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(54) **Title:** METHOD AND MEANS FOR ENHANCING THERAPEUTIC ANTIBODIES

(57) **Abstract:** The invention relates to a pharmaceutical composition comprising an IgM antibody or a fragment thereof and a therapeutic antibody, wherein the IgM antibody specifically binds to the therapeutic antibody. The invention further relates to a method of treatment of a disease or disorder, the method comprising the steps of: a) administering an effective dose of a therapeutic antibody; and b) administering a corresponding dose of an IgM antibody or a fragment thereof, wherein the IgM antibody specifically binds to the therapeutic antibody.

METHOD AND MEANS FOR ENHANCING THERAPEUTIC ANTIBODIES

FIELD OF THE INVENTION

The invention relates to a pharmaceutical composition comprising an IgM antibody or a fragment thereof and a therapeutic antibody, wherein the IgM antibody specifically binds to the therapeutic antibody. The invention further relates to a method of treatment of a disease or disorder, the method comprising the steps of: a) administering an effective dose of a therapeutic antibody; and b) administering a corresponding dose of an IgM antibody or a fragment thereof, wherein the IgM antibody specifically binds to the therapeutic antibody.

DESCRIPTION

Self-tolerance is crucial for maintaining physiological integrity by avoiding autoimmune reactions. Currently, absolute central and peripheral tolerance are believed to control the B cell receptor (BCR) repertoire during B cell development thereby preventing positive selection of self-reactive B cells [1,2,4]. It is assumed that central tolerance forces deletion of autoreactive B cells during early B cell development in the bone marrow [2,5–7]. Furthermore, autoreactive B cells escaping clonal deletion are subjected to receptor editing resulting in non-autoreactive BCR specificities [8–10]. Self-reactive B cells that circumvent central tolerance and migrate to the periphery are counteracted by clonal anergy (peripheral tolerance) leading to unresponsiveness mainly by downmodulation of IgM BCR expression [1,11–13]. However, the finding that the vast majority of serum IgM is autoreactive seems to contrast the concept of general elimination of autoreactivity [14]. In fact, the so-called natural polyreactive IgM plays important roles in homeostasis [15] arguing against the absolute elimination of autoreactive antibodies.

Interestingly, it has been shown that disease-specific autoreactive B cells are present within the pre-immune repertoire and that germinal centers (GC) specific for insulin, a common autoantigen, can be formed in wildtype mice contradicting the concept of central B cell tolerance [16,17].

In the past decades, B cell autoimmunity research focused largely on transgenic mouse models [1,2,5,18,19]. The usefulness of these models for studying autoimmunity has been heavily debated for several reasons [20]. Replacement of the germline configuration by a high-affinity mutated autoreactive BCR not only leads to an atypical situation during

B cell development, it also generates a monospecific repertoire [1,5,19]. Moreover, the characteristics of these antigens with regard to their availability, valency and form (soluble vs. membrane-bound) have not been adequately addressed [5, 18]. Furthermore, the antigens themselves do not have any relevance to known autoimmune diseases [21, 22].

Epidemiological studies show that up to 5% of the population in industrialized countries suffers from autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or type-1-diabetes (T1D) [21]. Notably, autoantibodies are present in the vast majority of autoimmune diseases and often are the driving force of pathogenesis [22].

Hence, there is a continued need to develop approaches for a controllable modulation of antibody based therapies in order to detect or treat or avoid conditions and or diseases.

The above technical problem is solved by the embodiments disclosed herein and as defined in the claims.

Accordingly, the invention relates to, inter alia, the following embodiments:

1. A pharmaceutical composition comprising an IgM antibody or a fragment thereof and a therapeutic antibody, wherein the IgM antibody specifically binds to the therapeutic antibody.
2. The pharmaceutical composition of embodiment 1, wherein the IgM antibody and the therapeutic antibody are comprised in a molar ratio of 5:1 to 1:10, preferably 2:1 to 1:5.
3. A method of treatment of a disease or disorder, the method comprising the steps of:
   a) administering an effective dose of a therapeutic antibody; and
   b) administering a corresponding dose of an IgM antibody or a fragment thereof, wherein the IgM antibody specifically binds to the therapeutic antibody, preferably wherein the corresponding dose of the IgM antibody is between 10% and 400% of the effective dose of the therapeutic antibody, more preferably 20% and 200% of the effective dose of the therapeutic antibody.
4. The pharmaceutical composition of embodiment 1 or 2 or the method of treatment of embodiment 3, wherein a half-live of the therapeutic antibody is prolonged by the binding of the IgM antibody.
5. The pharmaceutical composition of any one of the embodiments 1, 2 or 4 or the method of treatment of embodiment 3 or 4, wherein the IgM antibody binds to the therapeutic antibody with a KD of at least 10-8, preferably measured with Biolayer Interferometry.

6. The pharmaceutical composition of any one of the embodiments 1, 2, 4 or 5 or the method of treatment of any one of the embodiments 3 to 5, wherein the therapeutic antibody is an anti-rheumatoid arthritis antibody.
7. The pharmaceutical composition of any one of the embodiments 1, 2, 4 to 6 or the method of treatment of any one of the embodiments 3 to 6, wherein the therapeutic antibody is an anti-CD20 antibody.
8. The pharmaceutical composition of embodiment 5 or the method of treatment of embodiment 7, wherein the therapeutic antibody is Rituximab.
9. The pharmaceutical composition of any one of the embodiments 1 to 7 for use in treatment of an autoimmune disease or disorder.
10. The pharmaceutical composition for use of embodiment 8, wherein the autoimmune disease or disorder is multiple sclerosis or rheumatoid arthritis.
11. The method of treatment of anyone of the embodiments 3 to 8, wherein the disease or disorder is an autoimmune disease or disorder.
12. The method of treatment of embodiment 11, wherein the autoimmune disease or disorder is multiple sclerosis or rheumatoid arthritis.
13. A method for obtaining a protective-regulative antibody comprising the steps of:

(a) providing a blood sample of a subject, wherein the subject experienced elicitation of an IgG and oligomeric antibody response by a target antigen; and

(b) enriching a maturated oligomeric antibody, wherein

(i) the binding of the oligomeric antibody is more specific for the target antigen than the IgG-type antibody, preferably wherein the oligomeric antibody is monospecific for the target antigen; and/or

(ii) the binding affinity of the oligomeric antibody to the target antigen is equal or higher than the IgG-type antibody, preferably wherein the protective-regulative antibody binds to the target antigen with K_d of less than 10^{-7}, preferably of less than 10^{-8}, more preferably of less than 10^{-9} and most preferably in the range of about 10^{-10} to about 10^{-12},

(c) isolating the enriched maturated oligomeric antibody to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

14. The method according to embodiment 13, wherein the subject experienced elicitation of the IgG and oligomeric antibody response by the target antigen at least 7 days ago, preferably at least 14 days ago, more preferably at least 27 days ago.

15. A method for obtaining a degrading oligomeric antibody comprising the steps of:

(a) providing a blood sample of a subject, wherein the subject experienced elicitation of an IgG and oligomeric antibody response by a target antigen; and

(b) enriching a primary oligomeric antibody, wherein

(i) the binding of the oligomeric antibody is equally or less specific for the target antigen than the IgG-type antibody, preferably wherein the oligomeric antibody is cross-specific for the target antigen and DNA; and/or

(ii) the binding affinity of the oligomeric antibody to the target antigen is lower than the IgG-type antibody, preferably wherein the protective-regulative antibody binds to the target antigen with K_d of more than 10^{-7},

(c) isolating the enriched primary oligomeric antibody to obtain the degrading antibody that can form immune-degradable complexes with the target antigen.

16. The method according to any one of the embodiments 13 to 15, wherein the blood sample is selected from the group consisting of whole blood, plasma and serum sample, preferably serum sample.

17. The method according to any one of the embodiments 13 to 16, wherein isolating an oligomeric antibody comprises mass- and/or affinity-related isolation.

18. The method according to any one of the embodiments 13 to 17, wherein enriching an oligomeric antibody comprises immunoprecipitation of the oligomeric antibody.

19. The method according to any one of the embodiments 13 to 18, wherein the oligomeric antibody is an IgM antibody.

20. The pharmaceutical composition of any one of the embodiments 1, 2, 4 to 8, the

pharmaceutical composition for use of any one of the embodiments 9 to 12 or the method of treatment of any one of the embodiments 3 to 8, wherein the IgM antibody comprises:

a variable heavy (VH) chain comprising CDR1 sequence as encoded by SEQ ID NO: 60, CDR2 sequence as encoded by SEQ ID NO: 61 and CDR3 sequence as encoded by SEQ ID NO: 62 and a variable light (VL) chain comprising CDR1 sequence as encoded by SEQ ID NO: 57, CDR2 sequence as encoded by GGTGCATCC and CDR3 sequence as encoded by SEQ ID NO: 58.

21. The pharmaceutical composition of embodiment 20, the pharmaceutical composition for use of embodiment 20 or the method of treatment of embodiment 20, wherein the IgM antibody comprises:

a variable heavy (VH) chain sequence comprising the amino acid sequence encoded by the sequence as defined by SEQ ID NO: 59 or by a sequence having at least 90% sequence identity to SEQ ID NO: 59, preferably at least 95% sequence identity to SEQ ID NO: 59; and

a variable light (VL) chain sequence comprising the amino acid sequence encoded by the sequence as defined by SEQ ID NO: 56 or by a sequence having at least 90% sequence identity to SEQ ID NO: 56, preferably at least 95% sequence identity to SEQ ID NO: 56.

