049] In other embodiments, a polynucleotide can be RNA, for example, in the form of messenger RNA (mRNA), transfer RNA, or ribosomal RNA.

[0050] Polynucleotide and nucleic acid coding regions can be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide as disclosed herein. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells can have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, can be used. For example, the wild-type leader sequence can be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse ß-glucuronidase.

[0051] As used herein, the term “CD20” refers to a membrane protein expressed on the surface of B lymphocytes. The CD20 protein is referred to in the literature by other names, *e.g.*, B-lymphocyte antigen CD20, B-lymphocyte cell-surface antigen B1, Bp35, CVID5, LEU-16, Membrane-spanning 4-domains subfamily A member 1, or MS4A2. Certain multivalent anti-CD20 binding molecules, *e.g.*, IgM antibodies or fragments thereof as disclosed herein are further described in PCT Publication No. WO/2016/141303, which is incorporated herein by reference in its entirety.

[0052] Disclosed herein are certain binding molecules, or antigen-binding fragments, variants, or derivatives thereof. Unless specifically referring to full-sized antibodies, the term "binding molecule" includes full-sized antibodies as well as antigen-binding subunits, fragments, variants, analogs, or derivatives of such antibodies, *e.g.*, engineered antibody molecules or fragments that bind antigen in a manner similar to antibody molecules, but which use a different scaffold.

[0053] As used herein, the term “binding molecule” refers in its broadest sense to a molecule that specifically binds to a target or molecular determinant, *e.g.*, an epitope or an antigenic determinant. As described further herein, a binding molecule can comprise one or more “antigen binding domains” described herein. A non-limiting example of a binding molecule is an antibody or fragment thereof that retains antigen-specific binding.

[0054] As used herein, the terms “binding domain” or “antigen binding domain” refer to a region of a binding molecule that is sufficient to specifically bind to an epitope. For example, an “Fv,” *e.g.*, a variable heavy chain and variable light chain of an antibody, either as two separate polypeptide subunits or as a single chain, is considered to be a “binding domain.” Other antigen binding domains include, without limitation, the variable heavy chain (VHH) of an antibody derived from a camelid species, or six immunoglobulin complementarity determining regions (CDRs) expressed in a fibronectin scaffold. A “binding molecule” as described herein can include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more “antigen binding domains.”

[0055] The terms "antibody" and "immunoglobulin" can be used interchangeably herein. An antibody (or a fragment, variant, or derivative thereof as disclosed herein) includes at least the variable domain of a heavy chain (for camelid species) or at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood. (*See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd ed. 1988). Unless otherwise stated, the term “antibody” encompasses anything ranging from a small antigen-binding fragment of an antibody to a full sized antibody, *e.g.*, an IgG antibody that includes two complete heavy chains and two complete light chains, an IgA antibody that includes four complete heavy chains and four complete light chains and can include a J-chain and/or a secretory component, or an IgM antibody that includes ten or twelve complete heavy chains and ten or twelve complete light chains and can include a J-chain.

[0056] As will be discussed in more detail below, the term “immunoglobulin” comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, $\gamma1$-$\gamma4$ or $\alpha1$-$\alpha2$)). It is the nature of this chain that determines the "isotype" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (subtypes) *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, IgA_2, are well characterized and are known to confer functional specialization. Modified versions of each of these immunoglobulins are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of this disclosure. In certain embodiments, this disclosure provides modified human IgM antibodies.

[0057] Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are expressed, *e.g.*, by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. The basic structure of certain antibodies, *e.g.*, IgG antibodies, includes two heavy chain subunits and two light chain subunits covalently connected via disulfide bonds to form a “Y” structure, also referred to herein as an “H2L2” structure, or a “binding unit.”

[0058] The term “binding unit” is used herein to refer to the portion of a binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof, which corresponds to a standard immunoglobulin structure, *e.g.*, two heavy chains or fragments thereof and two light chains or fragments thereof, or two heavy chains or fragments thereof derived, *e.g.*, from a camelid or condricthoid antibody. In certain aspects, *e.g.*, where the binding molecule is a bivalent IgG antibody or antigen-binding fragment thereof, the terms “binding molecule” and “binding unit” are equivalent. In other aspects, *e.g.*, where the binding molecule is an IgM pentamer or an IgM hexamer, the binding molecule comprises two or more “binding units,” five or six in the case of an IgM pentamer or hexamer, respectively. A binding unit need not include full-length antibody heavy and light chains, but will typically be bivalent, *i.e.*, will include two “antigen binding domains,” as defined below. Certain IgM-derived binding molecules provided in this disclosure are pentameric or hexameric and include five or six bivalent binding units that include IgM constant regions, *e.g.*, modified human IgM constant regions, or fragments thereof. As used herein, a binding molecule comprising two or more binding units, *e.g.*, five or six binding units, can be referred to as “multimeric.”

[0059] The terms “J-chain,” “native sequence J-chain” or “native J-chain” as used herein refers to J-chain of native sequence IgM or IgA antibodies of any animal species, including mature human J-chain, the amino acid sequence of which is presented as SEQ ID NO: 7.

[0060] The term “modified J-chain” is used herein to refer to variants of native sequence J- chain polypeptides comprising a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain introduced into the native sequence. The introduction can be achieved by any means, including direct or indirect fusion of the heterologous polypeptide or other moiety or by attachment through a peptide or chemical linker. The term “modified human J-chain” encompasses, without limitation, a native sequence human J-chain of the amino acid sequence of SEQ ID NO: 7 or functional fragment thereof modified by the introduction of a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain. In certain aspects the heterologous moiety does not interfere with efficient polymerization of IgM into a pentamer and binding of such polymers to a target. Exemplary modified J-chains can be found, *e.g.*, in PCT Publication No. WO 2015/153912, in PCT Publication No. WO/2017/059387, and in PCT Publication No. WO/2017/059380, each of which is incorporated herein by reference in its entirety.

[0061] The terms “valency,” “bivalent,” “multivalent” and grammatical equivalents, refer to the number of antigen binding domains in given binding molecule or binding unit. As such, the terms “bivalent”, “tetravalent”, and “hexavalent” in reference to a given binding molecule, *e.g.*, an IgM antibody or fragment thereof, denote the presence of two antigen binding domains, four antigen binding domains, and six antigen binding domains, respectively. In a typical IgM-derived binding molecule where each binding unit is bivalent, the binding molecule itself can have 10 or 12 valencies. A bivalent or multivalent binding molecule can be monospecific, *i.e.*, all of the antigen binding domains are the same, or can be bispecific or multispecific, *e.g.*, where two or more antigen binding domains are different, *e.g.*, bind to different epitopes on the same antigen, or bind to entirely different antigens.

[0062] The term “epitope” includes any molecular determinant capable of specific binding to an antibody. In certain aspects, an epitope can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain aspects, can have three-dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of a target that is bound by an antibody.

[0063] The term “target” is used in the broadest sense to include substances that can be bound by a binding molecule. A target can be, *e.g.*, a polypeptide, a nucleic acid, a carbohydrate, a lipid, or other molecule. Moreover, a “target” can, for example, be a cell, an organ, or an organism that comprises an epitope bound that can be bound by a binding molecule.

[0064] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the variable light (VL) and variable heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant regions of the light chain (CL) and the heavy chain (*e.g.*, CH1, CH2, CH3, or CH4) confer biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 (or CH4 in the case of IgM) and CL domains are at the carboxy-terminus of the heavy and light chain, respectively.

[0065] A “full length IgM antibody heavy chain” is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable domain (V_H), an antibody constant heavy chain constant domain 1 (CM1 or Cμ1), an antibody heavy chain constant domain 2 (CM2 or Cμ2), an antibody heavy chain constant domain 3 (CM3 or Cμ3), and an antibody heavy chain constant domain 4 (CM4 or Cμ4) that can include a tailpiece.

[0066] As indicated above, a variable region (*i.e.*, the “antigen binding domain”) allows a binding molecule to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain (or just a VH domain for camelid or condricthoid antibodies (designated as VHH)), or subset of the complementarity determining regions (CDRs), of a binding molecule, *e.g.*, an antibody, can combine to form the antigen binding domain. More specifically, an antigen binding domain can be defined by three CDRs on each of the VH and VL chains (or 3 CDRs on a VHH). Certain antibodies form larger structures. For example, IgM can form a dimeric, pentameric, or hexameric molecule that includes two, five, or six H2L2 binding units and optionally a J-chain covalently connected via disulfide bonds.

[0067] The six “complementarity determining regions” or “CDRs” present in an antibody antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three-dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domain, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids that make up the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been defined in various different ways (*see*, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, *196*:901-917 (1987), which are incorporated herein by reference in their entireties).

[0068] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These regions have been described, for example, by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference. The Kabat and Chothia definitions include overlapping or subsets of amino acids when compared against each other. Nevertheless, application of either definition (or other definitions known to those of ordinary skill in the art) to refer to a CDR of an antibody or variant thereof is intended to be within the scope of the term as defined and used herein, unless otherwise indicated. The appropriate amino acids which encompass the CDRs as defined by each of the above cited references are set forth below in **Table 1** as a comparison. The exact amino acid numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which amino acids comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1 CDR Definitions*

	Kabat	**Chothia**
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

*Numbering of all CDR definitions in **Table 1** is according to the numbering conventions set forth by Kabat *et al.* (see below).

[0069] Immunoglobulin variable domains can also be analyzed, *e.g.*, using the IMGT information system (www://imgt.cines.fr/) (IMGT®/V-Quest) to identify variable region segments, including CDRs. *See, e.g.*, Brochet, X. *et al.*, *Nucl. Acids Res. 36*:W503-508 (2008).

[0070] Kabat *et al.* also defined a numbering system for variable and constant region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless use of the Kabat numbering system is explicitly noted, however, consecutive numbering is used for amino acid sequences in this disclosure. Another numbering scheme is the Eu numbering system for IgG (Edelman, GM, *et al.*, *Proc. Natl. Acad. Sci. USA 63*:78-85 (1969)), which has been adapted for other immunoglobulins including IgM (see, *e.g.*, Arya, S., *et al.*, *J. Immunol. 152*: 1206-1212 (1994)).

[0071] Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof include, but are not limited to, polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')$_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

[0072] By "specifically binds," it is generally meant that a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof binds to an epitope via its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, a binding molecule is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain binding molecule binds to a certain epitope. For example, binding molecule "A" can be deemed to have a higher specificity for a given epitope than binding molecule "B," or binding molecule "A" can be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0073] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof disclosed herein can be said to bind a target antigen with an off rate (k(off)) of less than or equal to 5 X 10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5 X 10^{-3} sec^{-1}, 10^{-3} sec^{-1}, 5 X 10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5 X 10^{-5} sec^{-1}, or 10^{-5} sec^{-1} 5 X 10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5 X 10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0074] A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target antigen with an on rate (k(on)) of greater than or equal to 10^3 M^{-1} sec^{-1}, 5 X 10^3 M^{-1} sec^{-1}, 10^4 M^{-1} sec^{-1}, 5 X 10^4 M^{-1} sec^{-1}, 10^5 M^{-1} sec^{-1}, 5 X 10^5 M^{-1} sec^{-1}, 10^6 M^{-1} sec^{-1}, or 5 X 10^6 M^{-1} sec^{-1} or 10^7 M^{-1} sec^{-1}.

[0075] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof is said to competitively inhibit binding of a reference antibody or antigen binding fragment to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody or antigen binding fragment to the epitope. Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays. A binding molecule can be said to competitively inhibit binding of the reference antibody or antigen binding fragment to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0076] As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with one or more antigen binding domains, *e.g.*, of an immunoglobulin molecule. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of antigen binding domains and an antigen. *See*, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual antigen binding domains in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity. An interaction between a between a bivalent monoclonal antibody with a receptor present at a high density on a cell surface would also be of high avidity.

[0077] Binding molecules or antigen-binding fragments, variants or derivatives thereof as disclosed herein can also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, a binding molecule is cross reactive if it binds to an epitope other than the one that induced its formation. The cross-reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, can actually fit better than the original.

[0078] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can also be described or specified in terms of their binding affinity to an antigen. For example, a binding molecule can bind to an antigen with a dissociation constant or K_D no greater than 5 x 10^{-2} M, 10^{-2} M, 5 x 10^{-3} M, 10^{-3} M, 5 x 10^{-4} M, 10^{-4} M, 5 x 10^{-5} M, 10^{-5} M, 5 x 10^{-6} M, 10^{-6} M, 5 x 10^{-7} M, 10^{-7} M, 5 x 10^{-8} M, 10^{-8} M, 5 x 10^{-9} M, 10^{-9} M, 5 x 10^{-10} M, 10^{-10} M, 5 x 10^{-11} M, 10^{-11} M, 5 x 10^{-12} M, 10^{-12} M, 5 x 10^{-13} M, 10^{-13} M, 5 x 10^{-14} M, 10^{-14} M, 5 x 10^{-15} M, or 10^{-15} M.

