

Modified Fc Region

This application claims priority from SG 10201810463Y filed 22 November 2018, the contents and elements of which are herein incorporated by reference for all purposes.

5 Field of the Invention

The present invention relates to the fields of molecular biology, more specifically antibody technology. The present invention also relates to methods of medical treatment and prophylaxis.

Background to the Invention

- 10 Tripartite motif-containing 21 (TRIM21) is a highly-conserved, ubiquitously-expressed cytosolic protein which defends against pathogenic agents that enter the cytosol as immune complexes. TRIM21 has been shown to bind to the Fc region of immune complexes of intracellular antibodies specific for adenoviruses. This leads to TRIM21 ubiquitination, which in turn targets the immune complexes for proteasomal degradation (Mallery et al., 2010). TRIM21-mediated pathogen degradation in non-immune cells has
- 15 been shown for adenoviruses (Mallery et al., 2010), human rhinovirus (Watkinson et al., 2015), picornaviruses (Fan et al., 2016), Salmonella (Rakebrandt et al., 2014), and the Alzheimer's Disease pathogenic protein, Tau (McEwan et al., 2017), and is termed 'antibody-dependent intracellular neutralization' (ADIN) (McEwan et al., 2011). During proteasomal degradation, TRIM21 releases free ubiquitin chains that initiate pro-inflammatory signalling through NFκB, AP-1 and IRF signalling pathways
- 20 (McEwan et al., 2013; Fletcher et al., 2015). Hence TRIM21 sets the cell into an anti-pathogen state by mediating pathogen degradation and triggering pro-inflammatory pathways.

- Alongside ADIN, the generation of pathogen-derived peptides during proteasomal degradation can have important immune consequences. Mammalian cells routinely present proteasome-generated peptides on
- 25 major histocompatibility complex I (MHC class I) for screening by CD8 T cells; when these T cells recognize the peptides, they kill the infected cell and thereby control infections. For this to occur, CD8 T cells must first be stimulated by recognition of their cognate peptide presented by MHC class I on an activated dendritic cell (DC) (Joffre et al., 2012). Accordingly, many pathogens have developed immune evasion strategies for this pathway, leading to chronic infection. For example, in Human
- 30 Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) infections, expression of T cell-stimulatory molecules by DCs is suppressed by viral products (Granelli-piperno et al., 2004; Zimmermann et al., 2008), and production of the anti-inflammatory cytokine IL-10 is increased (Saito et al., 2008), leading to reduced activation of CD8 T cells (Liu et al., 2009). Therapeutic vaccination to boost antigen-specific CD8 T cell responses is therefore a promising treatment for these chronic diseases. Induction of effective
- 35 immunity following prophylactic vaccination also relies on efficient cross-presentation of exogenous vaccine antigen to stimulate optimal CD8 T cell responses. However, vaccines that can activate an effective CD8 T cell response remain challenging to design.

Summary of the Invention

In a first aspect, the present invention provides an Fc region, optionally isolated, comprising modification to increase the affinity of association between the Fc region and TRIM21.

- 5 In some embodiments, the modification comprises one or more substitutions to the amino acid sequence of a polypeptide of the Fc region. In some embodiments, the polypeptide comprises substitution at one or more positions corresponding to the following positions of IGHG1: 252, 253, 254, 256, 309, 310, 311, 314, 315, 345, 428, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439 or 440. In some embodiments, the polypeptide comprises substitution at one or more positions corresponding to the following positions of
- 10 IGHG1: 253, 256, 433, 434, 435, 436 or 440. In some embodiments, the Fc region according to any one of claims 1 to 4, wherein the Fc region comprises a polypeptide comprising: I or L at the position corresponding to position 253; P, A, T, V, G, I, K, N or S at the position corresponding to position 256; S, V, H, A, D, P, Q, T or I at the position corresponding to position 433; H, R, N, I, L or M at the position corresponding to position 434; H or L at the position corresponding to position 435; Y, F, T, L, W or S at
- 15 the position corresponding to position 436; and S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C at the position corresponding to position 440.

- Unless stated otherwise, positions in polypeptides of Fc regions herein are numbered according to the EU numbering system as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed.
- 20 Public Health Service, National Institutes of Health, Bethesda, MD, 1991 (hereby incorporated by reference in its entirety).

- In some embodiments, the Fc region comprises a polypeptide comprising an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:167 to 175. In some embodiments, the Fc region
- 25 comprises a polypeptide comprising an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:32 to 166. The present invention also provides a polypeptide, optionally isolated, comprising: an amino acid sequence having at least 60% sequence identity to SEQ ID NO:10, wherein the polypeptide comprises the following amino acid residues at the specified positions numbered relative to SEQ ID NO:10: I or L at the position corresponding to position 26; P, A, T, V, G, I, K, N or S at the
- 30 position corresponding to position 29; S, V, H, A, D, P, Q, T or I at the position corresponding to position 206; H, R, N, I, L or M at the position corresponding to position 207; H or L at the position corresponding to position 208; Y, F, T, L, W or S at the position corresponding to position 209; and S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C at the position corresponding to position 213; and wherein the amino acid sequence of the polypeptide is not identical to the amino acid sequence of a constituent polypeptide of
- 35 the Fc region of a wildtype immunoglobulin.

- In some embodiments the polypeptide comprises an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:167 to 175. In some embodiments the polypeptide comprises an amino acid sequence having at least 60% sequence identity to one of SEQ ID NOs:32 to 166

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The present invention also provides a polypeptide, optionally isolated, comprising the amino acid sequence of one of SEQ ID NOs:32 to 166.

5 The present invention also provides an Fc region, optionally isolated, comprising a polypeptide according to the present invention.

The present invention also provides a polypeptide complex, optionally isolated, comprising an Fc region according to the present invention, or a polypeptide according to the present invention.

10 The present invention also provides an antigen-binding molecule comprising an antigen-binding domain capable of specific binding to a target antigen and an Fc region according to the present invention, a polypeptide according to the present invention, or a polypeptide complex according to the present invention.

15 In some embodiments, the target antigen is an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen. In some embodiments, the antigen-binding molecule comprises an antigen-binding domain capable of specific binding to an endocytosis receptor. In some embodiments, the antigen-binding molecule is a multispecific antigen-binding molecule. In some embodiments, the antigen-binding molecule further comprises an antigenic sequence of a target antigen.

20 The present invention also provides an immunogen comprising an antigenic sequence of a target antigen and an Fc region according to the invention, a polypeptide according to the invention, or a polypeptide complex according to the invention.

25 In some embodiments, the target antigen is an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen. In some embodiments, the immunogen additionally comprises an antigen-presenting cell (APC)-targeting region. In some embodiments, the APC-targeting region comprises or consists of a moiety capable of specific binding to an endocytosis receptor. In some embodiments the immunogen further comprises an antigen-binding domain capable of specific binding to a target antigen.

30 The present invention also provides a nucleic acid, or a plurality of nucleic acids, optionally isolated, encoding an Fc region, a polypeptide, a polypeptide complex, an antigen-binding molecule or an immunogen according to the present invention.

35 The present invention also provides an expression vector, or a plurality of expression vectors, comprising a nucleic acid or a plurality of nucleic acids according to the present invention.

40 The present invention also provides a cell comprising an Fc region, a polypeptide, a polypeptide complex, an antigen-binding molecule, an immunogen, a nucleic acid or a plurality of nucleic acids or an expression vector or a plurality of expression vectors according to the present invention.

The present invention also provides a method comprising culturing a cell comprising a nucleic acid or a plurality of nucleic acids or an expression vector or a plurality of expression vectors according to the invention under conditions suitable for expression of the Fc region, polypeptide, antigen-binding molecule or immunogen from the nucleic acid(s) or expression vector(s).

The present invention also provides a composition comprising an Fc region, a polypeptide, a polypeptide complex, an antigen-binding molecule, an immunogen, a nucleic acid or a plurality of nucleic acids, an expression vector or a plurality of expression vectors, or a cell according to the present invention.

The present invention also provides a polypeptide complex, optionally isolated, comprising an antigen-binding molecule or the immunogen according to the invention bound to the target antigen or a fragment thereof.

The present invention also provides a pharmaceutical composition comprising an antigen-binding molecule, an immunogen or a polypeptide complex according to the present invention, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

The present invention also provides an antigen-binding molecule, an immunogen, a polypeptide complex, or a pharmaceutical composition according to the present invention, for use in a method of medical treatment or prophylaxis.

The present invention also provides an antigen-binding molecule, an immunogen, a polypeptide complex, or a pharmaceutical composition according to the present invention for use in a method of medical treatment or prevention of an infectious disease, a cancer or an autoimmune disease.

The present invention also provides the use of an antigen-binding molecule, an immunogen, a polypeptide complex, or a pharmaceutical composition according to the present invention in the manufacture of a medicament for use in a method of treatment or prevention of an infectious disease, a cancer or an autoimmune disease.

The present invention also provides a method of treating or preventing an infectious disease, a cancer or an autoimmune disease, comprising administering to a subject a therapeutically or prophylactically effective amount of an antigen-binding molecule, an immunogen, a polypeptide complex, or a pharmaceutical composition according to the present invention.

The present invention also provides an immunogen or a polypeptide complex according to the present invention, for use in a method for inducing or enhancing an immune response to a target antigen in a subject.

The present invention also provides the use of an immunogen or a polypeptide complex according to the present invention in the manufacture of a medicament for use in a method for inducing or enhancing an immune response to a target antigen in a subject.

- 5 The present invention also provides a method for inducing or enhancing an immune response to a target antigen in a subject, comprising administering an immunogen or a polypeptide complex according to the present invention to the subject.

10 The present invention also provides a method comprising contacting a population of antigen-presenting cells with an immunogen or a polypeptide complex according to the present invention.

The present invention also provides a method for generating or expanding a population of immune cells specific for a target antigen, comprising contacting a population of immune cells with an antigen-presenting cell obtained by the method according to the invention.

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Description

The present invention provides an Fc regions engineered for improved affinity to TRIM21, constituent polypeptides thereof, and larger molecules and complexes comprising the engineered Fc regions.

- 20 Improved affinity for TRIM21 is demonstrated to provide advantageous properties relevant to therapeutic and prophylactic applications of molecules and complexes comprising the modified Fc regions. In particular, antigen-presenting cells stimulated with molecules/complexes comprising Fc regions modified for increased affinity to TRIM21 are shown to be capable of stimulating an improved cell-mediated immune response to target antigen.

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TRIM21

- Human tripartite motif containing-21 (TRIM21; also known as RNF81, RO52, SSA) is the protein identified by UniProt P19474. Alternative splicing of mRNA encoded by the human *TRIM21* gene yields two isoforms: isoform 1 (UniProt: P19474-1, v1; SEQ ID NO:1) and isoform 2 (UniProt: P19474-2; SEQ ID NO:2), which lacks the amino acid sequence corresponding to positions 169 to 245 of SEQ ID NO:1.

- The structure and function of TRIM21 is reviewed e.g. in Foss et al., Immunol Rev. (2015) 268(1):328-39, which is hereby incorporated by reference in its entirety. TRIM21 is a cytosolic Fc receptor that is structurally unrelated to all other classes of Fc receptors (James et al., Proc Natl Acad Sci U S A. (2007) 104(15):6200-5). It is part of the TRIM family of proteins, which have cellular roles antiviral defence. Like other TRIM proteins, TRIM21 comprises an N-terminal RING domain (shown in SEQ ID NO:3) which has E3 ubiquitin ligase activity, a B-box (shown in SEQ ID NO:4), and a central coiled-coil domain (shown in SEQ ID NO:5). The C-terminal domain determines ligand specificity and function, and is referred to as the PRYSPRY domain (shown in SEQ ID NO:6). The PRYSPRY domain of TRIM21 contains the antibody binding site, and is a globular fold comprising a β -sandwich of two antiparallel β -sheets connected by flexible loops (James et al., Proc Natl Acad Sci U S A. (2007) 104(15):6200-5). TRIM21 exists as a dimer

in solution and forms stable 1:1 complexes with human IgG1; the two PRYSPRY domains of a dimeric TRIM21 molecule may bind simultaneously to one IgG Fc (Mallery et al., Proc Natl Acad Sci U S A. (2010) 107(46):19985-90).

- 5 Antibody-mediated protection against intracellular pathogens such as viruses occurs through binding of neutralising antibody to epitopes of surface-exposed viral proteins. In recent years, it has become clear that the antiviral function of antibodies extends into the cytosolic compartment of cells, and is orchestrated by TRIM21. Engagement of TRIM21 results in rapid postentry elimination of antibody:virus complexes via recruitment of the proteasomal machinery (Mallery et al., Proc Natl Acad Sci U S A. (2010) 107(46):19985-90), in a mechanism termed antibody-dependent cellular neutralization (ADIN).
 10 Inflammatory signalling is also induced (McEwan et al., Nat Immunol. 2013 Apr; 14(4):327-36). In this way, antibodies that have failed to block entry of a virus particle into the cell and which are not intercepted by other antibody-mediated effector functions (such as antibody-dependent cellular phagocytosis or antibody-dependent cellular cytotoxicity) may still be protective in the cytosolic compartment.
- 15 TRIM21 shows remarkably broad antibody specificity as it can activate its functions upon binding to IgG, IgM or IgA (Mallery et al., Proc Natl Acad Sci U S A. (2010) 107(46):19985-90 and Bidgood et al., Proc Natl Acad Sci U S A. 2014 Sep 16; 111(37):13463-8). TRIM21 is also expressed by cells of most lineages (Reymond et al., EMBO J. (2001) 20(9):2140-51), suggesting that a susceptible pathogen may be
 20 targeted by TRIM21 independently of the site of infection and local distribution of antibody isotypes.

In this specification 'TRIM21' refers to TRIM21 from any species and includes TRIM21 isoforms, fragments, variants or homologues from any species.

- 25 As used herein, a 'fragment', 'variant' or 'homologue' of a protein may optionally be characterised as having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of the reference protein (e.g. a reference isoform). In some embodiments fragments, variants, isoforms and homologues of a reference protein may be characterised by ability to perform a function performed by the reference
 30 protein.

- A 'fragment' generally refers to a fraction of the reference protein. A 'variant' generally refers to a protein having an amino acid sequence comprising one or more amino acid substitutions, insertions, deletions or other modifications relative to the amino acid sequence of the reference protein, but retaining a
 35 considerable degree of sequence identity (e.g. at least 60%) to the amino acid sequence of the reference protein. An 'isoform' generally refers to a variant of the reference protein expressed by the same species as the species of the reference protein (e.g. TRIM21 isoforms 1 and 2 are isoforms of one another). A 'homologue' generally refers to a variant of the reference protein produced by a different species as compared to the species of the reference protein. For example, human TRIM21 isoform 1 (UniProt: P19474-1, v1; SEQ ID NO:1) and Rhesus macaque TRIM21 (UniProt: F7C1A0-1) are homologues of one
 40 another. Homologues include orthologues.

A 'fragment' of a reference protein may be of any length (by number of amino acids), although may optionally be at least 25% of the length of the reference protein (that is, the protein from which the fragment is derived) and may have a maximum length of one of 50%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the length of the reference protein.

A fragment of TRIM21 may have a minimum length of one of 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, or 450 amino acids, and may have a maximum length of one of 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, or 450 amino acids.

In some embodiments, the TRIM21 is TRIM21 from a mammal (e.g. a primate (rhesus, cynomolgous, non-human primate or human) and/or a rodent (e.g. rat or murine) TRIM21). Isoforms, fragments, variants or homologues of TRIM21 may optionally be characterised as having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of a TRIM21 isoform from a given species, e.g. human.

Isoforms, fragments, variants or homologues may optionally be functional isoforms, fragments, variants or homologues, e.g. having a functional property/activity of the reference TRIM21 (e.g. human TRIM21 isoform 1), as determined by analysis by a suitable assay for the functional property/activity. For example, an isoform, fragment, variant or homologue of TRIM21 may display association with one or more of IgG, IgM or IgA, and/or may potentiate proteasomal degradation of antibody complexes.

In some embodiments, the TRIM21 comprises, or consists of, an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:1 or 2.

Fc region

Fc regions comprise two polypeptide regions comprising immunoglobulin heavy chain constant regions. The two polypeptide regions associate to form a dimer.

In some embodiments of the Fc region of the present invention, the two polypeptides regions are provided on separate polypeptide chains, which associate to form the Fc region. In some embodiments the two polypeptide regions are provided within the same polypeptide chain. In such embodiments the two polypeptide regions may be connected by a linker sequence, e.g. as described herein below. It will be appreciated that in such embodiments the linker sequence is of sufficient length and flexibility to allow association of the two polypeptide regions to form the Fc region.

Fc regions of IgG, IgA and IgD molecules comprise dimers of polypeptides comprising constant heavy chain domains 2 and 3 (i.e. CH2 and CH3). Fc regions of IgM and IgE molecules comprise dimers of polypeptides comprising CH2, CH3 and CH4 domains.

An Fc region according to the present disclosure may be derived from an Fc region of any wildtype immunoglobulin isotype, e.g. IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE or IgM. In some embodiments, an Fc region may be derived from an Fc region of a human IgG1 allotype (e.g. G1m1, G1m2, G1m3 or G1m17).

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As used herein, an Fc region which is 'derived from' a reference Fc region comprises a polypeptide having an amino acid sequence having at least 60%, sequence identity to the amino acid sequence of a polypeptide of the reference Fc region. For example, an Fc region which is derived from the Fc region of IgG1 comprises a polypeptide having at least 60% sequence identity to SEQ ID NO:10. It will be appreciated that because Fc regions are dimers, an Fc region which is 'derived from' a reference Fc region comprises two polypeptides each having at least 60% sequence identity to the amino acid sequence of a polypeptide of the reference Fc region.

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The Fc region of human immunoglobulin G 1 (IGHG1; UniProt: P01857-1, v1; SEQ ID NO:7) comprises polypeptides comprising the CH2-CH3 region sequence shown in SEQ ID NO:10. The Fc region of human immunoglobulin G 2 constant (IGHG2; UniProt: P01859-1, v2; SEQ ID NO:11) comprises polypeptides comprising the CH2-CH3 region sequence shown in SEQ ID NO:14. The Fc region of human immunoglobulin G 3 constant (IGHG3; UniProt: P01860-1, v2; SEQ ID NO:15) comprises polypeptides comprising the CH2-CH3 region sequence shown in SEQ ID NO:18. The Fc region of human immunoglobulin G 4 constant (IGHG4; UniProt: P01861-1, v1; SEQ ID NO:19) comprises polypeptides comprising the CH2-CH3 region sequence shown in SEQ ID NO:22.

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The Fc region of human immunoglobulin A 1 constant (IGHA1; UniProt: P01876-1, v2; SEQ ID NO:23) comprises polypeptides comprising the CH2-CH3 region sequence shown in SEQ ID NO:180. The Fc region of human immunoglobulin A 2 constant (IGHA1; UniProt: P01877-1, v4; SEQ ID NO:24) comprises polypeptides comprising the CH2-CH3 region sequence shown in SEQ ID NO:181.

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The Fc region of human immunoglobulin D constant (IGHD; UniProt: P01880-1, v3; SEQ ID NO:25) comprises polypeptides comprising the CH2-CH3 region sequence shown in SEQ ID NO:182.

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The Fc region of human immunoglobulin E constant (IGHE; UniProt: P01854-1, v1; SEQ ID NO:26) comprises polypeptides comprising the CH2-CH3-CH4 region sequence shown in SEQ ID NO:183.

The Fc region of human immunoglobulin M constant (IGHM; UniProt: P01871-1, v4; SEQ ID NO:27) comprises polypeptides comprising the CH2-CH3-CH4 region sequence shown in SEQ ID NO:31.

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In some embodiments the Fc region of the present invention comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:10. In some embodiments the Fc region comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%,

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92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:14. In some embodiments the Fc region comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID

5 NO:18. In some embodiments the Fc region comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID

10 NO:22. In some embodiments the Fc region comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID

15 NO:180. In some embodiments the Fc region comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID

20 NO:181. In some embodiments the Fc region comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID

NO:182. In some embodiments the Fc region comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID

NO:183. In some embodiments the Fc region comprises one or more polypeptides comprising an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID

NO:31.

25 Modified Fc regions

Aspects of the present invention relate to Fc regions comprising modification to increase the affinity of association with TRIM21.

30 Herein, a 'modified' Fc region refers to an Fc region comprising one or more polypeptides comprising a modification relative to the polypeptides of a reference Fc region not comprising the modification(s). A reference Fc region may be a wildtype Fc region, e.g. the Fc region of an IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE or IgM. Modification may e.g. be to one or both of the polypeptides of the reference Fc region. Modification may be to one or more amino acids of the polypeptides of the reference Fc region. Modification may introduce a moiety/amino acid side chain/structural motif which

35 increases/stabilises interaction with TRIM21. Modification may remove a moiety/amino acid side chain/structural motif which decreases/destabilises interaction with TRIM21.

Modifications which increase the affinity of association between an Fc region and TRIM21 can be identified using a suitable assay, such as Surface Plasmon Resonance (SPR; see e.g. Hearty et al.,

40 Methods Mol Biol (2012) 907:411-442), Bio-Layer Interferometry (see e.g. Lad et al., (2015) J Biomol Screen 20(4): 498-507), flow cytometry, or Enzyme-linked immunosorbent assay. The affinity of

association between two molecules can be analysed and quantified using such methods. Such assays may be performed e.g. using full-length recombinant TRIM21, or recombinant PRYSPRY domain of TRIM21.

- 5 In some embodiments the modification increases the affinity of association between the modified Fc region and TRIM21 to more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times, ≥ 200 times, ≥ 300 times, ≥ 400 times, ≥ 500 times, ≥ 600 times, ≥ 700 times, ≥ 800 times, ≥ 900 times or ≥ 1000 times the affinity of association between TRIM21 and the equivalent Fc region lacking the modification (i.e. the reference, unmodified Fc region).

- 15 In some embodiments the modification comprises substitution of one or more amino acids of one or more of the polypeptides of the reference Fc region. In such embodiments the one or more amino acid substitutions result in increased affinity of association between the modified Fc region and TRIM21. By way of illustration, in the experimental examples of the present disclosure the inventors demonstrate that an Fc region comprising substitutions at positions 433, 434, 436, 440 and 256 numbered according to the EU numbering system (described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991) has increased affinity for TRIM21.

- 25 In some embodiments the modified Fc region comprises modification to one or more positions of the Fc region polypeptides which are known or predicted to be important for interaction of the Fc region with TRIM21. Such positions may participate directly in the interaction with TRIM21, or may be involved in forming the three dimensional structure necessary for interaction with TRIM21. Such positions can be identified with reference e.g. to the crystal structure of the human IgG Fc-TRIM21 complex, described in James et al., Proc Natl Acad Sci U S A. (2007) 104(15):6200-5, which is hereby incorporated by reference in its entirety. Positions may be predicted/known to contact TRIM21, or may be predicted/known to be present in a surface which is in close proximity to TRIM21.

- 35 The three-dimensional structure of an Fc region and/or an Fc region in complex with TRIM21 can be determined by analysis using methods known to the skilled person, described for example in Berg et al., Biochemistry, 5th Edn., Chapter 4, which is hereby incorporated by reference in its entirety. Such methods include X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, etc. The structure of an Fc region and/or an Fc region in complex with TRIM21 can also be modelled or predicted using methods known to the skilled person, reviewed in Zhang, Curr Opin Struct Biol, 2008, 18(3) 342-348, which is hereby incorporated by reference in its entirety. Such methods include homology modelling, protein threading and fold recognition analysis. Homology modelling uses known three-dimensional structures of amino acid sequences having homology to an amino acid sequence of interest as the basis for predicting the three-dimensional structure of the amino acid sequence. Homology modelling can be

performed, for example, using the methods described in Marti-Renom et al., *Annu Rev Biophys Biomol Struct*, 2000, 29: 291-325, which is hereby incorporated by reference in its entirety.

5 In some embodiments the modified Fc region comprises modification to one or more positions which are predicted in a resolved/predicted three-dimensional structure of the Fc region interacting with TRIM21 (e.g. the structure described in James et al., *supra*) to be: within 50Å (e.g. within 40Å, 30Å, 20Å, 10Å or 5Å) of TRIM21; present in a surface facing TRIM21; and/or orientated with their side chains facing towards TRIM21.

10 Positions which are important for interaction of an Fc region with TRIM21 can also be functional analysis. For example, candidate positions of Fc region polypeptides can be substituted, and the effect of the substitution on interaction between the Fc region and TRIM21 can be analysed, e.g. using a suitable assay, e.g. SPR or Bio-Layer Interferometry. A position which is important for interaction of an Fc region with TRIM21 is identified by determination of a change (i.e. a decrease or increase) in the level of
15 interaction between the Fc region and TRIM21 associated with substitution at the relevant position.

In some embodiments the modified Fc region comprises one or more polypeptides comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more positions. Substitution may be with any naturally- or non-naturally-occurring amino acid. Naturally-occurring amino acids include
20 alanine (Ala/A), arginine (Arg/R), asparagine (Asn/N), aspartic acid (Asp/D), cysteine (Cys/C), glycine (Gly/G), glutamine (Gln/Q), glutamic acid (Glu/E), histidine (His/H), isoleucine (Ile/I), leucine (Leu/L), lysine (Lys/K), methionine (Met/M), phenylalanine (Phe/F), proline (Pro/P), serine (Ser/S), threonine (Thr/T), tryptophan (Trp/W), tyrosine (Tyr/Y) and valine (Val/V). Non-natural amino acids are described e.g. in Saladino et al., *Mini-Reviews in Medicinal Chemistry* (2012), 12(4) 227-300 (hereby incorporated
25 by reference in its entirety), and include β -3 and β 2 amino acids, homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids and *N*-methyl amino acids.

In some embodiments the modified Fc region comprises one or more polypeptides comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 252, 253, 254, 256,
30 309, 310, 311, 314, 315, 345, 428, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439 or 440 (see e.g. Example 2.1). In some embodiments the modified Fc region comprises one or more polypeptides comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more
35 positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 252, 253, 254, 256, 309, 310, 311, 314, 315, 345, 428, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439 or 440.