22. A host cell comprising a polynucleotide having

a) a sequence as defined by SEQ ID NO: 59 or a sequence having at least 90% sequence identity to SEQ ID NO: 59, preferably at least 95% sequence identity to SEQ ID NO: 59; and/or

b) a sequence as defined by SEQ ID NO: 56 or a sequence having at least 90% sequence identity to SEQ ID NO: 56, preferably at least 95% sequence identity to SEQ ID NO: 56;

wherein the polynucleotide further encodes an IgM constant region and/or wherein the host cell comprises a further polynucleotide encoding an IgM constant region.

23. A method for producing an IgM antibody, the method comprising the steps of:

a) culturing the host cell according to embodiment 22,

b) isolating an IgM antibody.

In the following, the elements of the invention will be described. These elements are listed with specific embodiments; however, it should be understood that they may be combined

in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine two or more of the explicitly described embodiments or which combine the one or more of the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Accordingly, in one embodiment, the invention relates to a pharmaceutical composition comprising an IgM antibody or a fragment thereof and a therapeutic antibody, wherein the IgM antibody specifically binds to the therapeutic antibody.

The inventors identified IgM antibodies and ways to obtain IgM antibodies that stabilize IgG and intensify its effect *in vivo* upon binding thereto. This stabilizing effect is dependent on the affinity of the IgM antibody for IgG. Without being bound by theory, the effect can be independent of the pathogenic or beneficial nature of the target IgG. In fact, the inventors found that high affinity IgM stabilizes the autoreactive insulin-specific IgG and induces higher blood glucose levels prolonging the hyperglycemic state.

These findings are in sharp contrast to the current view proposing that autoantibodies develop in consequence to defects in central and peripheral tolerance mechanisms which in healthy conditions should prevent the development of autoreactive B cells.

Accordingly, the invention is at least in part based on the protecting and regulating properties of IgM antibodies on IgG antibodies, which can improve the treatment of improving the efficacy of IgG treatments such as therapeutic antibodies.

In some embodiments, the IgM antibody in the pharmaceutical composition of the invention is recombinantly produced. In some embodiments, the IgM antibody in the pharmaceutical composition of the invention is isolated from human blood, e.g., human plasma.

The fragment in the pharmaceutical composition of the invention is preferably an antigen binding fragment of the IgM antibody having similar, the same or substantially the same binding properties as the parent IgM antibody.

The phrase " IgM antibody specifically binds to the therapeutic antibody", as used herein, refers to an IgM antibody or a fragment thereof that is capable of binding the therapeutic antibody with sufficient affinity such that the therapeutic antibody is more useful as a preventive, diagnostic and/or therapeutic agent for the desired purpose. In some

embodiments, the IgM antibody in the pharmaceutical composition of the invention binds to the therapeutic antibody with a K_d of less than 10^{-7}, preferably of less than 10^{-8}, more preferably of less than 10^{-9} and most preferably in the range of about 10^{-10} to about 10^{-12}. In certain embodiments, the K_d is measured with Biolayer Interferometry.

The terms "RF" and "rheumatoid factor" are used herein interchangeably and refer to an IgM antibody binding IgG if not stated otherwise.

The IgM antibody in the pharmaceutical composition of the invention preferably binds to a region, which does not or not substantially hinder the target binding activity of the IgG antibody, e.g. the Fc region of the IgG antibody. In some embodiments, the IgM antibody described herein a recombinant antibody and is not or not substantially glycosylated. Endogenous IgM antibodies are typically glycosylated. However, the inventors found, that IgM antibodies do not require the glycosylation for their protective function.

In some embodiments, the IgM antibody described herein is an antibody selected from the group of: monomeric IgM antibody, dimeric IgM antibody, trimeric IgM antibody, quatromeric IgM antibody, pentameric IgM antibody and hexameric IgM antibody.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention, wherein the IgM antibody and the therapeutic antibody are comprised in a molar ratio of about 10:1 to about 1:100, or about 7:1 to about 1:50 or about 5:1 to about 1:10, or about 2:1 to about 1:5, or about 1:1.

The ratio of the IgM antibody and the therapeutic antibody can depend on the number of binding sites of the IgM antibody.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention, wherein the IgM antibody is a monomeric antibody and wherein the IgM antibody and the therapeutic antibody are comprised in a molar ratio of about 10:1 to about 1:10, or about 7:1 to about 1:5 or about 5:1 to about 1:2, or about 2:1 to about 1:1, or about 1:1.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention, wherein the IgM antibody is a pentameric antibody and wherein the IgM antibody and the therapeutic antibody are comprised in a molar ratio of about 10:1 to about 1:50, or about 3:1 to about 1:20 or about 2:1 to about 1:10, or about 1:1 to about 1:5, or about 1:1.

In certain embodiments, the invention relates to a method of treatment of a disease or disorder, the method comprising the steps of: a) administering an effective dose of a therapeutic antibody; and b) administering a corresponding dose of an IgM antibody or a fragment thereof, wherein the IgM antibody specifically binds to the therapeutic antibody

Various factors can influence the actual effective amount used for a particular application. For example, the frequency of administration, duration of treatment, use of multiple treatment agents, route of administration, and severity of the disease, disorder and/or condition may require an increase or decrease in the actual effective amount administered.

The administration of the therapeutic antibody and the IgM antibody can be done sequentially or simultaneously. Typically, the therapeutic antibody and the IgM antibody are administered in a way such that they are present at the same time in substantial amounts in the body of a subject. For example, the therapeutic antibody and the IgM antibody can be administered within one, two, three or four half-live period(s) of the therapeutic antibody and/or the IgM antibody. In some embodiments, therapeutic antibody and the IgM antibody described herein are brought into contact before administration such that a protecting binding can occur before exposure to the body of the subject.

In certain embodiments, the invention relates to a method of treatment of a disease or disorder, the method comprising the steps of: a) administering an effective dose of a therapeutic antibody; and b) administering a corresponding dose of an IgM antibody or a fragment thereof, wherein the IgM antibody specifically binds to the therapeutic antibody and wherein the corresponding dose of the IgM antibody is between 10% and 400% of the effective dose of the therapeutic antibody, preferably 20% and 200% of the effective dose of the therapeutic antibody.

Monomeric IgM antibodies typically benefit from an equal molar amount, or an excess compared to the therapeutic antibody. Oligomeric, e.g. pentameric IgM antibodies typically require less amounts of IgM antibodies e.g. 20% to 100% of the molar amount of therapeutic antibody.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention or the method of treatment of the invention, wherein a half-live of the therapeutic antibody is prolonged by the binding of the IgM antibody.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention or the method of treatment of the invention, wherein the therapeutic antibody is an anti-rheumatoid arthritis antibody.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention or the method of treatment of the invention, wherein the therapeutic antibody is an anti-CD20 antibody.

In certain embodiments, the invention relates to the pharmaceutical composition of the

invention or the method of treatment of the invention, wherein the therapeutic antibody is Rituximab.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention for use in treatment of an autoimmune disease or disorder.

The fact that low affinity RFs are found in healthy individuals and regulate half-life of IgG suggests that IgG homeostasis is controlled by such RFs and that defects in generating RF^{low} might be an important trigger for the development of autoimmune diseases. The means and method described herein have an effect in or on the disturbed homeostasis and are therefore particularly useful in the context of an autoimmune disease.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein the autoimmune disease or disorder is multiple sclerosis or rheumatoid arthritis.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the disease or disorder is an autoimmune disease or disorder.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the autoimmune disease or disorder is multiple sclerosis or rheumatoid arthritis.

In certain embodiments, the invention relates to a composition, comprising: (i) a monovalent antigen particle comprising an antigenic portion comprising not more than one antigenic structure capable of inducing an antibody mediated immune response against a target antigen; and (ii) a polyvalent antigen particle comprising an antigenic portion comprising more than one antigenic structures capable of inducing an antibody mediated immune response against the target antigen and wherein the more than one antigenic structure is cross-linked.

The term “valent” as used within the current application denotes the presence of a specified number of binding sites in an antibody or antigen, respectively, molecule. As such a binding site of an antibody is a paratope, whereas a binding site in the antigen is generally referred to as epitope. A natural antibody for example or a full length antibody according to the invention has two binding sites and is bivalent. Antigen proteins are monovalent (when present as monomers), however, if such antigen proteins are provided as multimers they may comprise more than one identical epitope and therefore are polyvalent, which may be bivalent, trivalent, tetravalent etc. As such, the terms “trivalent", denote the presence of three binding sites in an antibody molecule. As such, the terms "tetravalent", denote the presence of four binding sites in an antibody molecule.

The term “monovalent antigen particle” shall in context of the herein disclosed invention refer to a molecule or molecule-complex, such as a protein, or protein complexes, which are antigenic, and therefore capable of stimulating an immune response in a vertebrate. Typically, a monovalent antigen particle is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against such antigenic structure. As used herein, the term "antigenic structure” refers to fragment of an antigenic protein that retains the capacity of stimulating an antibody mediated immune response. Such an antigenic structure is understood to provide the antigenic determinant or “epitope” which refers to the region of a molecule that specifically reacts with an antibody, more specifically that reacts with a paratope of an antibody. In preferred embodiments of the invention a monovalent antigen particle of the invention comprises not more than one copy of one specific epitope of the antigenic structure. Hence, preferably only one antibody molecule of a certain antibody species having a specific paratope may bind to a monovalent antigen particle according to the invention.