[0079] Antibody fragments including single-chain antibodies or other antigen binding domains can exist alone or in combination with one or more of the following: hinge region, CH1, CH2, CH3, or CH4 domains, J-chain, or secretory component. Also included are antigen-binding fragments that can include any combination of variable region(s) with one or more of a hinge region, CH1, CH2, CH3, or CH4 domains, a J-chain, or a secretory component. Binding molecules, *e.g.*, antibodies, or antigen-binding fragments thereof can be from any animal origin including birds and mammals. The antibodies can be human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region can be condricthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and can in some instances express endogenous immunoglobulins and some not, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.*

[0080] As used herein, the term “heavy chain subunit” includes amino acid sequences derived from an immunoglobulin heavy chain, a binding molecule, *e.g.*, an antibody comprising a heavy chain subunit can include at least one of: a VH domain, a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH4 domain, or a variant or fragment thereof. For example, a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can include, without limitation, in addition to a VH domain: a CH1 domain; a CH1 domain, a hinge, and a CH2 domain; a CH1 domain and a CH3 domain; a CH1 domain, a hinge, and a CH3 domain; or a CH1 domain, a hinge domain, a CH2 domain, and a CH3 domain. In certain aspects a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can include, in addition to a VH domain, a CH3 domain and a CH4 domain; or a CH3 domain, a CH4 domain, and a J-chain. Further, a binding molecule for use in the disclosure can lack certain constant region portions, *e.g.*, all or part of a CH2 domain. It will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain subunit) can be modified such that they vary in amino acid sequence from the original immunoglobulin molecule.

[0081] As used herein, the term “light chain subunit” includes amino acid sequences derived from an immunoglobulin light chain. The light chain subunit includes at least a VL, and can further include a CL (*e.g.*, Cκ or Cλ) domain.

[0082] Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof can be described or specified in terms of the epitope(s) or portion(s) of an antigen that they recognize or specifically bind. The portion of a target antigen that specifically interacts with the antigen binding domain of an antibody is an "epitope," or an "antigenic determinant." A target antigen can comprise a single epitope or at least two epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen.

[0083] As used herein the term “disulfide bond” includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group.

[0084] As used herein, the term “chimeric antibody” refers to an antibody in which the immunoreactive region or site is obtained or derived from a first species and the constant region (which can be intact, partial or modified) is obtained from a second species. In some embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0085] The term "multispecific antibody, *e.g.*, "bispecific antibody" refers to an antibody that has antigen binding domains for two or more different epitopes within a single antibody molecule. Other binding molecules in addition to the canonical antibody structure can be constructed with two binding specificities. Epitope binding by bispecific or multispecific antibodies can be simultaneous or sequential. Triomas and hybrid hybridomas are two examples of cell lines that can secrete bispecific antibodies. Bispecific antibodies can also be constructed by recombinant means. (Ströhlein and Heiss, *Future Oncol. 6*:1387-94 (2010); Mabry and Snavely, *IDrugs. 13*:543-9 (2010)). A bispecific antibody can also be a diabody.

[0086] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain and/or constant region in either the heavy and light chain or both is altered by at least partial replacement of one or more amino acids in the CDR, framework, and/or constant regions. In certain aspects entire CDRs from an antibody of known specificity can be grafted into the framework regions of a heterologous antibody. Although alternate CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, CDRs can also be derived from an antibody of different class, *e.g.*, from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity are grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." In certain aspects, not all of the CDRs are replaced with the complete CDRs from the donor variable region and yet the antigen binding capacity of the donor can still be transferred to the recipient variable domains. Given the explanations set forth in, *e.g.*, U. S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of those skilled in the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional engineered or humanized antibody.

[0087] As used herein the term "engineered" includes manipulation of nucleic acid or polypeptide molecules by synthetic means (*e.g.* by recombinant techniques, *in vitro* peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques).

[0088] As used herein, the terms "linked," "fused" or "fusion" or other grammatical equivalents can be used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the translational reading frame of the original ORFs. Thus, a recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments can be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region can be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide.

[0089] In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which amino acids that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A portion of a polypeptide that is “amino-terminal” or “N-terminal” to another portion of a polypeptide is that portion that comes earlier in the sequential polypeptide chain. Similarly, a portion of a polypeptide that is “carboxy-terminal” or “C-terminal” to another portion of a polypeptide is that portion that comes later in the sequential polypeptide chain. For example, in a typical antibody, the variable domain is “N-terminal” to the constant region, and the constant region is “C-terminal” to the variable domain.

[0090] The term “expression” as used herein refers to a process by which a gene produces a biochemical, for example, a polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into RNA, *e.g.*, messenger RNA (mRNA), and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide that is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

[0091] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt or slow the progression of an existing diagnosed pathologic condition or disorder. Terms such as "prevent," "prevention," "avoid," "deterrence" and the like refer to prophylactic or preventative measures that prevent the development of an undiagnosed targeted pathologic condition or disorder. Thus, "those in need of treatment" can include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

[0092] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

[0093] As used herein, phrases such as "a subject that would benefit from therapy" and "an animal in need of treatment" includes subjects, such as mammalian subjects, that would benefit from administration of a binding molecule such as an antibody, comprising one or more antigen binding domains. Such binding molecules, *e.g.*, antibodies, can be used, *e.g.*, for diagnostic procedures and/or for treatment or prevention of a disease.

IgM Binding Molecules

[0094] IgM is the first immunoglobulin produced by B cells in response to stimulation by antigen and is present at around 1.5 mg/ml in serum with a half-life of 5 days. IgM is typically a pentameric or hexameric molecule. An IgM binding unit typically includes two light chains and two heavy chains. While IgG contains three heavy chain constant domains (CH1, CH2 and CH3), the heavy (μ) chain of IgM additionally contains a fourth constant domain (CH4), that includes a C-terminal "tailpiece." The human IgM constant region typically comprises the amino acid sequence SEQ ID NO: 1 (see Table 3). The human Cμ1 region ranges from about amino acid 5 to about amino acid 102 of SEQ ID NO: 1; the human Cμ2 region ranges from about amino acid 114 to about amino acid 205 of SEQ ID NO: 1, the human Cμ3 region ranges from about amino acid 224 to about amino acid 319 of SEQ ID NO: 1, the Cμ4 region ranges from about amino acid 329 to about amino acid 430 of

SEQ ID NO: 1, and the tailpiece ranges from about amino acid 431 to about amino acid 453 of SEQ ID NO: 1.

[0095] Five IgM binding units can form a complex with an additional small polypeptide chain (the J-chain) to form an IgM antibody. The precursor human J-chain comprises the amino acid sequence SEQ ID NO: 7 (Table 3). The first 22 amino acids of SEQ ID NO: 7 is the secretory signal peptide and the mature human J-chain starts at amino acid 23 of SEQ ID NO: 7. Without the J-chain, IgM binding units typically assemble into a hexamer. While not wishing to be bound by theory, the assembly of IgM binding units into a hexameric or pentameric binding molecule is thought to involve the Cμ3, Cμ4 and tailpiece domains. Accordingly, a hexameric or pentameric binding molecule provided in this disclosure typically includes IgM constant regions that include at least the Cμ3, Cμ4, and tailpiece domains.

[0096] An IgM heavy chain constant region can additionally include a Cμ2 domain or a fragment thereof, a Cμ1 domain or a fragment thereof, and/or other IgM heavy chain domains. In certain aspects, a binding molecule as provided herein can include a complete IgM heavy (μ) chain constant region (*e.g.*, SEQ ID NO: 1), or a variant, derivative, or analog thereof.

The Complement System and Complement-Dependent Cytotoxicity

[0097] The complement system comprises more than 30 glycoproteins, from which 20 are present in plasma and 10 are cell-associated regulators or receptors. Activation of the complement cascade induces diverse immune effector functions including cell lysis, phagocytosis, chemotaxis and immune cell activation. Complement can be activated by 3 different pathways: the classical, the alternative and the mannose-binding lectin pathway which all converge on the level of C3 protein and lead to complement-dependent cytotoxicity of the target cell by the formation of the membrane-attack complex (MAC). IgM and certain subclasses of IgG antibodies in immune complexes activate the classical complement pathway. The formation of an antigen-antibody complex induces conformational changes in the Fc portion of the IgM or IgG molecule that expose a binding site for the C1 component of the complement system. C1 in serum is a macromolecular complex consisting of C1q and two molecules each of C1r and C1s. Binding of C1q to Fc-binding sites induces a conformational change in C1r and C1s. Subsequently, proteolytically activated C1r cleaves C1s resulting in C1s activation. C1s then cleaves C2 and C4 to generate C2a, C2b, C4a, and C4b. C2a and C4b together form the C3 convertase. The cleavage of the central component C3 by the C3 convertase leads to formation of C3b, some of which binds to the plasma membrane of the target cell. Membrane-bound C3b interacts with the C3 convertase, leading to the formation of the C5 convertase. Consequently, C5 is cleaved into C5a and C5b. The activation of the terminal pathway leads to the deposition of the components C5b-C9 into the opsonized target cell membrane forming the membrane-attack complex, eventually causing complement dependent cytotoxicity (CDC).

[0098] The end result of complement dependent cytolysis is the formation of a pore in the lipid bilayer membrane of a cell that destroys membrane integrity. Complement-dependent cytotoxicity assays (CDC assays) test the efficacy of antibodies to activate the complement immune pathway to initiate a membrane-attack complex and lysis of targeted cells. One method for a CDC assay is to mix target cells bound by the antibody being evaluated with an additive, *e.g.*, serum, that contains the components of the complement system and then measure cell death. Cell death can be measured, *e.g.*, by pre-loading the target cells with a radioactive compound, which upon death is released from the cells, and the efficacy of the antibody to mediate cell death is measured by radioactivity level. Non-radioactive CDC assays, *e.g.*, the CELLTITER-GLO® assay available from Promega, measure the release of abundant cell components, like ATP, with fluorescent or luminescent measurements. In the event of CDC, the mixture results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture.

Modified Human IgM Constant Regions with Reduced CDC Activity

[0099] This disclosure provides a modified human IgM constant region that, when expressed as part of a modified target-specific human IgM antibody directed to a target antigen expressed on a cell, exhibits reduced CDC activity of the cell in the presence of complement, relative to a corresponding wild-type human IgM antibody. By "corresponding wild-type human IgM antibody" is meant a wild-type IgM antibody that is identical to the antibody comprising the modified human IgM constant region except for the modification or modifications in the constant region affecting CDC activity. For example, the "corresponding wild-type human IgM antibody" will comprise identical VH and VL regions and any other modifications or truncations that that the modified human IgM antibody might have other than the modifications affecting CDC activity. In certain aspects, the modified human IgM constant region comprises one or more amino acid substitutions, *e.g.*, in or close to the Cμ3 domain, *e.g.*, in the region extending from about amino acid T302 of SEQ ID NO: 1 to about amino acid K322 of SEQ ID NO: 1, *e.g.*, at least one, at least two, or at least three or more amino acid substitutions at position T302, C303, T304, V305, T306, H307, T308, D309, L310, P311, S312, P313, L314, K315, Q316, T317, I318, S319, R320, P321, and/or K322 of SEQ ID NO: 1 relative to a wild-type human IgM constant region. While not wishing to be bound by theory, the C1q component of complement is thought to associate with the human IgM constant region at least through certain amino acid residues present in the Cμ3 domain. Assays for measuring CDC are well known to those of ordinary skill in the art, and exemplary assays are described herein.

[0100] In certain aspects, a modified human IgM constant region as provided herein comprises a substitution relative to a wild-type human IgM constant region at position P311 of SEQ ID NO: 1. In other aspects a modified IgM constant region as provided herein comprises a substitution relative to a wild-type human IgM constant region at position P313 of SEQ ID NO: 1. In other aspects a modified IgM constant region as provided herein comprises a substitution relative to a wild-type human IgM constant region at position L310 of SEQ ID NO: 1. In other aspects a modified IgM constant region as provided herein comprises a substitution relative to a wild-type human IgM constant region at position K315 of SEQ ID NO: 1. In other aspects a modified IgM constant region as provided herein contains a combination of substitutions relative to a wild-type human IgM constant region at two or more of positions L310 of SEQ ID NO: 1, P311 of SEQ ID NO: 1, P313 of SEQ ID NO: 1, and/or K315 of SEQ ID NO: 1. A modified IgM constant region as provided herein can be substituted at amino acid position P311 of SEQ ID NO: 1 with, *e.g.*, alanine (P311A) (SEQ ID NO: 2), serine (P311S), or glycine (P311G). A modified IgM constant region as provided herein can be substituted at amino acid position P313 of SEQ ID NO: 1 with, *e.g.*, alanine (P313A), serine (P313S) (SEQ ID NO: 3), or glycine (P313G). A modified IgM constant region as provided herein can be substituted at amino acid position L310 of SEQ ID NO: 1 with, *e.g.*, alanine (L310A) (SEQ ID NO: 15), serine (L310S), glycine (L310G), or aspartic acid (L310D) (SEQ ID NO: 23). A modified IgM constant region as provided herein can be substituted at amino acid position K315 of SEQ ID NO: 1 with, *e.g.*, alanine (K315A) (SEQ ID NO: 16), serine (K315S), glycine (K315G), aspartic acid (K315D) (SEQ ID NO: 24), or glutamine (K315Q) (SEQ ID NO: 25). A modified IgM constant region as provided herein can be substituted at amino acid positions P311 and P313 of SEQ ID NO: 1 with, *e.g.*, alanine (P311A) and serine (P313S), respectively (SEQ ID NO: 4), or any combination of alanine, serine, and/or glycine. A modified IgM constant region as provided herein can be substituted at amino acid positions L310 and K315 of SEQ ID NO: 1 with, *e.g.*, alanine (L310A, K315A) (SEQ ID NO: 17), or serine (L310S, K315S) (SEQ ID NO: 18), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine. A modified IgM constant region as provided herein can be substituted at amino acid positions L310 and P311 of SEQ ID NO: 1 with, *e.g.*, alanine (L310A, P311A) (SEQ ID NO: 19), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine. A modified IgM constant region as provided herein can be substituted at amino acid positions L310 and P313 of SEQ ID NO: 1 with, *e.g.*, alanine and serine, respectively (L310A, P313S) (SEQ ID NO: 20), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine. A modified IgM constant region as provided herein can be substituted at amino acid positions P311 and K315 of SEQ ID NO: 1 with, *e.g.*, alanine (P311A, K315A) (SEQ ID NO: 21), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine. A modified IgM constant region as provided herein can be substituted at amino acid positions P313 and K315 of SEQ ID NO: 1 with, *e.g.*, serine and alanine, respectively (P313S, K315A) (SEQ ID NO: 22), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine.