It will be appreciated that positions 252, 253, 254, 256, 309, 310, 311, 314, 315, 345, 428, 430, 431, 432,
40 433, 434, 435, 436, 437, 438, 439 or 440 numbered according to the EU numbering system correspond

respectively to positions 135, 136, 137, 139, 192, 193, 194, 197, 198, 228, 311, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322 and 323 of SEQ ID NO:7.

In some embodiments the modified Fc region comprises one or more polypeptides comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 253, 256, 433, 434, 435, 436, or 440.

The skilled person able to identify positions of a given amino acid sequence 'corresponding to' positions of a reference amino acid sequence e.g. by alignment of the amino acid sequence to the reference sequence, for example using publicly available computer software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960).

By way of example, an alignment of the amino acid sequences encoding IGHG1 (SEQ ID NO:7), IGHG2 (SEQ ID NO:11), IGHG3 (SEQ ID NO:15), IGHG4 (SEQ ID NO:19), IGHA1 (SEQ ID NO:23), IGHA2 (SEQ ID NO:24), IGHD (SEQ ID NO:25), IGHE (SEQ ID NO:26) and IGHM (SEQ ID NO:27) is shown in Figure 9. It is clear from the alignment of Figure 9 that e.g. position 312 of SEQ ID NO:11 (IGHG2) corresponds to position 316 of SEQ ID NO:7 (IGHG1) (which is position 433 according to EU numbering). The skilled person is also able to identify positions of a given amino acid sequence 'corresponding to' positions of a reference amino acid sequence e.g. by comparison of known/predicted three-dimensional structures for the amino acid sequences.

Where a heavy chain Fc region/polypeptide is described herein as comprising specified amino acid(s) at position(s) "corresponding to" reference position(s), or is described as comprising specified substitutions "corresponding to" reference substitution(s), equivalent position(s)/substitution(s) in homologous Fc regions/polypeptides are contemplated.

By way of illustration (with reference to Figure 9), the substitution in IGHG3 which corresponds to Y436T in IGHG1 (EU numbering) is the substitution Phe>Thr at position 366 numbered according to SEQ ID NO:15.

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Positions in IGHG2, IGHG3, IGHG4, IGHA1, IGHA2, IGHD, IGHE and IGHM corresponding to positions of particular interest in IGHG1, as determined from the alignment shown in Figure 9, are shown below:

IGHG1 (Eu numbering)	IGHG1 (SEQ ID NO:7)	IGHG2 (SEQ ID NO:11)	IGHG3 (SEQ ID NO:15)	IGHG4 (SEQ ID NO:19)	IGHA1 (SEQ ID NO:23)	IGHA2 (SEQ ID NO:24)	IGHD (SEQ ID NO:25)	IGHE (SEQ ID NO:26)	IGHM (SEQ ID NO:27)
M252	M135	M131	M182	M132	L138	L125	W181	F230	F235
I253	I136	I132	I183	I133	L139	L126	L182	I231	L236
S254	S137	S133	S184	S134	G140	G127	R183	R232	T237
T256	T139	T135	T186	T136	E142	E129	K185	S234	S239
L309	L192	V188	L239	L189	C192	C179	P237	G287	C291
H310	H193	H189	H240	H190	A193	A180	R238	T288	E292

Q311	Q194	Q190	Q241	Q191	E194	Q181	S239	R289	D293
L314	L197	L193	L244	L194	N197	N184	N242	I292	N296
N315	N198	N194	N245	N195	H198	H185	A243	E293	S297
E345	E228	E224	E275	E225	R227	R214	K274	A323	R328
M428	M311	M307	M358	M308	G316	G303	S358	V408	A416
E430	E313	E309	E360	E310	E318	E305	E360	E410	E418
A431	A314	A310	A361	A311	A319	A306	D361	A411	A419
L432	L315	L311	L362	L312	L320	L307	S362	A412	L420
H433	H316	H312	H363	H313	P321	P308	R363	S413	P421
N434	N317	N313	N364	N314	L322	L309	T364	P414	N422
H435	H318	H314	R365	H315	A323	A310	L366	Q416	R423
Y436	Y319	Y315	F366	Y316	F324	F311	N367	T417	V424
T437	T320	T316	T367	T317	T325	T312	A368	V418	T425
Q438	Q321	Q317	Q368	Q318	Q326	Q313	S369	Q419	E426
K439	K322	K318	K369	K319	K327	K314	R370	R420	R427
S440	S323	S319	S370	S319	T328	T315	S371	A421	T428

In some embodiments the modified Fc region comprises one or more polypeptides comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 256, 433, 434, 435, 436, 438, 439 or 440. In some embodiments the modified Fc region comprises one or more polypeptides comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 433, 434, 435, 436, 438, 439 or 440. In some embodiments the modified Fc region comprises one or more polypeptides comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 433, 434, 436, or 440. In some embodiments the modified Fc region comprises a polypeptide comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 256, 433, 434, 436, or 440. In some embodiments the modified Fc region comprises a polypeptide comprising amino acid substitution(s) (relative to the reference, unmodified Fc region) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 256, 433 or 434.

In some embodiments the amino acid at the position corresponding to position 253 is I or L. In some embodiments the amino acid at the position corresponding to position 253 is I.

In some embodiments the amino acid at the position corresponding to position 256 is P, A, T, V, G, I, K, N or S. In some embodiments the amino acid at the position corresponding to position 256 is P, A or S. In some embodiments the amino acid at the position corresponding to position 256 is P, A or T. In some embodiments the amino acid at the position corresponding to position 256 is P or T. In some

embodiments the amino acid at the position corresponding to position 256 is P. In some embodiments the amino acid at the position corresponding to position 256 is A. In some embodiments the amino acid at the position corresponding to position 256 is T, V, G, I, K, N or S.

- 5 In some embodiments the amino acid at the position corresponding to position 433 is S, V, H, A, D, P, Q, T or I. In some embodiments the amino acid at the position corresponding to position 433 is S. In some embodiments the amino acid at the position corresponding to position 433 is V. In some embodiments the amino acid at the position corresponding to position 433 is H, A, D, P, Q or T. In some embodiments the amino acid at the position corresponding to position 433 is T or H. In some embodiments the amino acid at the position corresponding to position 433 is V or I. In some embodiments the amino acid at the position corresponding to position 433 is H.

- In some embodiments the amino acid at the position corresponding to position 434 is H, R, N, I, L or M. In some embodiments the amino acid at the position corresponding to position 434 is H. In some
15 embodiments the amino acid at the position corresponding to position 434 is R or N. In some embodiments the amino acid at the position corresponding to position 434 is R. In some embodiments the amino acid at the position corresponding to position 434 is N, I, L or M. In some embodiments the amino acid at the position corresponding to position 434 is N or M. In some embodiments the amino acid at the position corresponding to position 434 is N, L or M.

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In some embodiments the amino acid at the position corresponding to position 435 is H or L. In some embodiments the amino acid at the position corresponding to position 435 is H.

- In some embodiments the amino acid at the position corresponding to position 436 is Y, F, T, L, W or S.
25 In some embodiments the amino acid at the position corresponding to position 436 is Y or F. In some embodiments the amino acid at the position corresponding to position 436 is Y, T or F. In some embodiments the amino acid at the position corresponding to position 436 is Y, F, L or W. In some embodiments the amino acid at the position corresponding to position 436 is Y, F, L or T. In some embodiments the amino acid at the position corresponding to position 436 is F, T, Y, S or L. In some
30 embodiments the amino acid at the position corresponding to position 436 is F, T or Y. In some embodiments the amino acid at the position corresponding to position 436 is Y, F, T or S.

- In some embodiments the amino acid at the position corresponding to position 440 is S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C. In some embodiments the amino acid at the position corresponding to
35 position 440 is G, S, I, R, Y or N. In some embodiments the amino acid at the position corresponding to position 440 is S, R, V or G. In some embodiments the amino acid at the position corresponding to position 440 is S, R, I, N or T. In some embodiments the amino acid at the position corresponding to position 440 is S, I or G. In some embodiments the amino acid at the position corresponding to position 440 is S, G, R, P, N, I or V. In some embodiments the amino acid at the position corresponding to
40 position 440 is R, T, S, D, W, M, A, K, N, Y or I. In some embodiments the amino acid at the position

corresponding to position 440 is M, S, F, I, G, D or N. In some embodiments the amino acid at the position corresponding to position 440 is A, I, T, R, S, E, C, Y, D, N, G, V or M.

5 In some embodiments the Fc region of the invention comprises one or more polypeptides comprising, or consisting of, the amino acid sequence of one of SEQ ID NO:167 to 175, or an amino acid sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of one of SEQ ID NOs:167 to 175.

10 In some embodiments the Fc region of the invention comprises one or more polypeptides comprising, or consisting of, the amino acid sequence of one of SEQ ID NOs:32 to 166, or an amino acid sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of one of SEQ ID NOs:32 to 166.

15 Fc regions provide for interaction with Fc receptors and other molecules of the immune system to bring about functional effects. IgG Fc-mediated effector functions are reviewed e.g. in Jefferis et al., Immunol Rev 1998 163:59-76 (hereby incorporated by reference in its entirety), and are brought about through Fc-mediated recruitment and activation of immune cells (e.g. macrophages, dendritic cells, NK cells and T
20 cells) through interaction between the Fc region and Fc receptors expressed by the immune cells, recruitment of complement pathway components through binding of the Fc region to complement protein C1q, and consequent activation of the complement cascade. Fc-mediated functions include Fc receptor binding, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), formation of the membrane attack complex (MAC),
25 cell degranulation, cytokine and/or chemokine production, and antigen processing and presentation.

Modifications to antibody Fc regions that influence Fc-mediated functions are known in the art, such as those described e.g. in Wang et al., Protein Cell (2018) 9(1):63-73, which is hereby incorporated by reference in its entirety. In particular, exemplary Fc region modifications known to influence antibody
30 effector function are summarised in Table 1 of Wang et al., Protein Cell (2018) 9(1):63-73. In some embodiments the polypeptide comprises any one of the following amino acid substitutions/combinations of amino acid substitutions (or corresponding substitutions): F243L/R292P/Y300L/V305I/P396L; S239D/I332E; S239D/I332E/A330L; S298A/E333A/K334A; L234Y/L235Q/G236W/S239M/H268D/D270E/S298A; D270E/K326D/A330M/K334E;
35 G236A/S239D/I332E; K326W/E333S; S267E/H268F/S324T; E345R/E430G/S440Y; M252Y/S254T/T256E; and M428L/N434S.

In some embodiments, the antigen-binding molecule of the present invention comprises an Fc region comprising modification in one or more of the CH2 and CH3 regions promoting association of the Fc
40 region. Recombinant co-expression of constituent polypeptides of an antigen-binding molecule and subsequent association leads to several possible combinations. To improve the yield of the desired

combinations of polypeptides in antigen-binding molecules in recombinant production, it is advantageous to introduce in the Fc regions modification(s) promoting association of the desired combination of heavy chain polypeptides. Modifications may promote e.g. hydrophobic and/or electrostatic interaction between CH2 and/or CH3 regions of different polypeptide chains. Suitable modifications are described e.g. in Ha et al., Front. Immunol (2016) 7:394, which is hereby incorporated by reference in its entirety.

In some embodiments the Fc region comprises one or more paired substitutions for stabilising association between the constituent polypeptides. In some embodiments the Fc region comprises polypeptides comprising paired substitutions at positions corresponding to those indicated in Table 1 of Ha et al., Front. Immunol (2016) 7:394 (hereby incorporated by reference in its entirety), for the following formats: KiH, KiH_{s-s}, HA-TF, ZW1, 7.8.60, DD-KK, EW-RVT, EW-RVT_{s-s}, SEED or A107.

It will be appreciated that the present invention relates to modified, i.e. non-wildtype Fc regions. Accordingly, in some embodiments the Fc region of the invention does not consist of polypeptides having amino acid sequences which are identical to those of wildtype IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE or IgM.

In some embodiments the Fc region lacks a polypeptide comprising an amino acid sequence which is identical to the amino acid sequence of the CH2-CH3 region of a wildtype IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2) or IgD. In some embodiments the Fc region lacks a polypeptide comprising an amino acid sequence which is identical to the amino acid sequence of the CH2-CH3-CH4 region of a wildtype IgE or IgM.

In some embodiments the Fc region of the present invention does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:10. In some embodiments the Fc region does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:14. In some embodiments the Fc region does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:18. In some embodiments the Fc region does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:22. In some embodiments the Fc region does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:180. In some embodiments the Fc region does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:181. In some embodiments the Fc region does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:182. In some embodiments the Fc region does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:183. In some embodiments the Fc region does not comprise a polypeptide comprising an the amino acid sequence of SEQ ID NO:31.

It will also be appreciated that an Fc region according to the present can be a component of a larger molecule, e.g. an antigen-binding molecule or an immunogen as described herein. The Fc region may be covalently or non-covalently associated with the other components of the larger molecule.

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Polypeptides

The present invention also provides constituent polypeptides of Fc regions according to the invention. The polypeptides may be provided in isolated or substantially purified form. The Fc region of the invention may be, or may comprise, a complex (e.g. a non-covalent complex) of the polypeptides according to the invention.

As used herein, a 'peptide' is a chain of two or more amino acid monomers linked by peptide bonds. A peptide typically has a length in the region of about 2 to 50 amino acids. A 'polypeptide' is a polymer chain of two or more peptides. Polypeptides typically have a length greater than about 50 amino acids.

The present invention provides a polypeptide comprising immunoglobulin heavy chain constant regions capable of forming an Fc region. In some embodiments the polypeptide comprises heavy chain constant regions derived from CH2 and CH3 of IgG (e.g. IgG1, IgG2, IgG3 or IgG4). In some embodiments the polypeptide comprises heavy chain constant regions derived from CH2 and CH3 of IgA (e.g. IgA1 or IgA2). In some embodiments the polypeptide comprises heavy chain constant regions derived from CH2 and CH3 of IgD. In some embodiments the polypeptide comprises heavy chain constant regions derived from CH2, CH3 and CH4 of IgM. In some embodiments the polypeptide comprises heavy chain constant regions derived from CH2, CH3 and CH4 of IgE. As used herein a heavy chain constant region which is 'derived from' a reference heavy chain constant region has an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of the reference heavy chain constant region.

In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:10. In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:14. In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:18. In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:22. In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:180. In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:181. In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence

of SEQ ID NO:182. In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:183. In some embodiments the polypeptide comprises, or consists of, an amino acid sequence having at least
5 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO:31.

In some embodiments the polypeptide comprises amino acid substitution(s) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system):
10 252, 253, 254, 309, 310, 311, 314, 315, 345, 428, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439 or 440 (see e.g. Example 2.1). In some embodiments the polypeptide comprises amino acid substitution(s) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 252, 253, 254, 256, 309, 310, 311, 314, 315, 345, 428, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439 or 440.

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In some embodiments the polypeptide comprises amino acid substitution(s) at one or more positions corresponding to the following positions of IGHG1 (numbered according to the EU numbering system): 253, 256, 433, 434, 435, 436 or 440.

20 In some embodiments the polypeptide comprises an amino acid sequence having at least 60% sequence identity to SEQ ID NO:10, wherein the polypeptide comprises amino acid substitution(s) at one or more positions corresponding to the following positions numbered relative to SEQ ID NO:10: 26, 29, 206, 207, 208, 209 and 213.

In some embodiments the amino acid at the position corresponding to position 26 of SEQ ID NO:10 is I or
25 L. In some embodiments the amino acid at the position corresponding to position 26 of SEQ ID NO:10 is I.

In some embodiments the amino acid at the position corresponding to position 29 of SEQ ID NO:10 is P, A, T, V, G, I, K, N or S. In some embodiments the amino acid at the position corresponding to position 29
30 of SEQ ID NO:10 is P, A or S. In some embodiments the amino acid at the position corresponding to position 29 of SEQ ID NO:10 is P, A or T. In some embodiments the amino acid at the position corresponding to position 29 of SEQ ID NO:10 is P or T. In some embodiments the amino acid at the position corresponding to position 29 of SEQ ID NO:10 is P. In some embodiments the amino acid at the position corresponding to position 29 of SEQ ID NO:10 is A. In some embodiments the amino acid at the
35 position corresponding to position 29 of SEQ ID NO:10 is T, V, G, I, K, N or S.

In some embodiments the amino acid at the position corresponding to position 206 of SEQ ID NO:10 is S, V, H, A, D, P, Q, T or I. In some embodiments the amino acid at the position corresponding to position
40 206 of SEQ ID NO:10 is S. In some embodiments the amino acid at the position corresponding to position 206 of SEQ ID NO:10 is V. In some embodiments the amino acid at the position corresponding to position 206 of SEQ ID NO:10 is H, A, D, P, Q or T. In some embodiments the amino acid at the position

corresponding to position 206 of SEQ ID NO:10 is T or H. In some embodiments the amino acid at the position corresponding to position 206 of SEQ ID NO:10 is V or I. In some embodiments the amino acid at the position corresponding to position 206 of SEQ ID NO:10 is H.

- 5 In some embodiments the amino acid at the position corresponding to position 207 of SEQ ID NO:10 is H, R, N, I, L or M. In some embodiments the amino acid at the position corresponding to position 207 of SEQ ID NO:10 is H. In some embodiments the amino acid at the position corresponding to position 207 of SEQ ID NO:10 is R or N. In some embodiments the amino acid at the position corresponding to position 207 of SEQ ID NO:10 is R. In some embodiments the amino acid at the position corresponding to position 207 of SEQ ID NO:10 is N, I, L or M. In some embodiments the amino acid at the position corresponding to position 207 of SEQ ID NO:10 is N or M. In some embodiments the amino acid at the position corresponding to position 207 of SEQ ID NO:10 is N, L or M.

- 15 In some embodiments the amino acid at the position corresponding to position 208 of SEQ ID NO:10 is H or L. In some embodiments the amino acid at the position corresponding to position 208 of SEQ ID NO:10 is H.

- 20 In some embodiments the amino acid at the position corresponding to position 209 of SEQ ID NO:10 is Y, F, T, L, W or S. In some embodiments the amino acid at the position corresponding to position 209 of SEQ ID NO:10 is Y or F. In some embodiments the amino acid at the position corresponding to position 209 of SEQ ID NO:10 is Y, T or F. In some embodiments the amino acid at the position corresponding to position 209 of SEQ ID NO:10 is Y, F, L or W. In some embodiments the amino acid at the position corresponding to position 209 of SEQ ID NO:10 is Y, F, L or T. In some embodiments the amino acid at the position corresponding to position 209 of SEQ ID NO:10 is F, T, Y, S or L. In some embodiments the amino acid at the position corresponding to position 209 of SEQ ID NO:10 is F, T or Y. In some embodiments the amino acid at the position corresponding to position 209 of SEQ ID NO:10 is Y, F, T or S.

- 30 In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C. In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is G, S, I, R, Y or N. In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is S, R, V or G. In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is S, R, I, N or T. In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is S, I or G.
- 35 In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is S, G, R, P, N, I or V. In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is R, T, S, D, W, M, A, K, N, Y or I. In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is M, S, F, I, G, D or N. In some embodiments the amino acid at the position corresponding to position 213 of SEQ ID NO:10 is A, I, T, R, S, E, C, Y, D, N, G, V or M.
- 40

In some embodiments a polypeptide comprises, or consists of, the amino acid sequence of one of SEQ ID NOs:167 to 175, or an amino acid sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of one of SEQ ID NOs:167 to 175.

In some embodiments a polypeptide comprises, or consists of, the amino acid sequence of one of SEQ ID NOs:32 to 166, or an amino acid sequence having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of one of SEQ ID NOs:32 to 166.

In some embodiments polypeptide comprises any one of the following amino acid substitutions/combinations of amino acid substitutions (shown e.g. in Table 1 of Ha et al., Front. Immunol (2016) 7:394, incorporated by reference hereinabove; numbered according to the EU numbering system): T366W; T366S, L368A and Y407V; T366W and S354C; T366S, L368A, Y407V and Y349C; S364H and F405A; Y349T and T394F; T350V, L351Y, F405A and Y407V; T350V, T366L, K392L and T394W; K360D, D399M and Y407A; E345R, Q347R, T366V and K409V; K409D and K392D; D399K and E356K; K360E and K409W; Q347R, D399V and F405T; K360E, K409W and Y349C; Q347R, D399V, F405T and S354C; K370E and K409W; and E357N, D399V and F405T.

Modifications to antibody Fc regions that influence Fc-mediated functions are known in the art, such as those described e.g. in Wang et al., Protein Cell (2018) 9(1):63-73, which is hereby incorporated by reference in its entirety. Exemplary Fc region modifications known to influence antibody effector function are summarised in Table 1 of Wang et al., Protein Cell (2018) 9(1):63-73. In some embodiments the polypeptide comprises any one of the following amino acid substitutions/combinations of amino acid substitutions (or corresponding substitutions): F243L/R292P/Y300L/V305I/P396L; S239D/I332E; S239D/I332E/A330L; S298A/E333A/K334A; L234Y/L235Q/G236W/S239M/H268D/D270E/S298A; D270E/K326D/A330M/K334E; G236A/S239D/I332E; K326W/E333S; S267E/H268F/S324T; E345R/E430G/S440Y; M252Y/S254T/T256E; and M428L/N434S.

In some embodiments the amino acid sequence of the polypeptide of the present invention is not identical to the amino acid sequence of a constituent polypeptide of the Fc region of a wildtype immunoglobulin (e.g. IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE or IgM).

In some embodiments the polypeptide does not comprise an amino acid sequence which is identical to the amino acid sequence of the CH2-CH3 region of a wildtype IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2) or IgD. In some embodiments the polypeptide does not comprise an amino acid sequence which is identical to the amino acid sequence of the CH2-CH3-CH4 region of a wildtype IgE or IgM.

In some embodiments the polypeptide of the invention does not comprise the amino acid sequence of SEQ ID NO:10. In some embodiments the polypeptide does not comprise the amino acid sequence of SEQ ID NO:14. In some embodiments the polypeptide does not comprise the amino acid sequence of SEQ ID NO:18. In some embodiments the polypeptide does not comprise the amino acid sequence of
5 SEQ ID NO:22. In some embodiments the polypeptide does not comprise the amino acid sequence of SEQ ID NO:180. In some embodiments the polypeptide does not comprise the amino acid sequence of SEQ ID NO:181. In some embodiments the polypeptide does not comprise the amino acid sequence of SEQ ID NO:182. In some embodiments the polypeptide does not comprise the amino acid sequence of SEQ ID NO:183. In some embodiments the polypeptide does not comprise the amino acid sequence of
10 SEQ ID NO:31.

In some embodiments the polypeptide additionally comprises amino acid sequence encoding one or more further functional regions. For example, in some embodiments the polypeptide additionally comprises an amino acid sequence forming an antigen-binding domain according to the present disclosure (e.g. scFv
15 specific for a target antigen, or a peptide aptamer specific for a target antigen). In some embodiments the polypeptide additionally comprises an amino acid sequence forming part of an antigen-binding domain according to the present disclosure (e.g. VL or VH of an antigen-binding moiety specific for a target antigen). In some embodiments the polypeptide additionally comprises an amino acid sequence forming all or part of an antigenic sequence according to the present disclosure.

20

Polypeptide complexes

The present invention also provides polypeptide complexes comprising the Fc regions and/or the polypeptides of the invention.

25 As used herein, a 'polypeptide complex' refers to a complex comprising a polypeptide associated with another molecule. The association may involve covalent interaction (e.g. disulfide bonding) and/or non-covalent interaction (e.g. electrostatic interaction (e.g. ionic bonding, hydrogen bonding), Van der Waals forces) between the polypeptide and another molecule.

30 In some embodiments, a polypeptide complex may comprise, or consist of, a complex of more than one polypeptide (e.g. 2, 3, 4, 6, or 8 polypeptides). For example, polypeptide complexes include IgG-like antigen-binding molecules, which comprise heavy and light chain polypeptides associated into a polypeptide complex.

Polypeptide complexes include e.g. antigen-binding molecules as described herein, immunogens
35 described herein and complexes of antigen-binding molecules/immunogens with cognate target antigen.

Antigen-binding molecules

The present invention also provides antigen-binding molecules comprising the Fc regions and/or the polypeptides of the invention.

40

An 'antigen-binding molecule' refers to a molecule which is capable of binding to a target antigen, and encompasses monoclonal antibodies, polyclonal antibodies, monospecific and multispecific antibodies (e.g., bispecific antibodies, trispecific antibodies, etc.).

5 The antigen-binding molecule of the present invention comprises an antigen-binding domain. The antigen-binding domain may comprise, or consist of, a moiety capable of specific binding to a target antigen. In some embodiments, the moiety capable of binding to a target antigen comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL) of an antibody capable of specific binding to the target antigen. An antigen-binding domain formed by a VH and a VL may also be
10 referred to as an Fv region. In some embodiments the antigen-binding domain comprises, or consists of, an antibody fragment of an antibody capable of specific binding to the target antigen (e.g. Fv, scFv, Fab, scFab, F(ab')₂, Fab₂, diabody, triabody, scFv-Fc, minibody, single domain antibody (e.g. VhH), etc.). In some embodiments the antigen-binding molecule is a human, humanised or chimeric (e.g. mouse/human chimeric) antigen-binding molecule.

15 In some embodiments, the moiety capable of binding to a target antigen comprises or consists of an aptamer capable of binding to the target antigen, e.g. a nucleic acid aptamer (reviewed, for example, in Zhou and Rossi *Nat Rev Drug Discov.* 2017 16(3):181-202, which is hereby incorporated by reference in its entirety). 'Nucleic acid' refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either
20 single-, double- or multiple-stranded form, or complements thereof. The term 'polynucleotide' refers to a linear sequence of nucleotides. The term 'nucleotide' typically refers to a single unit of a polynucleotide, i.e., a monomer. Nucleotides can be ribonucleotides, deoxyribonucleotides, or modified versions thereof.

In some embodiments, the moiety capable of binding to a target antigen comprises or consists of an
25 antigen-binding peptide/polypeptide, e.g. a peptide aptamer, thioredoxin, monobody, anticalin, Kunitz domain, avimer, knottin, fynomer, atrimer, DARPin, affibody, nanobody (i.e. a single-domain antibody (sdAb)) affilin, armadillo repeat protein (ArmRP), OBody or fibronectin – reviewed e.g. in Reverdatto et al., *Curr Top Med Chem.* 2015; 15(12): 1082–1101, which is hereby incorporated by reference in its entirety (see also e.g. Boersma et al., *J Biol Chem* (2011) 286:41273-85 and Emanuel et al., *Mabs* (2011)
30 3:38-48).