The term “polyvalent antigen particle” shall in context of the herein disclosed invention refer to a molecule or molecule-complex, such as a protein, or protein complexes, which are antigenic, and therefore capable of stimulating an immune response in a vertebrate. In the invention, unlike monovalent antigenic particles, a polyvalent antigenic particle is composed of an antigenic portion comprising more than one of an antigenic structure capable of inducing an antibody mediated immune response. In preferred embodiments of the invention a polyvalent antigen particle of the invention comprises more than one copy of one specific epitope of the antigenic structure. Hence, preferably more than one antibody molecule of a certain antibody species having a specific paratope may bind to a monovalent antigen particle according to the invention. Such polyvalent antigen particle may have a structure that the more than one of an antigenic structure are covalently or non-covalently cross-linked with each other. Preferably, the more than one of an antigenic structure comprised in the antigenic portion of the polyvalent antigen particle comprises multiple identical antigenic structures.

In context of the invention the monovalent antigen particle of the invention is often referred to as “soluble” particle or antigen whereas the polyvalent antigen particle is referred to as “complexed” particle or antigen.

The term “antigen” may refer to any, preferably disease associated, molecule or structure that comprises an antigenic structure. Preferably an antigen described herein is an

autoantigen, a cancer associated antigen, or a pathogen associated antigen. In one very specific exemplary embodiment of the invention the antigen is insulin and the associated disease is diabetes. Human insulin protein is produced as proinsulin comprising a c-peptide, insulin B chain and the active insulin peptide. The amino acid sequence and further characteristics are well known to the skilled artisan and can be derived under accession no. P01308 in the UniProt database in the Version of January 27, 2020 (https://www.uniprot.org/uniprot/P01308).

The target antigen of the invention is preferably an antigen which is associated with a disease or condition, preferably a disease or condition the subject suffers or is suspected to suffer from. Such disease, as mentioned, may be pathogen associated, autoimmune associated, might by associated with a treatment, for example when using an antigenic protein as therapeutic such as a therapeutic antibody, or cancer associated or the like. A target antigen of the invention can be a natural or synthetic immunogenic substance, such as a complete, fragment or portion of an immunogenic substance, and wherein the immunogenic substance may be selected from a nucleic acid, a carbohydrate, a peptide, a hapten, or any combination thereof.

In context of the present invention, it is distinguished between monovalent antigenic particles opposed to multivalent antigenic particles. Each particle is considered as a single molecular entity, which may comprise covalently or non-covalently connected portions. However, according to the present invention each particle has an immunogenic activity towards a certain antigen. The monovalent antigen particle is therefore understood to comprise only a single antigenic structure that is able to elicit an immune response to the antigen whereas the multivalent antigen particle comprises multiple copies of such antigenic structure. In context of the present invention sometimes also the terms “soluble” antigen is used for the monovalent antigen particle opposed to “complex” antigen for the polyvalent antigen particle. It is understood that in most instances the antigenic structure comprises or consists of an epitope that elicits an antibody-mediated immune response, and in turn is a binding site for an antibody produced upon a cell-mediated immune response as defined herein elsewhere. In other words, the invention distinguishes between a presentation of immune eliciting epitopes as soluble single epitope or in a complexed array identical epitope.

The term “cross-link”, as used herein, refers to a bond that links at least two antigenic structures with each other, wherein the cross-linked complex has different physical properties than the separated antigenic structures. In some embodiments the cross-

linked complex is less soluble than the separated antigenic structures. In some embodiments, the cross-link described herein comprises at least one covalent bond. In some embodiments, the cross-link described herein comprises at least one ionic bond.

The present invention is predicated upon the surprising finding that antigens may induce different immune responses depending on whether they are presented to immune cells as soluble antigens or as complexed multivalent antigens. The latter in particular lead to strong and memory IgG antibody responses, whereas the former may repress such IgG response and induce a protective IgM (or an IgA) antibody response.

Accordingly, the invention is at least in part based on the surprising finding that the composition of the invention can modulate the immune response to a target antigen as described herein. Hence, the invention suggests to modulate the ratio soluble to complexed immune responses in order control the focus of B-cell immunity. The approach may be used in novel controlled vaccination treatments or for tackling autoimmune diseases such as diabetes.

In certain embodiments, the invention relates to the composition of the invention, wherein the more than one antigenic structures comprise multiple identical antigenic structures.

In preferred embodiments of the invention a polyvalent antigen particle of the invention comprises more than one copy of one specific epitope of the antigenic structure. Hence, preferably more than one antibody molecule of a certain antibody species having a specific paratope may bind to a monovalent antigen particle according to the invention. Such polyvalent antigen particle may have a structure that the more than one of an antigenic structure are covalently or non-covalently cross-linked with each other. A polyvalent antigen particle therefore, in preferred embodiments comprises complex comprising at least two identical, at least three or at least four epitopes, which allow for a binding of two antibodies to the polyvalent antigen particle at the same time. Preferably, the more than one of an antigenic structure comprised in the antigenic portion of the polyvalent antigen particle comprises multiple identical antigenic structures.

A polyvalent antigen particle therefore, in preferred embodiments comprises complex comprising at least two, at least three or at least four identical epitopes, which allow for a binding of two antibodies to the polyvalent antigen particle at the same time.

The composition comprising such particles (see e.g. Fig. 2 a, Fig. 21) invention can modulate an immune response (see e.g. Fig. 18).

Accordingly, the invention is at least in part based on the surprising finding that a plurality

of linked identical structures can modulate the immune response to a target antigen as described herein.

In certain embodiments, the invention relates to the composition of the invention, wherein the monovalent antigen particle further comprises a carrier portion which is coupled to the antigenic portion and wherein the carrier does not comprise another copy of the antigenic structure.

In some embodiments of the invention, the monovalent-antigen particle further comprises a carrier portion which is coupled to the antigenic portion, optionally via a linker, and wherein the carrier, and optionally the linker, does not comprise another copy of the antigenic structure, and wherein the carrier portion, and optionally the linker, is/are not capable of eliciting a cell-mediated immune response against the target antigen. In another alternative or additional embodiment of the invention, the polyvalent-antigen particle further comprises a carrier portion which is coupled to the antigenic portion, optionally via a linker. A "linker" in context of the present invention may comprise any molecule, or molecules, proteins or peptides which may be used to covalently or non-covalently connect two portions of the compounds of the invention with each other.

The term "carrier portion" in context of the herein disclosed invention preferably relates to a substance or structure that presents or comprises the antigenic structures of the particles of the invention. A carrier portion is preferably a substance or structure selected from immunogenic or non-immunogenic polypeptides, immune CpG islands, limpet hemocyanin (KLH), tetanus toxoid (TT), cholera toxin subunit B (CTB), bacteria or bacterial ghosts, liposome, chitosome, virosomes, microspheres, dendritic cells, particles, microparticles, nanoparticles, or beads.

Preferably, neither the carrier portion, and optionally also not the linker, is (are) capable of eliciting a cell-mediated immune response against the target antigen, such as the antigen associated with an autoimmune disorder.

A "linker" in context of the invention is preferably peptide linker which may have any size and length suitable for a given application in context of the invention. Linkers may have a length or 1-100 amino acids, preferably of 2 to 50 amino acids. A linker could be a typical 4GS linker in 2, 3, 4, 5, 6 or more repeats.

The carrier portion can facilitate presentation of the antigen to the immune system and improve stability of the particle.

Accordingly, the invention is at least in part based on the surprising finding that a carrier linked to the antigenic portion can improve the antigenic, pharmacologic and/or pharmacokinetic properties of the monovalent antigen particle and therefore influence the modulation of the immune response to a target antigen as described herein.

In certain embodiments, the invention relates to the composition of the invention, wherein the polyvalent antigen particle further comprises a carrier portion which is coupled to the antigenic portion.

The carrier portion can facilitate presentation of the antigen to the immune system and improve stability of the particle.

Accordingly, the invention is at least in part based on the surprising finding that a carrier linked to the antigenic portion can improve the antigenic, pharmacologic and/or pharmacokinetic properties of the polyvalent antigen particle and therefore influence the modulation of the immune response to a target antigen as described herein.

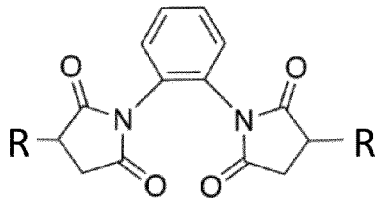
In certain embodiments, the invention relates to the composition of the invention, wherein the carrier portion comprises a structure selected from the group of polypeptides, immune CpG islands, limpet hemocyanin (KLH), tetanus toxoid (TT), cholera toxin subunit B (CTB), bacteria or bacterial ghosts, liposome, chitosome, virosomes, microspheres, dendritic cells, particles, microparticles, nanoparticles, or beads.

A carrier portion is preferably a substance or structure selected from immunogenic or non-immunogenic polypeptides, immune CpG islands, limpet hemocyanin (KLH), tetanus toxoid (TT), cholera toxin subunit B (CTB), bacteria or bacterial ghosts, liposome, chitosome, virosomes, microspheres, dendritic cells, particles, microparticles, nanoparticles, or beads.