[0101] In certain aspects, the complement-dependent cytotoxicity activity (CDC) of a target-specific IgM antibody comprising a modified human IgM constant region as provided herein comprising, *e.g.*, an amino acid substitution at L310, P311, P313, and/or K315, *e.g.*, L310A, L310S, L310G, L310D, P311A, P311S, P311G, P313A, P313S, P313G, K315A, K315S, K315G, K315D, and/or K315Q, or any combination thereof, can be reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a corresponding wild-type IgM antibody, *i.e.*, a wild-type target-specific IgM antibody that is identical except for the modified human IgM constant region.

[0102] In certain aspects, the antibody concentration effecting 50% CDC activity (EC_{50}) of a target-specific IgM antibody comprising a modified human IgM constant region as provided herein comprising, *e.g.*, an amino acid substitution at L310, P311, P313, and/or K315, *e.g.*, L310A, L310S, L310G, L310D, P311A, P311S, P311G, P313A, P313S, P313G, K315A, K315S, K315G, K315D, and/or K315Q, or any combination thereof, can be increased by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, and least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold relative to a corresponding wild-type IgM antibody, *i.e.*, a wild-type target-specific IgM antibody that is identical except for the modified human IgM constant region.

[0103] Modified human IgM constant regions as provided herein can be constructed by standard mutagenesis methods well known to those of ordinary skill in the art or can be obtained from a variety of commercial vendors. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New York); Kunkel, Proc. Natl. Acad. Sci. USA 82:488-492 (1985); Kunkel *et al.*, Methods Enzymol. 154:367-382 (1987); Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, N.Y.); U.S. Pat. No. 4,873,192; and the references cited therein; herein incorporated by reference. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the polypeptide of interest can be found in the model of Dayhoff *et al.* (1978) in Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.), pp. 345-352, herein incorporated by reference in its entirety. The model of Dayhoff *et al.* uses the Point Accepted Mutation (PAM) amino acid similarity matrix (PAM 250 matrix) to determine suitable conservative amino acid substitutions. In certain aspects, conservative substitutions, such as exchanging one amino acid with another having similar properties are used. Examples of conservative amino acid substitutions as taught by the PAM 250 matrix of the Dayhoff *et al.* model include, but are not limited to, Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln, and Phe↔Trp↔Tyr.

Pentameric or Hexameric Modified Human IgM Antibodies

[0104] In another aspect, this disclosure provides a modified human IgM antibody comprising a modified human IgM constant region as provided herein, and further comprising a heavy chain variable region (VH) situated amino terminal to the modified human IgM constant region that specifically binds to a target antigen (alone or in combination with light chain variable region (VL)) and exhibits decreased complement-dependent cytotoxicity (CDC) of cells expressing the target antigen and/or an increased antibody concentration effecting 50% CDC activity (EC_{50}) relative to a corresponding wild-type human IgM antibody, *i.e.*, a wild-type target-specific IgM antibody that is identical except for the modified human IgM constant region. In certain aspects, the modified human IgM antibody comprises one or more amino acid substitutions, *e.g.*, in or close to the Cμ3 domain, *e.g.*, in the region extending from about amino acid T302 of SEQ ID NO: 1 to about amino acid K322 of SEQ ID NO: 1, *e.g.*, at least one, at least two, or at least three or more amino acid substitutions at position T302, C303, T304, V305, T306, H307, T308, D309, L310, P311, S312, P313, L314, K315, Q316, T317, I318, S319, R320, P321, and/or K322 of SEQ ID NO: 1 relative to a wild-type human IgM constant region. While not wishing to be bound by theory, the C1q component of complement is thought to associate with the human IgM constant region at least through certain amino acid residues present in the Cμ3 domain. Assays for measuring CDC are well known to those of ordinary skill in the art, and exemplary assays are described herein.

[0105] In certain aspects, a modified human IgM antibody as provided herein comprises a substitution relative to a wild-type human IgM antibody at position P311 of SEQ ID NO: 1. In other aspects, a modified human IgM antibody as provided herein contains a substitution relative to a wild-type human IgM antibody at position P313 of SEQ ID NO: 1. In other aspects a modified human IgM antibody as provided herein contains a substitution relative to a wild-type human IgM antibody at position L310 of SEQ ID NO: 1. In other aspects a modified human IgM antibody as provided herein contains a substitution relative to a wild-type human IgM antibody at position K315 of SEQ ID NO: 1. In other aspects a modified IgM antibody as provided herein contains a combination of substitutions relative to a wild-type human IgM antibody at two or more of positions L310 of SEQ ID NO: 1, P311 of SEQ ID NO: 1, P313 of SEQ ID NO: 1, and/or K315 of SEQ ID NO: 1. A modified IgM antibody as provided herein can be substituted at amino acid position P311 of SEQ ID NO: 1 with, *e.g.*, alanine (P311A) (SEQ ID NO: 2), serine (P311S), or glycine (P311G). A modified IgM antibody as provided herein can be substituted at amino acid position P313 of SEQ ID NO: 1 with, *e.g.*, alanine (P313A), serine (P313S) (SEQ ID NO: 3), or glycine (P313G). A modified IgM antibody as provided herein can be substituted at amino acid position L310 of SEQ ID NO: 1 with, *e.g.*, alanine (L310A) (SEQ ID NO: 15), serine (L310S), glycine (L310G) or aspartic acid (L310D) (SEQ ID NO: 23). A modified IgM antibody as provided herein can be substituted at amino acid position K315 of SEQ ID NO: 1 with, *e.g.*, alanine (K315A) (SEQ ID NO: 16), serine (K315S), glycine (K315G), aspartic acid (K315D) (SEQ ID NO: 24), or glutamine (K315Q) (SEQ ID NO: 25). A modified IgM antibody as provided herein can be substituted at amino acid positions P311 and P313 of SEQ ID NO: 1 with, *e.g.*, alanine (P311A) and serine (P313S), respectively (SEQ ID NO: 4), or any combination of alanine, serine, and/or glycine. A modified IgM antibody as provided herein can be substituted at amino acid positions L310 and K315 of SEQ ID NO: 1 with, *e.g.*, alanine (L310A, K315A) (SEQ ID NO: 17), or serine (L310S, K315S) (SEQ ID NO: 18), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine. A modified IgM antibody as provided herein can be substituted at amino acid positions L310 and P311 of SEQ ID NO: 1 with, *e.g.*, alanine (L310A, P311A) (SEQ ID NO: 19), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine. A modified IgM antibody as provided herein can be substituted at amino acid positions L310 and P313 of SEQ ID NO: 1 with, *e.g.*, alanine and serine, respectively (L310A, P313S) (SEQ ID NO: 20), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine. A modified IgM antibody as provided herein can be substituted at amino acid positions P311 and K315 of SEQ ID NO: 1 with, *e.g.*, alanine (P311A, K315A) (SEQ ID NO: 21), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine. A modified IgM antibody as provided herein can be substituted at amino acid positions P313 and K315 of SEQ ID NO: 1 with, *e.g.*, serine and alanine, respectively (P313S, K315A) (SEQ ID NO: 22), or any combination of alanine, serine, aspartic acid, glutamine, and/or glycine.

[0106] In certain aspects, the complement-dependent cytotoxicity activity (CDC) of the modified IgM antibody as provided herein comprising, *e.g.*, an amino acid substitution at L310, P311, P313, and/or K315, *e.g.*, L310A, L310S, L310G, L310D, P311A, P311S, P311G, P313A, P313S, P313G, K315A, K315S, K315G, K315D, and/or K315Q, or any combination thereof, can be reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a corresponding wild-type IgM antibody, *i.e.*, a wild-type target-specific IgM antibody that is identical except for the modified human IgM constant region.

[0107] In certain aspects, antibody concentration effecting 50% CDC activity (EC_{50}) of the IgM antibody as provided herein, comprising, *e.g.*, an amino acid substitution at L310, P311, P313, and/or K315, *e.g.*, L310A, L310S, L310G, L310D, P311A, P311S, P311G, P313A, P313S, P313G, K315A, K315S, K315G, K315D, and/or K315Q, or any combination thereof, can be increased by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, and least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold relative to a corresponding wild-type IgM antibody, *i.e.*, a wild-type target-specific IgM antibody that is identical except for the modified human IgM constant region.

[0108] A modified human IgM antibody as provided herein can be hexameric or pentameric, comprising five or six IgM "binding units" as defined herein, one or more of which can specifically bind to a target antigen of interest. The disclosure encompasses a modified human IgM antibody that specifically binds to any target antigen. In certain aspects the target antigen is capable of expression on the surface of a cell, *e.g.*, a eukaryotic cell. Target antigens can include, without limitation, tumor antigens, other oncologic targets, immuno-oncologic targets such as immune checkpoint inhibitors, infectious disease antigens, such as viral antigens expressed on the surface of infected cells, target antigens involved in blood-brain-barrier transport, target antigens involved in neurodegenerative diseases and neuroinflammatory diseases, and any combination thereof. Non-limiting examples of target antigens, as well as non-limiting examples of antibody binding domains that bind to such target antigens can be found, *e.g.*, in PCT Publication Nos. WO 2016/141303, WO 2016/168758, WO 2016/154593, WO 2016/118641, WO 2015/153912, WO 2015/053887, WO 2013/120012, WO/2017/059387, WO/2017/059380, WO/2018/017888, WO/2018/017889, WO/2018/017761, and WO/2018/017763, the disclosures of which are incorporated herein by reference in their entireties.

[0109] In certain aspects, the disclosure provides a pentameric or hexameric modified human IgM antibody comprising five or six bivalent binding units, respectively, where each binding unit includes two modified human IgM heavy chain constant regions or fragments thereof as provided herein.

[0110] Where the modified human IgM antibody provided herein is pentameric, the antibody can further comprise a J-chain, or functional fragment thereof, or variant thereof. In certain aspects, the J-chain is a modified J-chain comprising a heterologous moiety or one or more heterologous moieties, *e.g.*, a heterologous polypeptide sequence, *e.g.*, an extraneous binding domain introduced into the native sequence. In certain aspects the extraneous binding domain specifically binds to CD3, *e.g.*, CD3ε. In certain aspects the modified J-chain comprises V15J (SEQ ID NO: 9) or J15V (SEQ ID NO: 11).

[0111] An IgM heavy chain constant region can include one or more of a Cμ1 domain, a Cμ2 domain, a Cμ3 domain (as provided herein, the Cμ3 can comprise one or more amino acid substitutions), and/or a Cμ4 domain, provided that the constant region can serve a desired function in the modified human IgM antibody, *e.g.*, associate with second IgM constant region to form an binding unit, or associate with other binding units to form a hexamer or a pentamer. In certain aspects the two modified human IgM heavy chain constant regions or fragments thereof within an individual binding unit each comprise a modified Cμ3 domain (*e.g.*, with substitutions at one or more of L310 of SEQ ID NO: 1, P311 of SEQ ID NO: 1, P313 of SEQ ID NO: 1, K315 of SEQ ID NO: 1, and/or a combination thereof) or fragment thereof, a Cμ4 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a modified Cμ3 domain a Cμ4 domain, and a TP or fragment thereof. In certain aspects the two modified human IgM heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cμ2 domain or fragment thereof, a Cμ1 domain or fragment thereof, or a Cμ1 domain or fragment thereof and a Cμ2 domain or fragment thereof.

[0112] In certain aspects each of the two modified human IgM heavy chain constant regions in a given binding unit is associated with an antigen binding domain, for example a heavy chain variable region (VH) or an Fv portion of an antibody, *e.g.*, a VH and a VL of an antibody.

Modified J-Chains

[0113] In certain aspects a modified human IgM antibody as provided herein can be bispecific, incorporating a modified J-chain. As provided herein and in PCT Publication Nos. WO 2015/153912 WO/2017/059387, and WO/2017/059380, a modified J-chain can comprise a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an extraneous binding domain, which can include, for example, a polypeptide binding domain capable of specifically binding to a target. The binding domain can be, for example, an antibody or antigen-binding fragment thereof, an antibody-drug conjugate or antigen-binding fragment thereof, or an antibody-like molecule. A polypeptide binding domain can be introduced into a J-chain by appropriately selecting the location and type of addition (*e.g.* direct or indirect fusion, chemical tethering, etc.).