An antigen-binding molecule preferably displays specific binding to a target antigen. As used herein, 'specific binding' refers to binding which is selective for the target antigen, and which can be discriminated from non-specific binding to non-target antigen. An antigen-binding molecule that specifically binds to a
35 target antigen preferably binds the target with greater affinity, and/or with greater duration than it binds to other, non-target antigen. The ability of a given antigen-binding molecule to bind specifically to a target can be determined by analysis according to methods known in the art, such as by ELISA, Surface Plasmon Resonance (SPR; see e.g. Hearty et al., *Methods Mol Biol* (2012) 907:411-442), Bio-Layer Interferometry (see e.g. Lad et al., (2015) *J Biomol Screen* 20(4): 498-507), flow cytometry, or by a
40 radiolabeled antigen-binding assay (RIA) enzyme-linked immunosorbent assay. Through such analysis binding to a given target can be detected and quantified.

Antigen-binding molecule valency refers to the number of binding sites provided, and specificity refers to the number of different target antigens for which the antigen-binding molecule comprises an antigen-binding domain.

5

The antigen-binding molecule of the present invention comprises at least one antigen-binding domain. In some embodiments the antigen-binding molecules comprise e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 antigen-binding domains. In embodiments where the antigen-binding molecule of the present invention comprises more than one antigen-binding domain, the antigen-binding domains may be the same or different. Where

10 the antigen-binding molecule comprises two or more different (i.e. non-identical) antigen-binding domains, the antigen-binding molecule is multispecific. The non-identical antigen-binding domains of a multispecific antigen-binding molecule may preferably be specific for non-identical target antigens. In some embodiments the antigen-binding molecule of the present invention is monospecific. In some embodiments the antigen-binding molecule is multispecific (e.g. bispecific, trispecific, etc.).

15

The antigen-binding molecule of the present invention is at least monovalent. In some embodiments the antigen-binding molecule of the present invention is multivalent (e.g. bivalent, trivalent, tetravalent, etc.).

20

An antigen-binding molecule may be, or may comprise, an antigen-binding polypeptide, or an antigen-binding polypeptide complex. An antigen-binding molecule may comprise more than one polypeptide which together form an antigen-binding domain. The polypeptides may associate covalently or non-covalently. An antigen-binding molecule may refer to a non-covalent or covalent complex of more than one polypeptide (e.g. 2, 3, 4, 6, or 8 polypeptides), e.g. an IgG-like antigen-binding molecule comprising two heavy chain polypeptides and two light chain polypeptides.

25

The antigen-binding domain of the antigen-binding molecule of the present invention may be specific for any target antigen(s) of interest. In some embodiments the antigen-binding domain is specific for an antigen which is an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen.

30

In some embodiments the target antigen is an antigen of a pathogen. In some embodiments the pathogen may be prokaryotic (bacteria), eukaryotic (e.g. protozoan, helminth, fungus), virus or prion. In some embodiments, the pathogen is an intracellular pathogen. In some embodiments, the pathogen is a parasite.

35

In some embodiments the pathogen is a virus. A virus may be a dsDNA virus (e.g. adenovirus, herpesvirus, poxvirus), ssRNA virus (e.g. parvovirus), dsRNA virus (e.g. reovirus), (+)ssRNA virus (e.g. picornavirus, togavirus), (-)ssRNA virus (e.g. orthomyxovirus, rhabdovirus), ssRNA-RT virus (e.g. retrovirus) or dsDNA-RT virus (e.g. hepadnavirus). In particular, the present disclosure contemplates

40 viruses of the families adenoviridae, herpesviridae, papillomaviridae, polyomaviridae, poxviridae, hepadnaviridae, parvoviridae, astroviridae, caliciviridae, picornaviridae, coronaviridae, flaviviridae,

togaviridae, hepeviridae, retroviridae, orthomyxoviridae, arenaviridae, bunyaviridae, filoviridae, paramyxoviridae, rhabdoviridae and reoviridae. In some embodiments the virus is selected from adenovirus, Herpes simplex type 1 virus, Herpes simplex type 2 virus, Varicella-zoster virus, Epstein-barr virus, Human cytomegalovirus, Human herpesvirus type 8, Human papillomavirus, BK virus, JC virus, Smallpox, Hepatitis B virus, Parvovirus B19, Human Astrovirus, Norwalk virus, coxsackievirus, hepatitis A virus, poliovirus, rhinovirus, severe acute respiratory syndrome virus, Hepatitis C virus, yellow fever virus, dengue virus, West Nile virus, TBE virus, Rubella virus, Hepatitis E virus, Human immunodeficiency virus, influenza virus, lassa virus, Crimean-Congo hemorrhagic fever virus, Hantaan virus, ebola virus, Marburg virus, measles virus, mumps virus, parainfluenza virus, picornavirus, respiratory syncytial virus, rabies virus, hepatitis D virus, rotavirus, orbivirus, coltivirus, and banna virus.

In some embodiments the pathogen is a bacterium. The bacterium may be gram positive or gram negative. In particular, the present disclosure contemplates bacteria of the genera *Bacillus*, *Bartonella*, *Bordetella*, *Borrelia*, *Brucella*, *Campylobacter*, *Chlamydia*, and, *Chlamydophila*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Escherichia*, *Francisella*, *Haemophilus*, *Helicobacter*, *Legionella*, *Leptospira*, *Listeria*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Ureaplasma*, *Vibrio* and *Yersinia*.

In some embodiments the pathogen is protozoan. In particular, the present disclosure contemplates protozoa of the genera *Entamoeba*, *Plasmodium*, *Giardia*, *Trypanosoma*, *Leishmania*, *Besnoitia* and *Toxoplasma*.

In some embodiments the pathogen is a fungus. In particular, the present disclosure contemplates fungi of the genera *Candida*, *Aspergillus*, *Blastomyces*, *Coccidioides*, *Sporothrix*, *Cryptococcus*, *Histoplasma*, *Pneumocystis*, *Stachybotrys*, *Rhizopus*, *Mucor*, *Cunninghamella*, *Apophysomyces*, *Trichophyton*, *Microsporum*, *Epidermophyton*, *Fusarium*, and *Lichtheimia*.

In some embodiments the target antigen is a cancer-associated antigen. In some embodiments the cancer-associated antigen is an antigen whose expression is associated with the development, progression or severity of symptoms of a cancer. The cancer-associated antigen may be associated with the cause or pathology of the cancer, or may be expressed abnormally as a consequence of the cancer. In some embodiments, the cancer-associated antigen is an antigen whose expression is upregulated (e.g. at the RNA and/or protein level) by cells of a cancer, e.g. as compared to the level of expression of by comparable non-cancerous cells (e.g. non-cancerous cells derived from the same tissue/cell type).

In some embodiments, the cancer-associated antigen may be preferentially expressed by cancerous cells, and not expressed by comparable non-cancerous cells (e.g. non-cancerous cells derived from the same tissue/cell type). In some embodiments, the cancer-associated antigen may be the product of a mutated oncogene or mutated tumor suppressor gene. In some embodiments, the cancer-associated antigen may be the product of an overexpressed cellular protein, a cancer antigen produced by an

oncogenic virus, an oncofetal antigen, or a cell surface glycolipid or glycoprotein. GPC3 is an exemplary cancer-associated antigen.

Cancer-associated antigens are reviewed by Zarour HM, DeLeo A, Finn OJ, et al. Categories of Tumor Antigens. In: Kufe DW, Pollock RE, Weichselbaum RR, et al., editors. Holland-Frei Cancer Medicine. 6th edition. Hamilton (ON): BC Decker; 2003. Cancer-associated antigens include oncofetal antigens: CEA, Immature laminin receptor, TAG-72; oncoviral antigens such as HPV E6 and E7; overexpressed proteins: BING-4, calcium-activated chloride channel 2, cyclin-B1, 9D7, Ep-CAM, EphA3, Her2/neu, telomerase, mesothelin, SAP-1, surviving; cancer-testis antigens: BAGE, CAGE, GAGE, MAGE, SAGE, XAGE, CT9, CT10, NY-ESO-1, PRAME, SSX-2; lineage restricted antigens: MART1, Gp100, tyrosinase, TRP-1/2, MC1R, prostate specific antigen; mutated antigens: β -catenin, BRCA1/2, CDK4, CML66, Fibronectin, MART-2, p53, Ras, TGF- β RII; post-translationally altered antigens: MUC1, idiotypic antigens: Ig, TCR. Other cancer-associated antigens include heat-shock protein 70 (HSP70), heat-shock protein 90 (HSP90), glucose-regulated protein 78 (GRP78), vimentin, nucleolin, feto-acinar pancreatic protein (FAPP), alkaline phosphatase placental-like 2 (ALPPL-2), siglec-5, stress-induced phosphoprotein 1 (STIP1), protein tyrosine kinase 7 (PTK7), and cyclophilin B.

In some embodiments the target antigen is an antigen of an adenovirus, e.g. adenovirus 5. In some embodiments the target antigen adenovirus 5 hexon.

In some embodiments the antigen-binding molecule of the present invention comprises the CDRs and/or the VH and VL domains of an adenovirus 5 hexon-binding antibody, e.g. 9C12 (described in Varghese et al., 2004a).

In some embodiments the antigen-binding molecule of the present invention comprises an antigen-binding domain capable of specific binding to an endocytosis receptor. As used herein, an 'endocytosis receptor' refers to receptor expressed at the cell surface which is capable of internalisation by the cell expressing the receptor, e.g. following binding of a molecule to the receptor. In this way the endocytosis receptor facilitates cellular uptake of molecules which bind to the receptor. Endocytosis is described e.g. in Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell, 4th Edn. (New York: Garland Science; 2002) at chapter 13, subsection entitled 'Transport into the Cell from the Plasma Membrane: Endocytosis', which is hereby incorporated by reference in its entirety. Briefly, endocytosis refers to uptake of extracellular material by cells through binding of the material to receptors expressed at the cell surface, and subsequent invagination of the plasma membrane and internalization of the material in a membrane-bound vesicle. Endocytosis receptors include e.g. DEC-205, CD11c/CD18, DC-SIGN, Toll-like receptors (TLRs) and phagocytosis receptors. Phagocytosis receptors are expressed at the cell surface of phagocytic cells and include e.g. Fc γ RIIa, Fc γ RIIb, CR3, SRAI, MARCO, CD36, Dectin-1, MICL, CD206 and CD169. Phagocytosis is described e.g. in Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell, 4th Edn. (New York: Garland Science; 2002) at Chapter 25, which is hereby incorporated by reference in its entirety.

In some embodiments the antigen-binding molecule comprises an antigen-binding domain capable of specific binding to an endocytosis receptor expressed by an antigen presenting cell (APC). APCs according to the present disclosure may be professional APCs. Professional APCs are specialised for presenting antigen to T cells; they are efficient at processing and presenting MHC-peptide complexes at the cell surface, and express high levels of costimulatory molecules. Professional APCs include dendritic cells (DCs), macrophages, and B cells. Non-professional APCs are other cells capable of presenting MHC-peptide complexes to T cells, in particular MHC Class I-peptide complexes to CD8+ T cells.

In some embodiments the APC is an APC capable of cross-presentation on MHC class I of antigen internalised by the APC (e.g. taken-up by endocytosis/phagocytosis). Cross-presentation on MHC class I of internalized antigens to CD8+ T cells is described e.g. in Alloatti et al., *Immunological Reviews* (2016), 272(1): 97-108 and Gros and Amigorena, *Frontiers in Immunology* (2019) 10:41, both of which are hereby incorporated by reference in their entirety. APCs capable of cross-presentation include e.g. dendritic cells (DCs), macrophages, B cells and sinusoidal endothelial cells. In some embodiments the antigen-binding molecule is specific for an endocytosis receptor expressed by a dendritic cell (DC).

In some embodiments the antigen-binding molecule of the present invention comprises an antigen-binding domain capable of specific binding to protein expressed at the cell surface of a dendritic cell, e.g. DEC-205 or CLEC9A. In some embodiments the antigen-binding molecule of the present invention comprises an antigen-binding domain capable of specific binding to DEC-205. In some embodiments the antigen-binding molecule of the present invention comprises the CDRs and/or the VH and VL domains of a DEC-205-binding antibody, e.g. 3G9-2D2 (described in Cheong et al., 2010).

In some embodiments the antigen-binding molecule of the present invention comprises (i) an antigen-binding domain specific for an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen, (ii) an antigen-binding domain capable of specific binding to an endocytosis receptor, and (iii) an Fc region or polypeptide according to the invention. The skilled person is able to design and prepare such multispecific antigen-binding molecules with reference e.g. to Brinkmann and Kontermann MABs (2017) 9(2): 182-212, which is hereby incorporated by reference in its entirety. Suitable formats include those formats comprising an Fc region which are shown in Figure 2 of Brinkmann and Kontermann MABs (2017) 9(2): 182-212.

In some embodiments, the antigen-binding molecule of the present invention further comprises an antigenic sequence of a target antigen, e.g. according to an embodiment described herein.

It will be appreciated that the antigenic sequence of a target antigen is comprised in a peptide or polypeptide. The peptide/polypeptide comprising the antigenic sequence of a target antigen may be covalently or non-covalently associated with the antigen-binding molecule.

In some embodiments the peptide/polypeptide comprising the antigenic sequence of a target antigen is joined to the antigen-binding molecule via a linker sequence (e.g. a linker sequence as described herein).

In some embodiments the peptide/polypeptide comprising the antigenic sequence of a target antigen is joined to the antigen-binding molecule via chemical conjugation, e.g. via click chemistry or via maleimide-thiol conjugation chemistry. Click chemistry and its use to produce conjugates of biomolecules is described in Nwe and Brechbiel Cancer Biother Radiopharm. (2009) 24(3):289-302, which is hereby
5 incorporated by reference in its entirety.

The peptide/polypeptide comprising the antigenic sequence of a target antigen may be associated to any suitable part of the antigen-binding molecule.

10 In some embodiments, the peptide/polypeptide comprising the antigenic sequence of a target antigen is joined via a peptide bond or a peptide linker to N- or C-terminus of a constituent polypeptide of the antigen-binding molecule. In some embodiments, the peptide/polypeptide comprising the antigenic sequence of a target antigen is joined via chemical conjugation to the N- or C-terminus of a constituent polypeptide of the antigen-binding molecule. In some embodiments, the peptide/polypeptide comprising
15 the antigenic sequence of a target antigen is joined via chemical conjugation to the hinge region sequence or a cysteine residue of a constituent polypeptide of the antigen-binding molecule.

The present invention also provides a polypeptide complex comprising an antigen-binding molecule of the present invention and the target antigen or a fragment thereof. The target antigen is the target antigen for
20 which the antigen-binding molecule comprises a specific antigen-binding domain. The polypeptide complex is preferably a non-covalent complex. The complex is preferably formed by interaction between the CDRs of the antigen-binding molecule and the epitope of the target antigen. In some embodiments the polypeptide complex may be provided in isolated or substantially purified form.

25 Where the polypeptide complex of the invention comprises a fragment of the target antigen, it will be appreciated that the fragment comprises the epitope for the antigen-binding domain of the antigen-binding molecule.

Immunogens

30 The present invention also provides immunogens comprising the Fc regions and/or the polypeptides of the invention.

In the present disclosure, an 'immunogen' is a molecule capable of stimulating an adaptive immune response, e.g. a B cell or T cell response. In some embodiments an immunogen is a molecule comprising
35 an antigenic sequence. As used herein, an 'antigenic sequence' refers to an amino acid sequence which is capable of being specifically recognised by an antibody or, capable of being specifically recognised by an immune cell receptor (e.g. a T cell receptor), e.g. when presented by an appropriate MHC molecule.

An immunogen may be, or may comprise, a polypeptide complex. An immunogen may comprise more
40 than one polypeptide which together form the immunogen. The polypeptides may associate covalently or

non-covalently. An immunogen may refer to a non-covalent or covalent complex of more than one polypeptide (e.g. 2, 3, 4, 6, or 8 polypeptides).

5 The 'antigenic sequence' may be, or may be derived from, an amino acid sequence of an antigen, e.g. an antigen described herein (e.g. an antigen of a pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen). An antigenic sequence which is 'derived from' an amino acid sequence of a reference antigen has an amino acid sequence having at least 60%, e.g. one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of the reference antigen.

10

In some embodiments the antigenic sequence comprises, or consists of, 5 to 500, 5 to 400, 5 to 300, 5 to 200, 5 to 100, 5 to 90, 5 to 80, 5 to 70, 5 to 60, 5 to 50, 5 to 40, 5 to 35, 5 to 30, 5 to 25, 5 to 20, 5 to 19, 5 to 18, 5 to 17, 5 to 16, 5 to 15, 5 to 14, 5 to 13, 5 to 12, 5 to 11, or 5 to 10 contiguous amino acids of the amino acid sequence of the antigen. In some embodiments the antigenic sequence comprises, or consist
15 of, the entire amino acid sequence of the antigen.

The immunogen of the present invention accordingly comprises at least an antigenic sequence and an Fc region and/or polypeptide according to the present invention.

20 In some embodiments the immunogen additionally comprises an APC-targeting region. An 'APC-targeting region' is capable of facilitating localisation to and/or uptake of the immunogen by an APC. The APC may be a professional or non-professional APC. In some embodiments the APC-targeting region is a DC-targeting region, a macrophage-targeting region or a B cell-targeting region.

25 In some embodiments the APC-targeting region comprises or consists of a moiety capable of binding to a receptor expressed on the surface of an APC, e.g. a moiety for capable of binding to an endocytosis receptor (e.g. an endocytosis receptor described herein). In some embodiments a moiety capable of specific binding to a receptor comprises the VH and VL of an antibody capable of specific binding to the receptor, a nucleic acid aptamer capable of binding to the receptor, or a receptor-binding
30 peptide/polypeptide. In some embodiments the APC-targeting region comprises or consists of a ligand for the receptor.

In some embodiments the APC-targeting region comprises or consists of a moiety capable of binding to DEC-205.

35

In some embodiments the immunogen additionally comprises one or more carrier sequences. A carrier sequence may comprise, or consist of, the amino acid sequence of a carrier protein, e.g. selected from Keyhole Limpet Hemocyanin (KLH), Concholepas Concholepas Hemocyanin (CCH), Bovine Serum Albumin (BSA), or Ovalbumin (OVA).

40

In some embodiments, the immunogen of the present invention further comprises an antigen-binding domain capable of specific binding to a target antigen, e.g. according to an embodiment described herein. The antigen-binding domain may be covalently or non-covalently associated with the immunogen.

- 5 In some embodiments the antigen-binding domain is joined to the immunogen via a linker sequence (e.g. a linker sequence as described herein). In some embodiments the antigen-binding domain is joined to the immunogen via chemical conjugation, e.g. via click chemistry or maleimide-thiol conjugation chemistry.

The antigen-binding domain may be associated to any suitable part of the immunogen.

10

In some embodiments, the antigen-binding domain is joined via a peptide bond or a peptide linker to N- or C-terminus of a constituent polypeptide of the immunogen. In some embodiments, the antigen-binding domain is joined via chemical conjugation to the N- or C-terminus or to the side chains of a constituent polypeptide of the immunogen.

15

- It will be appreciated that an immunogen of the present invention is useful in methods for generating and/or enhancing an immune response to the antigenic sequence (and thus also to the antigen). By way of illustration, Dhodapkar et al., Sci. Transl. Med. (2014) 6:232ra51 (hereby incorporated by reference in entirety) describes the production and characterisation of an antibody-antigen fusion protein comprising an antigen-binding molecule specific for human DEC-205 fused to the tumor antigen NY-ESO-1. The authors demonstrated that this molecule was capable of inducing humoral and cellular immunity to NY-ESO-1. Similarly, Bozzacco et al., Proc Natl Acad Sci U S A. (2007) 104:1289-1294 (hereby incorporated by reference in entirety) describes the production and characterisation of an antibody-antigen fusion protein comprising an antigen-binding molecule specific for human DEC-205 fused to HIV p24 gag protein. The authors demonstrated that this molecule was capable of stimulating proliferation and IFN-gamma production by CD8⁺ T cells isolated from the blood of HIV-infected donors.
- 20
- 25

Accordingly the present invention provides the immunogen of the present invention for use in prophylactic and therapeutic applications, and also in methods for generating/expanding populations of immune cells specific for an antigen.

30

Particular exemplary molecules contemplated

- (1) An antigen-binding molecule comprising: (i) an Fc region according to the present invention, (ii) an antigen-binding domain specific for an endocytosis receptor expressed by a dendritic cell (e.g. DEC-205), and (iii) a peptide/polypeptide comprising an antigenic sequence of a disease-associated antigen.
- 35

(2) An antigen-binding molecule comprising: (i) an Fc region according to the present invention, (ii) an antigen-binding domain specific for an endocytosis receptor expressed by a dendritic cell (e.g. DEC-205), and (iii) a peptide/polypeptide comprising an antigenic sequence of a disease-associated antigen;

wherein the peptide/polypeptide comprising an antigenic sequence of a disease-associated antigen is joined via a peptide bond, peptide linker or chemical conjugation to the C-terminus of one or more of the CH3 domains of the Fc region.

- 5 (3) An antigen-binding molecule comprising: (i) an Fc region according to the present invention, (ii) an antigen-binding domain specific for an endocytosis receptor expressed by a dendritic cell (e.g. DEC-205), and (iii) a peptide/polypeptide comprising an antigenic sequence of a disease-associated antigen;
wherein the peptide/polypeptide comprising an antigenic sequence of a disease-associated
antigen is joined via a peptide bond, peptide linker or chemical conjugation N-terminal to one or more of
10 the CH2 domains of the Fc region.

(4) An antigen-binding molecule comprising an Fc region according to the present invention, and an antigen-binding domain specific for a disease-associated target antigen, (e.g. GPC3, or an antigen of a
15 parasite).

(5) An antigen-binding molecule comprising: (i) an Fc region according to the present invention, (ii) an antigen-binding domain specific for a disease-associated target antigen, (e.g. GPC3, or an antigen of a
parasite), and (iii) an antigen-binding domain specific for an endocytosis receptor expressed by a
dendritic cell (e.g. DEC-205).

20 (6) An immunogen comprising an Fc region according to the present invention, and a peptide/polypeptide comprising an antigenic sequence of a disease-associated antigen (e.g. GPC3).

(7) An immunogen comprising: (i) an Fc region according to the present invention, (ii) a
25 peptide/polypeptide comprising an antigenic sequence of a disease-associated antigen (e.g. GPC3), and (iii) an antigen-binding domain specific for an endocytosis receptor expressed by a dendritic cell (e.g. DEC-205).

Additional sequences

- 30 The antigen-binding molecules, immunogens and polypeptides according to the present invention may additionally comprise further amino acids or sequences of amino acids.

For example, the antigen-binding molecules may comprise one or more linker sequences between the antigen-binding domain and the Fc region. The linker sequence(s) may be provided in one or more of the
35 polypeptides of the antigen-binding molecule. Similarly, the immunogens may comprise one or more linker sequences between the antigenic sequence and the Fc region.

Linker sequences are known to the skilled person, and are described, for example in Chen et al., Adv Drug Deliv Rev (2013) 65(10): 1357-1369, which is hereby incorporated by reference in its entirety. In
40 some embodiments, a linker sequence may be a flexible linker sequence. Flexible linker sequences allow for relative movement of the amino acid sequences which are linked by the linker sequence. Flexible

linkers are known to the skilled person, and several are identified in Chen et al., Adv Drug Deliv Rev (2013) 65(10): 1357-1369. Flexible linker sequences often comprise high proportions of glycine and/or serine residues. In some embodiments, the linker sequence comprises at least one glycine residue and/or at least one serine residue. In some embodiments the linker sequence consists of glycine and serine residues. In some embodiments, the linker sequence has a length of 1-2, 1-3, 1-4, 1-5 or 1-10 amino acids.

The antigen-binding molecules, immunogens and polypeptides of the invention may comprise amino acid sequence(s) to facilitate expression, folding, trafficking, processing, purification or detection of the antigen-binding molecule/polypeptide. For example, the antigen-binding molecule/polypeptide may comprise a sequence encoding a His, (e.g. 6XHis), Myc, GST, MBP, FLAG, HA, E, or Biotin tag, optionally at the N- or C- terminus of the antigen-binding molecule/immunogen/polypeptide. In some embodiments the antigen-binding molecule/polypeptide comprises a detectable moiety, e.g. a fluorescent, luminescent, immuno-detectable, radio, chemical, nucleic acid or enzymatic label.

The antigen-binding molecules, immunogens and polypeptides of the present invention may additionally comprise a signal peptide (also known as a leader sequence or signal sequence). Signal peptides normally consist of a sequence of 5-30 hydrophobic amino acids, which form a single alpha helix. Secreted proteins and proteins expressed at the cell surface often comprise signal peptides. A signal peptide may be present at the N-terminus of the antigen-binding molecule/polypeptide, and may be present in the newly synthesised antigen-binding molecule/polypeptide. The signal peptide provides for efficient trafficking and/or secretion of the antigen-binding molecule/polypeptide. Signal peptides are often removed by cleavage, and thus are not comprised in the mature antigen-binding molecule/polypeptide. Signal peptides are known for many proteins, and are recorded in databases such as GenBank, UniProt, Swiss-Prot, TrEMBL, Protein Information Resource, Protein Data Bank, Ensembl, and InterPro, and/or can be identified/predicted e.g. using amino acid sequence analysis tools such as SignalP (Petersen et al., 2011 Nature Methods 8: 785-786) or Signal-BLAST (Frank and Sippl, 2008 Bioinformatics 24: 2172-2176).

30 Functional properties

The antigen-binding molecules, polypeptide complexes and immunogens of the present invention may be defined by reference to certain functional properties.

In some embodiments, a molecule comprising a modified Fc region according to the present invention may display increased affinity for TRIM21 and/or increased binding to TRIM21 as compared to an equivalent molecule comprising an unmodified Fc region. The molecule comprising a modified Fc region may e.g. be an antigen-binding molecule or a polypeptide complex of an antigen-binding molecule and target antigen/fragment thereof. An equivalent molecule comprises all of the same features as the test molecule, the only difference being the modification(s) to the Fc region.