Certain carrier portions are particularly useful for presentation of the antigen to the immune system and/or for improvement stability of the particle, while being biologically tolerated.

Accordingly, the invention is at least in part based on the surprising finding that certain specific carriers linked to the antigenic portion can improve the antigenic, pharmacologic and/or pharmacokinetic properties of the monovalent antigen particle and therefore influence the modulation of the immune response to a target antigen as described herein.

In certain embodiments, the invention relates to the composition of the invention, wherein the polyvalent-antigen particle comprises a complex of the following formula A-L-A, wherein A is a target antigen comprising portion, and wherein L is the linker of the cross link, preferably wherein L is a bismaleimide, and most preferably the complex is of the following structure (I), wherein R is a target antigen comprising portion:



(I).

Preferably, neither the carrier portion, and optionally also not the linker, is (are) capable of eliciting an antibody-mediated immune response against the target antigen.

The carrier portion can facilitate presentation of the antigen to the immune system and improve stability of the particle.

Accordingly, the invention is at least in part based on the surprising finding that a carrier linked to the antigenic portion can improve the antigenic, pharmacologic and/or pharmacokinetic properties of the polyvalent antigen particle and therefore influence the modulation of the immune response to a target antigen as described herein.

In certain embodiments, the invention relates to the composition of the invention, wherein the polyvalent-antigen particle comprises a linker with a crosslink reactive group for protein conjugation.

The term “crosslink reactive group for protein conjugation”, as used herein, refers to any chemical group or structure that enables creating a link between the antigen particles described herein and a protein. Such crosslink reactive groups ant the preparation thereof a well known to the person skilled in the art (see e.g. Brinkley, M., 1992, Bioconjugate chemistry, 3(1), 2-13; Kluger, R., & Alagic, A, 2004, Bioorganic chemistry 32.6 (2004): 451-472.; Stephanopoulos, N.; Francis, M. B., 2011, Nature Chemical Biology. 7 (12): 876–884.).

The inventors found that a linker that is linked to the antigen particle described herein (e.g. the polyvalent antigen particle) and that comprises a crosslink reactive group to bind

to endogenous protein in a subject can enhance the immune response (see e.g. Figure 34 – 36, Example 12, 13, 15).

In certain embodiments, the invention relates to the composition of the invention, wherein the polyvalent-antigen particle comprises a linker with a crosslink reactive group for stable protein conjugation.

The term "stable protein conjugation", as used herein, refers to a covalent protein conjugation that is not an S-S binding. In some embodiments, the stable protein conjugation described herein is hydrolytically stable. In some embodiments, the stable protein conjugation described herein is an irreversible binding.

The inventors found that stable binding to endogenous proteins can enhance the immune reaction against the antigen particles described herein (Example 14).

In certain embodiments, the invention relates to the composition of the invention, wherein the crosslink reactive group couples to a protein with at least one selected from the group of lysine amino acid residue, cysteine residue, tyrosine residues, tryptophan residues, N-terminus and C- terminus.

In certain embodiments, the invention relates to the composition of the invention, wherein the crosslink reactive group is a group selected from carboxyl-to-amine reactive groups, amine-reactive groups, sulfhydryl-reactive groups, aldehyde-reactive groups and photoreactive groups.

In certain embodiments, the invention relates to the composition of the invention, wherein the crosslink reactive group is a group selected from carbodiimide, NHS ester, imidoester, pentafluorophenyl ester, hydroxymethyl phosphine, maleimide, haloacetyl, hydrazide, alkoxyamine, diazirine and aryl azide.

Accordingly, the invention is at least in part based on the enhancement of the immune response by binding to endogenous proteins.

In certain embodiments, the invention relates to the composition of the invention, wherein the polyvalent antigen particle is linked to an adjuvant, preferably wherein the polyvalent particle is covalently linked to an adjuvant.

The term “adjuvant”, as used herein, refers to an agent that does not comprise the target antigen and can enhance the immune response to the antigen particles described herein. In some embodiments, the adjuvant described herein comprises at least one adjuvant selected from the group of oils (e.g., paraffin oil, peanut oil), bacterial products, saponins, cytokines (e.g., IL-1, IL-2, IL-12), squalene and IgG, preferably wherein the adjuvant comprises a free SH-group.

The inventors found that linking the antigen particles described herein to adjuvants can enhance the immune response, in particular the immune response induce by the polyvalent antibody (Figure 36D and E, Figure 34). This linking to adjuvants reduces the necessity of formulating the antigen particles described herein with substantially larger amounts of non-linked adjuvants. Furthermore, the adjuvants can increase the stability of the antigen particles described herein.

Accordingly, the invention is at least in part based on the finding that linking of the antigen particles described herein to adjuvants can enhance the elicited immune response.

In certain embodiments, the invention relates to the composition of the invention, wherein the polyvalent antigen particle comprises the at least two copies of the antigenic structure in spatial proximity to each other.

A polyvalent-antigen particle of the invention preferably comprises the at least two copies of the antigenic structure in spatial proximity to each other, preferably within a nanometer range selected from the ranges 1 nm to 10 µm, more preferably 1nm to 5µm, 1nm to 1000nm, 1nm to 500nm, 1nm to 100nm, 1nm to 50nm and 1nm to 10nm.

The term “spatial proximity”, as used herein, refers to being on the same antigen particle and sufficiently close distance to modulate the immune response. The “sufficiently close” depends on the size and structure of the polyvalent antigen particle itself and the size of the antigenic structure. In some embodiments, the distance between two copies of the antigenic structure is within a range of 3 nm to 20 nm.

In some embodiments, the at least two copies of the antigenic structure are in a spatial proximity in the range of about 1 nm to about 1000 nm, preferably about 1 nm to about 500 nm, preferably about 1nm to about 100 nm, preferably about 1 nm to about 50 nm, preferably about 1 nm to about 20 nm, or preferably about 3 nm to about 20 nm

Methods for measurement of spatial proximity are known to the person skilled in the art

(see e.g. F. Schueder et al., 2021, Angew. Chem. Int. Ed. 2021, 60, 716; Erickson, D. et al., 2008, Microfluidics and nanofluidics, 4(1-2), 33-52; Turkowyd, B., et al., 2016, Anal Bioanal Chem 408, 6885–6911).

The inventors found that the polyvalent particles in a certain size range are particularly effective in electing certain immune responses.

Accordingly, the invention is at least in part based on the surprising finding that the size of the antigenic particle and/or the spatial proximity can influence the modulation of the immune response to a target antigen as described herein.

In certain embodiments, the invention relates to the composition of the invention, wherein the target antigen comprises at least one agent selected from the group of nucleic acid, carbohydrate, peptide, and hapten.

The term “hapten”, as used herein, refers to a small molecule which elicits a detectable immune response when attached to a carrier moiety. Haptens described herein can also include an immunogenic group. In some cases, the immunogenic group comprises a fluorescent group, an enzyme or fragment thereof, a peptide or fragment thereof, or biotin. In some instances, the immunogenic groups are selected from the list comprising biotin, fluorescein, digoxigenin or dinitrophenyl.

Nucleic acids, carbohydrates, peptides, and/or haptens are useful structures to copy or mimic endogenous or pathologic antigen patterns. Furthermore, they can be designed to elicit a specific immune response without substantial side effects.

Accordingly, the invention is at least in part based on the surprising finding that certain antigen types can influence the modulation of the immune response to a target antigen as described herein.

In certain embodiments, the invention relates to the composition of the invention, wherein the ratio of monovalent antigen particle:polyvalent antigen particle is greater than 1, preferably greater than 10^1, more preferably greater than 10^2, more preferably greater than 10^3, more preferably greater than 10^4.

In context of the present invention, it was found that a specific ratio of monovalent and polyvalent antigen can modulate antibody-mediated immune responses mediated by B-cells. Hence, it is a preferred embodiment of the invention the composition comprising the monovalent-antigen particle and the polyvalent-antigen particle comprises a specific antigen-ratio, which is preferably a ratio of monovalent-antigen particle to polyvalent-

antigen particle. In particular of such preferred embodiments modulating the cell-mediated target antigen-specific immune response in the subject constitutes a control of an IgG-type (and/or IgM) target antigen-specific B-cell response in the subject by contacting one or more of the B-cells of the subject with a composition comprising a specific antigen-ratio which is greater than 1, preferably greater than 10^1, 10^2, 10^3, 10^4 or more. In other embodiments of the invention the contacting one or more of the B-cells of the subject with the composition involves administering to the subject an amount of monovalent-antigen particle which is effective to generate in the subject a specific antigen-ratio which is greater than 1, preferably greater than 10^1, 10^2, 10^3, 10^4 or more.

The inventors found, that the ratio of monovalent antigen particle:polyvalent antigen particle can be used to modulate the immune response (see e.g. Fig. 18). A higher ratio of monovalent antigen particle:polyvalent antigen particle can reduce the polyvalent antigen particle-induced IgG antibody production (see e.g. Fig. 1b, d) and improve the production of protective-regulative IgM antibody production(see e.g. Fig. 7, 11). A higher ratio of monovalent antigen particle:polyvalent antigen particle can protect the function of a target antigen against an immune response(see e.g. Fig. 16).

Accordingly, the invention is at least in part based on the surprising finding that the modulation of the immune response to a target antigen depends on the monovalent antigen particle:polyvalent antigen particle ratio.