[0114] In certain aspects, the binding domain can be an antibody or an antigen-binding fragment of an antibody, including monospecific, bispecific, and multi-specific antibodies and antibody fragments. The antibody fragment can be, without limitation, a Fab fragment, a Fab' fragment, a F(ab')$_2$ fragment, an scFv, (scFv)$_2$ fragment, single-chain antibody molecules, minibodies, or multispecific antibodies formed from antibody fragments. In certain aspects, the antibody fragment is a scFv.

[0115] In other aspects, the binding domain can be an antibody-like molecule, for example, a human domain antibody (dAb), Dual-Affinity Re-Targeting (DART) molecule, a diabody, a di-diabody, dual-variable domain antibody, a Stacked Variable Domain antibody, a Small Modular Immuno Pharmaceutical (SMIP), a Surrobody, a strand-exchange engineered domain (SEED)-body, or TandAb.

[0116] The binding domain can be introduced into the native J-chain sequence at any location that allows the binding of the binding domain to its binding target without interfering with the binding of a recipient IgM molecule to its binding target or binding targets or the ability of the J-chain to effectively incorporate into an IgM pentamer. In certain aspects the binding domain can be inserted at or near the C-terminus, at or near the mature N-terminus (*e.g.*, amino acid number 23 of SEQ ID NO: 7 following cleavage of the signal peptide) or at an internal location that, based on the three-dimensional structure of the J-chain is accessible. In certain aspects, the binding domain can be introduced into the native sequence J-chain without about 10 residues from the C-terminus or without about 10 amino acid residues from the mature N-terminus (amino acid 23) of the human J-chain of SEQ ID NO: 7. In another aspect, the binding domain can be introduced into the native sequence human J-chain of SEQ ID NO: 7 in between cysteine residues 113 and 122 of SEQ ID NO: 7, or at an equivalent location of another native sequence J-chain. In a further aspect, the binding domain can be introduced into a native sequence J-chain, such as a J-chain of SEQ ID NO: 7, at or near a glycosylation site. In certain aspects, the binding domain can be introduced into the native sequence human J-chain of SEQ ID NO: 7 within about 10 amino acid residues from the C-terminus.

[0117] Introduction can be accomplished by direct or indirect fusion, *i.e.* by the combination of the J-chain and binding domain in one polypeptide chain by in-frame combination of their coding nucleotide sequences, with or without a peptide linker. The peptide linker (indirect fusion), if used, can be about 1 to 50, or about 1 to 40, or about 1 to 30, or about 1 to 20, or about 1 to 10, or about 10 to 20 amino acids in length, and can be present at one or both ends of the binding domain to be introduced into the J-chain sequence. In certain aspects, the peptide linker is about 10 to 20, or 10 to 15 amino acids long. In certain aspects the peptide linker is 15 amino acids long. In certain aspects the peptide linker is $(GGGGS)_3$ (SEQ ID NO: 12).

[0118] It is also possible to introduce more than one heterologous polypeptide, *e.g.*, more than one binding domain or other moiety, into a J-chain.

[0119] The modified J-chain can be produced by well-known techniques of recombinant DNA technology, by expressing a nucleic acid encoding the modified J-chain in a suitable prokaryotic or eukaryotic host organism.

[0120] The modified J-chain can also be co-expressed with the heavy and light chains of a recipient modified human IgM antibody as described elsewhere herein. The recipient antibody, prior to modified J-chain incorporation, can be monospecific, bispecific or multi-specific, *e.g.*, a monospecific, bispecific, or multispecific modified human IgM antibody. Bispecific and multi-specific IgM antibodies, including antibodies, are described, for example, in PCT Publication Nos. WO 2015/053887, and WO 2015/120474, the entire contents of which are hereby expressly incorporated by reference.

[0121] In certain aspects, a modified human IgM antibody as described herein can include a modified J-chain with binding specificity for an immune effector cell, such as a T-cell, NK-cell, a macrophage, or a neutrophil. In certain aspects the effector cell is a T-cell and the binding target is CD3 (discussed below). By activating and redirecting effector cells, *e.g.* effector T-cells such as cytotoxic T-cells (CTLs), to a cell expressing a target antigen of interest, a modified human IgM antibody as provided herein can produce an enhanced effector response against the target antigen, thereby further increasing potency and efficacy. In certain aspects, the ability of a bispecific modified human IgM antibody as provided herein to elicit T-cell mediated cytotoxicity through binding to CD3 via a modified J-chain is unaffected by the modifications in the IgM constant region that reduce CDC relative to a corresponding wild-type IgM antibody.

[0122] In the case of T-cells, cluster of differentiation 3 (CD3) is a multimeric protein complex, known historically as the T3 complex, and is composed of four distinct polypeptide chains (ε, γ, δ, ζ) that assemble and function as three pairs of dimers (εγ, εδ, ζζ). The CD3 complex serves as a T-cell co-receptor that associates non-covalently with the T-cell receptor (TCR). Components of this CD3 complex, especially CD3ε, can be targets for a modified J-chain of a bispecific IgM or IgA binding molecule provided herein.

[0123] In certain aspects, a bispecific modified human IgM antibody is provided where the J-chain is modified to bind to CD3ε.

[0124] In certain aspects the anti-CD3ε binding domain of a modified J-chain provided herein is a scFv. The anti CD3ε scFv can be fused at or near the N-terminus of the J-chain, or at or near the C-terminus of the J-chain either directly or indirectly with a synthetic linker introduced in between the scFv and the J-chain sequences, *e.g.*, a $(GGGGS)_3$ linker (SEQ

ID NO: 12). In certain aspects the scFv comprises the VH and VL regions of visilizumab (Nuvion). In certain aspects the modified J-chain comprises a scFv comprising the VH of visilizumab, a $(GGGGS)_3$ linker, and the VL of visilizumab.

[0125] In certain aspects the modified J-chain comprises a scFv of visilizumab fused to the N-terminus of the human J-chain through a 15-amino acid $(GGGGS)_3$ linker, a modified J-chain referred to herein as V15J. V15J can further include a signal peptide to facilitate transport and assembly into a modified human IgM antibody as provided herein. The mature V15J protein is presented as SEQ ID NO: 9, the precursor version, comprising a 19-amino acid-immunoglobulin heavy chain signal peptide is presented as SEQ ID NO: 8. In certain aspects the modified J-chain comprises a scFv of visilizumab fused to the C-terminus of the human J-chain through a 15-amino acid $(GGGGS)_3$ linker, a modified J-chain referred to herein as J15V. J15V can further include a signal peptide to facilitate transport and assembly into a modified human IgM antibody as provided herein. The mature J15V protein is presented as SEQ ID NO: 11, the precursor version, comprising the 22-amino acid-human J-chain signal peptide is presented as SEQ ID NO: 10. In certain aspects, other signal peptides can be used. Selection and inclusion of suitable signal peptides to facilitate expression, secretion, and incorporation of a modified J-chain into a modified human IgM antibody as provided herein is well within the capabilities of a person of ordinary skill in the art.

[0126] This disclosure employs, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook *et al.*, ed. (1989) Molecular Cloning A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) Molecular Cloning: A Laboratory Manual, (Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) DNA Cloning, Volumes I and II; Gait, ed. (1984) Oligonucleotide Synthesis; Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) Nucleic Acid Hybridization; Hames and Higgins, eds. (1984) Transcription And Translation; Freshney (1987) Culture Of Animal Cells (Alan R. Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) A Practical Guide To Molecular Cloning; the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian Cells, (Cold Spring Harbor Laboratory); Wu *et al.*, eds., Methods In Enzymology, Vols. 154 and 155; Mayer and Walker, eds. (1987)

Immunochemical Methods In Cell And Molecular Biology (Academic Press, London); Weir and Blackwell, eds., (1986) Handbook Of Experimental Immunology, Volumes I-IV; Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel *et al.* (1989) Current Protocols in Molecular Biology (John Wiley and Sons, Baltimore, Md.).

[0127] General principles of antibody engineering are set forth in Borrebaeck, ed. (1995) Antibody Engineering (2nd ed.; Oxford Univ. Press). General principles of protein engineering are set forth in Rickwood *et al.*, eds. (1995) Protein Engineering, A Practical Approach (IRL Press at Oxford Univ. Press, Oxford, Eng.). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff (1984) Molecular Immunology (2nd ed.; Sinauer Associates, Sunderland, Mass.); and Steward (1984) Antibodies, Their Structure and Function (Chapman and Hall, New York, N.Y.). Additionally, standard methods in immunology known in the art and not specifically described can be followed as in Current Protocols in Immunology, John Wiley & Sons, New York; Stites *et al.*, eds. (1994) Basic and Clinical Immunology (8th ed; Appleton & Lange, Norwalk, Conn.) and Mishell and Shiigi (eds) (1980) Selected Methods in Cellular Immunology (W.H. Freeman and Co., NY).

[0128] Standard reference works setting forth general principles of immunology include Current Protocols in Immunology, John Wiley & Sons, New York; Klein (1982) J., Immunology: The Science of Self-Nonself Discrimination (John Wiley & Sons, NY); Kennett *et al.*, eds. (1980) Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses (Plenum Press, NY); Campbell (1984) "Monoclonal Antibody Technology" in Laboratory Techniques in Biochemistry and Molecular Biology, ed. Burden *et al.*, (Elsevier, Amsterdam); Goldsby *et al.*, eds. (2000) Kuby Immunology (4th ed.; W.H. Freeman and Co., NY); Roitt *et al.* (2001) Immunology (6th ed.; London: Mosby); Abbas *et al.* (2005) Cellular and Molecular Immunology (5th ed.; Elsevier Health Sciences Division); Kontermann and Dubel (2001) Antibody Engineering (Springer Verlag); Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press); Lewin (2003) Genes VIII (Prentice Hall, 2003); Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Press); Dieffenbach and Dveksler (2003) PCR Primer (Cold Spring Harbor Press).

[0129] All the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0130] The following examples are offered by way of illustration and not by way of limitation.

Examples

Example 1: Effects of Cμ3 Domain Mutations on IgM Assembly

[0131] Plasmid variants of pFUSEss-CHIg-hM*03-encoding modified human IgM constant regions with single site mutations in the Cμ3 domain, P311A (SEQ ID NO: 2), P313S (SEQ ID NO: 3), both P311A and P313S (SEQ ID NO: 4), D294G (SEQ ID NO: 5), and S283N (SEQ ID NO: 6), were designed and submitted to a commercial vendor for synthesis. Exemplary plasmid constructs that can express wild-type or modified human pentameric or hexameric IgM antibodies comprising the wild-type or modified IgM constant regions, and that can specifically bind to CD20, were produced by the following method.

[0132] DNA fragments encoding the VH and VL regions of 1.5.3 (SEQ ID NOs 13 and 14, respectively) were synthesized by a commercial vendor (Genescript), with an EcoRV restriction site on the '5 end and an XbaI restriction site on the 3' end for subcloning into heavy chain and light chain expression vectors. The synthesized DNA constructs were re-suspended in Tris-EDTA buffer at 1 μg/ml. DNA samples (1 μg) were digested with EcoRV and XbaI, and the synthesized VH and VL were separated from the carrier plasmid DNA by electrophoresis. The digested DNA was ligated to pre-digested plasmid DNA (pFUSEss-CHIg-hM*03 available from Invivogen or the modified constructs described above for μ chain, pFUSE2ss-CLIg-hk for kappa chain, available from Invivogen) by standard molecular biology techniques. The ligated DNAs were transformed into competent bacteria and plated on LB plates with multiple selective antibiotics. Several bacterial colonies were picked, and DNA preparations were made by standard molecular biology techniques. The constructs encoding the heavy chain and light chains were verified by sequencing.

[0133] The plasmid constructs encoding the IgM heavy chains and light chains, or the heavy chains, light chains, and J-chain were cotransfected into CHO cells, and cells that express wild-type or modified CD20 IgM antibodies, either with or without J-chain, were selected, all according to standard methods.

[0134] Antibodies present in the cell supernatants were recovered using Capture Select IgM (Catalog 2890.05, BAC, Thermo Fisher) according to the manufacturer's protocol. Antibodies were evaluated on SDS PAGE under non-reducing conditions to show assembly as previously described, *e.g.*, in PCT Publication No. WO 2016/141303 (**FIG. 1** and **FIG. 5**), or under reducing conditions for certain bispecific antibodies (**FIG. 5**).

[0135] Control antibodies (IgM (pure), IgM + J (pure) (an IgM antibody with wild-type J chain) and IgM (an IgM lacking a J chain)) and the IgM Cμ3 domain mutants, S283N, P313S, P311A, and D294G, were evaluated on SDS PAGE under non-reducing conditions to show assembly of the antibodies. NuPage LDS Sample Buffer (Life Technologies) was added to samples before loading onto a NativePage Novex 3-12% bis-Tris Gel (Life Technologies Catalog #BN1003). Novex Tris-Acetate SDS Running Buffer (Life Technologies Catalog #LA0041) was used for gel electrophoresis. The gel was run until the dye front reached the bottom of the gel. After electrophoresis, the gel was stained with Colloidal Blue Stain (Life Technologies Catalog #LC6025).