A "modified Fc region" may be any embodiment of an Fc region according to the present invention. An "unmodified Fc region" may be the wildtype Fc region of an IgG (e.g. IgG1, IgG2, IgG3, IgG4), IgA (e.g. IgA1, IgA2), IgD, IgE or IgM. In some embodiments, an unmodified Fc region is comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:10, 14, 18, 22, 180, 181 or 182. In some embodiments, an unmodified Fc region is comprised of CH2-CH3-CH4 having the amino acid sequence of SEQ ID NO:31 or 183. In particular embodiments, an unmodified Fc region is comprised of CH2-CH3 having the amino acid sequence of SEQ ID NO:10.

The affinity of association between a molecule comprising an Fc region and TRIM21 can be identified using a suitable assay, such as Surface Plasmon Resonance (SPR; see e.g. Hearty et al., *Methods Mol Biol* (2012) 907:411-442), Bio-Layer Interferometry (see e.g. Lad et al., (2015) *J Biomol Screen* 20(4): 498-507), flow cytometry, or Enzyme-linked immunosorbent assay. The affinity of association between two molecules can be analysed and quantified using such methods. The affinity of association between a molecule comprising an Fc region and TRIM21 can be analysed as described in Example 1.10.

In some embodiments the molecule comprising a modified Fc region binds to TRIM21 with an affinity which is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times, ≥ 200 times, ≥ 300 times, ≥ 400 times, ≥ 500 times, ≥ 600 times, ≥ 700 times, ≥ 800 times, ≥ 900 times or ≥ 1000 times the affinity of association between TRIM21 and the equivalent molecule comprising an unmodified Fc region.

In some embodiments, an antigen-binding molecule comprising a modified Fc region according to the present invention displays one or more of the following properties as compared to an equivalent antigen-binding molecule comprising an unmodified Fc region:

Increased affinity for TRIM21; and

Similar level of antibody-dependent intracellular neutralization (ADIN).

An equivalent antigen-binding molecule comprises all of the same features as the test antigen-binding molecule, the only difference being the modification(s) to the Fc region.

Analysis of ADIN can be performed using a suitable assay, e.g. as described in Example 1.11 herein, or in Watkinson et al., *J. Virol.* (2013) 87(13) 7309-7313, which is hereby incorporated by reference in its entirety. It will be appreciated that the assay employs a suitable target and antigen-binding domain, e.g. a target and an antigen-binding domain of an antibody known to participate in ADIN.

A similar level of ADIN may be a level of ADIN which is ≥ 0.75 times and ≤ 1.25 times, e.g. ≥ 0.8 times and ≤ 1.2 times, ≥ 0.85 times and ≤ 1.15 times, ≥ 0.9 times and ≤ 1.1 times, ≥ 0.91 times and ≤ 1.09 times, ≥ 0.92 times and ≤ 1.08 times, ≥ 0.93 times and ≤ 1.07 times, ≥ 0.94 times and ≤ 1.06 times, ≥ 0.95

times and ≤ 1.05 times, ≥ 0.96 times and ≤ 1.04 times, ≥ 0.97 times and ≤ 1.03 times, ≥ 0.98 times and ≤ 1.02 times, or ≥ 0.99 times and ≤ 1.01 times the level of ADIN displayed by an equivalent antigen-binding molecule comprising an unmodified Fc region.

- 5 In some embodiments, a polypeptide complex comprising an antigen-binding molecule according to the present invention and target antigen/fragment thereof is useful to stimulate dendritic cells (e.g. monocyte-derived dendritic cells) displaying one or more of the following properties as compared to dendritic cells stimulated with a polypeptide complex comprising an equivalent antigen-binding molecule having an unmodified Fc region and the target antigen/fragment thereof:
- 10 Increased expression of one or more markers of APC maturation;
 Increased expression of one or more costimulatory molecules;
 Increased expression of one or more Th1-associated factors;
 Increased ability to expand T cells (e.g. CD8⁺ T cells, e.g. antigen-specific CD8⁺ T cells);
 Increased ability to expand IFN γ -expressing T cells (e.g. IFN γ -expressing, CD8⁺ T cells, e.g.
- 15 IFN γ -expressing, antigen-specific CD8⁺ T cells); and
 Increased cross-presentation of a peptide of the target antigen.

As used herein, 'expression' may be gene expression or protein expression. Gene expression can be determined e.g. by detection of mRNA encoding the marker, for example by quantitative real-time PCR

20 (qRT-PCR), or by reporter-based methods. Protein expression can be determined e.g. by detection of the protein, for example by antibody-based methods which are well known to the skilled person, such as western blot, immunohistochemistry, immunocytochemistry, flow cytometry, and ELISA. Protein expression can be determined by reporter-based methods, e.g. assays for a function of the protein.

25 Assays for analysing expression of one or more factors by dendritic cells in response to stimulation with polypeptide complexes can be performed e.g. as described in Example 1.12. It will be appreciated that the assays employ a suitable target antigen/fragment and antigen-binding molecule combination, e.g. a combination known to induce maturation of dendritic cells and/or expression of Th1 factors by dendritic cells.

30 In some embodiments the one or more markers of APC maturation may be one or more markers of monocyte-derived dendritic cell (moDC) maturation. In some embodiments the one or more markers may be selected from CD80, CD83, CD86 and HLA-DR.

35 In some embodiments the one or more costimulatory molecules may be selected from CD80, CD83 and CD86.

Th1-associated factors are factors which promote differentiation of CD4⁺ helper T cells (i.e. Th cells) to a Th1 phenotype. In some embodiments the one or more Th1-associated factors are selected from CCL3

40 (MIP1- α), CCL4 (MIP-1 β), CCL5 (RANTES), TRAIL, IFN- γ , IL-6 and TNF- α .

- Increased expression may be a level of expression which is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times the level of expression of the relevant factor(s) by dendritic cells stimulated with a polypeptide complex comprising an equivalent antigen-binding molecule having an unmodified Fc region and the target antigen/fragment thereof.
- Expansion of a cell type can be analysed by monitoring cell number/proportion or cell division over a period of time. Cell numbers and proportions can be determined e.g. by flow cytometry analysis using antibodies allowing detection of various cell types. Cell division can be analysed, for example, by *in vitro* analysis of incorporation of ^3H -thymidine or by CFSE dilution assay, e.g. as described in Fulcher and Wong, *Immunol Cell Biol* (1999) 77(6): 559-564, hereby incorporated by reference in entirety. Proliferating cells may also be identified by analysis of incorporation of 5-ethynyl-2'-deoxyuridine (EdU) by an appropriate assay, as described e.g. in Buck et al., *Biotechniques*. 2008 Jun; 44(7):927-9, and Sali and Mitchison, *PNAS USA* 2008 Feb 19; 105(7): 2415-2420, both hereby incorporated by reference in their entirety.
- The ability of dendritic cells to stimulate expansion of a cell type of interest can be determined by analysis by flow cytometry following co-culture of a population of immune cells comprising the cell type of interest (e.g. PBMCs) in the presence of dendritic cells which have been stimulated with the polypeptide complex. Expansion of T cells may be analysed e.g. in an assay as described herein in Example 1.13. Expansion of antigen-specific T cells may be analysed e.g. in an assay as described herein in Example 1.14.
- Increased ability to expand T cells may be determined by detection of an increased number/proportion of T cells following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with a polypeptide complex according to the invention as compared to the number/proportion of T cells obtained following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with a polypeptide complex comprising an equivalent antigen-binding molecule having an unmodified Fc region and the target antigen/fragment thereof. In some embodiments the increased number/proportion is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times the number/proportion of T cells obtained following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with a polypeptide complex comprising an equivalent antigen-binding molecule having an unmodified Fc region and the target antigen/fragment thereof.

Increased ability to expand IFN- γ -expressing T cells (e.g. IFN- γ -expressing, CD8+ T cells, e.g. IFN- γ -expressing, antigen-specific CD8+ T cells) may be determined by detection of an increased number/proportion of IFN- γ -expressing T cells following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with a polypeptide complex according to the invention as compared to the number/proportion of IFN- γ -expressing T cells obtained following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with a polypeptide complex comprising an equivalent antigen-binding molecule having an unmodified Fc region and the target antigen/fragment thereof. In some embodiments the increased number/proportion is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times the number/proportion of IFN- γ -expressing T cells obtained following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with a polypeptide complex comprising an equivalent antigen-binding molecule having an unmodified Fc region and the target antigen/fragment thereof.

Cross-presentation refers to presentation of a peptide of the target antigen on an MHC class I molecule following internalisation and processing of the complex by the antigen-presenting cells (e.g. dendritic cells). Cross-presentation can be detected and quantified using methods capable of detecting MHC class I:target antigen peptide complexes expressed on the surface of the dendritic cells following incubation of the dendritic cells with polypeptide complexes of the antigen-binding molecule and the target antigen/fragment thereof.

Increased cross-presentation may be cross-presentation which is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times the level of cross-presentation by dendritic cells contacted with a polypeptide complex comprising an equivalent antigen-binding molecule having an unmodified Fc region and the target antigen/fragment thereof.

In some embodiments, an immunogen according to the present invention is useful to stimulate dendritic cells (e.g. monocyte-derived dendritic cells) displaying one or more of the following properties as compared to dendritic cells stimulated with a control immunogen comprising an unmodified Fc region:

- Increased expression of one or more markers of maturation;
- Increased expression of one or more Th1-associated factors;
- Increased ability to stimulate T cell proliferation (e.g. CD8+ T cell proliferation, e.g. antigen-specific CD8+ T cell proliferation);
- Increased ability to stimulate IFN- γ -expressing T cells (e.g. IFN- γ -expressing, CD8+ T cells, e.g. IFN- γ -expressing, antigen-specific CD8+ T cells); and

Increased cross-presentation of a peptide of the target antigen.

Increased expression may be a level of expression which is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times the level of expression of the relevant factor(s) by dendritic cells stimulated with an equivalent immunogen having an unmodified Fc region.

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Increased ability to expand T cells may be determined by detection of an increased number/proportion of T cells following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with an immunogen according to the invention as compared to the number/proportion of T cells obtained following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with a polypeptide complex comprising an equivalent immunogen having an unmodified Fc region. In some embodiments the increased number/proportion is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times the number/proportion of T cells obtained following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with an equivalent immunogen having an unmodified Fc region.

Increased ability to expand IFN- γ -expressing T cells (e.g. IFN- γ -expressing, CD8 $^{+}$ T cells, e.g. IFN- γ -expressing, antigen-specific CD8 $^{+}$ T cells) may be determined by detection of an increased number/proportion of IFN- γ -expressing T cells following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with an immunogen according to the invention as compared to the number/proportion of IFN- γ -expressing T cells obtained following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with an equivalent immunogen having an unmodified Fc region. In some embodiments the increased number/proportion is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times, ≥ 2 times, ≥ 3 times, ≥ 4 times, ≥ 5 times, ≥ 6 times, ≥ 7 times, ≥ 8 times, ≥ 9 times, ≥ 10 times, ≥ 20 times, ≥ 30 times, ≥ 40 times, ≥ 50 times, ≥ 60 times, ≥ 70 times, ≥ 80 times, ≥ 90 times, ≥ 100 times the number/proportion of IFN- γ -expressing T cells obtained following co-culture of a population of immune cells (e.g. PBMCs) with dendritic cells stimulated with an equivalent immunogen having an unmodified Fc region.

Increased cross-presentation may be cross-presentation which is more than 1 times, e.g. ≥ 1.01 times, ≥ 1.02 times, ≥ 1.03 times, ≥ 1.04 times, ≥ 1.05 times, ≥ 1.06 times, ≥ 1.07 times, ≥ 1.08 times, ≥ 1.09 times, ≥ 1.1 times, ≥ 1.2 times, ≥ 1.3 times, ≥ 1.4 times, ≥ 1.5 times, ≥ 1.6 times, ≥ 1.7 times, ≥ 1.8 times, ≥ 1.9 times,

≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times, ≥10 times, ≥20 times, ≥30 times, ≥40 times, ≥50 times, ≥60 times, ≥70 times, ≥80 times, ≥90 times, ≥100 times the level of cross-presentation by dendritic cells contacted with an equivalent immunogen having an unmodified Fc region.

5 Methods using the antigen-binding molecules, immunogens and polypeptide complexes

The antigen-binding molecules, immunogens and polypeptide complexes according to the present invention are useful in methods for producing antigen-presenting cells (e.g. dendritic cells) having desirable properties. The antigen-binding molecules, immunogens and polypeptide complexes according to the present invention are useful in methods for generating/expanding populations of immune cells of interest. The antigen-binding molecules, immunogens and polypeptide complexes according to the present invention are useful in methods for inducing or enhancing an immune response to a target antigen in a subject.

15 Antigen-presenting cells (e.g. dendritic cells) which are contacted/treated/stimulated with (and which subsequently internalise) the immunogens and polypeptide complexes according to the present invention are provided with desirable properties relevant to their use in methods for expanding populations of cells of interest (e.g. T cells, e.g. CD8+ T cells, e.g. antigen-specific CD8+ T cells), e.g. *in vivo*, *ex vivo* or *in vitro*.

20 Accordingly, the present invention provides a method comprising contacting a population of antigen-presenting cells (e.g. dendritic cells) with an immunogen according to the present invention or a polypeptide complex according to the invention (i.e. comprising an antigen-binding molecule according to the present invention and a target antigen/fragment thereof). The present invention also provides a population of antigen-presenting cells (e.g. dendritic cells) produced according to the method. Also provided is a method for generating or expanding a population of immune cells (e.g. T cells, e.g. CD8+ T cells) specific for a target antigen, comprising contacting a population of immune cells with an antigen-presenting cell produced according to the method.

30 Aspects of the present invention contemplate the use of the antigen-binding molecules, immunogens and polypeptide complexes of the present invention in methods for generating/stimulating/boosting/increasing an immune response (in particular a cell mediated-immune response, and in particular a T cell-mediated immune response (e.g. a CD8+ T cell-mediated immune response)) to a target antigen.

35 The antigen-presenting cells may be contacted/treated/stimulated with the immunogens/polypeptide complexes *in vitro* or *in vivo*. In some embodiments the polypeptide complexes may be formed *in vivo* following administration of an antigen-binding molecule to a subject infected with the relevant pathogen or expressing the relevant target antigen. In some embodiments the polypeptide complexes may be formed *in vitro* by contacting the antigen-binding molecule with the relevant target antigen/fragment thereof.

40 Culture of cells in accordance with the methods of the invention is performed using suitable medium and under suitable environmental conditions (e.g. temperature, pH, humidity, atmospheric conditions,

agitation etc.) for the *in vitro* and/or *ex vivo* culture of immune cells, which are well known to the person skilled in the art of cell culture. Conveniently, cultures of cells may be maintained at 37°C in a humidified atmosphere containing 5% CO₂. Cultures can be performed in any vessel suitable for the volume of the culture, e.g. in wells of a cell culture plate, cell culture flasks, a bioreactor, etc. The cell cultures can be established and/or maintained at any suitable density, as can readily be determined by the skilled person. For example, cultures may be established at an initial density of $\sim 0.5 \times 10^6$ to $\sim 5 \times 10^6$ cells/ml of the culture (e.g. $\sim 1 \times 10^6$ cells/ml). Cells may be cultured in any suitable cell culture vessel. In some embodiments of the methods according to the various aspects of the present invention, cells are cultured in a bioreactor.

Populations of cells of interest (e.g. T cells, e.g. CD8⁺ T cells, e.g. antigen-specific CD8⁺ T cells) may be generated/expanded according to the present invention from within a population of immune cells. It will be appreciated that the population of immune cells comprises the cell type of interest, e.g. at low frequency. The population of immune cells from which the cells of interest are generated/expanded according to the methods of the present invention comprise at least one cell of interest.

In some embodiments, populations of cells of interest may be generated/expanded from within a population of immune cells, e.g. peripheral blood mononuclear cells (PBMCs). For example, a population of T cells (e.g. CD8⁺ T cells, e.g. antigen-specific CD8⁺ T cells) may be generated/expanded from within a population of immune cells (e.g. PBMCs), by culture of the immune cells in the presence of antigen-presenting cells (APCs; e.g. dendritic cells) which have been contacted with an immunogen or polypeptide complex according to the present invention. In embodiments of the methods disclosed herein, the antigen-presenting cells may be obtained/derived from a population of PBMCs. In some embodiments the antigen-presenting cells and the population of immune cells are autologous (i.e. are derived/obtained from the same subject).

Nucleic acids and vectors

The present invention provides a nucleic acid, or a plurality of nucleic acids, encoding an antigen-binding molecule, immunogen or polypeptide according to the present invention.

In some embodiments, the nucleic acid is purified or isolated, e.g. from other nucleic acid, or naturally-occurring biological material. In some embodiments the nucleic acid(s) comprise or consist of DNA and/or RNA.

The present invention also provides a vector, or plurality of vectors, comprising the nucleic acid or plurality of nucleic acids according to the present invention.

The nucleotide sequence may be contained in a vector, e.g. an expression vector. A 'vector' as used herein is a nucleic acid molecule used as a vehicle to transfer exogenous nucleic acid into a cell. The vector may be a vector for expression of the nucleic acid in the cell. Such vectors may include a promoter sequence operably linked to the nucleotide sequence encoding the sequence to be expressed. A vector

may also include a termination codon and expression enhancers. Any suitable vectors, promoters, enhancers and termination codons known in the art may be used to express a peptide or polypeptide from a vector according to the invention.

- 5 The term 'operably linked' may include the situation where a selected nucleic acid sequence and regulatory nucleic acid sequence (e.g. promoter and/or enhancer) are covalently linked in such a way as to place the expression of nucleic acid sequence under the influence or control of the regulatory sequence (thereby forming an expression cassette). Thus a regulatory sequence is operably linked to the selected nucleic acid sequence if the regulatory sequence is capable of effecting transcription of the
10 nucleic acid sequence. The resulting transcript(s) may then be translated into a desired peptide(s)/polypeptide(s).

- Suitable vectors include plasmids, binary vectors, DNA vectors, mRNA vectors, viral vectors (e.g. gammaretroviral vectors (e.g. murine Leukemia virus (MLV)-derived vectors), lentiviral vectors,
15 adenovirus vectors, adeno-associated virus vectors, vaccinia virus vectors and herpesvirus vectors), transposon-based vectors, and artificial chromosomes (e.g. yeast artificial chromosomes).

- In some embodiments, the vector may be a eukaryotic vector, e.g. a vector comprising the elements necessary for expression of protein from the vector in a eukaryotic cell. In some embodiments, the vector
20 may be a mammalian vector, e.g. comprising a cytomegalovirus (CMV) or SV40 promoter to drive protein expression.

- Constituent polypeptides of an antigen-binding molecule/Fc region/immunogen according to the present invention may be encoded by different nucleic acids of the plurality of nucleic acids, or by different vectors
25 of the plurality of vectors.

Cells comprising/expressing the antigen-binding molecules, immunogens and polypeptides

- The present invention also provides a cell comprising or expressing an antigen-binding molecule, immunogen or polypeptide according to the present invention. Also provided is a cell comprising or
30 expressing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the invention.

- The cell may be a eukaryotic cell, e.g. a mammalian cell. The mammal may be a primate (rhesus, cynomolgous, non-human primate or human) or a non-human mammal (e.g. rabbit, guinea pig, rat,
35 mouse or other rodent (including any animal in the order Rodentia), cat, dog, pig, sheep, goat, cattle (including cows, e.g. dairy cows, or any animal in the order Bos), horse (including any animal in the order Equidae), donkey, and non-human primate).

- The present invention also provides a method for producing a cell comprising a nucleic acid(s) or
40 vector(s) according to the present invention, comprising introducing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the present invention into a cell. In some

embodiments, introducing an isolated nucleic acid(s) or vector(s) according to the invention into a cell comprises transformation, transfection, electroporation or transduction (e.g. retroviral transduction).

The present invention also provides a method for producing a cell expressing/comprising an antigen-binding molecule, immunogen or polypeptide according to the present invention, comprising introducing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the present invention in a cell. In some embodiments, the methods additionally comprise culturing the cell under conditions suitable for expression of the nucleic acid(s) or vector(s) by the cell. In some embodiments, the methods are performed *in vitro*.

The present invention also provides a method for producing an antigen-binding molecule, immunogen or polypeptide according to the present invention, comprising introducing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the present invention in a cell and culturing the cell under conditions suitable for expression of the nucleic acid(s) or vector(s) by the cell. In some embodiments, the methods additionally comprise isolating/purifying the expressed antigen-binding molecule(s)/immunogen(s)/polypeptide(s). In some embodiments, the methods are performed *in vitro*.

The present invention also provides cells obtained or obtainable by the methods according to the present invention.

Producing the antigen-binding molecules, immunogens and polypeptides

Antigen-binding molecules, immunogens and polypeptides according to the invention may be prepared according to methods for the production of polypeptides known to the skilled person.

Polypeptides may be prepared by chemical synthesis, e.g. liquid or solid phase synthesis. For example, peptides/polypeptides can be synthesised using the methods described in, for example, Chandrudu et al., Molecules (2013), 18: 4373-4388, which is hereby incorporated by reference in its entirety.

Alternatively, antigen-binding molecules, immunogens and polypeptides may be produced by recombinant expression. Molecular biology techniques suitable for recombinant production of polypeptides are well known in the art, such as those set out in Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th Edition), Cold Spring Harbor Press, 2012, and in Nat Methods. (2008); 5(2): 135-146 both of which are hereby incorporated by reference in their entirety. Methods for the recombinant production of antigen-binding molecules are also described in Frenzel et al., Front Immunol. (2013); 4: 217 and Kunert and Reinhart, Appl Microbiol Biotechnol. (2016) 100: 3451-3461, both of which are hereby incorporated by reference in their entirety.

In some cases the antigen-binding molecules of the present invention are comprised of more than one polypeptide chain. In such cases, production of the antigen-binding molecules may comprise transcription and translation of more than one polypeptide, and subsequent association of the polypeptide chains to form the antigen-binding molecule.

For recombinant production according to the invention, any cell suitable for the expression of polypeptides may be used. The cell may be a prokaryote or eukaryote. In some embodiments the cell is a prokaryotic cell, such as a cell of archaea or bacteria. In some embodiments the bacteria may be Gram-negative bacteria such as bacteria of the family Enterobacteriaceae, for example *Escherichia coli*. In
5 some embodiments, the cell is a eukaryotic cell such as a yeast cell, a plant cell, insect cell or a mammalian cell, e.g. CHO, HEK (e.g. HEK293), HeLa or COS cells. In some cases the cell is not a prokaryotic cell because some prokaryotic cells do not allow for the same folding or post-translational modifications as eukaryotic cells. In addition, very high expression levels are possible in eukaryotes and
10 proteins can be easier to purify from eukaryotes using appropriate tags. Specific plasmids may also be utilised which enhance secretion of the protein into the media.

In some embodiments polypeptides may be prepared by cell-free-protein synthesis (CFPS), e.g. according using a system described in Zemella et al. *Chembiochem* (2015) 16(17): 2420-2431, which is
15 hereby incorporated by reference in its entirety.

Production may involve culture or fermentation of a eukaryotic cell modified to express the polypeptide(s) of interest. The culture or fermentation may be performed in a bioreactor provided with an appropriate supply of nutrients, air/oxygen and/or growth factors. Secreted proteins can be collected by partitioning
20 culture media/fermentation broth from the cells, extracting the protein content, and separating individual proteins to isolate secreted polypeptide(s). Culture, fermentation and separation techniques are well known to those of skill in the art, and are described, for example, in Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (4th Edition; incorporated by reference herein above).

25 Bioreactors include one or more vessels in which cells may be cultured. Culture in the bioreactor may occur continuously, with a continuous flow of reactants into, and a continuous flow of cultured cells from, the reactor. Alternatively, the culture may occur in batches. The bioreactor monitors and controls environmental conditions such as pH, oxygen, flow rates into and out of, and agitation within the vessel such that optimum conditions are provided for the cells being cultured.

30 Following culturing the cells that express the antigen-binding molecule/immunogen/polypeptide(s), the polypeptide(s) of interest may be isolated. Any suitable method for separating proteins from cells known in the art may be used. In order to isolate the polypeptide it may be necessary to separate the cells from nutrient medium. If the polypeptide(s) are secreted from the cells, the cells may be separated by
35 centrifugation from the culture media that contains the secreted polypeptide(s) of interest. If the polypeptide(s) of interest collect within the cell, protein isolation may comprise centrifugation to separate cells from cell culture medium, treatment of the cell pellet with a lysis buffer, and cell disruption e.g. by sonification, rapid freeze-thaw or osmotic lysis.

40 It may then be desirable to isolate the polypeptide(s) of interest from the supernatant or culture medium, which may contain other protein and non-protein components. A common approach to separating protein

components from a supernatant or culture medium is by precipitation. Proteins of different solubilities are precipitated at different concentrations of precipitating agent such as ammonium sulfate. For example, at low concentrations of precipitating agent, water soluble proteins are extracted. Thus, by adding different increasing concentrations of precipitating agent, proteins of different solubilities may be distinguished.

5 Dialysis may be subsequently used to remove ammonium sulfate from the separated proteins.

Other methods for distinguishing different proteins are known in the art, for example ion exchange chromatography and size chromatography. These may be used as an alternative to precipitation, or may be performed subsequently to precipitation.

10

Once the polypeptide(s) of interest have been isolated from culture it may be desired or necessary to concentrate the polypeptide(s). A number of methods for concentrating proteins are known in the art, such as ultrafiltration or lyophilisation.

15 Compositions

The present invention also provides compositions comprising the antigen-binding molecules, immunogens, polypeptides, nucleic acids, expression vectors and cells described herein.

20 The antigen-binding molecules, immunogens, polypeptides, nucleic acids, expression vectors and cells described herein may be formulated as pharmaceutical compositions or medicaments for clinical use and may comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The composition may be formulated for topical, parenteral, systemic, intracavitary, intravenous, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, intratumoral, subcutaneous, intradermal, intrathecal, oral or transdermal routes of administration which may include injection or infusion.

25

Suitable formulations may comprise the antigen-binding molecule or immunogen in a sterile or isotonic medium. Medicaments and pharmaceutical compositions may be formulated in fluid, including gel, form. Fluid formulations may be formulated for administration by injection or infusion (e.g. via catheter) to a selected region of the human or animal body.