In certain embodiments, the invention relates to the composition of the invention, further comprising a pharmaceutically acceptable carrier and/or excipient.

The term "pharmaceutically acceptable carrier", as used herein, refers to an ingredient in the composition, other than the active ingredient(s), which is nontoxic to recipients at the dosages and concentrations employed.

Pharmaceutically acceptable carriers include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides,

and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US 2005/0260186 and US 2006/0104968.

The pharmaceutically acceptable carrier and/or excipient may facilitate stability, delivery and/or pharmacokinetic/pharmacodynamic properties of the composition of the invention.

In certain embodiments, the invention relates to a method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response, the method comprising the steps of: a) contacting one or more B-cells with the composition of the invention; and b) eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response.

A “cell-mediated target antigen-specific immune response” in context of the present invention shall refer to an immune response involving one or more B lymphocytes (B-cell), and preferably, a B-cell-mediated immune response.

The term “B lymphocyte” or “B cell”, as used herein, refers to a lymphocyte that plays a role in humoral immunity of the adaptive immune system, and which is characterised by the presence of the B cell receptor (BCR) on the cell surface. B cell types include plasma cells, memory B cells, B-1 cells, B-2 cells, marginal-zone B cells, follicular B cells, and regulatory B cells (B_{reg}). Further, the term “B cell” (also known as a “B lymphocyte”) refers to immune cells which express a cell surface immunoglobulin molecule and which, upon activation, terminally differentiate into cells, which secrete antibody. Accordingly, this includes, for example, convention B cells, CD5 B cells (also known as B-1 cells and transitional CD5 B cells). “B cell” should also be understood to encompass reference to B cell mutants. “Mutants” include, but are not limited to, B cells which have been naturally or non-naturally modified, such as cells which are genetically modified. Reference to “B cells” should also be understood to extend to B cells which exhibit commitment to the B cell image. These cells may be at any differentiative stage of development and therefore

may not necessarily express a surface immunoglobulin molecule. B cell commitment may be characterized by the onset of immunoglobulin gene re-arrangement or it may correspond to an earlier stage of commitment which is characterized by some other phenotypic or functional characteristic such as the cell surface expression of CD45R, MHCII, CD10, CD19 and CD38. Examples of B cells at various stages of differentiation include early B cell progenitors, early pro-B cells, late pro-B cells, pre-B cells, immature B cells, mature B cells, plasma cells, and memory (B) cells. In context of the present invention a B-cell can be seen as a non-maturated B-cell expressing mainly IgM type B-cell receptor, a maturated B-cell expressing mainly IgD type B-cell receptor or memory B-cell expressing IgG type B-cell receptor. The difference between the IgM type and IgD type B-cell receptor is the type of heavy chain sequence which either is of the μ or δ type. Methods to obtain genetically modified B-cells are known to the person skilled in the art (see e.g. Johnson, M. J., et al., 2018, Scientific reports, 8(1), 1-9.). Further methods to obtain cells which exhibit commitment to the B cell image are known to the person skilled in the art (see e.g. Brudno, J. N., 2018, Journal of Clinical Oncology, 36(22), 2267).

In context of the invention the term “cell-mediated target antigen-specific immune response” preferably pertains to a cellular immune type response involving an immune cell such as a lymphocyte, preferably a B lymphocyte (B-cell mediated immune response), preferably which comprises and/or expresses one or more antibody, or variants thereof, and/or B cell receptors, and/or variants thereof, which are specific for the target antigen. Preferably a cell-mediated target antigen-specific immune response involves a B cell expressing an Immunoglobulin (Ig) M, IgD, IgA or IgG type antibody and/or B-cell receptor.

In general, the term “contacting” shall be understood to present such antigen particles to the immune system of the subject in order to induce preferably a B-cell mediated immune response.

In some embodiments, the invention pertains to a method of eliciting and/or modulating a cell-mediated target antigen-specific immune response in a subject, the method comprising contacting one or more immune-cells (such as B-cells) of the subject with a composition comprising:

(i) a monovalent antigen particle which is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen, and

(ii) a polyvalent antigen particle which is composed of an antigenic portion comprising more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen and wherein the more than one of an antigenic structure are covalently or non-covalently cross-linked.

In some embodiments, which is an alternative to the first aspect, the invention pertains a composition for use in eliciting and/or modulating a cell-mediated target antigen-specific immune response in a subject, the composition comprising

(iii) a monovalent antigen particle which is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen, and

(iv) a polyvalent antigen particle which is composed of an antigenic portion comprising more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen and wherein the more than one of an antigenic structure are covalently or non-covalently cross-linked;

wherein the composition is used by contacting one or more immune-cells of the subject with the composition.

In preferred embodiments of the invention the contacting one or more immune-cells of the subject or patient with a composition comprising a monovalent-antigen particle and a polyvalent-antigen particle involves (i) administration of the monovalent-antigen particle to the subject, (ii) administration of the polyvalent-antigen particle to the subject, or (iii) administration of the monovalent-antigen particle and the polyvalent-antigen particle to the subject, wherein in (i), (ii) and (iii), the immune cells of the subject are as a result of the administration in contact with the composition the monovalent-antigen particle and the polyvalent-antigen particle. Preferably, in (i) the subject is characterized by the presence of the polyvalent-antigen particle before administration of the monovalent-antigen particle, and in (ii) the subject is characterized by the presence of the monovalent-antigen particle before administration of the polyvalent-antigen particle.

In further particular embodiments of the invention, the method is preferred wherein the contacting one or more of the B-cells of the subject with the amount of monovalent-

antigen particle is administered either with or without a direct combination of administering polyvalent-antigen particle to the subject.

In context of the present invention modulating the cell-mediated target antigen-specific immune response in the subject constitutes preferably an increasing of an IgG-type target antigen-specific B-cell response in the subject by contacting one or more of the B-cells of the subject with a composition comprising a specific antigen-ratio which is less than 1, preferably less than 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} or less. Preferably wherein the contacting one or more of the B-cells of the subject with the composition involves administering to the subject an amount of polyvalent-antigen particle which is effective to generate in the subject a specific antigen-ratio which is less than 1, preferably less than 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} or less.

It is preferred that the contacting one or more of the B-cells of the subject with the amount of polyvalent-antigen particle is administered either with or without a direct composition of administering monovalent-antigen particle to the subject.

In some embodiments, the method described herein is a non-therapeutic and non-surgical method. In this embodiment, the method of the invention is not for treating a subject but for inducing an immune response for, for example, the production and isolation of novel antibodies which are isolated in a subsequent step. In this embodiment, the subject is a generally healthy subject not suffering from any disease which is treated by performing the method. In this aspect the subject is preferably a non-human vertebrate.

In some embodiments, the method described herein is a method for diagnosis.

Accordingly, the invention is at least in part based on the surprising finding that the composition of the invention can be used to in a method to modulate B-cell immune responses.

In certain embodiments, the invention relates to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention, wherein the B-cell-mediated target antigen-specific immune response comprises one or more antibodies and/or B-cell receptors, and/or variants thereof, which are specific for the target antigen.

As used herein, the term “antibody” may be understood in the broadest sense as any immunoglobulin (Ig) that enables binding to its epitope. An antibody as such is a species

of an ABP. Full length “antibodies” or “immunoglobulins” are generally heterotetrameric glycoproteins of about 150 kDa, composed of two identical light and two identical heavy chains. Each light chain is linked to a heavy chain by one covalent disulphide bond, while the number of disulphide linkages varies between the heavy chain of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulphide bridges. Each heavy chain has an amino terminal variable domain (VH) followed by three carboxy terminal constant domains (CH). Each light chain has a variable N-terminal domain (VL) and a single C-terminal constant domain (CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to cells or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. Other forms of antibodies include heavy-chain antibodies, being those which consist only of two heavy chains and lack the two light chains usually found in antibodies. Heavy-chain antibodies include the hcIgG (IgG-like) antibodies of camelids such as dromedaries, camels, llamas and alpacas, and the IgNAR antibodies of cartilaginous fishes (for example sharks). And yet other forms of antibodies include single-domain antibodies (sdAb, called Nanobody by Ablynx, the developer) being an antibody fragment consisting of a single monomeric variable antibody domain. Single-domain antibodies are typically produced from heavy-chain antibodies, but may also be derived from conventional antibodies.

Typical antibody Ig variants discussed in context of the invention comprise IgG, IgM, IgE, IgA, or IgD antibodies.

The term “B-cell receptor”, as used herein, refers to a transmembrane protein on the surface of a B cell such as a membrane bound antibody.

The term “variant”, as used herein, refers to a first agent (e.g., a first molecule), that is related to a second agent (e.g., a parent molecule). The variant molecule (e.g. variant antibody, variant of a B-cell receptor) can be derived from, isolated from, based on, or homologous to the parent molecule. The term variant can be used to describe either

polynucleotides or polypeptides.

The method of the invention allows to induce an immune response comprising varying antibodies and/or B-cell receptors, and/or variants. Depending on properties of the composition of the invention (e.g. type of antigen, particle size, particle ratio, priming/boosting) and depending on the properties of the B-cell (cell type, maturation stadium, mutations) the immune response can be altered (see e.g. Fig. 1, 2, 3, 7, 8, 19, 20A).

Accordingly, the invention is at least in part based on the surprising finding that the composition of the invention can be used to in a method to modulate an antibody-mediated, B-cell receptor-mediated, and/or variant-mediated immune response to a target antigen.