[0136] The results, shown in **FIG. 1**, demonstrate that the IgM Cμ3 domain mutant antibodies assemble as well as control antibodies.

[0137] Control bispecific antibody (1.5.3 IgM V15J) and the 1.5.3 IgM V15J Cμ3 domain mutants, P313S, P311A, and the P311A/P313S double mutant, were evaluated on SDS PAGE under non-reducing ("hybrid") and reducing conditions to show assembly of the antibodies. NuPage LDS Sample Buffer (Life Technologies) was added to samples before loading onto a NativePage Novex 3-12% bis-Tris Gel (Life Technologies Catalog #BN1003). Novex Tris-Acetate SDS Running Buffer (Life Technologies Catalog #LA0041) was used for gel electrophoresis. The gel was run until the dye front reached the bottom of the gel. After electrophoresis, the gel was stained with Colloidal Blue Stain (Life Technologies Catalog #LC6025).

[0138] The results, shown in **FIG. 5**, demonstrate that the bispecific IgM Cμ3 domain mutant antibodies assemble as well as control antibody.

Example 2: Complement Dependent Cytotoxicity Activity of IgM Cμ3 Mutants

[0139] The CD20-expressing Ramos (ATCC cat. #CRL-1596), cell line was obtained from ATCC and DSMZ. 50,000 cells were seeded in a 96-well plate. Cells were treated with the wild-type control antibodies, 1.5.3 IgM, 1.5.3 IgG and 1.5.3 IgM x V15J, as well as the IgM Cμ3 domain single mutants (P311A, P313S, D294G, and S283N). Human serum complement (Quidel cat. #A113) was added to antibody-treated cells at a final concentration of 10%. The reaction mixtures were incubated at 37 °C for 4 hours. CELLTITER-GLO®

reagent (Promega cat. #G7572) was added at a volume equal to the volume of culture medium present in each well. The plate was shaken for 2 minutes, incubated for 10 minutes at room temperature, and luminescence was measured on a luminometer.

[0140] The results are shown in **FIG. 2**. Two IgM Cμ3 single mutants, P311A and P313S, exhibited approximately 50% less cell killing than the control 1.5.3 IgM antibodies in the presence of complement. IgM Cμ3 mutants, S283N and D294G, showed similar cell killing with complement to the control wild-type IgMs. In the absence of complement, minimal cell killing was observed for the 1.5.3 IgG and 1.5.3 IgM x V15J antibodies.

Example 3: Complement Dependent Cytotoxicity of Bispecific anti-CD20 x anti-CD3 IgM Cμ3 Mutants

[0141] The CD20-expressing Ramos (ATCC cat. #CRL-1596), cell line was obtained from ATCC and DSMZ. 50,000 cells were seeded in a 96-well plate. Cells were treated with control antibody, 1.5.3 IgM x V15J, and the bispecific IgM Cμ3 domain mutants (P311A x V15J, P313S x V15J, D294G x V15J, and S283N x V15J). All the antibodies included a J chain bispecific for CD20 and CD3 generated and expressed as previously described, see, PCT Publication No. WO 2016/141303. Human serum complement (Quidel cat. #A113) was added to the antibody-treated cells at a final concentration of 10%. The reaction mixtures were incubated at 37 °C for 4 hours. CELLTITER-GLO® reagent (Promega cat. #G7572) was added at a volume equal to the volume of culture medium present in each well. The plate was shaken for 2 minutes, incubated for 10 minutes at room temperature, and luminescence was measured on a luminometer.

[0142] The results are shown in **FIG. 3** and in **FIG. 6**. The bispecific IgM Cμ3 mutants, P311A and P313S, exhibited half maximal Ramos cell killing with complement, whereas control 1.5.3 IgM x V15J and the bispecific IgM Cμ3 mutants D294G and S283N achieved nearly maximal complement dependent cytotoxicity (90-100% effective) in the presence of complement. In the assay shown in **FIG. 6**, bispecific IgM Cμ3 mutant P311A exhibited maximum killing of about 46% with complement (EC_{50} was undefined), while the P313S mutant showed maximum killing of 31% with complement (EC_{50} was 243 pM). Complement-mediated killing was essentially eliminated in the double mutant, P311A/P313S (**FIG. 6**, see also **Table 2**).

Example 4: T-Cell Activation Potential of Bispecific anti-CD20 x anti-CD3 IgM Cμ3 Mutants

[0143] Engineered Jurkat T-cells (Promega CS176403) and RPMI8226 cells (ATCC CCL-155) were cultured in RPMI (Invitrogen) supplemented with 10% Fetal Bovine Serum (Invitrogen). Serial dilutions of bispecific 1.5.3 IgM x V15J antibody and the bispecific IgM Cμ3 mutants, D294G x V15J, S283N x V15J, P313S x V15J, P311A x V15J, P311A/P313S x V15J, and 1.5.3 IgM (without J-chain) were incubated with 7500 RPMI8226 cells in 20 μL in a white 384 well assay plate for 2h at 37 °C with 5% CO_2. The engineered Jurkat cells (25000) were added to mixture to final volume of 40 μL. The mixture was incubated for 5h at 37 °C with 5% CO_2. The cell mixtures were then mixed with 20 μL lysis buffer containing luciferin (Promega, CELLTITER-GLO®) to measure luciferase reporter activity. Light output was measured by EnVision plate reader. EC_{50} was determined by 4 parameter curve fit using Prism software.

[0144] The results are shown in **FIG. 4** and in **FIG.** 7. All of the bispecific IgM Cμ3 mutants activated T cells as effectively as the bispecific control antibody (1.5.3 IgM x V15J). No T cell activation was observed in the IgM control lacking the bispecific J chain (1.5.3 IgM).

Example 5: Complement Dependent Cytotoxicity of Additional Bispecific anti-CD20 x anti-CD3 IgM Cμ3 Mutants

[0145] To further evaluate the effect of Cμ3 mutations on CDC activity, systematic single amino acid ala substitutions were made at each position from T302 of SEQ ID NO: 1 to K322 of SEQ ID NO: 1. Each of these mutant IgM constant regions were assembled as 1.5.3 IgM V15J Cμ3 domain mutants as described in Example 1, and were tested for CDC activity as described in Example 3 and for T cell activation as described in Example 4. In addition to the previously evaluated mutations at P311 and P313, 1.5.3 IgM V15J Cμ3 domain ala substitutions at L310 (SEQ ID NO: 15) and K315 (SEQ ID NO: 16) modestly reduced CDC activity with an increased EC_{50}, as shown in **FIG. 8** and **Table 2**, without significantly affecting T cell activation (data not shown).

[0146] Various combinations of double substitutions at two of positions L310, P311, P313, and K315 were also constructed as described in Example 1 and were tested for CDC activity as described in Example 3. These included double mutants L310A, K315A (SEQ ID NO: 17), L310S, K315S (SEQ ID NO: 18), L310A, P311A (SEQ ID NO: 19), L310A,

P313S (SEQ ID NO: 20), P311A, K315A (SEQ ID NO: 21), and P313S, K315A (SEQ ID NO: 22). The CDC activity of these double mutants relative to wild-type human IgM is summarized in **Table 2**. The L310A, K315A double mutant shown an enhanced reduction in CDC activity relative to either of the single mutations.

[0147] Additional amino acid substitutions at positions L310 and K315 were evaluated to determine the effect of charged or polar amino acids on CDC activity. L310 was substituted with the negatively-charged aspartic acid (D, SEQ ID NO: 23), and K315 was substituted with aspartic acid (D, SEQ ID NO: 24) or the polar amino acid glutamine (Q, SEQ ID NO: 25). These mutants were tested for CDC activity as described in Example 3. The results are shown in **FIG. 9A** and **FIG. 9B**, and in **Table 2**. The introduction of negatively-charged amino acids at positions 310 or 315 disrupted CDC activity more than the neutral ala substitutions.

Table 2: CDC Activity of Human IgM Cμ3 Mutants

Substitution(s)	**Amino Acid Residue in Human IgM Heavy Chain Constant Region (SEQ ID NO: 1)**								**Fold EC_{50} Increase**	**%CDC Relative to Wild Type**
	309	310	311	312	313	314	315	316		
WT	D	L	P	S	P	L	K	Q	1	100%
L310A		A							2	95%
P311A			A						11	46%
P313S					S				6	83%
K315A							A		5	95%
L310D		D							ND	12%
K315D							D		7	50%
K315Q							Q		3	85%
L310A K315A		A					A		80	52%
L310S K315S		S					S		8	86%
L310A P311A		A	A						ND	18%
L310A P313S		A			S				6	39%
P311A K315A			A				A		100	29%
P313S K315A					S		**A**		**16**	**47%**
P311A P313S			**A**		S				**ND**	**25%**

Table 3: Sequences

SEQ ID NO	Short Name	Sequence
1	human IgM constant region AA	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
2	Modified human IgM constant region P311A	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLASPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
3	Modified human IgM constant region P313S	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSSLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
4	Modified human IgM constant region P311A/P313S	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLASSLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFS

SEQ ID NO	Short Name	Sequence
		PADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
5	Modified human IgM constant region D294G	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDGWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
6	Modified human IgM constant region S283N	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFNAVGEASICEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
7	J-chain AA	MKNHLLFWGVLAVFIKAVHVKAQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD
8	precursor modified J-chain sequence for V15J	MGWSYIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIKGGGGSGGGGSGGGGSQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEV

SEQ ID NO	Short Name	Sequence
		ELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD
9	mature modified J-chain sequence for V15J	QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIKGGGGSGGGGSGGGGSQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD
10	Precursor modified J-chain sequence for J15V	MKNHLLFWGVLAVFIKAVHVKAQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPDGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIK
11	mature modified J-chain sequence for J15V	QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPDGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIK
12	(GGGGS)$_3$ linker	GGGGSGGGGSGGGGS
13	1.5.3 VH	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSITTAYLQWSSLKASDTAMYYCARHPSYGSGSPNFDYWGQGTLVTVSS

SEQ ID NO	Short Name	Sequence
14	1.5.3 VL	DIVMTQTPLSSPVTLGQPASISCRSSQSLVYSDGNTYLSWLQQRPGQPPRLLIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCVQATQFPLTFGGGTKVEIK
15	Modified human IgM constant region L310A	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDAPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
16	Modified human IgM constant region K315A	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSPLAQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
17	Modified human IgM constant region L310A, K315A	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDAPSPLAQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
18	Modified human IgM constant region L310S, K315S	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDSPSPLSQTI

SEQ ID NO	Short Name	Sequence
		SRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
19	Modified human IgM constant region L310A, P311A	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDAASPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
20	Modified human IgM constant region L310A, P313S	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDAPSSLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
21	Modified human IgM constant region P311A, K315A	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLASPLAQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
22	Modified human IgM constant region P313S, K315A	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESH

SEQ ID NO	Short Name	Sequence
		PNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSSLAQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
23	Modified human IgM constant region L310D	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDDPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
24	Modified human IgM constant region K315D	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSPLDQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
25	Modified human IgM constant region K315Q	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSPLQQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

[0148] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary embodiments but should be defined only in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. A modified human IgM constant region comprising one or more amino acid substitutions relative to a wild-type human IgM constant region, wherein at least one amino acid substitution is at a position in the Cμ3 domain ranging from T302 of SEQ ID NO: 1 to K322 of SEQ ID NO: 1, and wherein a modified IgM antibody comprising the modified IgM constant region and a heavy chain variable region specific for a target antigen exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

2. The modified human IgM constant region of claim 1, wherein the at least one amino acid substitution is at amino acid T302, C303, T304, V305, T306, H307, T308, D309, L310, P311, S312, P313, L314, K315, Q316, T317, I318, S319, R320, P321, and/or K322 of SEQ ID NO: 1.

3. The modified human IgM constant region of claim 2 wherein the at least one amino acid substitution is at position L310 of SEQ ID NO: 1, position P311 of SEQ ID NO: 1, position P313 of SEQ ID NO: 1, position K315 of SEQ ID NO: 1, or any combination thereof.

4. The modified human IgM constant region of claim 3, wherein the amino acid substitution is at position L310 of SEQ ID NO: 1.

5. The modified human IgM constant region of claim 4, wherein L310 of SEQ ID NO: 1 is substituted with alanine (L310A), serine (L310S), aspartic acid (L310D) or glycine (L310G).

6. The modified human IgM constant region of claim 5, wherein L310 of SEQ ID NO: 1 is substituted with alanine (L310A).

7. The modified human IgM constant region of claim 6, comprising SEQ ID NO: 15.

8. The modified human IgM constant region of claim 5, wherein L310 of SEQ ID NO: 1 is substituted with aspartic acid (L310D).

9. The modified human IgM constant region of claim 8, comprising SEQ ID NO: 23.

10. The modified human IgM constant region of claim 3, wherein the amino acid substitution is at position P311 of SEQ ID NO: 1.

11. The modified human IgM constant region of claim 10, wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A), serine (P311S), or glycine (P311G).

12. The modified human IgM constant region of claim 11, wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A).

13. The modified human IgM constant region of claim 12, comprising SEQ ID NO: 2.

14. The modified human IgM constant region of claim 3, wherein the amino acid substitution is at position P313 of SEQ ID NO: 1.