30

In accordance with the invention described herein methods are also provided for the production of pharmaceutically useful compositions, such methods of production may comprise one or more steps selected from: producing an antigen-binding molecule, immunogen, polypeptide, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein; isolating an antigen-binding molecule, immunogen, polypeptide, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein; and/or mixing antigen-binding molecule, immunogen, polypeptide, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

35

40 For example, a further aspect the invention described herein relates to a method of formulating or producing a medicament or pharmaceutical composition for use in the treatment of a disease/condition,

the method comprising formulating a pharmaceutical composition or medicament by mixing an antigen-binding molecule, immunogen, polypeptide, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

5

Therapeutic and prophylactic applications

The antigen-binding molecules, polypeptide complexes, immunogens, polypeptides and pharmaceutical compositions described herein find use in therapeutic and prophylactic applications.

10 The present invention provides an antigen-binding molecule, immunogen, polypeptide complex, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein for use in a method of medical treatment or prophylaxis. Also provided is the use of an antigen-binding molecule, immunogen, polypeptide complex, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein in the manufacture of a medicament for treating or
15 preventing a disease or condition. Also provided is a method of treating or preventing a disease or condition, comprising administering to a subject a therapeutically or prophylactically effective amount of an antigen-binding molecule, immunogen, polypeptide complex, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein.

20 The methods may be effective to reduce the development or progression of a disease/condition, alleviation of the symptoms of a disease/condition or reduction in the pathology of a disease/condition. The methods may be effective to prevent progression of the disease/condition, e.g. to prevent worsening of, or to slow the rate of development of, the disease/condition. In some embodiments the methods may lead to an improvement in the disease/condition, e.g. a reduction in the symptoms of the
25 disease/condition or reduction in some other correlate of the severity/activity of the disease/condition. In some embodiments the methods may prevent development of the disease/condition at a later stage (e.g. a chronic stage or metastasis).

It will be appreciated that the antigen-binding molecules and polypeptide complexes of the present
30 invention may be used for the treatment/prevention of any disease/condition that would derive therapeutic or prophylactic benefit from a reduction in the number and/or activity of cells comprising/expressing the target antigen for which the antigen-binding molecule is specific.

Similarly, it will be appreciated that the immunogens of the present invention may be used for the
35 treatment/prevention of any disease/condition that would derive therapeutic or prophylactic benefit from a reduction in the number and/or activity of cells comprising/expressing the target antigen corresponding to the antigenic sequence of the immunogen.

For example, the disease/condition may be a disease/condition in which cells expressing the target
40 antigen are pathologically implicated, e.g. a disease/condition in which an increased number/proportion of cells expressing the target antigen is positively associated with the onset, development or progression of

the disease/condition, and/or severity of one or more symptoms of the disease/condition, or for which an increased number/proportion of cells expressing the target antigen, is a risk factor for the onset, development or progression of the disease/condition.

- 5 In some embodiments, the disease/condition to be treated/prevented in accordance with the present invention is a disease/condition characterised by an increase in the number/proportion/activity of cells expressing the target antigen, e.g. as compared to the number/proportion/activity of cells expressing the target antigen in the absence of the disease/condition.
- 10 The treatment/prevention may be aimed at one or more of: delaying/preventing the onset/progression of symptoms of disease/condition, reducing the severity of symptoms of the disease/condition, reducing the survival/growth/activity/number of effectors of the disease/condition, and/or increasing survival of the subject.
- 15 In some embodiments the target antigen is an antigen of a pathogen (e.g. as described hereinabove), and the disease/condition to be treated/prevented is a disease/condition which is caused or exacerbated by infection with the pathogen, a disease for which infection with the pathogen is a risk factor and/or a disease for which infection with the pathogen is positively associated with disease onset, development, progression and/or severity. A subject may be determined to be infected with the pathogen or to have the
- 20 have the disease/condition by analysis of a sample obtained from the subject.

- In some embodiments the target antigen is a cancer-associated antigen (e.g. as described hereinabove), and the disease/condition to be treated/prevented is a cancer. The cancer may be any unwanted cell proliferation (or any disease manifesting itself by unwanted cell proliferation), neoplasm or tumor. The
- 25 cancer may be benign or malignant and may be primary or secondary (metastatic). A neoplasm or tumor may be any abnormal growth or proliferation of cells and may be located in any tissue. The cancer may be of tissues/cells derived from e.g. the adrenal gland, adrenal medulla, anus, appendix, bladder, blood, bone, bone marrow, brain, breast, cecum, central nervous system (including or excluding the brain) cerebellum, cervix, colon, duodenum, endometrium, epithelial cells (e.g. renal epithelia), gallbladder,
 - 30 oesophagus, glial cells, heart, ileum, jejunum, kidney, lacrimal gland, larynx, liver, lung, lymph, lymph node, lymphoblast, maxilla, mediastinum, mesentery, myometrium, nasopharynx, omentum, oral cavity, ovary, pancreas, parotid gland, peripheral nervous system, peritoneum, pleura, prostate, salivary gland, sigmoid colon, skin, small intestine, soft tissues, spleen, stomach, testis, thymus, thyroid gland, tongue, tonsil, trachea, uterus, vulva, white blood cells. Tumors to be treated may be nervous or non-nervous
 - 35 system tumors. Nervous system tumors may originate either in the central or peripheral nervous system, e.g. glioma, medulloblastoma, meningioma, neurofibroma, ependymoma, Schwannoma, neurofibrosarcoma, astrocytoma and oligodendroglioma. Non-nervous system cancers/tumors may originate in any other non-nervous tissue, examples include melanoma, mesothelioma, lymphoma, myeloma, leukemia, Non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, chronic myelogenous
 - 40 leukemia (CML), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), cutaneous T-cell lymphoma (CTCL), chronic lymphocytic leukemia (CLL), hepatoma, epidermoid carcinoma, prostate

carcinoma, breast cancer, lung cancer, colon cancer, ovarian cancer, pancreatic cancer, thymic carcinoma, NSCLC, hematologic cancer and sarcoma. The treatment/prevention may be aimed at one or more of: delaying/preventing the onset/progression of symptoms of the cancer, reducing the severity of symptoms of the cancer, reducing the survival/growth/invasion/metastasis of cells of the cancer, reducing
5 the number of cells of the cancer and/or increasing survival of the subject.

In some embodiments, the cancer to be treated/prevented comprises cells expressing or overexpressing the target antigen. Overexpression can be determined by detection of a level of expression (gene/protein expression) of the target antigen which is greater than the level of expression by equivalent, non-
10 cancerous cells/non-tumor tissue. A subject may be determined to have a cancer expressing or overexpressing the target antigen by analysis of a sample obtained from the subject.

Administration of the articles of the present invention is preferably in a "therapeutically effective" or "prophylactically effective" amount, this being sufficient to show therapeutic or prophylactic benefit to the
15 subject. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease/condition and the particular article administered. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disease/disorder to be treated, the condition of the individual subject, the site of delivery, the method of administration and other factors known to
20 practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

Administration may be alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. The antigen-binding molecule or composition
25 described herein and a therapeutic agent may be administered simultaneously or sequentially.

In some embodiments, the methods comprise additional therapeutic or prophylactic intervention, e.g. where the methods is for the treatment/prevention of a cancer. In some embodiments, the additional therapeutic or prophylactic intervention is selected from chemotherapy, immunotherapy, radiotherapy,
30 surgery, vaccination and/or hormone therapy.

Multiple doses of the antigen-binding molecule, immunogen, polypeptide, polypeptide complex, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition according to the invention may be provided. One or more, or each, of the doses may be accompanied by simultaneous or
35 sequential administration of another therapeutic/prophylactic agent. Multiple doses may be separated by a predetermined time interval, which may be selected to be one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days, or 1, 2, 3, 4, 5, or 6 months. By way of example, doses may be given once every 7, 14, 21 or 28 days (plus or minus 3, 2, or 1 days).

40 The immunogens and polypeptide complexes according to the present invention are useful as vaccines. The immunogens and polypeptide complexes can be used to generate immunity to a disease/condition in

which the target antigen is implicated. Accordingly, the present invention provides a vaccine comprising, methods for vaccination using, and the use as a vaccine of, an immunogen or a polypeptide complex in accordance with any embodiment as described herein.

- 5 In some embodiments multiple, different immunogens/polypeptide complexes may be used in a vaccine or vaccination according to the present invention. Such vaccines/vaccination may therefore be useful to vaccinate against multiple diseases/conditions. The skilled person is readily able to determine suitable formulations for vaccines and schedules for vaccination in accordance with the present invention, e.g. by reference to Vaccines (6th Edn.) Plotkin et al. 2012, Elsevier Saunders, which is hereby incorporated by
10 reference in its entirety.

Subjects

- The subject in accordance with aspects the invention described herein may be any animal or human. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but
15 is more preferably human. The subject may be male or female. The subject may be a patient. A subject may have been diagnosed with a disease or condition requiring treatment (e.g. a cancer), may be suspected of having such a disease/condition, or may be at risk of developing/contracting such a disease/condition.
- 20 In embodiments according to the present invention the subject is preferably a human subject. In some embodiments, the subject to be treated according to a therapeutic or prophylactic method of the invention herein is a subject having, or at risk of developing, a disease/condition. In embodiments according to the present invention, a subject may be selected for treatment according to the methods based on characterisation for certain markers of such disease/condition.

25

Kits

- In some aspects of the invention described herein a kit of parts is provided. In some embodiments the kit may have at least one container having a predetermined quantity of an antigen-binding molecule, immunogen, polypeptide, polypeptide complex, nucleic acid (or plurality thereof), expression vector (or
30 plurality thereof), cell or composition described herein.

- In some embodiments, the kit may comprise materials for producing an antigen-binding molecule, immunogen, polypeptide, polypeptide complex, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein. In some embodiment the kit additionally comprises
35 instructions for administration to a patient in order to treat or prevent a specified disease/condition.

Sequence identity

- As used herein, 'sequence identity' refers to the percent of nucleotides/amino acid residues in a subject sequence that are identical to nucleotides/amino acid residues in a reference sequence, after aligning the
40 sequences and, if necessary, introducing gaps, to achieve the maximum percent sequence identity between the sequences. Pairwise and multiple sequence alignment for the purposes of determining

- percent sequence identity between two or more amino acid or nucleic acid sequences can be achieved in various ways known to a person of skill in the art, for instance, using publicly available computer software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960), T-coffee (Notredame et al. 2000, J. Mol. Biol. (2000) 302, 205-217), Kalign (Lassmann and Sonnhammer 2005, BMC Bioinformatics, 6(298)) and MAFFT (Kato and Standley 2013, Molecular Biology and Evolution, 30(4) 772–780 software. When using such software, the default parameters, e.g. for gap penalty and extension penalty, are preferably used.

Sequences

SEQ ID NO:	DESCRIPTION	SEQUENCE
1	Human TRIM21 isoform 1 (UniProt: P19474-1, v1)	MASAA RL TMMWEEVTCPICLDPFVEPVSI ECGHSFCQECISQVGKGGGSVCPVCRQRFL LK NLRPNRQLANMVNNLKEISQEAREGTQGGERCAVHGERLHLFCEKDGKALCWVCAQSRKHR DHAMVPLEEAAQEYQEKLQVALGELRRKQELAEKLEVEIAIKRADWKKTVETQKSRIHA EFV QQKNFLVEEEQRQLQLEKDEREQLRILGEKEAKLAQQSQALQELISELDRRCHSSALELLQ EVIIVLRSSESWN LKDL DITSPELRSVCHVPGLKKMLRTCAVHITLDPDTANPWLILSEDRRQ VRLGDTQQSIPGNEERFDSYPMVLGAQHFGHSGKHYWEVDVTGKEAWDLGVCRDSVRRKG HFLSSKSGFWTIWLWNKQKYEAGTYPQTPLHLQVPPCQVGIFLDYEAGMVSFYNITDHGS LIYSFSECAFTGPLRPFFSPGFNDGGKNTAPLTL CPLNIGSQGSTDY
2	Human TRIM21 isoform 2 (UniProt: P19474-2)	MASAA RL TMMWEEVTCPICLDPFVEPVSI ECGHSFCQECISQVGKGGGSVCPVCRQRFL LK NLRPNRQLANMVNNLKEISQEAREGTQGGERCAVHGERLHLFCEKDGKALCWVCAQSRKHR DHAMVPLEEAAQEYQEKLQVALGELRRKQELAEKLEVEIAIKRADWKEVIVLRSSESWN LK DLDITSPELRSVCHVPGLKKMLRTCAVHITLDPDTANPWLILSEDRRQVRLGDTQQSIPGNE ERFDSYPMVLGAQHFGHSGKHYWEVDVTGKEAWDLGVCRDSVRRKGHFLSSKSGFWTIW LWNKQKYEAGTYPQTPLHLQVPPCQVGIFLDYEAGMVSFYNITDHGSLIYSFSECAFTGPLR PFFSPGFNDGGKNTAPLTL CPLNIGSQGSTDY
3	TRIM21 RING domain (positions 16 to 55 of UniProt: P19474-1)	CPICLDPFVEPVSI ECGHSFCQECISQVGKGGGSVCPVCR
4	TRIM21 B-box (positions 92 to 123 of UniProt: P19474-1)	CAVHGERLHLFCEKDGKALCWVCAQSRKHRDH
5	TRIM21 Coiled coil domain (positions 128 to 238 of UniProt: P19474-1)	LEEAAQEYQEKLQVALGELRRKQELAEKLEVEIAIKRADWKKTVETQKSRIHA EFVQQKNFL VEEQRQLQLEKDEREQLRILGEKEAKLAQQSQALQELISELDRRCHS
6	TRIM21 PRYSPRY domain (positions 268 to 465 of UniProt: P19474-1)	ELRSVCHVPGLKKMLRTCAVHITLDPDTANPWLILSEDRRQVRLGDTQQSIPGNEERFDSY P MVLGAQHFGHSGKHYWEVDVTGKEAWDLGVCRDSVRRKGHFLSSKSGFWTIWLWNKQKY EAGTYPQTPLHLQVPPCQVGIFLDYEAGMVSFYNITDHGSLIYSFSECAFTGPLRPFFSPGF NDGGKNTAPLTL CPL
7	Human IgG1 constant region (IGHG1; UniProt:P01857-1, v1)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCPPCPAPELLGGPSV FLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
8	CH2 IgG1 (positions 111-223 of P01857-1, v1)	PCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
9	CH3 IgG1 (positions 224-330 of P01857-1, v1)	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS D GSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
10	CH2-CH3 IgG1 (positions 111-330 of P01857-1, v1)	PCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLT VDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
11	Human IgG2 constant region (IGHG2; UniProt:P01859-1, v2)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSVVTPSSNFGTQTYTCNVDHKPSNTKVDKTVKRCCKVECPCCPAPPVAGPSVFLF PPKPKD TLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDISVEWESNGQPENNYKTTTPMLDSGDSFFLYSKLTVDKSRWQQGNVFS C SVMHEALHNHYTQKSLSLSPGK
12	CH2 IgG2 (positions 111-	APPVAGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPR

	219 of P01859-1, v2)	EEQFNSTFRVSVSLTVVHQDWLNGKEYCKVSNKGLPAPIEKTISKTK
13	CH3 IgG2 (positions 220-326 of P01859-1, v2)	GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTTPMLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKLSLSLSPGK
14	CH2-CH3 IgG2 (positions 111-326 of P01859-1, v2)	APPVAGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTFRVSVSLTVVHQDWLNGKEYCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPS REEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTTPMLDSGDSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKLSLSLSPGK
15	Human IgG3 constant region (IGHG3; UniProt:P01860-1, v2)	ASTKGPSVFPLAPCSRSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSCDT PPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDITLM SRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVSVSLTVLHQDW LNGKEYCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESSGQPENNYNTTPMLDSGDSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNR FTQKLSLSLSPGK
16	CH2 IgG3 (positions 161-270 of P01860-1, v2)	APELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKP REEQYNSTFRVSVSLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKTK
17	CH3 IgG3 (positions 271-376 of P01860-1, v2)	GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLDS DGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKLSLSLSPG
18	CH2-CH3 IgG3 (positions 161-376 of P01860-1, v2)	APELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKP REEQYNSTFRVSVSLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKTKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLDSGDSFFLYSKLTVDK SRWQQGNIFSCSVMHEALHNRFTQKLSLSLSPG
19	Human IgG4 constant region (IGHG4; UniProt:P01861-1, v1)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLF PPKPKDITLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSSEEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSC SVMHEALHNHYTQKLSLSLGLK
20	CH2 IgG4 (positions 111-220 of P01861-1, v1)	APEFLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVSVSLTVLHQDWLNGKEYCKVSNKGLPSSIEKTIKAK
21	CH3 IgG4 (positions 221-327 of P01861-1, v1)	GQPREPQVYTLPPSSEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKLSLSLGLK
22	CH2-CH3 IgG4 (positions 111-327 of P01861-1, v1)	APEFLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVSVSLTVLHQDWLNGKEYCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPP SSEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDK SRWQEGNVFSCSVMHEALHNHYTQKLSLSLGLK
23	Human IgA1 constant region (IGHA1; UniProt:P01876-1, v2)	ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFPPQEPLSVTVWSESGQGVTVARNFPPSQDASG DLTYTSSQLTLPATQCCLAGKSVTCHVKHYTNPSQDVTVPCTPPTPTPTPTPTPTPTSCC HPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGPPERDLCGCYSV SSVSLPGCAEPWNHKGKFTTCTAAYPESTKPLTATLSKSGNTRFEVHLLPPPSEELALNELV TLTCLARGFSPKDVLRVWLQGSQELPREKYLTVASRQEPSQGTTFFAVTSILRVAEEDWKK GDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVMAEVDGTCY
24	Human IgA2 constant region (IGHA2; UniProt:P01877-1, v4)	ASPTSPKVFPLSLDSTPDQGNVVVACLVGQFFPQEPLSVTVWSESGQGVTVARNFPPSQDAS GDLYTSSQLTLPATQCPCDGSVTCVVKHYTNSSQDVTVPCRVPPPPPCCHPRLSLHRPAL EDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGPPERDLCGCYSVSSVSLPGCAQP WNHGETFTCTAAHPELKTPLTANITKSGNTRFEVHLLPPPSEELALNELVTLTCLARGFSPK DVLVRWLQGSQELPREKYLTVASRQEPSQGTTTYAVTSILRVAEEDWKKGETFSCMVGHE ALPLAFTQKTIDRMAGKPTHINVSVMMAEADGTCY
25	Human IgD constant region (IGHD; UniProt:P01880-1, v3)	APTKAPDVFPFIISGCRHPKDNSPVVLACLITGYHPTSVTVTWYMGTSQSPQRTFPEIQRRDS YYMTSSQLSTPLQQWRQGEYKCVVQHTASKSKKEIFRWEPSPKAQASSVPTAQPAQEGSL AKATTAPATNTNRDLGGEKKKEKEKEKEETTERTECPESQTLQGVYLLTPAVQDLWLRL DKATFTCFVVGSGDKDAHLTWEVAGKEVPTGGVEEGLERHSNGSQSHSRLTLPRSLWNA GTSVTCTLNHPSLPPQRLMALREPAQAQPVKLSNLLASSDPPEAASWLLCEVSGFSPPNIL LMWLEDQREVNTSGFAPARPPPPQRSTTFWAWWSLVRVAPPSPQPATYTCVVSHEDSRTL LNASRLEVSVDTHGPMK
26	Human IgE constant region (IGHE; UniProt:P01854-1, v1)	ASTQSPSVFPLTRCKNIPSNATSVTLGCLATGYFPEPVMVTVWDTGSLNGTMTTLPATTLTL SGHYATISLLTVSGAWAKQMFTCRVAHTPSSTDWVDNKTFSVCSRDFTPPTVKILQSSCDG GGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVLDSTASTTQEGELASTQSELTLSQKHV LSDRTYTCQVTYQGHTFEDSTKCKADSNPRGVSAYLSRSPFDLFIKRSPTITCLVDLAPS KGTVNLTVWSRASGKPVNHSTRKEKQKRNGLTVTSLTPVGRDWDIEGETYQCRVTHPLP RALMRSTTKTSGPRAAPEVYAFATPEWPGRSDKRTLACLIQNFMPEDISVQWLHNEVQLPD ARHSTTPRRTKSGSGFFVFSRLEVTRAWEQKDEFICRAVEAASPSQTVQRAVSVNPGK
27	Human IgM constant region (IGHM; UniProt:P01871-1, v4)	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRG GKYAATSQVLLPSKDVMQQTDHEHVVKVQHPNGNEKNVLPVIAELPPKVSFVPPRDGF FGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSVTTDQVQAEAKESGPTTYKYVTSTLTIK ESDWLQGSMTCTCRVDHRLTFFQQNASMCPVDPQDTAIRVFAIPPSFASIFLTKSTKLTLCTV LTLTYDSVTISWTRQNGEAVKHTNISESHPNATFSVAEASICEDDWSNGERFCTVTHTD LPSPKQTIISRPKGVALHRPDVYLLPPAREQLNRESATICTLVTFSPADVFQVQMQRGQ

		LSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKS TGKPTLYNVSLVMSDTAGTCY
28	CH2 IgM (positions 106-217 of P01871-1, v4)	IAELPPKVSFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVSGSVTTDQVQA EAKESGPTTYKVTSTLTIKESDWLGQSMFTCRVDHRLTFQQNASSMCPD
29	CH3 IgM (positions 218-323 of P01871-1, v4)	QDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTYYDSVTISWTRQNGEAVKTHTNISESHPNATF SAVGEASICEDDWNSGERFTCTVTHTDLPSPKQTIISRPKG
30	CH4 IgM (positions 324-452 of P01871-1, v4)	VALHRPDVYLLPPAREQLNLRRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMP EPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHEALPNRVTERTVDKSTGKPTLYNVSLVMS SDTAGTC
31	CH2-CH3-CH4 IgM (positions 106-452 of P01871-1, v4)	IAELPPKVSFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVSGSVTTDQVQA EAKESGPTTYKVTSTLTIKESDWLGQSMFTCRVDHRLTFQQNASSMCPDQDTAIRVFAIP PSFASIFLTKSTKLTCLVTDLTYYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICE DDWNSGERFTCTVTHTDLPSPKQTIISRPKGVALHRPDVYLLPPAREQLNLRRESATITCLVT GFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCV VAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTC
32	CH2-CH3 IgG1_256P/433S/434H/44 0G	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALSHHYTQKLSLSPGK
33	CH2-CH3 IgG1_256P/433S/434H	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALSHHYTQKLSLSPGK
34	CH2-CH3 IgG1_256P/433S/434H/44 0I	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALSHHYTQKLSLSPGK
35	CH2-CH3 IgG1_256P/433S/434H/44 0R	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALSHHYTQKLSLSPGK
36	CH2-CH3 IgG1_256P/433S/434H/43 5L/440Y	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALSHLYTQKLSLSPGK
37	CH2-CH3 IgG1_256P/433S/434H/43 6F	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALSHHYTQKLSLSPGK
38	CH2-CH3 IgG1_256A/433S/434H	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALSHHYTQKLSLSPGK
39	CH2-CH3 IgG1_256S/433S/434H/44 0N	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALSHHYTQKLSLSPGK
40	CH2-CH3 IgG1_256P/433V/434H	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALVHHYTQKLSLSPGK
41	CH2-CH3 IgG1_256P/433V/434H/44 0R	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALVHHYTQKLSLSPGK
42	CH2-CH3 IgG1_256P/433V/434H/43 6F	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALVHHYTQKLSLSPGK
43	CH2-CH3 IgG1_256P/433V/434H/44 0V	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALVHHYTQKLSLSPGK
44	CH2-CH3 IgG1_256A/433V/434H/44 0G	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVHLQDVLNGKEYCKKVSNNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALVHHYTQKLSLSPGK
45	CH2-CH3	PCPAPELLGGPSVFLFPPKPKDLMISRPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK

	IgG1_433V/434H/436F	TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVHHFTQKSLSLSPGK
46	CH2-CH3 IgG1_256P/434H	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHHHYTQKSLSLSPGK
47	CH2-CH3 IgG1_256P/434H/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHHHYTQKRLSLSPGK
48	CH2-CH3 IgG1_256P/434H/440I	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHHHYTQKILSLSPGK
49	CH2-CH3 IgG1_256P/434H/436T/440N	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHHHTTQKNLSLSPGK
50	CH2-CH3 IgG1_256P/434H/440T	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHHHYTQKTLSLSPGK
51	CH2-CH3 IgG1_256P/433A/434H/440T	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALAHHYTQKTLSLSPGK
52	CH2-CH3 IgG1_256P/433D/434H/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALDHHYTQKRLSLSPGK
53	CH2-CH3 IgG1_256P/433D/434H	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALDHHYTQKSLSLSPGK
54	CH2-CH3 IgG1_256P/433D/434H/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALDHHFTQKSLSLSPGK
55	CH2-CH3 IgG1_256P/433P/434H	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALPHHYTQKSLSLSPGK
56	CH2-CH3 IgG1_256P/433Q/434H	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALQHHTTQKSLSLSPGK
57	CH2-CH3 IgG1_256P/433T/434H	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHHHTTQKSLSLSPGK
58	CH2-CH3 IgG1_256A/434H	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHHHTTQKSLSLSPGK
59	CH2-CH3 IgG1_256A/433Q/434H/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALQHHTTQKSLSLSPGK
60	CH2-CH3 IgG1_256A/433T/434H	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTHHTTQKSLSLSPGK
61	CH2-CH3 IgG1_433T/434H/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTHHTTQKSLSLSPGK
62	CH2-CH3	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK

	IgG1_433T/434H	TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
63	CH2-CH3 IgG1_256P/433T/434R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
64	CH2-CH3 IgG1_256P/433T/434R/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
65	CH2-CH3 IgG1_256P/433T/434R/436L	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
66	CH2-CH3 IgG1_256P/433T/434R/440I	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
67	CH2-CH3 IgG1_256P/433T/434R/436F/440G	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
68	CH2-CH3 IgG1_433T/434R/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
69	CH2-CH3 IgG1_433T/434R	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
70	CH2-CH3 IgG1_433T/434R/436W	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
71	CH2-CH3 IgG1_256P/433T/434R/436F/440I (also referred to herein as "PN04-90")	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
72	CH2-CH3 IgG1_433T/434R/436F/440I (also referred to herein as "V5")	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
73	CH2-CH3 IgG1_256P/434R/436F/440I (also referred to herein as "V1")	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
74	CH2-CH3 IgG1_256P/433T/436F/440I (also referred to herein as "V2")	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
75	CH2-CH3 IgG1_256P/433T/434R/440I (also referred to herein as "V3")	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
76	CH2-CH3 IgG1_256P/433T/434R/436F (also referred to herein as "V4")	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALTRHHTQKSLSLSPGK
77	CH2-CH3 IgG1_256P/433V/434R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHHTQKSLSLSPGK
78	CH2-CH3 IgG1_256P/433V/434R/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHHTQKSLSLSPGK
79	CH2-CH3	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK

	IgG1_256P/433V/434R/440G	TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHYTQKLSLSPGK
80	CH2-CH3 IgG1_256P/433V/434R/436L	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHLTQKLSLSPGK
81	CH2-CH3 IgG1_256P/433V/434R/436F/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHFTQKRLSLSPGK
82	CH2-CH3 IgG1_256P/433V/434R/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHYTQKRLSLSPGK
83	CH2-CH3 IgG1_256P/433V/434R/440P	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHYTQKPLSLSPGK
84	CH2-CH3 IgG1_256A/433V/434R/436T/440N	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHTTQKNLSLSPGK
85	CH2-CH3 IgG1_256A/433V/434R/440I	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHYTQKILSLSPGK
86	CH2-CH3 IgG1_433V/434R/436T/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHTTQKRLSLSPGK
87	CH2-CH3 IgG1_433V/434R/440I	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHYTQKILSLSPGK
88	CH2-CH3 IgG1_433V/434R/436T	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHTTQKLSLSPGK
89	CH2-CH3 IgG1_433V/434R/440V	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHYTQKVLSPGK
90	CH2-CH3 IgG1_433V/434R	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHYTQKLSLSPGK
91	CH2-CH3 IgG1_433V/434R/435L	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRLYTQKLSLSPGK
92	CH2-CH3 IgG1_433V/434R/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALVRHYTQKRLSLSPGK
93	CH2-CH3 IgG1_256P/433I/434R/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALIRHFTQKLSLSPGK
94	CH2-CH3 IgG1_256P/433I/434R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALIRHYTQKLSLSPGK
95	CH2-CH3 IgG1_256P/436F/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
96	CH2-CH3	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK

	IgG1_256P/436F/440T	TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKTLSPGK
97	CH2-CH3 IgG1_256P/436T/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
98	CH2-CH3 IgG1_256P	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKSLSLSPGK
99	CH2-CH3 IgG1_256P/436T	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKSLSLSPGK
100	CH2-CH3 IgG1_256P/436T/440T	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKTLSPGK
101	CH2-CH3 IgG1_256P/440D	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKDLSPGK
102	CH2-CH3 IgG1_256P/436S/440W	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHSTQKWLSPGK
103	CH2-CH3 IgG1_256P/436T/440M	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKMLSPGK
104	CH2-CH3 IgG1_256P/436T/440A	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKALSLSPGK
105	CH2-CH3 IgG1_256P/436T/440D	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKDLSPGK
106	CH2-CH3 IgG1_256P/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
107	CH2-CH3 IgG1_256P/440A	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKALSLSPGK
108	CH2-CH3 IgG1_256P/436L	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKSLSLSPGK
109	CH2-CH3 IgG1_256P/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKSLSLSPGK
110	CH2-CH3 IgG1_256P/440T	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKTLSPGK
111	CH2-CH3 IgG1_256P/436F/440I	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKILSLSPGK
112	CH2-CH3 IgG1_256P/436T/440N	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKNLSLSPGK
113	CH2-CH3	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK

	IgG1_256P/440K	TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHYTQKKLSLSPGK
114	CH2-CH3 IgG1_256P/436S	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHSTQKLSLSPGK
115	CH2-CH3 IgG1_256P/434I	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHIIHTTQKLSLSPGK
116	CH2-CH3 IgG1_256P/434I/436F/440M	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHIIHTTQKMLSLSPGK
117	CH2-CH3 IgG1_256P/434L/436T	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHIIHTTQKLSLSPGK
118	CH2-CH3 IgG1_256P/434L	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHIIHTTQKLSLSPGK
119	CH2-CH3 IgG1_256P/434M/436T/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHMHHTTQKRLSLSPGK
120	CH2-CH3 IgG1_256P/434M	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHMHHTTQKLSLSPGK
121	CH2-CH3 IgG1_256P/436L/440N	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHHTTQKLSLSPGK
122	CH2-CH3 IgG1_256P/436T/440Y	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHHTTQKLSLSPGK
123	CH2-CH3 IgG1_256A/436F/440M	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHHTTQKLSLSPGK
124	CH2-CH3 IgG1_256A/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHHTTQKLSLSPGK
125	CH2-CH3 IgG1_256A/436T	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHHTTQKLSLSPGK
126	CH2-CH3 IgG1_256A/436T/440F	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHHTTQKFLSLSPGK
127	CH2-CH3 IgG1_256A	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHYTQKLSLSPGK
128	CH2-CH3 IgG1_256A/440I	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHYTQKILSLSPGK
129	CH2-CH3 IgG1_256A/440G	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHYTQKGLSLSPGK
130	CH2-CH3	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK

[illegible]

	IgG1_436F/440R	TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
148	CH2-CH3 IgG1_436T	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
149	CH2-CH3 IgG1_434L/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHLLHFTQKRLSLSPGK
150	CH2-CH3 IgG1_434L	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHLLHYTQKRLSLSPGK
151	CH2-CH3 IgG1_434L/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHLLHYTQKRLSLSPGK
152	CH2-CH3 IgG1_434L/436F/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHLLHFTQKRLSLSPGK
153	CH2-CH3 IgG1_434L/435L/440G	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHLLHYTQKRLSLSPGK
154	CH2-CH3 IgG1_434L/440T	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHLLHYTQKRLSLSPGK
155	CH2-CH3 IgG1_434L/440V	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHLLHYTQKRLSLSPGK
156	CH2-CH3 IgG1_434M	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHMHYTQKRLSLSPGK
157	CH2-CH3 IgG1_256V/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRVPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
158	CH2-CH3 IgG1_256G/440M	PCPAPELLGGPSVFLFPPKPKDTLMISRGPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
159	CH2-CH3 IgG1_256I/440N	PCPAPELLGGPSVFLFPPKPKDTLMISRIPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
160	CH2-CH3 IgG1_256K/436F	PCPAPELLGGPSVFLFPPKPKDTLMISRKPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
161	CH2-CH3 IgG1_256N/436T/440N	PCPAPELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
162	CH2-CH3 IgG1_256N	PCPAPELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
163	CH2-CH3 IgG1_256N/440R	PCPAPELLGGPSVFLFPPKPKDTLMISRNPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGQNVFSCSVMEALHNHFTQKRLSLSPGK
164	CH2-CH3 IgG1_256S	PCPAPELLGGPSVFLFPPKPKDTLMISRSPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK

		TKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
165	CH2-CH3 IgG1_256S/436S	PCPAPELLGGPSVFLFPPKPKDTLMISRSPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHSTQKSLSLSPGK
166	CH2-CH3 IgG1_256S/440A	PCPAPELLGGPSVFLFPPKPKDTLMISRSPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKALSLSLSPGK
167	CH2-CH3 IgG1_1-8con	PCPAPELLGGPSVFLFPPKPKDTLMX ₁ SRX ₂ PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALX ₃ X ₄ X ₅ X ₆ TQKX ₇ LSLSLSPGK wherein X ₁ = I or L; X ₂ = P, A, T, V, G, I, K, N or S; X ₃ = S, V, H, A, D, P, Q, T or I; X ₄ = H, R, N, I, L or M; X ₅ = H or L; X ₆ = Y, F, T, L, W or S; and X ₇ = S, G, I, R, Y, N, V, T, P, D, W, M, A, K, F, E or C.
168	CH2-CH3 IgG1_1con	PCPAPELLGGPSVFLFPPKPKDTLMISRX ₈ PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALSHX ₉ X ₁₀ TQKX ₁₁ LSLSLSPGK wherein X ₈ = P, A or S; X ₉ = H or L; X ₁₀ = Y or F; and X ₁₁ = G, S, I, R, Y or N.
169	CH2-CH3 IgG1_2con	PCPAPELLGGPSVFLFPPKPKDTLMISRX ₁₂ PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALVHHX ₁₃ TQKX ₁₄ LSLSLSPGK wherein X ₁₂ = P, A or T; X ₁₃ = Y or F; and X ₁₄ = S, R, V or G.
170	CH2-CH3 IgG1_3con	PCPAPELLGGPSVFLFPPKPKDTLMISRX ₁₅ PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALX ₁₆ HXX ₁₇ TQKX ₁₈ LSLSLSPGK wherein X ₁₅ = P, A or T; X ₁₆ = H, A, D, P, Q or T; X ₁₇ = Y, T or F; and X ₁₈ = S, R, I, N or T.
171	CH2-CH3 IgG1_4con	PCPAPELLGGPSVFLFPPKPKDTLMISRX ₁₉ PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALX ₂₀ X ₂₁ HXX ₂₂ TQKX ₂₃ LSLSLSPGK wherein X ₁₉ = P or T; X ₂₀ = T or H; X ₂₁ = R or N; X ₂₂ = Y, F, L or W; and X ₂₃ = S, I or G.
172	CH2-CH3 IgG1_5con	PCPAPELLGGPSVFLFPPKPKDTLMISRX ₂₄ PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALX ₂₅ RX ₂₆ X ₂₇ TQKX ₂₈ LSLSLSPGK wherein X ₂₄ = P, A or T; X ₂₅ = V or I; X ₂₆ = H or L; X ₂₇ = Y, F, L or T; and X ₂₈ = S, G, R, P, N, I or V.
173	CH2-CH3 IgG1_6con	PCPAPELLGGPSVFLFPPKPKDTLMISRPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHX ₂₉ HXX ₃₀ TQKX ₃₁ LSLSLSPGK wherein X ₂₉ = N, I, L or M; X ₃₀ = F, T, Y, S or L; and X ₃₁ = R, T, S, D, W, M, A, K, N, Y or I.
174	CH2-CH3 IgG1_7con	PCPAPELLGGPSVFLFPPKPKDTLMISRAPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHX ₃₂ HXX ₃₃ TQKX ₃₄ LSLSLSPGK wherein X ₃₂ = N or M; X ₃₃ = F, T or Y; and X ₃₄ = M, S, F, I, G, D or N.
175	CH2-CH3 IgG1_8con	PCPAPELLGGPSVFLFPPKPKDTLMX ₃₅ SRX ₃₆ PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHX ₃₇ X ₃₈ X ₃₉ TQKX ₄₀ LSLSLSPGK wherein X ₃₅ = I or L; X ₃₆ = T, V, G, I, K, N or S; X ₃₇ = N, L or M; X ₃₈ = H or L; X ₃₉ = Y, F, T or

		S _i and X ₄₀ = A, I, T, R, S, E, C, Y, D, N, G, V or M.
176	HLA-A*2401-Ad5 epitope	TYFSLNNKF
177	HLA-A*0201-Ad5 epitope	YVLFEDVDV
178	scrambled peptide	LAVFEDYVAF
179	HLA-A2-HIV epitope	SLYNTVATL
180	CH2-CH3 IgA1	PRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVFTFTWTPSSGKSAVQGPPERDLCGCYSV SSVLPGCAEPWNHGKFTTCTAAYPESKTPLTATLSPEVHLLPPPSEELALNELVTLTCLARG FSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTFFAVTSILRVAEDWKKGDTFSCM VGHEALPLAFTQKTID
181	CH2-CH3 IgA2	PRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVFTFTWTPSSGKSAVQGPPERDLCGCYSV SSVLPGCAEPWNHGKFTTCTAAYPESKTPLTANITPEVHLLPPPSEELALNELVTLTCLARGF SPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTFYAVTSILRVAEDWKKGETFSCMV GHEALPLAFTQKTID
182	CH2-CH3 IgD	PAVQDLWRDKATFTCFVVGSDLDKDAHLTWEVAGKVPTGGVEEGLLERHSHNGSQSHRSL TLPRSLWNAGTSVTCTLNHPSLPQRLMALREPAQAQPVKLSNLLASSDPPEAASWLLCE VSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPRSTTFWAWSVLRVPAPPSPQPATYTC VSHEDSRTLLNAGRSLE
183	CH2-CH3-CH4 IgE	PTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELAST QSELTLSQKHWSLDRYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSFDFIRKSP TITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTSTLPVGTRDWIEGETY QCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQ WLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTV QRAVS
184	TRIM21 PRYSPRY domain insert sequence	VHITLDPDTANPWLILSEDRRQVRLGDTQQSIPGNEERFDSYPMVLGAQHFSHGKHYWEVD VTGKEAWDLGVCRRDSVRRKGHFLSSKSGFWTIWLWNKQKYEAGTYPQTPLHLQVPPCQV GIFLDYEAGMVSYFNITDHGSLIYSFSECAFTGPLRPFSPGFNDGGKNTAPLTLCLPLNIGSQ GSTDY
185	hIgG1 G1m3 allotype CH2-CH3 region	SVFLFPPKPKDLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFPYSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMEALHNHYTQKSLSLSPGK

The invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

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The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word 'comprise,' and variations such as 'comprises' and 'comprising,' will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms 'a,' 'an,' and 'the' include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from 'about' one particular value, and/or to 'about' another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular

value. Similarly, when values are expressed as approximations, by the use of the antecedent 'about,' it will be understood that the particular value forms another embodiment.

Where a nucleic acid sequence is disclosed herein, the reverse complement thereof is also expressly contemplated.

Methods described herein may preferably performed *in vitro*. The term '*in vitro*' is intended to encompass procedures performed with cells in culture whereas the term '*in vivo*' is intended to encompass procedures with/on intact multi-cellular organisms.

Brief Description of the Figures

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures.

Figure 1A to 1I. Images, schematics, tables and schematics relating to analysis of the effect of Fc modifications on Fc affinity for TRIM21. **(1A)** Crystal structure of TRIM21 PRYSPRY domain in complex with Fc region; the position of the amino acids selected for randomization is indicated. **(1B)** shows the amino acid sequence of the CH2-CH3 region; amino acids selected for randomization are underlined. **(1C)** Frequencies of the different clones identified after Round 2 and Round 3 of biopanning. **(1D)** Schematic representation of the two sets of antibodies: anti-DEC205 human IgG1 and anti-Ad5 chimeric IgG1, with modification (squares) or without modification to the Fc region. Figures **(1E-1I)** show the binding and dissociation of TRIM21 to Fc-modified human IgG1 **(1E)**, Fc-modified chimeric IgG1 **(1F)**, Fc-unmodified human IgG1 **(1G)**, Fc-unmodified chimeric IgG1 **(1H)**, and reverse mutant **(1I)** which is the Fc-modified chimeric IgG1 but with the spurious mutation T256P corrected back to threonine. Raw data and curve fittings using Langmuir 1:1 stoichiometry are shown.

Figures 2A to 2C. Histograms, graphs and image showing the results of analysis of the effect of Fc modification on ADIN. **(2A)** Flow cytometry histograms showing the percentage of GFP+ HeLa cells and moDCs 48 h after treatment with no virus, virus alone or virus with antibodies. For HeLa cells an MOI of 1.2 was used and an antibody concentration of 1.25 pM was used. For moDCs, an MOI of 400 was used and an antibody concentration of 55 nM was used. **(2B)** Graphs showing infection of HeLa cells and moDCs with adenovirus (MOI 1.2 and 400 respectively) in the presence of different concentrations of antibodies. The graphs shows the mean relative infection and the SEM of three different donors. **(2C)** Image of western blot detection for human Fab (hFab), TRIM21, and GAPDH in the lysate (L), unbound (U), or eluate (E) fractions of untreated or immune complex-treated moDC (from Donor C103) following coimmunoprecipitation with beads conjugated with anti-human Fab. The result shown is representative of three independent experiments.

Figure 3A to 3D. Bar charts and histograms showing the results of analysis of the effect of Fc modification on moDC maturation. moDCs were treated with Ad5 alone (virus alone), Fc-unmodified immune complexes (unmod IC) or Fc-modified immune complexes (mod IC) at an MOI of 0, 25, 50, 100,

200 and 400 and analysed 24 h after treatment for expression of maturation markers. Immune complexes were formed by pre-incubating the virus and antibody at 5×10^8 virus and 20 μ g antibody per ml. Thereafter the amount of immune complex was added based on the MOI of the virus. LPS (1 μ g/ml) was used as a positive control. **(3A)** Bar charts showing the Median Fluorescent Intensities (MFIs) of the different maturation markers for one representative donor (Donor 22). **(3B)** Histograms showing the Median Fluorescent Intensity MFIs of the different maturation markers when moDCs were treated at the highest dose of 400 MOI virus alone, 55 nM antibody alone or immune complexes of the corresponding dosages for one donor (Donor 21) representative of five donors that responded to the Fc-modified immune complex, and Donor PAT37 that did not respond to the Fc-modified immune complex. **(3C)** Bar charts showing fold increase in MFI relative to treatment with virus alone for moDCs derived from 3 different donors. Each donor is represented by a circle. Error bar shows the SEM. Statistical analyses were performed using an ordinary one-way ANOVA. The adjusted P values are represented by * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. **(3D)** Bar charts showing MFIs of various different maturation markers from 6 different donors. Each donor is represented by a circle. Statistical analyses (repeated measure of ANOVA with Dunnett correction on log of MFI) were performed for treatments indicated with lines. The adjusted P values are represented by * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

Figure 4A to 4D. Heatmap and graphs showing the results of analysis of the effect of Fc modification on expression of soluble factors by moDCs. **(4A)** Fold change in cytokine and chemokine concentration is shown relative to treatment with unmodified immune complex detected in the culture supernatant. The heat map shows the cytokines and chemokines assayed using human Milliplex MAP kits immunology panel 1 and 2, which includes a total of 64 targets. The cytokines and chemokines are ranked by the Spearman rho correlation (for fold change versus dosages). The top nine factors (I.e, CCL3 to TNF- α) show a Spearman rho of > 0.3 at $p < 0.05$. **(4B and 4C)** Concentrations of the chemokines and cytokines CCL3, CCL4, CCL5, TRAIL, IFN- γ , IL-6, TNF- α , SDF-1a+b and MCP-3 are shown. Each donor is represented by a circle. IC: immune complexes. Graphs shows mean and SEM of three donors. Statistical analyses were performed using ordinary one-way ANOVA. For statistical analysis, the adjusted p-values are represented by **** for $p < 0.0001$, *** for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$. **(4D)** Concentration of the seven chemokines and cytokines (CCL3, CCL4, CCL5, CCL7, IL-6, TNF-a, and IFN-g) for 6 donors. Each donor is represented by a circle. Repeated measure of ANOVA with Dunnett correction was performed on the logarithm of the cytokine/chemokine concentrations. Statistical analyses were performed for treatments with lines, and the adjusted P values are represented by * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$ and **** for $p < 0.0001$.

Figure 5A to 5E. Scatterplots and graphs showing the results of analysis of the effect of Fc modification on ability of moDCs to stimulate T cell expansion. **(5A)** Results of flow cytometry analysis of CD4+ and CD8+ cells in moDC:CD14- co-cultures at 13 days post infection of moDC with virus alone, immune complexes or controls. Cells were gated by singlets/live/CD3+CD56-. The numbers in the plots show the percentages of CD4 T cells and CD8 T cells in the total T cell population. **(5B)** Actual numbers of CD4 and CD8 T cells at 13 days post infection. Each point is a replicate of the experiment. Results show the mean and SEM of replicates for one donor (Donor 33). **(5C)** Fold change in T cell count relative

to the PBS treatment for CD4 T cells and CD8 T cells. Each point represents one donor and the line represents the mean of 7 donors. **(5D)** Two groups of donors respond differently to the treatments. Type 1 donors are strong responders. Type 2 donors are poor responders to virus-alone treatment but they show a significant increase in CD8 T cell count when treated with modified Fc-immune complexes.

- 5 Numbers in brackets indicate the mean fold change relative to treatment with PBS. Each point represents one donor. Statistical analyses were performed using ordinary one-way ANOVA. LPS and TransACT were omitted from statistical analysis. **(5E)** Fold change in CD4 T cell count and CD8 T cell count relative to treatment with PBS, unmodified Ab, or Fc-modified Ab, respectively. Each dot represents one donor. Friedman with Dunn correction was performed on the fold change of cell count. Statistical analyses were performed for treatments with lines. For all statistical results, **** represents $p < 0.0001$, *** represents $p < 0.001$, ** represents $p < 0.01$ and * represents $p < 0.05$ and ns is not significant with $p > 0.05$.

- Figure 6A and 6B.** Bar charts and graphs showing the results of analysis of the effect of Fc modification on ability of moDCs to stimulate IFN- γ production in a moDC:CD14- PBMC co-culture. **(6A)** Results of an ELISA analysis at 4, 7 and 13 days post infection by virus, immune complexes or controls. Each graph shows the mean and SEM of triplicates for a responding donor. Bars for PBS to TransACT are shown from left to right, for each period. Statistical analyses were performed using an ANOVA. LPS and TransACT are omitted from all statistical analysis. **(6B)** Levels of IFN- γ for the 7 donors at 7 days post infection for Type 1 donors (showing 3-fold or more increase in CD8 count when treated with virus alone) and Type 2 donors (showing a better response to Fc-modified immune complex than to virus alone), and donor LCY05, displaying high IFN- γ even in response to PBS treatment, and no expansion in CD8 count to all treatments. Each dot represents one donor and the line represents the mean of 7 donors. Repeated measure of ANOVA with Dunnett correction was performed on the logarithm of the IFN- γ concentrations. Statistical analyses were performed for treatments with lines. For all statistical results, **** represents $p < 0.0001$, *** represents $p < 0.001$, ** represents $p < 0.01$ and * represents $p < 0.05$ and ns is not significant with $p > 0.05$.

- Figure 7.** Scatterplots showing CD4 and CD8 T cell responses of donor LCY02 following co-culture for 11 days of CD14- PBMCs with MoDCs which pre-treated with PBS, antibodies, virus alone or immune complexes. After 11 days, co-cultures that received the modified-Fc immune complex treatment display significant increase in the percentage of CD8 T cells.

- Figures 8A to 8D.** Bar charts and scatterplots showing the results of analysis of the effect of Fc modification on ability of moDCs to stimulate expansion of antigen-specific CD8+ T cells. **(8A and 8B)** Results of an ELISA detecting the level of IFN- γ in the supernatant of moDC:CD14- PBMCs co-cultures that were re-stimulated for 16 h with autologous moDCs pulsed with peptides. Results show the means and SEM of duplicates, or of a single well (for A*24-Ad5, Scrambled and HIV gag) of donor LCY02. **(8A)** A*24-Ad5 and A*2-Ad5 are peptides from Adenovirus that are presented by HLA-A24 and HLA-A2 haplotypes, respectively. Scrambled and HIV gag are negative controls. **(8B)** Results of ELISA detecting the level of IFN- γ in the supernatant when moDCs were pulsed with peptide library of 15-mer peptides from the hexon protein of Adenovirus (Ad5 hexon) or the human protein NY-ESO-1 (negative control).

(8C) Results of analysis by flow cytometry of donor LCY02's CD4 and CD8 T cells from CD14- PBMC co-cultures with autologous moDCs that were untreated or treated with modified Fc immune complexes for 11 days, re-stimulated with moDCs pulsed with different peptides for 16 h, and then treated for 5 h with Brefeldin A. Numbers at the bottom show percentages of CFSE-diluted cells in a population of CD8 or CD4 T cells. Numbers at the top show percentage of IFN- γ high cells (responding cells) in the population of CFSE-diluted CD8 or CD4 T cells. (8D) Results of analysis by flow cytometry analysis of CD4 and CD8 T cells from donors LCY10, PAT35, and LCY25, from CD14- PBMC co-cultures with autologous moDC that were treated with modified Fc immune complexes for 11 days, and then re-stimulated for 16 h with moDC pulsed with different peptides in the presence of Brefeldin A. Numbers at the top show percentage of IFN- γ high cells (responding cells) in a population of CFSE-diluted CD8 or CD4 T cells. Asterisks indicated responses that are specific to restimulation by the Ag. (8E) Results of analysis by flow cytometry analysis of CFSE-dilution in CD8 T cells following 11 d of coculture with moDC (Donor LCY25). The moDC were either pretreated with medium alone or medium with MG132 for 1 h and then subjected to a 4-h treatment in medium with PBS, unmodified Abs (unmod Ab), or Fc-modified Abs (Fc-mod Ab) in the presence or absence of adenovirus. TransACT is used as a positive control for T cell proliferation.

Figures 9A and 9B. Alignment of the amino acid sequences for IGHG1, IGHG2, IGHG3, IGHG4, IGHA1, IGHA2, IGHD, IGHE and IGHM performed using ClustalOmega software (Söding, J. 2005, Bioinformatics 21, 951-960).

Figure 10. Bar chart showing relative binding to TRIM21 PRYSPRY domain by Fc molecules having different sequences expressed from the indicated clones, as determined by ELISA. Binding by 4 clones identified by biopanning experiment 1 (see Example 4) to bind TRIM21 PRYSPRY domain with greater affinity than wildtype IgG1 Fc (wildtype Fc) is shown. Binding signal for PN04-90 Fc (modified Fc) is also shown. Filled bars show the signal for binding to 5 μ g/ml biotinylated PRYSPRY domain. Open bars show the signal for binding to 5 μ g/ml anti-FLAG.

Figure 11. Bar chart showing relative binding to TRIM21 PRYSPRY domain by Fc molecules having different sequences expressed from the indicated clones, as determined by ELISA. Binding by 11 clones identified by biopanning experiment 2 (see Example 4) to bind TRIM21 PRYSPRY domain with greater affinity than wildtype IgG1 Fc (wildtype Fc) is shown. Binding signal for PN04-90 Fc (modified Fc) is also shown. Filled bars show the signal for binding to 5 μ g/ml biotinylated PRYSPRY domain. Open bars show the signal for binding to 5 μ g/ml anti-FLAG.