In certain embodiments, the invention relates to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention, wherein the B-cell-mediated target antigen-specific immune response involves a B cell expressing an Immunoglobulin (Ig) M, IgD, IgA or IgG type antibody and/or B-cell receptor.

In certain embodiments, the invention relates to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention, wherein the B-cell-mediated target antigen-specific immune response involves a B cell expressing an Immunoglobulin (Ig) M, IgA and/or IgG type antibody and/or B-cell receptor.

In certain embodiments, the invention relates to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention, wherein the B-cell-mediated target antigen-specific immune response involves a B cell expressing an Immunoglobulin (Ig) M and/or IgG type antibody.

As used herein, the term “IgG” has its general meaning in the art and refers to an immunoglobulin that possesses heavy g-chains. Produced as part of the secondary immune response to an antigen, this class of immunoglobulin constitutes approximately 75% of total serum Ig. IgG is the only class of Ig that can cross the placenta in humans, and it is largely responsible for protection of the newborn during the first months of life.

IgG is the major immunoglobulin in blood, lymph fluid, cerebrospinal fluid and peritoneal fluid and a key player in the humoral immune response. Serum IgG in healthy humans presents approximately 15% of total protein beside albumins, enzymes, other globulins and many more. There are four IgG subclasses described in human, mouse and rat (e.g. IgGl, IgG2, IgG3, and IgG4 in humans). The subclasses differ in the number of disulfide bonds and the length and flexibility of the hinge region. Except for their variable regions, all immunoglobulins within one class share about 90% homology, but only 60% among classes. IgG1 comprises 60 to 65% of the total main subclass IgG, and is predominantly responsible for the thymus-mediated immune response against proteins and polypeptide antigens. IgG1 binds to the Fc-receptor of phagocytic cells and can activate the complement cascade via binding to C1 complex. IgG1 immune response can already be measured in newborns and reaches its typical concentration in infancy. IgG2, the second largest of IgG isotypes, comprises 20 to 25% of the main subclass and is the prevalent immune response against carbohydrate/polysaccharide antigens. “Adult” concentrations are usually reached by 6 or 7 years old. IgG3 comprises around 5 to 10% of total IgG and plays a major role in the immune responses against protein or polypeptide antigens. The affinity of IgG3 can be higher than that of IgG1. Comprising usually less than 4% of total IgG, IgG4 does not bind to polysaccharides. In the past, testing for IgG4 has been associated with food allergies, and recent studies have shown that elevated serum levels of IgG4 are found in patients suffering from sclerosing pancreatitis, cholangitis and interstitial pneumonia caused by infiltrating IgG4 positive plasma cells.

As used herein, the term “IgM” has its general meaning in the art and refers to an immunoglobulin that possesses heavy m-chains. Serum IgM exists as a pentamer (or hexamer) in mammals and comprises approximately 10% of normal human serum Ig content. It predominates in primary immune responses to most antigens and is the most efficient complement-fixing immunoglobulin. IgM is also expressed on the plasma membrane of B lymphocytes as membrane-associated immunoglobulin (which can be organized as multiprotein cluster in the membrane). In this form, it is a B-cell antigen receptor, with the H chains each containing an additional hydrophobic domain for anchoring in the membrane. Monomers of serum IgM are bound together by disulfide bonds and a joining (J) chain. Each of the five monomers within the pentamer structure is composed of two light chains (either kappa or lambda) and two heavy chains. Unlike in IgG (and the generalized structure shown above), the heavy chain in IgM monomers is composed of one variable and four constant regions, with the additional constant domain replacing the hinge region. IgM can recognize epitopes on invading microorganisms,

leading to cell agglutination. This antibody-antigen immune complex is then destroyed by complement fixation or receptor-mediated endocytosis by macrophages. IgM is the first immunoglobulin class to be synthesized by the neonate and plays a role in the pathogenesis of some autoimmune diseases. Immunoglobulin M is the third most common serum Ig and takes one of two forms: a pentamer (or hexamer under some circumstances) where all heavy chains are identical and all light chains are identical. The membrane-associated form is a monomer (e.g., found on B lymphocytes as B cell receptors) that can form multimeric clusters on the membrane.

IgM is the first antibody built during an immune response. It is responsible for agglutination and cytolytic reactions since in theory, its pentameric structure gives it 10 free antigen-binding sites as well as it possesses a high avidity. Due to conformational constraints among the 10 Fab portions, IgM only has a valence of 5. Additionally, IgM is not as versatile as IgG. However, it is of vital importance in complement activation and agglutination. IgM is predominantly found in the lymph fluid and blood and is a very effective neutralizing agent in the early stages of disease. Elevated levels can be a sign of recent infection or exposure to antigen.

As used herein, the term “IgA” has its general meaning in the art and refers to an immunoglobulin that possesses heavy a-chains. IgA comprises approximately 15% of all immunoglobulins in healthy serum. IgA in serum is mainly monomeric, but in secretions, such as saliva, tears, colostrums, mucus, sweat, and gastric fluid, IgA is found as a dimer connected by a joining peptide. Most IgA is present in secreted form. This is believed to be due to its properties in preventing invading pathogens by attaching and penetrating epithelial surfaces. IgA is a very weak complement-activating antibody; hence, it does not induce bacterial cell lysis via the complement system. However, secretory IgA works together with lysozymes (also present in many secreted fluids), which can hydrolyse carbohydrates in bacterial cell walls thereby enabling the immune system to clear the infection. IgA is predominantly found on epithelial cell surfaces where it acts as a neutralizing antibody. Two IgA subtypes exist in humans, IgA1 und IgA2, while mice have only one subclass. They differ in the molecular mass of the heavy chains and in their concentration in serum. IgA1 comprises approximately 85% of total IgA concentration in serum. Although IgA1 shows a broad resistance against several proteases, there are some that can affect/splice on the hinge region. IgA1 shows a good immune response to protein antigens and, to a lesser degree, polysaccharides and lipopolysaccharides. IgA2, representing only up to 15% of total IgA in serum, plays a crucial role in the mucosa of the airways, eyes and the gastrointestinal tract to fight against polysaccharide and

lipopolysaccharide antigens. It also shows good resistance to proteolysis and many bacterial proteases, supporting the importance of IgA2 in fighting bacterial infections.

As used herein, the term "IgD" has its general meaning in the art and refers to an immunoglobulin that possesses heavy d-chains. IgD is an immunoglobulin which makes up about 1% of proteins in the plasma membranes of immature B-lymphocytes where it is usually co-expressed with another cell surface antibody IgM. IgD is also produced in a secreted form that is found in very small amounts in blood serum, representing 0.25% of immunoglobulins in serum. Secreted IgD is produced as a monomeric antibody with two heavy chains of the delta (δ) class, and two Ig light chains.

The method of the invention allows to elicit and/or modulate an immune response comprising certain antibody types and/or certain ratios of antibody types. Depending on properties of the composition of the invention (e.g. type of antigen, particle size, particle ratio, priming/boosting) and depending on the properties of the B-cell (cell type, maturation stadium, mutations) the immune response can be altered (see e.g. Fig. 1, 2, 3, 7, 8).

Accordingly, the invention is at least in part based on the surprising finding that the composition of the invention can be used to in a method to modulate an immune response to a target antigen mediated by IgM, IgD, IgA or IgG type antibodies and/or B-cell receptors.

In certain embodiments, the invention relates to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention, wherein the elicited B-cell-mediated target antigen-specific immune response comprises eliciting of at least one IgG-type antibody and at least one oligomeric antibody.

The method of the invention allows to elicit and/or modulate an immune response comprising IgG-type antibodies and IgM-type antibodies. Depending on properties of the composition of the invention (e.g. type of antigen, particle size, particle ratio, priming/boosting) and depending on the properties of the B-cell (cell type, maturation stadium, mutations) the immune response can be altered for example in that IgG-type antibodies are suppressed and IgM-type antibodies increased (see e.g. Fig. 1, 2, 3, 7, 8).

Accordingly, the invention is at least in part based on the surprising finding that the

composition of the invention can be used to in a method to modulate an immune response to a target antigen mediated by IgM and IgG type antibodies.

In certain embodiments, the invention relates to a method for obtaining a protective-regulative antibody comprising the steps of: (a) eliciting at least one IgG-type antibody and at least one oligomeric antibody according to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention; and (b) isolating a maturated oligomeric antibody, wherein the binding affinity of the oligomeric antibody to the target antigen is equal or higher than the IgG-type antibody, to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

The method in such an embodiment the method is preferably a non-medical method such as an in-vitro method.

The term “protective-regulative for the function of the target antigen”, as used herein refers to modulating the function of the target antigen. In some embodiments, the invention relates to the method for obtaining a protective-regulative antibody, wherein the function of the target antigen is prolonged (e.g. by hindering a degrading immune response) by the protective-regulative antibody. In some embodiments, the invention relates to the method for obtaining a protective-regulative antibody, wherein the function of the target antigen is prolonged by the protective-regulative antibody by prolonging the half live of the target antigen.

In certain embodiments, the invention relates to a method for obtaining a protective-regulative antibody comprising the steps of: (a) eliciting at least one IgG-type antibody and at least one oligomeric antibody according to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention; and (b) isolating a maturated oligomeric antibody, wherein (i) the binding of the oligomeric antibody is more specific for the target antigen than the IgG-type antibody, to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

The method in such an embodiment the method is preferably a non-medical method such as an in-vitro method.