15. The modified human IgM constant region of claim 14, wherein P313 of SEQ ID NO: 1 is substituted with alanine (P313A), serine (P313S), or glycine (P313G).

16. The modified human IgM constant region of claim 15, wherein P313 of SEQ ID NO: 1 is substituted with serine (P313S).

17. The modified human IgM constant region of claim 16, comprising SEQ ID NO: 3.

18. The modified human IgM constant region of claim 3, wherein the amino acid substitution is at position K315 of SEQ ID NO: 1.

19. The modified human IgM constant region of claim 18, wherein K315 of SEQ ID NO: 1 is substituted with alanine (K315A), serine (K315S), aspartic acid (K315D), glutamine (K315Q), or glycine (P313G).

20. The modified human IgM constant region of claim 19, wherein K315 of SEQ ID NO: 1 is substituted with alanine (K315A).

21. The modified human IgM constant region of claim 20, comprising SEQ ID NO: 16.

22. The modified human IgM constant region of claim 19, wherein K315 of SEQ ID NO: 1 is substituted with aspartic acid (K315D).

23. The modified human IgM constant region of claim 22, comprising SEQ ID NO: 24.

24. The modified human IgM constant region of claim 19, wherein K315 of SEQ ID NO: 1 is substituted with glutamine (K315Q).

25. The modified human IgM constant region of claim 24, comprising SEQ ID NO: 25.

26. The modified human IgM constant region of claim 3, comprising amino acid substitutions at positions P311 and P313 of SEQ ID NO: 1.

27. The modified human IgM constant region of claim 26, wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A), serine (P311S), or glycine (P311G), and

wherein P313 of SEQ ID NO: 1 is substituted with alanine (P313A), serine (P313S), or glycine (P313G).

28. The modified human IgM constant region of claim 27, wherein P311 of SEQ ID NO: 1 is substituted with alanine (P311A) and P313 of SEQ ID NO: 1 is substituted with serine (P313S).

29. The modified human IgM constant region of claim 28, comprising SEQ ID NO: 4.

30. The modified human IgM constant region of claim 3, comprising amino acid substitutions at positions L310 and K315 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) or serine (L310S) and K315 is substituted with alanine (K315A) or serine (K315S).

31. The modified human IgM constant region of claim 30, comprising SEQ ID NO: 17 or SEQ ID NO: 18.

32. The modified human IgM constant region of claim 3, comprising amino acid substitutions at positions L310 and P311 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) and P311 is substituted with alanine (P311A).

33. The modified human IgM constant region of claim 32, comprising SEQ ID NO: 19.

34. The modified human IgM constant region of claim 3, comprising amino acid substitutions at positions L310 and P313 of SEQ ID NO: 1, wherein L310 is substituted with alanine (L310A) and P313 is substituted with serine (P313S).

35. The modified human IgM constant region of claim 34, comprising SEQ ID NO: 20.

36. The modified human IgM constant region of claim 3, comprising amino acid substitutions at positions P311 and K315 of SEQ ID NO: 1, wherein P311 is substituted with alanine (P311A) and K315 is substituted with alanine (K315A).

37. The modified human IgM constant region of claim 36, comprising SEQ ID NO: 21.

38. The modified human IgM constant region of claim 3, comprising amino acid substitutions at positions P313 and K315 of SEQ ID NO: 1, wherein P313 is substituted with serine (P313S) and K315 is substituted with alanine (K315A).

39. The modified human IgM constant region of claim 38, comprising SEQ ID NO: 22.

40. The modified human IgM constant region of any one of claims 1 to 39, wherein the maximum CDC activity achieved by a target-specific IgM antibody comprising the modified human IgM constant region in a dose-response assay is reduced by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

41. The modified human IgM constant region of any one of claims 1 to 39, wherein the antibody concentration of a target-specific IgM antibody comprising the modified human IgM constant region effecting 50% CDC activity (EC50) is increased by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, and least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, or at least 100-fold relative to a corresponding wild-type IgM antibody identical except for the modified human IgM constant region.

42. A modified human IgM antibody comprising the modified human IgM constant region of any one of claims 1 to 41, and a heavy chain variable region (VH) situated amino terminal to the modified human IgM constant region, wherein the modified human IgM antibody specifically binds to a target antigen and exhibits reduced complement-dependent cytotoxicity (CDC) of cells expressing the target antigen relative to a corresponding wild-type human IgM antibody.

43. The modified human IgM antibody of claim 42, which is a pentameric or a hexameric antibody comprising five or six bivalent IgM binding units, respectively, wherein each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the modified human IgM constant region, and two immunoglobulin light chains each comprising a light chain variable domain (VL) situated amino terminal to a human immunoglobulin light chain constant region.

44. The modified human IgM antibody of claim 43, which is a pentameric, and further comprises a J-chain, or functional fragment thereof, or a functional variant thereof.

45. The modified human IgM antibody of claim 44, wherein J-chain or fragment thereof comprises amino acids 23 to 158 of SEQ ID NO: 7 or a functional fragment thereof, or a functional variant thereof.

46. The modified human IgM antibody of claim 44 or claim 45, wherein the J-chain or fragment or variant thereof is a modified J-chain further comprising a heterologous

polypeptide, wherein the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment or variant thereof.

47. The modified human IgM antibody of claim 46, wherein the heterologous polypeptide is fused to the J-chain or fragment thereof via a peptide linker.

48. The modified human IgM antibody of claim 47, wherein the peptide linker comprises at least 5 amino acids, but no more than 25 amino acids.

49. The modified human IgM antibody of claim 48, wherein the peptide linker consists of GGGGSGGGGSGGGGS (SEQ ID NO: 12).

50. The modified human IgM antibody of any one of claims 46 to 49, wherein the heterologous polypeptide is fused to the N-terminus of the J-chain or fragment or variant thereof, the C-terminus of the J-chain or fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or fragment or variant thereof.

51. The modified human IgM antibody of any one of claims 46 to 50, wherein the heterologous polypeptide comprises a binding domain.

52. The modified human IgM antibody of claim 51, wherein the binding domain of the heterologous polypeptide is an antibody or antigen-binding fragment thereof.

53. The modified human IgM antibody of claim 52, wherein the antigen-binding fragment comprises an Fab fragment, an Fab' fragment, an F(ab')2 fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) fragment, a disulfide-linked Fv (sdFv) fragment, or any combination thereof.

54. The modified human IgM antibody of claim 53, wherein the antigen-binding fragment is a scFv fragment.

55. The modified human IgM antibody of any one of claims 51 to 54 wherein the heterologous polypeptide can specifically bind to CD3ε.

56. The modified human IgM antibody of claim 55, wherein the modified J-chain comprises the amino acid sequence SEQ ID NO: 9 (V15J) or SEQ ID NO: 11 (J15V).

57. The modified human IgM antibody of claim 56, wherein the modified J-chain further comprises a signal peptide.

58. The modified human IgM antibody of claim 57 wherein the modified J-chain comprises the amino acid sequence SEQ ID NO: 8 (V15J) or SEQ ID NO: 10 (J15V).

59. The modified human IgM antibody of any one of claims 55 to 58, which can direct T-cell-mediated killing of a cell expressing the target antigen equivalent to that of a

corresponding IgM antibody that is identical to the modified IgM antibody except for the modified IgM constant region.

60. The modified human IgM antibody of any one of claims 42 to 59, wherein the cell expressing the target antigen is a eukaryotic cell.

61. A polynucleotide comprising a nucleic acid sequence that encodes the modified human IgM constant region of any one of claims 1 to 40, or a heavy chain polypeptide subunit of the modified human IgM antibody of any one of claims 42 to 59.

62. A composition comprising the polynucleotide of claim 61.

63. The composition of claim 62, further comprising a nucleic acid sequence that encodes a light chain polypeptide subunit.

64. The composition of claim 63, wherein the light chain polypeptide subunit comprises a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL.

65. The composition of claim 63 or claim 64, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit are on separate vectors.

66. The composition of claim 63 or claim 64, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit and the nucleic acid sequence encoding the light chain polypeptide subunit are on a single vector.

67. The composition of any one of claims 62 to 66, further comprising a nucleic acid sequence that encodes a J-chain, or functional fragment thereof, or a functional variant thereof.

68. The composition of claim 67, wherein the J-chain or fragment thereof is a modified J-chain that further comprises a heterologous polypeptide, wherein the heterologous polypeptide is directly or indirectly fused to the J-chain or fragment thereof.

69. The composition of claim 67 or claim 68, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain are on a single vector.

70. The composition of claim 67 or claim 68, wherein the nucleic acid sequence encoding the heavy chain polypeptide subunit, the nucleic acid sequence encoding the light chain polypeptide subunit, and the nucleic acid sequence encoding the J-chain are each on separate vectors.

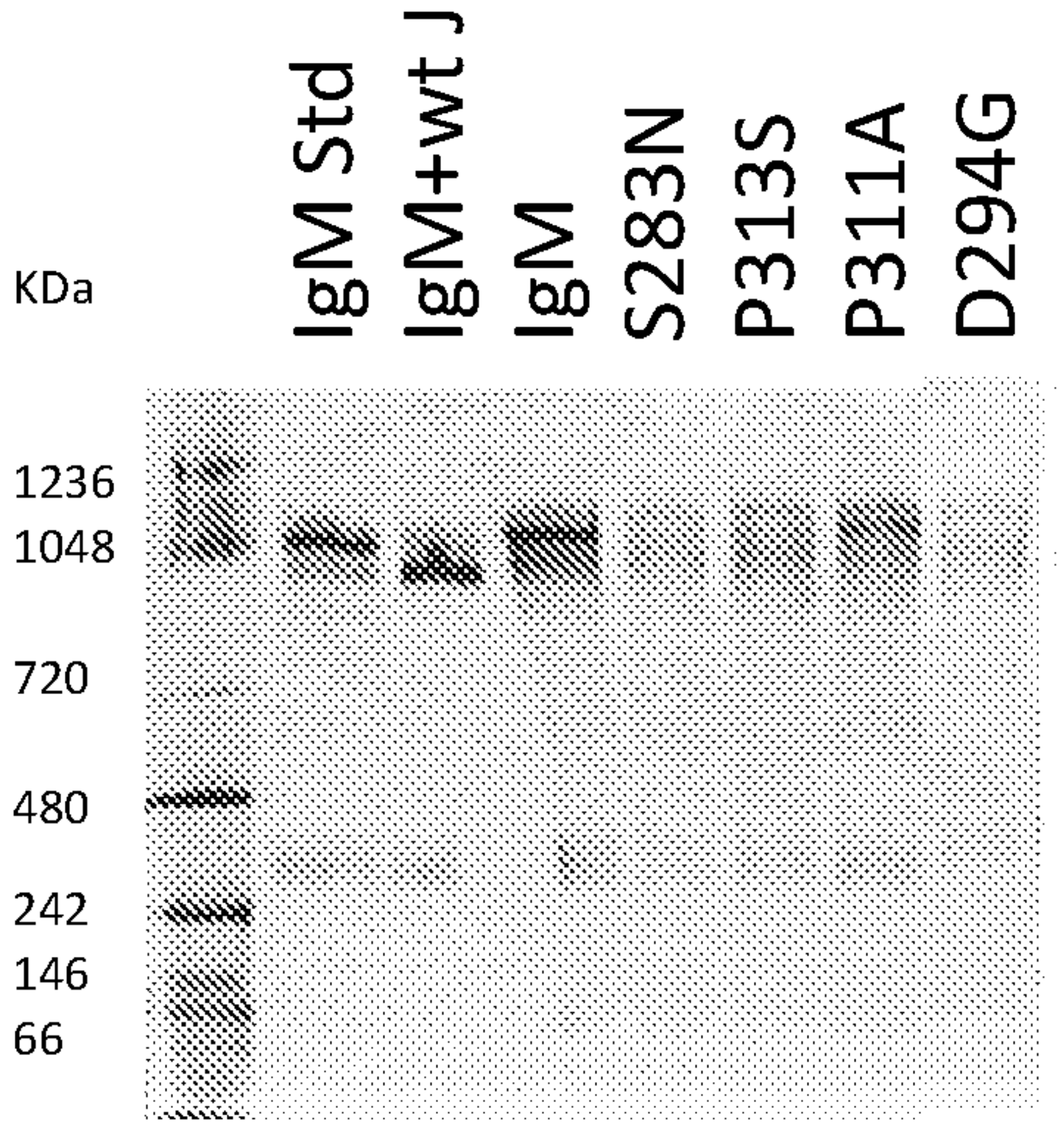
71. A vector of claim 66 or claim 69.

72. The vectors of claim 65 or claim 70.

73. A host cell comprising the polynucleotide of claim 61, the composition of any one of claims 62 to 70, or the vector or vectors of claim 71 or claim 72, wherein the host cell can express the modified human IgM constant region of any one of claims 1 to 40, or the modified human IgM antibody of any one of claims 42 to 59 or a functional fragment thereof.

74. A method of producing the modified human IgM constant region of any one of claims 1 to 40, or the modified human IgM antibody of any one of claims 42 to 60, comprising culturing the host cell of claim 73, and recovering the constant region or antibody.