Figures 12A to 12H. Bar charts showing relative binding to TRIM21 PRYSPRY domain by Fc molecules having different sequences expressed from the indicated clones, as determined by ELISA. Binding by 293 clones identified by biopanning experiment 3 (see Example 4) to bind TRIM21 PRYSPRY domain with greater affinity than wildtype IgG1 Fc (wildtype Fc) is shown. Binding signal for PN04-90 Fc (modified Fc) is also shown. Filled bars show the signal for binding to 0.125 μ g/ml biotinylated PRYSPRY domain. Open bars show the signal for binding to 5 μ g/ml anti-FLAG. **12A to 12H** show the results of different ELISAs.

Figures 13A and 13B. Table summarising the sequence information for Fc molecules produced by clones identified as binding to TRIM21 PRYSPRY domain with greater affinity than wildtype IgG1 Fc. The affinity of binding to TRIM21 PRYSPRY domain as determined by Surface Plasmon Resonance analysis for certain of the Fc molecules is also shown.

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Figure 14. Bar charts showing the results of analysis of the effect of Fc modification on moDC maturation. moDCs were treated with PBS, (PBS; negative control), Ad5-specific antibody comprising wildtype IgG1 Fc (WT only), Ad5-specific antibody comprising V1 Fc (V1 only), Ad5-specific antibody comprising PN04-90 Fc (PN04-90 only), Ad5 only (Virus only), Immunocomplex comprising Ad5-specific antibody comprising wildtype IgG1 Fc + Ad5 (WT + Virus), Immunocomplex comprising Ad5-specific antibody comprising V1 Fc + Ad5 (V1 + Virus), Immunocomplex comprising Ad5-specific antibody comprising PN04-90 Fc + Ad5 (PN04-90 + Virus) or LPS only (LPS; positive control), and analysed 24 h after treatment for expression of moDC maturation markers. Bar chart showing the Median Fluorescent Intensities (MFIs) of the different maturation markers for 3 representative donors.

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Figures 15A and 15B. Histograms and graph showing the results of analysis of antibody-dependent intracellular neutralization mediated by unmodified and Fc-modified antibody, as determined by flow cytometry. **(15A)** Expression of eGFP by moDCs in the absence of replication-defective Ad5 carrying eGFP reporter, following infection with the virus at different MOI (multiplicity of infection), or following treatment with immune complexes comprising 9C12-wildtype hlgG1 Fc + virus (Unmod Ab) or 9C12-PN04-90 Fc + virus (Mod A)b at different MOI, for Donor C03. **(15B)** Percentage of eGFP-positive moDCs 48 h after infection with adenovirus alone, or with the immune complexes at different MOI. The MOI is based on the number of infectious units when the virus is added to HEK293T cells. Graph shows the mean percentage and SD for three independent donors (Donors C01, C02 and C03).

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Figures 16A to 16C. Histograms, graphs and intensity plots relating to analysis of the effect of different dosages of Fc-modified immune complexes on moDC phenotype. **(16A)** Median Fluorescent Intensity (MFI) of CD80, CD83, CD86, HLA-DR, CD206 and CCR7 on moDC was analysed using flow cytometry. MoDC were treated for 24 h with different dosages immune complexes with Fc-unmodified or Fc-modified antibodies, or with virus alone. Treatment with PBS or antibodies alone are used as negative controls, and treatment with LPS are used as positive controls. Results from one representative donor (Donor 22) are shown. **(16B)** Differences between surface expression of CD83, CD206 and CCR7 by moDCs subjected to different treatments were not statistically significant. Repeated measure ANOVA with Dunnett correction was performed on the log of the MFI. Statistical analyses were performed only for treatments indicated with lines; n.s. means not significant ($p > 0.05$). **(16C)** Cell purity of CD14⁺ cells after purification. PBMCs from three donors were subjected to CD14⁺ isolation. Cells before and after purification were stained for Live/Dead and CD14 and analysed by flow cytometry. Cells were gated for singlets/live/CD14⁺. The percentage of CD14⁺ cells in the sample before and after purification is shown in the box.

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Figures 17A to 17C. Scatterplots, histograms and bar charts relating to CD4 and CD8 T cell responses. **(17A)** CFSE dilution in T cells following coculture with moDC (Donor PAT35). TransACT is a positive control that stimulates T cells proliferation non-specifically. Results show CFSE dilution for T cells that are co-culture with moDC that were pretreated with virus and Fc-modified antibodies. Re-stimulation of the T cells for 16 h did not lead to further cell division. **(17B)** Viability of CD14- PBMCs after 16 h of Brefeldin A exposure. CD14- PBMCs from Donor PAT43 were cultured either in the presence or absence of TransACT for 2 days to generate activated or non-activated cells respectively. Thereafter, the cells were treated with or without Brefeldin A (BFA) for 16 h and were analysed using Live/Dead stain on flow cytometry. The graph shows the mean number of live cells from triplicate wells. Error bars show the SEM. The numbers of live cells in BFA-treatments are also expressed as a percentage of the no-BFA treatments. **(17C)** Viability of moDCs one day after MG132 treatment. The cells were incubated with or without MG132 for 1 h. After incubation, the cells were washed and resuspended in fresh medium without MG132 and cultured for one day before analysis using Live/Dead stain on flow cytometry. The graph shows the mean number of live cells from triplicate wells. Error bars show the SEM. The number of live cells in MG132-treatment is also expressed as a percentage of the no-MG132 treatment.

Examples

In the following Examples, the inventors describe the engineering of Immunoglobulin Fc to increase its affinity for TRIM21, and analysis of the effect of this improved affinity for TRIM21-mediated functions.

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The entire contents of Ng et al., J Immunol. (2019) 202(8):2307-2319 is specifically incorporated by reference in its entirety.

Example 1: Materials and Methods

25 1.1 PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from apheresis blood of healthy donors using Ficoll-Paque Premium (GE Healthcare).

1.2 MoDCs

30 Monocytes were isolated from PBMCs using CD14 Microbeads (Miltenyi). Monocytes were differentiated into moDC by culture in the presence of 100ng/ml GM-CSF (premium grade, Miltenyi) and 100ng/ml IL-4 (premium grade, Miltenyi) in RPMI-1640 with 25mM HEPES and L-Glutamine (Hyclone) further supplemented with 10% FBS (South American origin, Gibco) and antibiotics comprising 100U/ml Penicillin and 100ug/ml Streptomycin (Gibco). On the fourth day of the culture, one third of the culture medium was replaced with fresh culture medium with the same supplements. After 7 days, moDC (floating cells) from the cell culture were harvested for use in experiments.

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1.3 Cell lines

40 HEK293T cells and HeLa cells were maintained in 1g/L glucose DMEM (Hyclone) supplemented with 10% FBS (South American origin, Gibco) and antibiotics (100U/ml Penicillin, 100ug/ml Streptomycin, Gibco).

1.4 Viruses

Purified E1- and E3-deleted replication-deficient adenovirus type 5 with the eGFP reporter gene (VQAdCMV eGFP, ViraQuest) was used for the antibody-dependent intracellular neutralization (ADIN) assays. The same strain of adenovirus with no eGFP gene (VQAd EMPTY, ViraQuest) was used for all other assays. To titer the virus, HEK293T cells were seeded onto 0.01% poly-L-lysine (Sigma) coated 24-well plates at a cell density of 2.5×10^5 cells/ml. Once the cells had adhered, 10-fold serial dilutions of virus were added to the cell culture and incubated for 2 days. Viral titer was calculated from the number of infected cells as detected by the Adeno-X Rapid Titer Kit (Clontech).

1.5 Human TRIM21 (PRYSPRY domain) protein.

The human recombinant TRIM21 PRYSPRY domain sequence shown in SEQ ID NO:184 was cloned into the 3'-end of a His-tag in the pQE-2 bacteria expression vector, and expressed in *E. coli* BL21 (DE3). A 20 ml overnight culture was inoculated into 380 ml fresh growth medium and grown at 37°C until the OD₆₀₀ reached 1.0. Protein expression was induced with 1 mM IPTG for 4.5 hours at room temperature. The expressed protein was then purified from cell lysates using Ni-NTA resin (Qiagen), followed by gel filtration in PBS using a Superdex 75 chromatography column (GE Healthcare).

1.6 Antibodies

The variable domains of the chimeric anti-adenovirus antibody were cloned from hybridoma 9C12 (TC31-9C12.C9)(Varghese et al., 2004b), obtained from the Developmental Studies Hybridoma bank (NICHD/University of Iowa). The variable domains of the human anti-DEC205 antibody were synthesized based on the published sequence of clone 3G9-2D2 (US patent 8,236,318 B2). The variable domains were cloned into the N-terminal of the constant domains of the human IgG1 in the pTT5 vector. For the Fc-modified antibodies, the 5 amino acid modifications in the constant regions were modified using the Quikchange Lightning Multi Site-Directed Mutagenesis Kit (Agilent Technologies). Antibodies were expressed in HEK293-6E cells or in CHO cells, and purified from their supernatants using Protein G beads. The purified antibodies were buffer-exchanged into 20 mM His, 150 mM NaCl, pH 6.0, then filtered and assayed for endotoxin content using the Limulus Amoebocyte Lysate-QCL1000 (Lonza). Only antibody preparations with an endotoxin content of <1EU/μg were used for cell-based assays.

1.7 Peptides

Peptides were synthesized according to the following sequences: TYFSLNNKF (SEQ ID NO:176; HLA-A*2401-Ad5 epitope), YVLFEVFDVV (SEQ ID NO:177; HLA-A*0201-Ad5 epitope), LAVFEDYVAF (SEQ ID NO:178; scrambled peptide) and SLYNTVATL (SEQ ID NO:179; HLA-A2-HIV epitope).

1.8 Phage display library construction

The PN04-44AD phagemid has the human IgG1 Fc gene encoding amino acid positions 239 to 447 (EU numbering) fused to an amber stop (TAG) followed by a truncated gIII. PN04-44AD was used as the template for making the phage display library using a Kunkel reaction-based site-directed mutagenesis strategy previously described (Tonikian et al., 2007): briefly, a single-stranded template was used for

annealing of oligonucleotides carrying the library sequences; double-stranded DNA was then produced and electroporated into E. coli TG-1 cells that were pre-infected with helper phage. The electroporated TG-1 cells were enumerated by plating dilutions of an aliquot onto 2YT plates with ampicillin and kanamycin. The remaining electroporated TG-1 were cultured at 37°C, overnight, in 2YT medium with ampicillin and kanamycin for the production of bacteriophages. The next day, bacteriophages were harvested from the cell culture supernatant and stored in PBS with 20% glycerol until use.

1.9 Biopanning of the Phage display library

Recombinant human TRIM21 PRYSPRY domain was biotinylated and then used as bait for biopanning. In the first round of biopanning, 2×10^{12} bacteriophages were incubated with 5 nM of bait protein for 1 hour to allow for interaction. The bait was then captured using DynaBeads M-280 Streptavidin (Invitrogen). The beads were then washed five times with casein, incubated for 30 min with 1 µM of soluble Fc and eluted using trypsin. In the second round of biopanning, the eluted bacteriophages were amplified and incubated first with empty streptavidin beads, and followed by incubation with 0.5 nM of bait protein. The bait was then captured using streptavidin beads. The beads were then washed ten times with casein and incubated with 1 µM soluble Fc for two rounds of 30 mins, before elution using trypsin. In the third round of biopanning, the eluted phages were amplified, incubated with empty streptavidin beads, and then incubated with 0.05 nM of bait protein. The bait was then captured using streptavidin beads. The beads were washed ten times, then incubated for 5 hours with 1 µM of soluble Fc, before elution using trypsin. 50 clones from each of rounds two and three were sequenced.

1.10 Surface Plasmon Resonance (SPR) analysis

The affinity constants were measured using ProteON XPR36 (Bio-Rad). Antibodies were immobilized onto a GLC sensor chip via amine coupling, and PBS with 0.05% Tween-20 was used as the running buffer. Curves were fitted with the ProteON Manager software using five concentrations of TRIM21 and based on a Langmuir 1:1 model.

1.11 Antibody-dependent intracellular neutralization (ADIN) Assays

For ADIN assays, 1.25×10^5 HeLa cells were seeded into 24-well plates and 1×10^5 moDC were seeded into 96-well plates for infection with immune complexes, virus alone, antibodies alone or medium alone for 48 h. As HeLa cells are highly permissive to adenovirus infection (Fechner et al., 2000), an MOI of 1.2 was used, while for moDCs, an MOI of 400 was used. Infected cells were detected by analysis of expression of eGFP by flow cytometry. For HeLa cells, immune complexes were pre-formed by incubation of virus with antibodies at concentrations ranging from 0.3 pM to 35 nM for 1 h at room temperature prior to use. Thereafter, the incubation mixture was diluted 55-fold into the HeLa cell culture to give a final concentration ranging from 0.006 pM to 627 pM. For moDCs, immune complexes were pre-formed by incubation of virus with antibodies at concentrations ranging from 0.17 nM to 690 nM for 1 h at room temperature prior to use. Thereafter, the incubation mixture was diluted by 12.5-fold into the moDC culture to give a final concentration ranging from 0.01 nM to 55 nM.

Coimmunoprecipitation: Ad5 was preincubated with Abs for 1 h at room temperature in PBS to form immune complexes. Immature moDC were then treated with the immune complexes in RPMI 1640 supplemented with 10% FBS at an MOI of 200 and incubated at 37°C with 5% CO₂ for 4 h. Cells were lysed on ice with RIPA buffer containing 1% Nonidet P-40 substitute (Sigma-Aldrich), 50 mM Tris-Cl (pH 7.6), 150 mM NaCl, 1 mM EDTA, 1% Phosphatase Inhibitor Cocktail 3 (Sigma-Aldrich), and 10% glycerol, supplemented with Protease Inhibitor Mini Tablets (Thermo Fisher Scientific), 1 mM PMSF (Roche), 10 mM MG132 (Sigma-Aldrich), and 20 mM N-ethylmaleimide (Sigma-Aldrich). Goat Fab anti-human Fab (Jackson ImmunoResearch) was coupled onto M-270 Epoxy Dynabeads (Thermo Fisher Scientific), following the manufacturer's instructions, in a Dynabeads Antibody Coupling Kit. Immunoprecipitations were performed by incubating cell lysates from 1.5 million cells with 1 mg of Dynabeads (prebound with anti-human Fab) overnight at 4°C. Beads were then washed with RIPA buffer, and immunoprecipitates were eluted with 0.1 M Glycine buffer (pH 2.7).

Western blot: Cell extracts and immunoprecipitates were resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane using Trans-Blot SD semi-dry transfer cell (Bio-Rad Laboratories), according to the manufacturer's protocols. After incubation with 5% nonfat milk in TBST (25 mM Tris [pH 7.2], 140 mM NaCl, and 3 mM KCl, 0.2% Tween 20) for 1 h, the membrane was incubated with Abs against IgG Fab (1:5000; Jackson ImmunoResearch), Ro52/TRIM21 (1:500, D-12; Santa Cruz Biotechnology), and GAPDH (1:10000, VPA00187; Bio-Rad Laboratories) overnight at 4°C. Membranes were washed with TBST four times, then incubated with HRP-conjugated anti-mouse (1:10000; Dako) or anti-rabbit (1:20000; Thermo Fisher Scientific) Abs for 1 h. Blots were washed with TBST four times and developed with ECL Plus Western Blotting Substrate (Thermo Fisher Scientific) according to the manufacturer's protocols. Blot images were acquired using the ChemiDoc imaging system (Bio-Rad Laboratories).

1.12 MoDC maturation Assay

1x10⁵ moDCs were incubated in 200 µl of cell culture medium in 96-well plates with either PBS, 4x10⁷ Ad5 virus, 1.6 µg antibodies or immune complexes made from 4x10⁷ Ad5 virus and 1.6 µg antibodies that were pre-incubated for 1 h at room temperature. All treatments were administered to the cell culture in a volume of between 8-80 µl, and medium was added accordingly to make the final cell culture volume up to 200 µl. For a positive control, 1 µg/ml LPS was used. The cells were analyzed by FACS after 24 h.

1.13 MoDC: autologous CD14⁺ PBMC Co-culture Assay

MoDCs were prepared and treated in the same way as in the moDC maturation for 4 h.

Treatment of moDC with MG132 is done by incubating moDC in a six-well plate with 10 µM of MG132 (Sigma-Aldrich) in medium for 1 h at 37°C, 5% CO₂. After 1 h, the cells were centrifuged to remove MG132. MG132-treated or -untreated moDC were resuspended in fresh medium and treated in the same way as in the moDC maturation for 4 h.

After 4 h, the cells were co-cultured in fresh medium with 5×10^5 autologous CD14⁺ PBMCs that had been labeled with 10 μ M CFSE using the Vybrant™ CFDA SE Cell Tracer kit (Life technologies): briefly, 100 million cells were labeled in 1 ml of 10 μ M CFSE in FBS-supplemented medium for 5 mins at 20°C. Excess dye was then removed by centrifugation at 10,000 x g for 1 min and the cells were washed three times with fresh medium. The co-culture was maintained for up to 13 days with replacement of one third of old medium with fresh medium on days 4 and 7. For positive controls, either 1 μ g/ml LPS was added to the moDCs, or 2 μ l T Cell TransACT™ (Miltenyi) was used.

1.14 Peptide re-stimulation assays

To prepare moDCs for pulsing of peptides, autologous moDCs were generated using the same method as above from frozen monocyte stock. After 6 days, moDCs were pulsed for a day with either 10 μ g/ml of sterile-filtered peptides, or 200 μ g peptide libraries (Miltenyi, PepTivator AdV5 Hexon or PepTivator NY-ESO-1) in medium containing 100 ng/ml GM-CSF (Miltenyi), 100 ng/ml IL-4 (Miltenyi) and 50 ng/ml TNF- α (Miltenyi). Thereafter, 1×10^5 peptide-pulsed moDCs were used to re-stimulate autologous 11 day old moDC:CD14⁺ PBMC co-culture, at a ratio of 1:5 peptide-pulsed moDCs to 11 day old co-culture. This was done by harvesting the 11-d coculture, resuspending the cells to a concentration of 5×10^6 cells/ml in fresh medium (with or without brefeldin A), and adding 100 μ l of cells (i.e., 5×10^5 cells) to the peptide-pulsed moDC in the 96-well, round-bottom plate. For donor LCY02, cells were re-stimulated for 16 hours and the supernatant of the re-stimulated cells was harvested for analysis by ELISA. The cells were then treated with fresh medium containing 1 μ g/ml Brefeldin A. After 5 h, cells were harvested and labelled for analysis by flow cytometry. For donors LCY10, PAT35 and LCY25 cells were re-stimulated for 16 hours in the presence of 1.5 μ g/ml Brefeldin A and then harvested for analysis by flow cytometry. For the 11 day old moDC:CD14⁺ PBMC co-cultures, replacement of one third old medium with fresh medium was performed at days 4 and 7 for LCY02, PAT35 and LCY25; and for LCY10, feeding was performed on day 4 followed by dilution of the co-culture into an equal volume of fresh medium containing 10 ng/mL IL-7 and IL-15 on days 7 and day 10.

1.15 Flow cytometry

In all assays, LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Invitrogen) was used to exclude dead cells. In the moDC maturation assay, cells were incubated with anti-FcR-blocking antibody (eBioscience, San Diego, CA, USA) and then labelled using combinations of the following antibodies: Pacific-Blue-anti-CD14 (M5E2), APC-anti-CD11c (S-HCL-3), Alexa Fluor700-anti-CD80 (L307.4), FITC-anti-CD83 (HB15e), PE-Cy7-anti-CD86 (FUN-1), APC-Cy7-anti-HLA-DR (L243), BV650-anti-CCR7 (G043H7) and PE-anti-CD206 (19.2). In the co-culture assay, cells were incubated with human FcR blocking reagent (Miltenyi) and then labelled using the following antibodies: Alexa Fluor647-anti-CD3 (SK1), PE-Cy7-anti-CD4 (OKT4), Pacific Blue-anti-CD8 (SK7), and PE-anti-CD56 (AF12-7H3). In the haplotyping of donors, the dyes used were Alexa Fluor 647-anti-HLA-A24 (17A10) and PE-anti-HLA-A2 (BB7.2). For haplotype controls, a HLA-A24⁺ cell line HT29 and HLA-A2⁺ cell line MDA-MB-231 were also labelled and included in the analysis. In the re-stimulation assays, the dyes used were Alexa Fluor647-anti-CD3 (SK7), PE-Cy7-anti-IFN- γ (4S.B3), Pacific Blue-anti-CD8 (SK1), and PE-anti-CD56 (AF12-7H3). After the surface markers are labelled, cells were fixed and permeabilized using BD Cytofix/Cytoperm solution followed by

PE-Cy7-anti-IFN- γ . For absolute cell counts 10 μ l of CountBright™ Absolute Counting Beads were added to cells. Samples were acquired using BD FACSDiva software on the LSRFortessa cell analyzer and FACSCanto II (BD Biosciences). Data were analyzed using FlowJo software (Tree Star, Ash-land, OR, USA).

5

1.16 Cytokine and chemokine analysis

Supernatants from the moDC maturation assays were analyzed by a multiplex analysis using human cytokine/chemokine bead panel 1 and 2, which measure a total of 64 targets (Milliplex MAP kits, Millipore) on a Flexmap 3D system (Luminex Corp, Texas, USA). Supernatants from the co-culture assays were

10 analyzed by ELISA for the level of IFN- γ using Human IFN- γ ELISA MAX™ Standard (Biolegend).

1.17 Statistical analysis

Statistical analyses were performed using GraphPad Prism 7.01 software using repeated measure ANOVA with Dunnett's multiple comparison testing, or Friedman with Dunn's multiple comparison testing.

15 The adjusted p-values are indicated in the Figures by asterisks.

Example 2: Results

2.1 A modified Fc exhibits increased affinity for TRIM21

The inventors first identified the amino acids in Fc region that are in contact with TRIM21, based on the crystal structure of the human IgG Fc-TRIM21 complex (Figure 1A) (James et al., 2007). Both the CH2 and CH3 regions of the Fc interact with TRIM21. Using a cut-off distance of 5Å, 21 potentially-interacting

20 amino acids were identified:

Amino acid (EU numbering)	CH Domain	Secondary Structure
252(MET)	CH2	Loop
253(ILE)		Loop
254(SER)		Loop
309(LEU)		Helix
310(HIS)		Helix
311(GLN)		Helix
314(LEU)		Helix
315(ASN)		Helix
345(GLU)	CH3	Loop
428(MET)		Sheet
430(GLU)		Loop
431(ALA)		Loop
432(LEU)		Loop
433(HIS)		Loop
434(ASN)		Loop
435(HIS)		Loop

436(TYR)		Sheet
437(THR)		Sheet
438(GLN)		Sheet
439(LYS)		Sheet
440(SER)		Sheet

The majority of the amino acids interacting with TRIM21 in the CH2 domain were in the α -helix, and the inventors reasoned that this region could be destabilised by modification. The inventors therefore instead focused on modification of the amino acids in the CH3 domain, and selected 7 amino acids whose side groups were proximal to and facing towards TRIM21 for modification (see Figure 1B).

A phage library of 2 billion bacteriophages displaying the human IgG1 Fc was generated with randomly-substituted amino acids in the selected 7 positions. Recombinant human TRIM21 PRYSPRY domain was used as bait for biopanning. After three sequential rounds of biopanning, approximately 50 clones from the output of Rounds 2 and 3 were sequenced. One sequence was found in ~10% of the clones analysed in Round 2; and in 94% of the clones analysed in Round 3 (Figure 1C, Clone #1). This variant (designated PN04-90) possessed five amino acid modifications relative to wildtype human IgG1: H433T, N434R, Y436F, S440I and T256P, the last of which is a mutation in the CH2 domain. The amino acid sequence of the CH2-CH3 region for PN04-90 is shown in SEQ ID NO:71. While the crystal structure of IgG1 Fc with TRIM21 places T256 out of direct contact with TRIM21, it is in close proximity to three of the contact points (M252, I253 and S254) (James et al., 2007), and is therefore likely to improve Fc binding by bringing the contact points closer to TRIM21.

The inventors next investigated whether antibodies bearing the modified Fc bound to TRIM21 with higher affinity than antibodies comprising unmodified Fc. Two sets of antibodies were constructed: the first set comprises the variable domains of human antibody, 3G9-2D2 (Cheong et al., 2010) which recognizes human DEC-205, joined to human IgG1 constant regions that have the modified or unmodified Fc; the second set comprises variable domains of the mouse antibody, 9C12 (Varghese et al., 2004a) which recognizes the Adenovirus Type 5 (Ad5) hexon, joined to human IgG1 constant regions that have the modified or unmodified Fc. The first set was designated 'human IgG1', and the second set was designated 'chimeric IgG1' (Figure 1D). This approach enabled the inventors to investigate whether the modified Fc could be applied to antibodies with different variable domains, and from different species.

The affinity of the unmodified and Fc-modified antibodies for TRIM21 was measured by surface plasmon resonance analysis. Modifying the Fc region of human IgG1 increased its affinity for the PRYSPRY domain of TRIM21 by at least 100 fold: the affinity constants (K_D) were 0.522 nM for the Fc-modified human IgG1 (Figure 1E) and 0.425 nM for the Fc-modified chimeric IgG1 (Figure 1F); while unmodified human (Figure 1G) and chimeric (Figure 1H) IgG1 bound with lower affinity (47.3 nM and 125 nM, respectively). The T256P substitution in the CH2 domain was found to contribute towards improved affinity of the modified Fc antibodies, because a variant Fc comprising substitutions H433T, N434R, Y436F and S440I but having Thr at position 256 (designated V5) was found to have reduced affinity for

TRIM21 as compared to the PN04-90 variant ($K_D = 23.5$ nM see Figure 1I). Therefore all five amino acid substitutions were incorporated into the chimeric, Ad5-specific Fc-modified antibodies that were used in subsequent functional characterization studies.