In certain embodiments, the invention relates to a method for obtaining a protective-regulative antibody comprising the steps of: (a) eliciting at least one IgG-type antibody and at least one oligomeric antibody according to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention; and (b) isolating a maturated oligomeric antibody, wherein (i) the binding of the oligomeric antibody is more specific for the target antigen than the IgG-type antibody, and (ii) the binding affinity of the oligomeric antibody to the target antigen is equal or higher than the IgG-type antibody, to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

The method in such an embodiment the method is preferably a non-medical method such as an in-vitro method.

In certain embodiments, the invention relates to a method for obtaining a protective-regulative antibody comprising the steps of: (a) eliciting at least one IgG-type antibody and at least one oligomeric antibody according to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention; and (b) isolating a maturated oligomeric antibody, wherein (i) the binding of the oligomeric antibody is more specific for the target antigen than the IgG-type antibody and wherein the oligomeric antibody is monospecific for the target antigen; and (ii) the binding affinity of the oligomeric antibody to the target antigen is equal or higher than the IgG-type antibody, to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

The method in such an embodiment the method is preferably a non-medical method such as an in-vitro method.

In some embodiments, the “oligomeric” antibody is an IgM-type antibody or an oligomeric antibody derived thereof. In some embodiments, the “oligomeric” antibody is an IgM-type antibody.

In certain embodiments, the invention relates to a method for obtaining a protective-regulative antibody comprising the steps of: (a) eliciting at least one IgG-type antibody and at least one oligomeric antibody according to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention; and (b) isolating a maturated oligomeric antibody, wherein (i) the binding of the oligomeric antibody is more specific for the target antigen than the IgG-

type antibody; and (ii) the binding affinity of the oligomeric antibody to the target antigen is equal or higher than the IgG-type antibody and wherein the protective-regulative antibody binds to the target antigen with K_d of less than 10^{-7}, preferably of less than 10^{-8}, more preferably of less than 10^{-9} and most preferably in the range of about 10^{-10} to about 10^{-12}, to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

In certain embodiments, the invention relates to a method for obtaining a protective-regulative antibody comprising the steps of: (a) eliciting at least one IgG-type antibody and at least one oligomeric antibody according to the method of eliciting and/or modulating a humoral and/or B-cell-mediated target antigen-specific immune response according to the invention; and (b) isolating a maturated oligomeric antibody, wherein (i) the binding of the oligomeric antibody is more specific for the target antigen than the IgG-type antibody, and wherein the oligomeric antibody is monospecific for the target antigen; and (ii) the binding affinity of the oligomeric antibody to the target antigen is equal or higher than the IgG-type antibody, and wherein the protective-regulative antibody binds to the target antigen with K_d of less than 10^{-7}, preferably of less than 10^{-8}, more preferably of less than 10^{-9} and most preferably in the range of about 10^{-10} to about 10^{-12}, to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

In certain embodiments, the invention relates to a method for obtaining a protective-regulative antibody comprising the steps of:(a) providing a blood sample of a subject, wherein the subject experienced elicitation of an IgG and oligomeric antibody response by a target antigen; and (b) enriching a maturated oligomeric antibody, wherein (i) the binding of the oligomeric antibody is more specific for the target antigen than the IgG-type antibody, preferably wherein the oligomeric antibody is monospecific for the target antigen; and/or (ii) the binding affinity of the oligomeric antibody to the target antigen is equal or higher than the IgG-type antibody, preferably wherein the protective-regulative antibody binds to the target antigen with K_d of less than 10^{-7}, preferably of less than 10^{-8}, more preferably of less than 10^{-9} and most preferably in the range of about 10^{-10} to about 10^{-12}, (c) isolating the enriched maturated oligomeric antibody to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

In certain embodiments, the invention relates to the method according to the invention, wherein the subject experienced elicitation of the IgG and oligomeric antibody response

by the target antigen at least 7 days ago, preferably at least 14 days ago, more preferably at least 27 days ago.

In certain embodiments, the invention relates to the method according to the invention, wherein the subject is a (healthy) subject with IgG and oligomeric antibody in the blood, e.g. plasma. The elicitation was therefore not actively induced, but is visible by the presence of IgG and oligomeric antibody in the blood, e.g. plasma.

As such the invention relates at least in part to the isolation of a new antibody fraction with distinct properties, particularly protective regulative high affinity IgM antibodies.

In certain embodiments, the invention relates to a method for obtaining a degrading oligomeric antibody comprising the steps of: (a) providing a blood sample of a subject, wherein the subject experienced elicitation of an IgG and oligomeric antibody response by a target antigen; and (b) enriching a primary oligomeric antibody, wherein (i) the binding of the oligomeric antibody is equally or less specific for the target antigen than the IgG-type antibody, preferably wherein the oligomeric antibody is cross-specific for the target antigen and DNA; and/or (ii) the binding affinity of the oligomeric antibody to the target antigen is lower than the IgG-type antibody, preferably wherein the protective-regulative antibody binds to the target antigen with K_d of more than 10^{-7}, (c) isolating the enriched primary oligomeric antibody to obtain the degrading antibody that can form immune-degradable complexes with the target antigen.

As such the invention relates at least in part to the isolation of a new antibody fraction with distinct properties, particularly degrading affinity IgM antibodies.

In certain embodiments, the invention relates to the method according to the invention, wherein the blood sample is selected from the group consisting of whole blood, plasma and serum sample, preferably serum sample.

In certain embodiments, the invention relates to the method according to the invention, wherein isolating an oligomeric antibody comprises mass- and/or affinity-related isolation.

In certain embodiments, the invention relates to the method according to the invention, wherein enriching an oligomeric antibody comprises immunoprecipitation of the oligomeric antibody.

In certain embodiments, the invention relates to the method according to the invention, wherein the oligomeric antibody is an IgM antibody.

The term “K_D”, as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of Kd to Ka (i. e., Kd/Ka) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art such as plasmon resonance (BIAcore®), Bio-Layer Interferometry (BLI), ELISA and KINEXA. A preferred method for determining the KD of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a BIAcore® system or by ELISA. "Ka" (or "K-assoc"), as used herein, refers broadly to the association rate of a particular antibody-antigen interaction, whereas the term "Kd" (or “K-diss"), as used herein, refers to the dissociation rate of a particular antibody-antigen interaction. Another preferred method is the use of BLI. The term “bio-layer interferometry” or “BLI” refers to an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on a biosensor tip, and an internal reference layer. Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time.

In some embodiments, an antibody is considered herein “more specific” based on at least one specificity assessment method. Specificity of an antibody, variant or fragment, may be tested, for example, by assessing binding of the antibody, variant or fragment, under conventional conditions (see, e.g., Harlow and Lane, 1988 Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, and Harlow and Lane, 1999 using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press).These methods may comprise, inter alia, binding studies, blocking and competition studies with structurally and/or functionally closely related molecules. These binding studies also comprise FACS analysis, surface plasmon resonance, analytical ultracentrifugation, isothermal titration calorimetry, fluorescence anisotropy, fluorescence spectroscopy or by radiolabeled ligand binding assays. (Cross-)specificity can be determined experimentally by methods known in the art and methods as described herein. Such methods comprise, but are not limited to Western Blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans.

The term “monospecific” in context of antibodies as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen. More importantly, the term “monospecific” in context of the present invention pertains to such an antibody which has a high affinity to one antigen and which does not bind

specifically to any other antigen. In this aspect a monospecific antibody binds to the antigen associated with an autoimmune disorder with a K_D of less than 10^{-7} nM, preferably of less than 10^{-8} nM, more preferably of less than 10^{-9} nM and most preferably of about 10^{-10} nM. Hence, such monoclonal IgM does not bind to an unrelated antigen, which is an antigen other than the antigen associated with the autoimmune disorder, and preferably the treatment if the invention therefore does not comprise the use of a polyspecific antibody specific for an unrelated antigen which is an antigen other than the antigen associated with the autoimmune disorder. In some embodiments, monospecificity of an antibody is defined in that it does not recognize dsDNA in ELISA and shows no binding in Hep-2 slides (see e.g. Example 4, Figure 16C, 16D and Material and Methods).

The term “maturated oligomeric antibody”, as used herein, refers to an oligomeric antibody that a) is monospecific for the target antigen b) binds to the target antigen with a K_D of less than 10^{-7} nM, preferably of less than 10^{-8} nM, more preferably of less than 10^{-9} nM, more preferably of less than 10^{-10} nM, more preferably of less than 10^{-11} nM and most preferably of about 10^{-12} nM; and/or c) underwent a maturation process. The maturation process of the oligomeric antibody can be modulated by the development stage of the B-cell, genetical modification (e.g. by absence of IgD see Fig. 25, 29) and/or contact with maturation altering cells or signal agents. In some embodiments the maturation process and/or completion thereof is defined by a number of mutations, preferably at least 1, at least 2, at least 3 mutations, at least 4 mutations, at least 50 mutations, at least 100 mutations or at least 500 mutations of the maturated oligomeric antibody compared to a first generation oligomeric antibody. In some embodiments the maturation process and/or completion thereof is defined by a certain time period, preferably more than 7 days, more than 8 days, more than 9 days, more than 10 days, more than 11 days, more than 12 days, more than 13 days, more than 14 days, more than 15 days, more than 16 days, more than 17 days, more than 18 days, more than 19 days, more than 20 days, more than 21 days, more than 22 days, more than 23 days, more than 24 days, more than 25 days, more than 26 days, more than 27 days, more than 28 days, more than 29 days, more than 30 days, more than 31 days, more than 32 days, more than 33 days, more than 34 days, more than 35 days, more than 36 days, more than 37 days, more than 38 days, more than 39 days, more than 40 days, more than 41 days, more than 42 days, more than 43 days, more than 44 days, more than 45 days, more than 46 days, more than 47 days, more than 48 days, more than 49 days, more than 50 days, more than 51 days, more than 52 days, more than 53 days, more than 54 days, more than 55 days, more than 56 days, more than 57 days, more than 58

days, more than 59 days, more than 60 days, more than 61 days, more than 62 days, more than 63 days, more than 64 days, more than 65 days, more than 66 days, more than 67 days, more than 68 days, more than 69 days, more than 70 days, more than 71 days, more than 72 days, more than 73 days, more than 74 days, more than 75 days, more than 76 days, more than 77 days, more than 78 days, more than 79 days, more than 80 days, more than 81 days, more than 82 days, more than 83 days, more than 84 days or more than 85 days.