FIGURE 1


KDa
IgM Std
IgM+wt J
IgM
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P313S
P311A
D294G
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1048
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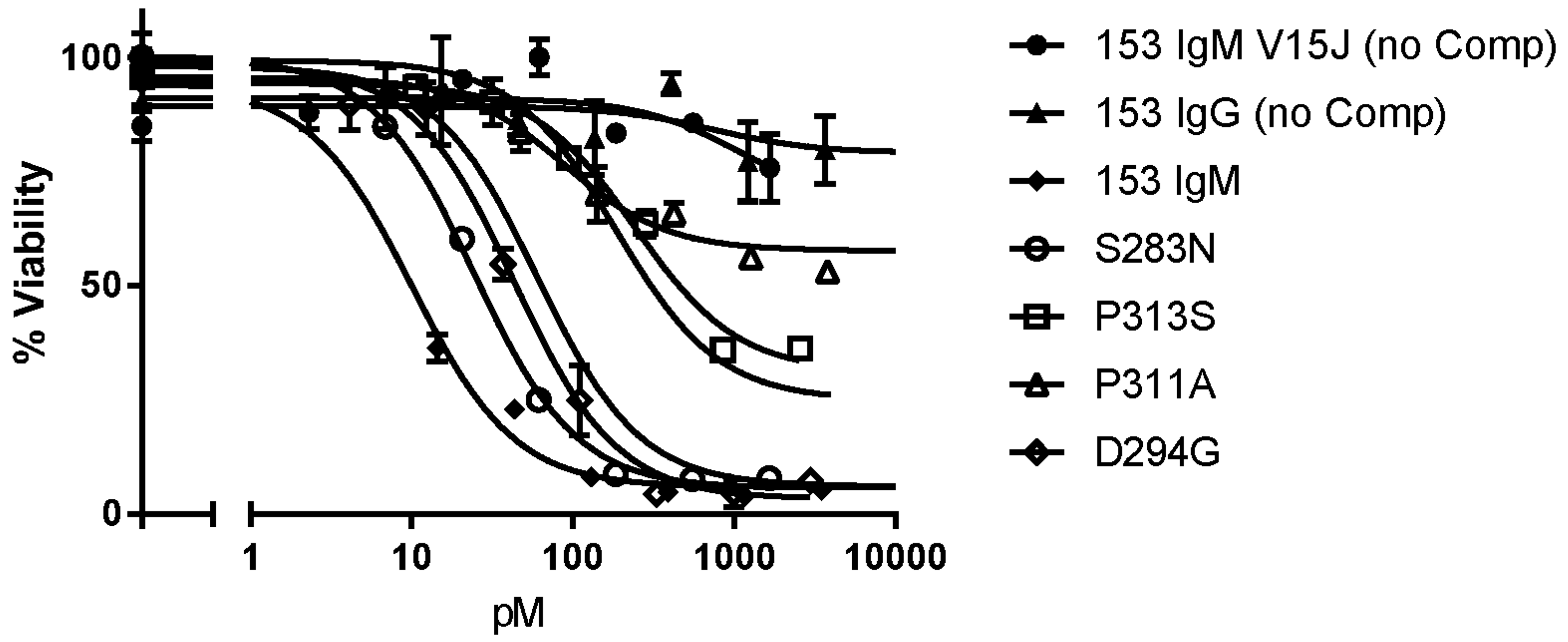
480
242
146
66

FIGURE 2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50
0
% Viability
1
10
100
1000
10000
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153 IgG (no Comp)
153 IgM
S283N
P313S
P311A
D294G

FIGURE 3

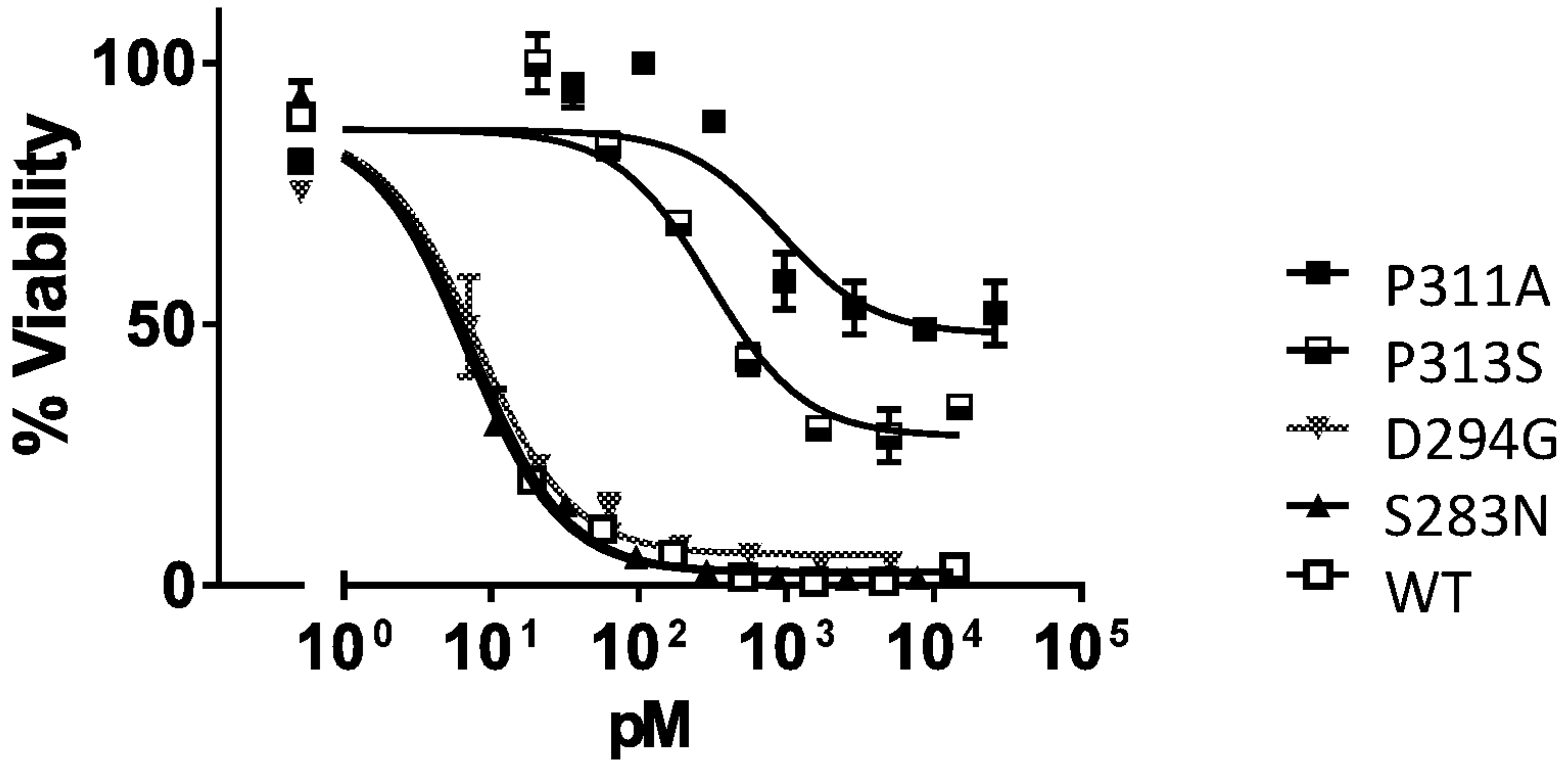
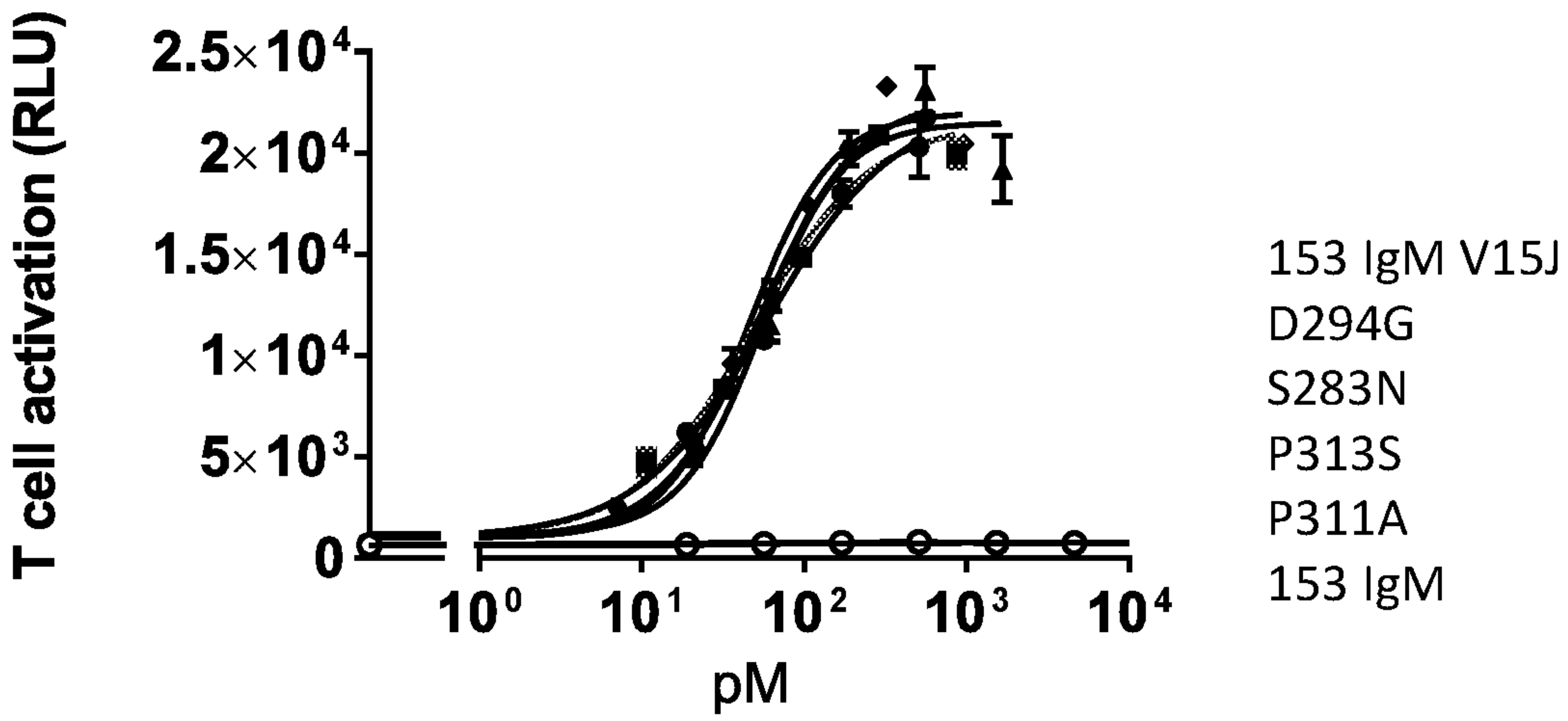

100
50
0
% Viability
10^0 10^1 10^2 10^3 10^4 10^5
pM
P311A
P313S
D294G
S283N
WT

FIGURE 4


T cell activation (RLU)
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2×10⁴
1.5×10⁴
1×10⁴
5×10³
0
10⁰
10¹
10²
10³
10⁴
pM
153 IgM V15J
D294G
S283N
P313S
P311A
153 IgM