- 5 To assess the involvement of each single mutation in the affinity increase for TRIM21, 5 mutants with reversion to wild-type amino-acid in 1 single position were derived from Fc-modified chimeric IgG1 and their affinity for TRIM21 domain PRYSPRY was measured (Table 1). Reversion of mutations in positions 256, 433 and 434 resulted in lower affinity for TRIM21, suggesting that modifications T256P, H433T and N434R are important for improved binding.

10

Table 1: Affinity of Fc-modified and unmodified chimeric IgG1 for TRIM21.

	Amino-acid position in IgG1 (EU numbering system)					Affinity K_D (M)
	256	433	434	436	440	
Wildtype chimeric IgG1	T	H	N	Y	S	125
PN04-90	P	T	R	F	I	4.25×10^{-10}
V1	P	H	R	F	I	1.08×10^{-8}
V2	P	T	N	F	I	1.62×10^{-8}
V3	P	T	R	Y	I	2.11×10^{-10}
V4	P	T	R	F	S	2.76×10^{-10}
V5	T	T	R	F	I	2.35×10^{-8}

15 2.2 Increasing Fc affinity for TRIM21 preserves ADIN

- Antibody-dependent intracellular neutralization (ADIN) was first demonstrated in HeLa cells infected with adenoviruses (Mallery et al., 2010). The hexon protein of adenovirus is recognized by the monoclonal antibody 9C12 (Varghese et al., 2004a), which was used in construction of the chimeric antibodies. 9C12 does not block viral entry, but mediates post-entry neutralization (Varghese et al., 2004b), in a TRIM21-dependent fashion (Mallery et al., 2010). While reducing the affinity of antibody for TRIM21 does not affect ADIN of adenovirus in HeLa cells (Foss et al., 2016), the effects of increasing affinity for TRIM21 have not been investigated. The inventors investigated how increasing Fc affinity for TRIM21 influences ADIN in both HeLa cells (non-immune cells) and monocyte-derived DCs (immune cells).

- 25 Different concentrations of Fc-modified antibodies comprising PN04-90 Fc and unmodified antibodies comprising wildtype human IgG1 Fc were incubated with replication-defective Ad5 which carries the eGFP (enhanced green fluorescent protein) reporter gene to form immune complexes, which were then added to HeLa cells or monocyte-derived DCs (moDCs). The frequency of infection was monitored after 48 h via analysis of eGFP expression (Figure 2A). In HeLa cells, antibodies bearing PN04-90 Fc or 30 unmodified Fc regions mediated ADIN of Ad5 infection equally well (Figure 2B) with an IC_{50} of 0.9 pM for a viral MOI of 1.2; similarly in moDCs, the IC_{50} of unmodified Fc and the PN04-90 Fc were very similar (3.0 nM versus 4.5 nM; see Figure 2B) at a viral MOI of 400. Increasing the affinity of Fc for TRIM21 was found not to disrupt ADIN in both non-immune and immune cells.

To verify that the Fc-modified Abs interact with TRIM21 in moDC, the treated moDC were lysed and beads conjugated with Fab anti-human Fab were used to immunoprecipitate the Fc-modified Abs. Western blot analysis showed that TRIM21 coimmunoprecipitated with modified Ab, suggesting that it binds to the internalized Fc-modified immune complexes (Figure 2C).

5

Figure 15A and 15B show the results obtained with moDCs when a range of different MOIs were used. An MOI of 100 gave ~46% infected moDC, and an MOI of 400 gave ~80% infected moDC (Figure 15B).

ADIN mediated by the unmodified Fc and modified Fc remained the same when tested at lower viral MOI (i.e. less than 400).

10 2.3 Increasing Fc affinity for TRIM21 promotes moDC maturation

Reducing the affinity of antibodies for TRIM21 has previously been shown to impair pro-inflammatory signalling in HEK293T cells (Foss et al., 2016). For moDCs, stimulation of a T cell response requires the simultaneous presentation of cognate antigen, and the provision of activating signals in the form of pro-inflammatory cytokines and co-stimulatory molecules on the DC surface. This process is often subverted by viruses, including adenoviruses (Newton et al., 2008). The inventors therefore investigated whether increasing Fc affinity for TRIM21 promoted moDC expression of co-stimulatory molecules and pro-inflammatory cytokines.

20 Incubating moDCs with Ad5 alone for 24 h did not increase expression of maturation markers, even at the highest dose of 400 MOI (Figures 3A and 3B), which resulted in an average of 69% of the moDCs being infected (Figure 2A, moDC panel). This is in agreement with previous reports that at 50 MOI or higher, E1- and E3- deleted adenoviruses do not upregulate moDC co-stimulatory molecules, and instead suppress responses to LPS or PHA stimulation (Newton et al., 2008). Accordingly, incubating moDCs
25 with wildtype IgG1 Fc (Fc-unmodified) Ad5 immune complexes also did not significantly increase the level of surface expression of CD80, CD83, CD86 or HLA-DR (Figures 3A and 3B, Figure 16A), even though at the highest dose (400 MOI and 55 nM) the same concentration of antibodies were highly functional when it comes to ADIN (Figure 2B, moDC panel). Only moDCs treated with PN04-90 Fc (Fc-modified) immune complexes significantly increased surface expression of CD80, CD83, CD86 and HLA-DR (Figure 3C,
30 Figure 16A), and this trend was dose-dependent (Figure 3A) and reproducible between different donors (Figure 3C, 3D).

2.4 Increasing Fc affinity for TRIM21 promotes moDC production of Th1-associated chemokines.

The inventors next investigated whether increasing Fc affinity for TRIM21 promoted expression of pro-inflammatory cytokines by moDCs. Using a multiplex bead-based assay, the inventors measured the
35 secretion of 64 cytokines and chemokines by moDCs after 24 h of incubation with immune complexes comprising the Fc-modified or unmodified antibodies. For each cytokine/chemokine, fold change in expression level between treatments with PN04-90 Fc (Fc-modified) and wildtype IgG1 Fc (unmodified) immune complexes was calculated and correlated to their dosages (Figure 4A). Nine proteins (CCL-3,-4,-
40 5,-7, CXCL12, TRAIL, IL-6, TNF- α and IFN- γ) were determined to have a fold-change to dosage correlation higher than spearman rho 0.3 with $p < 0.05$. Comparison of the concentrations of the molecules

under different moDC treatment conditions by ANOVA revealed that CCL3 (MIP1- α), CCL4 (MIP1- β), CCL5 (RANTES), CCL7, TNF- α and IL-6 production was significantly higher following moDC incubation with Fc-modified immune complexes as compared to other treatments (Figure 4B, 4C, 4D).

- 5 As IFN- γ is not a typical cytokine produced by moDCs, the slight increase in the levels of this cytokine most probably came from T cells and/or innate lymphoid cells that could be present in the CD14+ preparations, which had cell purity ranging from 81 to 91% (Figure 16C).

- 10 *In vivo*, early-maturing DCs in peripheral tissues are the main producers of CCL3, CCL4 and CCL5 (Sallusto et al., 1999, 2000), which attract immature DC and T cells that promote a T helper type 1 (Th1) response (Lebre et al., 2005). Taken together, these data show that exposure of moDCs to Ad5 immune complexes comprising antibodies with Fc modified for increased affinity to TRIM21 induced increased co-stimulatory marker expression and greater production of Th1-associated pro-inflammatory cytokines compared to either virus alone, or Fc-unmodified immune complexes.

15

2.5 Increasing Fc affinity for TRIM21 increases T cell expansion by moDC

- The inventors next assessed how Fc-modified immune complexes influenced CD8 T cell responses in PBMCs. moDCs were incubated either Ad5 alone, or with PN04-90 Fc (Fc-modified) or wildtype IgG1 Fc (unmodified) antibody-Ad5 immune complexes, and then co-cultured with autologous CD14- PBMCs for
20 13 days. LPS and TransACT (a CD3/CD28 agonist) were used as positive controls to non-specifically activate DCs and T cells respectively.

- After 13 days of co-culture, the relative proportions of CD4 and CD8 T cells were determined by flow cytometry. Cells were gated for singlets/live/CD3+CD56- cells to include T cells and exclude NKT cells.
25 CD8 T cells were found to be markedly enriched in co-cultures where moDC were treated with PN04-90 Fc (Fc-modified) immune complexes (Figure 5A). Furthermore, this relative enrichment was not due to CD4 T cell death, as the absolute number of CD4 T cells was not lower (Figure 5B); moreover, the absolute number of CD8 T cells was significantly higher in treatments with the modified Fc immune complexes compared to the other treatments (Figure 5B).

30

- Analyzing results from 7 different donors, the inventors observed that treatment of moDCs with Fc-modified immune complexes significantly increased CD8 T cell counts but not CD4 T cell counts in co-culture experiments, as compared to treatment with Fc-unmodified Ad5 immune complexes (Figure 5C); Interestingly, heterogeneity in the capacity of cells from different donors to respond to Ad5 was observed
35 and this could be separated into two groups: the first group (Type 1 donors) exhibited strong CD8 proliferation to virus alone (above 2-fold relative to no virus control) but a smaller increase in CD8 proliferation when moDCs were treated with the modified-Fc immune complexes; while donors that responded poorly to virus alone (below 2-fold relative to no virus control; Type 2 donors) showed a marked and significant increase of 5-fold when moDCs were treated with the modified-Fc immune
40 complexes (Figure 5D).

Treatment with Fc-modified immune complexes, compared with treatment with unmodified immune complex, resulted in an increase in cell count that was statistically significant for CD8 T cells but not CD4 T cells (Figure 5E). However, when compared with virus alone, the increase in CD8 T cell count was not statistically significant. This was believed to be due to donor variation. Three donors (LCY04, LCY06, LCY08) who responded very well to virus alone (>3-fold increase compared with buffer treatment) responded poorly to the immune complexes comprising unmodified Fc or modified Fc.

During T cell expansion, IFN- γ is produced and so the inventors measured secretion of IFN- γ at 4, 7 and 13 days of co-culture for each donor. As in the CD8 T cell response, treating moDCs with Fc-modified immune complexes induced significantly higher IFN- γ production in the moDCs:CD14- PBMC co-culture than did either virus alone or Fc-unmodified immune complexes at 4, 7 and 13 days post infection, as determined by ELISA of the donors that responded to the modified-Fc immune complexes (Figure 6A).

Analyzing the IFN- γ results from the same 7 donors again revealed a heterogeneity in response that could be grouped into the Type 1 and Type 2 donors (Figure 6B). Type 1 donors displayed higher IFN- γ in control treatments and did not increase much more upon treatment with Fc-modified immune complexes. Type 2 donors displayed much lower IFN- γ levels in control treatments, and an increase upon treatment with Fc-modified immune complexes.

Taken together, the data show that through modifying Fc for improved affinity to TRIM21, immune complexes can be generated which increase the ability of moDC to stimulate IFN- γ production by PBMCs, and to stimulate CD4 and CD8 T cell expansion. In the case of CD8 T cell count, this effect is particularly marked in the Type 2 subset of donors that do not respond well to virus alone.

2.6 Increasing Fc affinity for TRIM21 increases antigen-specific CD8 T cell proliferation induced by moDC incubated with immune complexes

To understand the extent to which the CD8 T cell response was antigen-specific, the inventors further investigated the response of donor LCY02; this donor was HLA-A24- and HLA-A2-positive, and exhibited a 2.7-fold increase in the percentage of CD8 T cells in moDC:CD14- PBMC co-cultures in response to PN04-90 Fc immune complexes (Figure 7). 11 day old moDC-CD14- PBMC co-cultures were re-stimulated with autologous moDCs that had been treated with TNF- α and pulsed with peptides representing an Adenovirus epitope for HLA-A24 haplotype (TYFSLNNKF), HLA-A2 haplotype (YVLFEVFDVV), and, as negative controls, a scrambled peptide (LAVFEDYVAF) or a HIV epitope (SLYNTVATL). The antigen-specific response was also investigated using a library of 15-mer peptides representing the hexon protein of Adenovirus or an irrelevant protein (Human NY-ESO-1 protein).

A marked and specific IFN- γ response to the HLA-A24-restricted-Adenovirus epitope was observed after 16 h of re-stimulation (Figure 8A). A strong IFN- γ response was also detected to the peptide library generated from the Adenovirus-hexon protein was detected, but not to the peptide library of the NY-ESO-1 protein (Figure 8B).

In the same experiment, CD14- PBMCs were labelled with CFSE to enable identification of proliferating cell populations. T cells that were cocultured with moDCs pretreated with the virus and Fc-modified Abs showed the most proliferation (Figure 17A). No such proliferation is seen when moDC were treated with PBS, virus alone, Abs alone, or virus with unmodified Abs.

5

After 11 days, 43% of the CD8 T cells, and 21% of the CD4 T cells had proliferated (Figure 8C). After 16 h of re-stimulation, Brefeldin A was added to the co-cultures to enable detection of individual cells that were producing IFN- γ , and also to understand their proliferative response to the different antigens. Both CD4 and CD8 T cell populations exhibited higher percentages of CFSE-diluted IFN- γ -producing cells when moDCs had been stimulated by the Ad5 hexon peptide library (4.73% and 3.46% respectively) than when stimulated by the NY-ESO-1 peptide library (1.12% and 1.16%).

10

In the single peptide experiments, the HLA-A24 adenovirus peptide stimulated 6.57% of the proliferated CD8 T cells to produce IFN- γ , while the response to the HLA-A2 adenovirus peptide was comparable to the negative controls (Figure 8C). Since the single peptide experiment used 9-mer peptides, restimulation of CD4 T cells was not expected; however, there was a small increase in the frequency of IFN- γ -producing CD4 T cells in co-cultures where moDCs were treated with the HLA-A24 peptide relative to negative controls. This may have been a bystander effect resulting from the production of cytokines by the CD8 T cells that were truly responding to the 9-mer peptide.

15

20

To eliminate the bystander effect of cytokines which could be released during re-stimulation, the experiment was repeated in the presence of Brefeldin A to stop cytokine release right at the start of re-stimulation. Only CD8 but not CD4 T cells were found to display antigen-specific re-stimulation (Figure 8D). Controls using CD3/CD28 agonist confirmed that the CD4 T cells were capable of responding to stimulation and are not intoxicated by the overnight treatment with Brefeldin A.

25

In a separate experiment, toxicity from overnight brefeldin A treatment was directly measured by comparing the number of live cells in CD14- PBMCs in the presence and absence of brefeldin A. LIVE/DEAD staining showed that after 16 h the viability of CD14- PBMCs with brefeldin A treatment was ~70 and 86% of that without brefeldin A treatment for inactivated and activated cells, respectively (Figure 17B). Notwithstanding the toxicity of brefeldin A, our results indicate that with overnight brefeldin A treatment T cell proliferation stimulated by Fc-modified immune complexes appears to be driven by Ag-specific CD8 T cells.

30

Taken together, these results show that Fc-modified immune complexes increase moDC stimulation of antigen-specific IFN- γ production and CD8 T cell proliferation.

35

The inventors next investigated whether the CD8 T cell proliferation stimulated by moDCs treated with Fc-modified immune complex were dependent on proteasomal degradation in the moDCs.

40

MoDCs were pretreated with MG132 for 1 h, then subjected to treatment with immune complexes or various controls and then cocultured with autologous CFSE-labeled CD14⁺ PBMCs for 11 days.

The results are shown in Figure 8E, and demonstrate that pretreatment of moDC with the proteasomal inhibitor MG132 resulted in the loss of CD8 T cell proliferation.

To ensure that the loss of proliferation was not solely because of MG132-associated toxicity to moDC, moDC viability was analysed 1 d after MG132 treatment. At 22 h posttreatment, the viability of moDC with MG132 treatment was 61% of that without MG132 treatment (Figure 17C). This indicated that there were still moDCs after MG132 pretreatment, but they were unable to mediate enhanced Ag cross-presentation because the Fc-modified immune complex is dependent on a proteasome-mediated pathway.

Example 3: Discussion

The inventors engineered the human IgG Fc region to increase its affinity for TRIM21 by 100-fold, and showed that the resulting antibodies directs viral antigens effectively into the cross-presentation pathway leading to the stimulation of antigen-specific CD8 T cells. This process is mediated by cross-presentation and not the classical MHC class I presentation of viral proteins because most of the endocytosed viruses are neutralized and therefore viral genes are not being expressed. Importantly, the cross-presentation process is remarkably enhanced by Fc-modification for increased TRIM binding, while the host-protective mechanism of ADIN is retained.

While both the Fcγ receptor (FcγR) and the neonatal Fc receptor (FcRn) have been shown to facilitate cross-presentation (Baker et al. 2011; Regnault et al. 1999), this is the first demonstration that TRIM21 also accesses this pathway. The involvement of TRIM21 with the proteasome has led to speculation that it regulates antigen-processing (signal 1) in DCs, but the inventors also found that TRIM21 ligation by Fc-modified immune complexes induces expression of co-stimulatory molecules (signal 2), and pro-inflammatory cytokine/chemokine release (signal 3) by moDCs. For cross-priming of CD8 T cells, all 3-signals are required. DC-targeted vaccine strategies often rely on TLR-stimulants, such as monophosphorylated lipid A, polyinosinic-polycytidylic acid, and CpG oligonucleotides, or a cocktail of four cytokines, IL1β, IL-6, TNF-α and Prostaglandin E2 (Castiello et al., 2011; de Jong et al., 2002; Han et al., 2009), to provide signals 2 and 3 to DCs. Fc-modified immune complexes could potentially provide all three signals, removing the need to optimize the timing for the adding of antigen (before or after the addition of the maturation cocktail, depending on whether antigen-internalization is required) and dosages of the different components; and also overcomes the issue of ensuring all four components reach the DCs with the correct timing *in vivo*. In other words, the modified Fc would render *in vivo* application of DC-targeted vaccines more feasible.

MoDCs treated with Fc-modified immune complexes consistently upregulated maturation marker expression in five out of six donors, and a sub-population of donors (60%) exhibit markedly increased CD8 T cell expansion. The differences are likely to be due to the level and timing of previous exposure to

Ad5 for the donors. Donors with prior exposure to adenovirus may have memory T cells capable of responding to viral antigens in the absence of co-stimulatory molecule expression by DC. In this case, moDCs treated with virus alone would be expected to outperform moDCs treated with immune complexes, due to increased expression of viral proteins: at MOI 400 the replication-deficient Ad5 infects 5 69% of the moDC, likely leading to expression of viral proteins at a level equivalent to that of a replication-competent adenovirus at an MOI of 1 (Saha and Parks, 2017). Thus in the absence of antibodies (and therefore ADIN), there is a larger population of moDCs (69% versus 4% in the presence of antibody) producing viral proteins, albeit without upregulating their co-stimulatory markers, and able to stimulate memory CD8 T cells in the subpopulation of donors who have them. Importantly, prophylactic vaccines 10 need to stimulate naïve T cells rather than memory T cells, since they are meant to protect individuals not having had prior exposure to the virus while therapeutic vaccines need to revive a virally-damped immune response, which means that the DC might need a 'boost' to upregulate their stimulatory status again. In both cases, the ability of the modified-Fc to enhance moDC-cross-priming of CD8 T cells is advantageous.

15 Ad5 immune complexes were used to demonstrate the potential of modified-Fc immune complexes to induce and enhance CD8 T cell responses to viral antigen, but these findings are of relevance to other pathogens and diseases. Ad5 immune complexes reach TRIM21 in the cytosol of moDC because adenoviruses release protein VI, which lyses the endosome (Wiethoff et al., 2005; Greber et al., 1993).

20 Antibody-antigen fusion proteins targeting DCs such as anti-DEC205-NY-ESO-1 (Dhodapkar et al., 2014) and anti-DEC-HIV gag (Bozzacco et al., 2007) have been shown to stimulate anti-cancer and anti-HIV CD8 T cell responses. Also, antibodies can simply be designed to target antigens of interest to form immune complexes that are taken into the DC via the Fc receptor. Fc receptor-mediated antigen 25 internalization in DCs is known to be channelled to a special transport pathway which allows the antigen efficient access to the cytosol (Amigorena, 2002). HRP-anti-HRP immune complexes were detectable by HRP substrate (DAB) and anti-rabbit IgG Fabs suggesting that both antigen and antibody remains mostly intact in the cytosol (Rodriguez et al., 1999, Gros and Amigorena, 2019). Given that an Fc receptor (TRIM21) and an Ag-processing enzyme (proteasome) both exist in the cytosol, immune complexes may 30 have privileged access to deliver themselves and their cargo completely intact to the cytosol.

In summary, the inventors have identified a readily-adaptable method of Fc modification for targeting endocytosed antigen in immune complexes to MHC class I cross-presentation pathway via TRIM21, and have shown its potential to provide all the signals necessary for the stimulation of a potent CD8 T cell and 35 cytokine response to specific antigen.

Example 4: Further Fc variants

Further variant Fc regions having improved affinity for TRIM21 as compared to wildtype human IgG1 Fc were identified.

40

Briefly, an Fc Phage display library was generated, essentially as described in Example 1.8, comprising 200 million Fc variants having amino acids randomised at positions 256, 433, 434, 436 and 440 of human IgG1.

- 5 The Fc Phage display was used in three biopanning experiments performed as described in Example 1.9, as summarised in Table 2 below. Binding to the PRYSPRY domain of TRIM21 was analysed by ELISA.

Table 2. Biopanning with an Fc Phage display library randomised at positions 256, 433, 434, 436 and 440 to identify variants with improved binding to TRIM21

Expt	Biopanning conditions	Host cells for phage amplification	Host cells for Fc expression for ELISA	No. clones picked for ELISA	No. clones with improved binding to TRIM21 as determined by ELISA	No. of new sequences
1	Low stringency approach (3 rounds of panning)	TG1	HB2151	94	4 (only 2 have PCR insert)	2
2	Low stringency approach (repeated pan 3)	TG1	HB2151	24	11	8
3	High stringency approach (2 rounds of panning)	XL1-Blue	XL1-Blue	372	293	125

10

Figures 10 to 12 show the binding of the variant Fc regions to the PRYSPRY domain of TRIM21 (filled bars), and to anti-FLAG (open bars; the Fc regions include a FLAG tag).

Figure 10 shows the results for the 4 clones obtained from biopanning experiment 1.

15

Figure 11 shows the results for the 11 clones obtained from biopanning experiment 2.

Figures 12A to 12H show the results of the 293 clones obtained from biopanning experiment 3.

- 20 A total of 306 clones were identified as expressing Fc regions which bound to the PRYSPRY domain of TRIM21 with greater affinity to wildtype Fc.

The 306 clones were sequenced, and identifying 128 new, unique sequences (SNs. 1 to 8, 10 to 39, and 46 to 135 of Figure 13A). A further sequence that was not identified in the biopanning experiments was also prepared (see SN.9 of Figure 13A).

25

The 135 sequences were divided into 8 subgroups based on their sequence pattern (see Figures 13A and 13B). Sequences comprising the combinations of substitutions shown in Table 1 of Example 2.1 are also shown in Figure 13 (SNs.40-45 of Figure 13A).

5

Fc regions comprising sequences corresponding to 8 of the 129 new sequences were analysed for binding to TRIM21 PRYSPRY domain by Surface Plasmon Resonance, which was performed as described in Example 1.10.

10 The results are summarised below:

SN (of Figure 13)	Amino-acid (EU numbering system)					Ka (1/Ms)	Kd (1/s)	KD (M)
	256	433	434	436	440			
Wildtype IgG1 Fc	T	H	N	Y	S	6.47×10^5	8.12×10^{-2}	1.26×10^{-7}
1	P	S	H	Y	G	7.05×10^6	3.88×10^{-4}	5.5×10^{-11}
2	P	S	H	Y	S	4.66×10^6	7.27×10^{-4}	1.56×10^{-10}
10	P	V	H	Y	R	5.15×10^5	4.60×10^{-4}	8.92×10^{-10}
9	P	V	H	Y	S	5.03×10^6	4.81×10^{-4}	9.57×10^{-11}
15	P	H	H	Y	S	7.84×10^6	1.65×10^{-2}	2.11×10^{-9}
32	P	T	R	Y	S	1.06×10^6	5.30×10^{-4}	4.98×10^{-10}
46	P	V	R	Y	S	7.30×10^5	1.24×10^{-4}	1.69×10^{-10}
92	A	H	N	F	M	3.94×10^6	1.45×10^{-2}	3.68×10^{-9}

Example 2.3 and Figure 3 demonstrate that virus-antibody immune complexes comprising PN04-90 Fc, which binds to TRIM21 PRYSPRY domain with an affinity of $K_D = 4.25 \times 10^{-10}$ M, stimulate maturation of moDC to a greater extent than equivalent virus-antibody immune complexes comprising wildtype human IgG1 Fc.

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The inventors next investigated whether the improved ability to stimulate moDC maturation was also possessed by virus-antibody immune complexes comprising variant Fc having a more modest improvement in the affinity of binding to TRIM21 PRYSPRY domain relative to wildtype human IgG1 Fc.

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The inventors therefore investigated the co-stimulatory molecule expression by DCs following co-culture with immune complexes comprising V1 Fc. V1 Fc binds to TRIM21 PRYSPRY domain with an affinity of $K_D = 1.08 \times 10^{-8}$ M (see Example 2.1 above).

25

A moDC maturation assay was performed as described in Example 1.12, using moDCs from PBMCs obtained from three different donors. The following test conditions were investigated:

- PBS only (PBS; negative control);
- Ad5-specific antibody comprising wildtype IgG1 Fc (WT only)
- Ad5-specific antibody comprising V1 Fc (V1 only)
- Ad5-specific antibody comprising PN04-90 Fc (PN04-90 only)

30

- Ad5 only (Virus only)
- Immunocomplex comprising Ad5-specific antibody comprising wildtype IgG1 Fc + Ad5 (WT + Virus)
- Immunocomplex comprising Ad5-specific antibody comprising V1 Fc + Ad5 (V1 + Virus)
- 5 • Immunocomplex comprising Ad5-specific antibody comprising PN04-90 Fc + Ad5 (PN04-90 + Virus)
- LPS only (LPS; positive control)

10 The results are shown in Figure 14. V1 Fc + Ad5 complexes promoted significantly greater moDC maturation than wildtype IgG1 Fc + Ad5 complexes.

Based on these results it can be concluded that even a modest improvement in affinity for TRIM21 provides functional effects relevant to therapeutic and prophylactic applications of molecules and complexes comprising Fc regions.

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