FIGURE 5

1. 1.5.3 IgM V15J
2. 1.5.3 IgM V15J P311A
3. 1.5.3 IgM V15J P313S
4. 1.5.3 IgM V15J P311A P313S

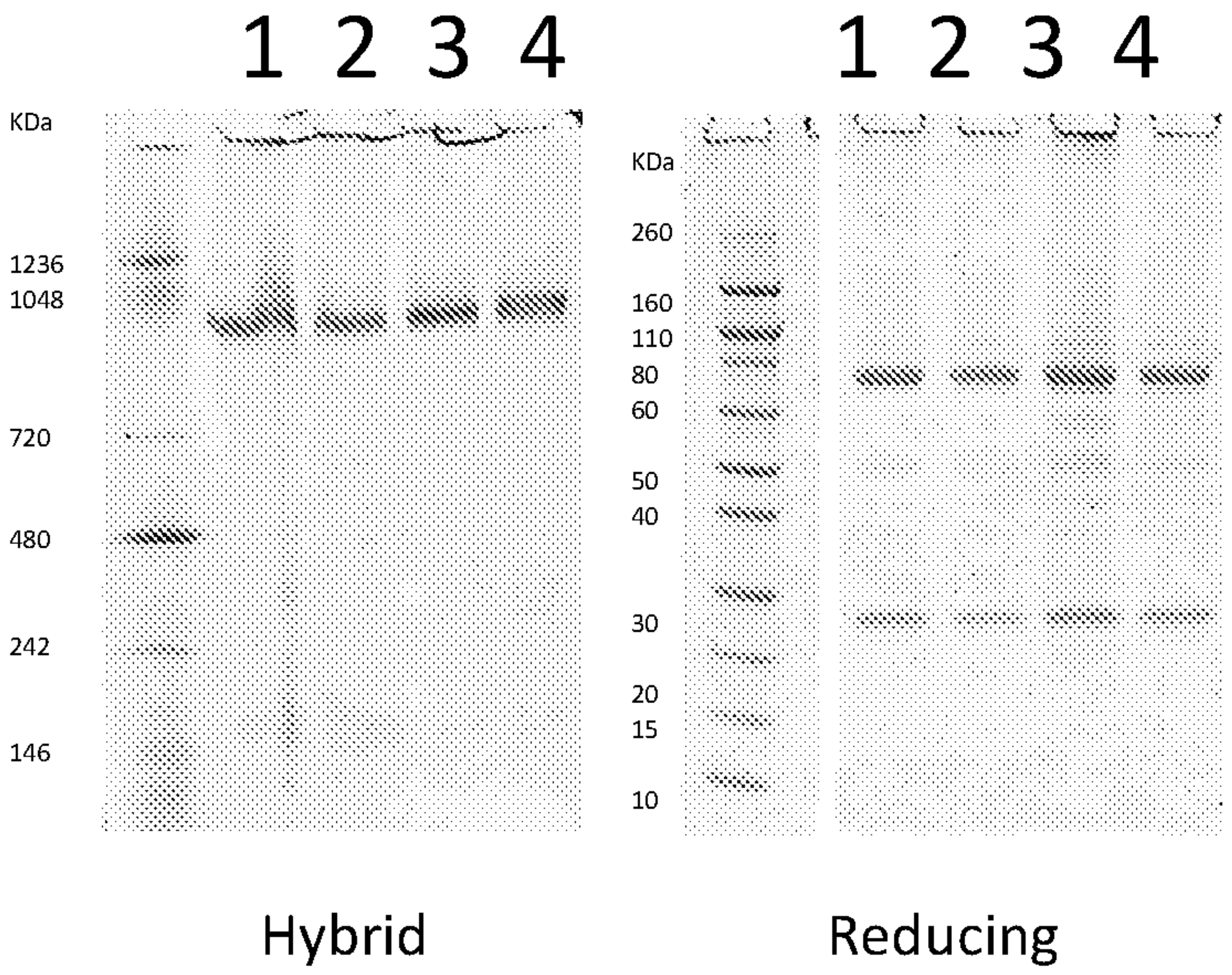


FIGURE 6

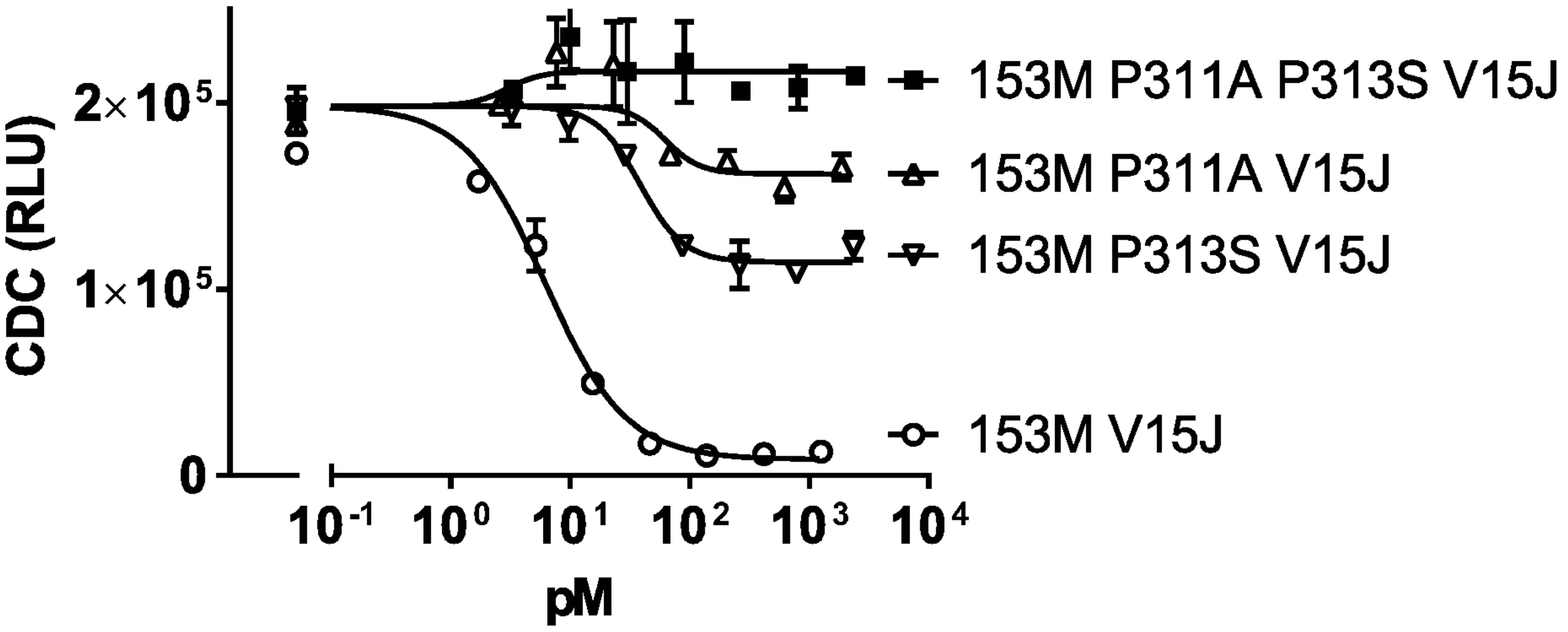
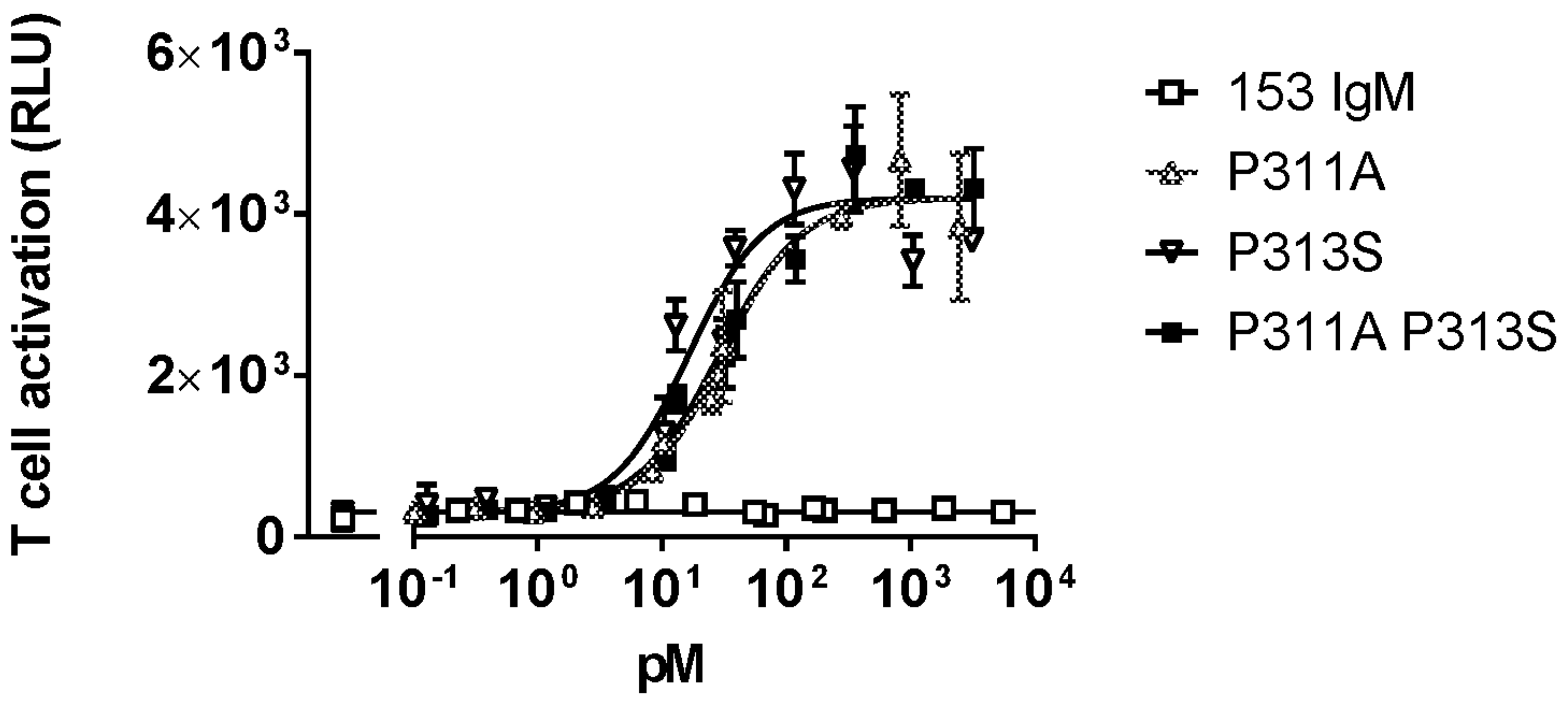

CDC (RLU)
2×10⁵
1×10⁵
0
10⁻¹
10⁰
10¹
10²
10³
10⁴
pM
153M P311A P313S V15J
153M P311A V15J
153M P313S V15J
153M V15J

FIGURE 7



	HillSlope	IC50
P311A	1.4	31 nM
P313S	1.5	16 nM
P311A P313S	1.3	29 nM

FIGURE 8

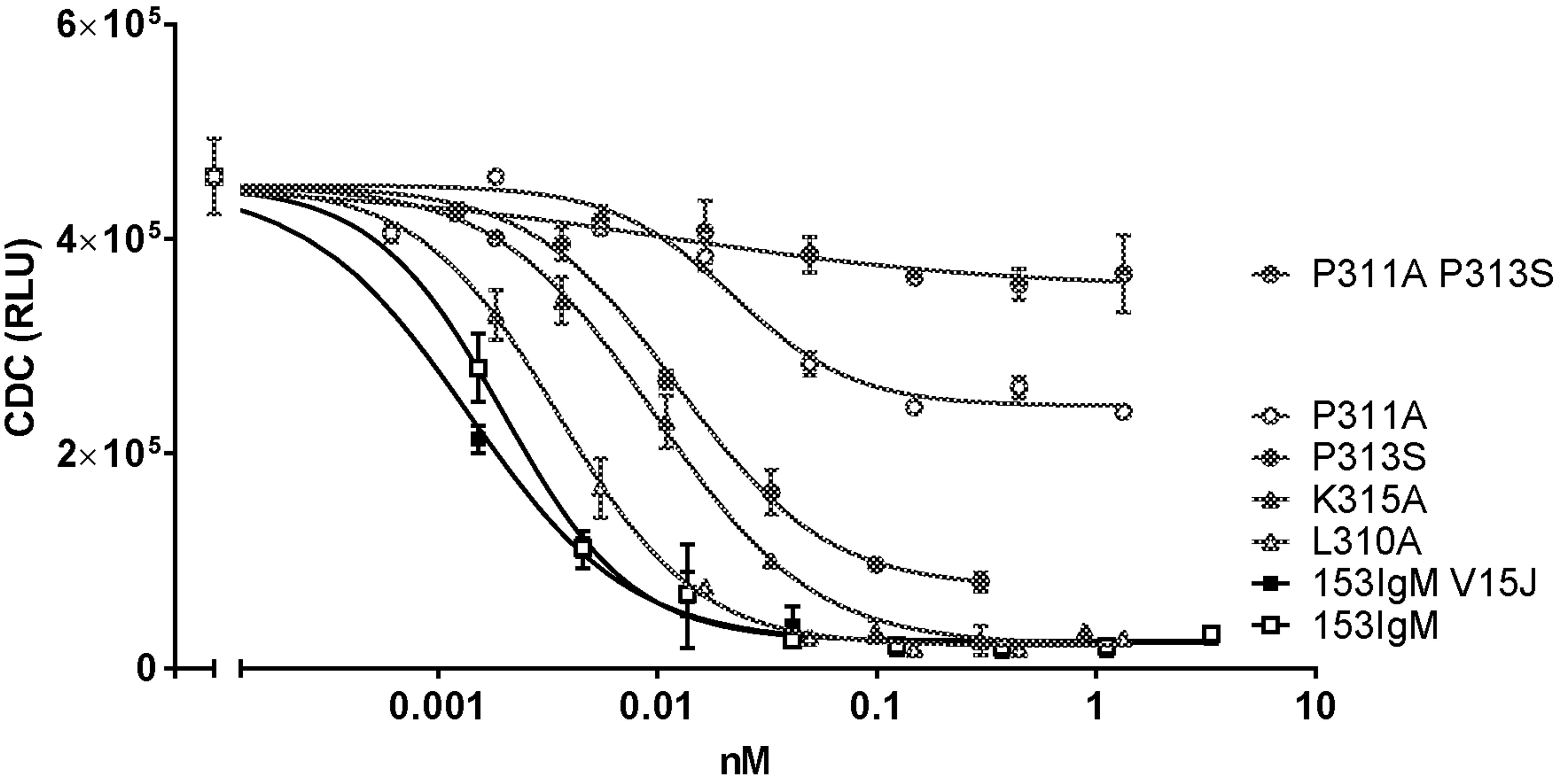

6×10⁵
4×10⁵
2×10⁵
0
CDC (RLU)
0.001
0.01
0.1
1
10
nM
P311A P313S
P311A
P313S
K315A
L310A
153IgM V15J
153IgM

FIGURE 9A and 9B