Methods for the selective isolation of antibodies are known to the person skilled in the art (see e.g. Huang J, Doria-Rose NA, et al., 2013, Nat Protoc. Oct;8(10):1907-15.)

Any method known to the person skilled in the art can be used to isolate the maturated antibody. In some embodiments, isolating a maturated oligomeric antibody as described herein comprises at least one method selected from the group of physicochemical fractionation, class-specific affinity and antigen-specific affinity. For example an antibody can be isolated as described herein in the Isolation of Insulin-specific serum immunoglobulins of the material and method section.

Accordingly, the invention is at least in part based on the surprising finding that the method of the invention can be used to obtain antibodies variants or fragments that protect and/or regulate the function of the antigen by competing with the binding of antigen-function limiting antigen-binding agents.

In certain embodiments, the invention relates to a protective-regulative antibody obtainable according to the method for obtaining a protective-regulative antibody according to the invention or a variant or fragment thereof that is protective-regulative for the function of the target antigen.

The term “fragment” of an antibody, as used herein, refers to an antibody fragment capable of binding to the same antigen like its antibody counterpart. Such fragments can be simply identified by the skilled person and comprise, as an example, Fab fragment (e.g., by papain digestion), Fab' fragment (e.g., by pepsin digestion and partial reduction), F(ab')2 fragment (e.g., by pepsin digestion), Facb (e.g., by plasmin digestion), Fa (e.g., by pepsin digestion, partial reduction and reaggregation), and also scFv (single chain Fv; e.g., by molecular biology techniques) fragment are encompassed by the invention.

In some embodiments, the protective-regulative antibody of the invention is an oligomeric antibody, preferably a monospecific IgM-type antibody.

In another embodiment, the protective-regulative antibody, variant or fragment of the invention, preferably the monospecific IgM-type antibody, or variant thereof, of the invention is not a polyclonal antibody, or the antigen binding fragment is not a fragment of a polyclonal antibody. In more specific embodiments, the protective-regulative antibody, variant or fragment of the invention, preferably the monospecific IgM-type antibody, or variant thereof, of the invention is not a primary (polyspecific) IgM-type antibody.

In an alternative, and preferred, embodiment the protective-regulative antibody, variant or fragment of the invention preferably the monospecific IgM-type antibody, or variant thereof, is an antibody or an antigen binding fragment thereof, and the antibody is a monoclonal antibody, or wherein the antigen binding fragment is a fragment of a monoclonal antibody.

The term “monoclonal antibody” or “mAb” as used herein refers to an antibody obtained from a population of substantially identical antibodies based on their amino acid sequence. Monoclonal antibodies are typically highly specific. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (e.g. epitopes) of an antigen, each mAb is typically directed against a single determinant on the antigen. In addition to their specificity, mAbs are advantageous in that they can be synthesized by cell culture (hybridomas, recombinant cells or the like) uncontaminated by other immunoglobulins. The mAbs herein include for example chimeric, humanized or human antibodies or antibody fragments.

Monoclonal IgM antibodies in accordance with the present invention may be prepared by methods well known to those skilled in the art. For example, mice, rats, goats, camels, alpacas, llamas or rabbits may be immunized with an antigen of interest (or a nucleic acid encoding an antigen of interest) together with adjuvant. Splenocytes are harvested as a pool from the animals that are administered several immunisations at certain intervals with test bleeds performed to assess for serum antibody titers. Splenocytes are prepared that are either used immediately in fusion experiments or stored in liquid nitrogen for use in future fusions. Fusion experiments are then performed according to the procedure of Stewart & Fuller, J. Immunol. Methods 1989, 123:45-53. Supernatants from wells with growing hybrids are screened by eg enzyme-linked immunosorbent assay (ELISA) for mAb secretors. ELISA-positive cultures are cloned either by limiting dilutions or fluorescence-activated cell sorting, typically resulting in hybridomas established from

single colonies. The ability of an antibody, including an antibody fragment or sub-fragment, to bind to a specific antigen can be determined by binding assays known in the art, for example, using the antigen of interest as the binding partner. Alternatively, splenic B cells that bind to the immunizing antigen are sorted as single cells and subsequently the cDNA encoding the heavy and light chain is cloned from single cells. The cloned cDNA is then used for in vitro production of monoclonal recombinant antibodies which are further characterized based on their specificity and affinity to the immunizing antigen.

A monospecific oligomeric antibody, or variant thereof, in accordance with the present invention may be prepared by genetic immunisation methods in which native proteins are expressed in vivo with normal post-transcriptional modifications, avoiding antigen isolation or synthesis. For example, hydrodynamic tail or limb vein delivery of naked plasmid DNA expression vectors can be used to produce the antigen of interest in vivo in mice, rats, and rabbits and thereby induce antigen-specific antibodies (Tang et al, Nature 356: 152 (1992); Tighe et al, Immunol. Today 19: 89 (1998); Bates et al, Biotechniques, 40:199 (2006); Aldevron-Genovac, Freiburg DE). This allows the efficient generation of high-titre, antigen-specific antibodies which may be particularly useful for diagnostic and/or research purposes. For such genetic immunisation, a variety of gene delivery methods can be used, including direct injection of naked plasmid DNA into skeletal muscle, lymph nodes, or the dermis, electroporation, ballistic (gene gun) delivery, and viral vector delivery.

In a further preferred embodiment, a monospecific oligomeric antibody, or variant thereof, of the invention is an antibody or an antigen binding fragment thereof, wherein the antibody is a human antibody a humanised antibody or a chimeric-human antibody, or wherein the antigen binding fragment is a fragment of a human antibody a humanised antibody or a chimeric-human antibody.

Human antibodies can also be derived by in vitro methods. Suitable examples include but are not limited to phage display (CAT, Morphosys, Dyax, Biosite/Medarex, Xoma, Yumab, Symphogen, Alexion, Affimed) and the like. In phage display, a polynucleotide encoding a single Fab or Fv antibody fragment is expressed on the surface of a phage particle (see e.g., Hoogenboom et al., J. Mol. Biol., 227: 381 (1991); Marks et al., J Mol Biol 222: 581 (1991); U.S. Patent No. 5,885,793). Phage are “screened” to identify those antibody fragments having affinity for target. Thus, certain such processes mimic immune selection through the display of antibody fragment repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to target. In certain

such procedures, high affinity functional neutralizing antibody fragments are isolated. A complete repertoire of human antibody genes may thus be created by cloning naturally rearranged human V genes from peripheral blood lymphocytes (see, e.g., Mullinax et al., Proc Natl Acad Sci (USA), 87: 8095-8099 (1990)) or by generating fully synthetic or semi-synthetic phage display libraries with human antibody sequences (see Knappik et al 2000; J Mol Biol 296:57; de Kruif et al, 1995; J Mol Biol 248):97).

The antibodies described herein may alternatively be prepared through the utilization of the XenoMouse® technology. Such mice are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. In particular, a preferred embodiment of transgenic production of mice and antibodies is disclosed in U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996 and International Patent Application Nos. WO 98/24893, published June 11, 1998 and WO 00/76310, published December 21, 2000. See also Mendez et al., Nature Genetics, 15:146-156 (1997). Through the use of such technology, fully human monoclonal antibodies to a variety of antigens have been produced. Essentially, XenoMouse® lines of mice are immunized with an antigen of interest. e.g. IGSF11 (VSIG3), lymphatic cells (such as B-cells) are recovered from the hyper-immunized mice, and the recovered lymphocytes are fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. These hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. Other “humanised” mice are also commercially available: eg, Medarex - HuMab mouse, Kymab – Kymouse, Regeneron – Velocimmune mouse, Kirin – TC mouse, Trianni – Trianni mouse, OmniAb – OmniMouse, Harbour Antibodies – H2L2 mouse, Merus – MeMo mouse. Also are available are “humanised” other species: rats: OmniAb – OmniRat, OMT – UniRat. Chicken: OmniAb – OmniChicken.

The term “humanised antibody” according to the present invention refers to immunoglobulin chains or fragments thereof (such as Fab, Fab', F(ab')2, Fv, or other antigen-binding sub-sequences of antibodies), which contain minimal sequence (but typically, still at least a portion) derived from non-human immunoglobulin. For the most part, humanised antibodies are human immunoglobulins (the recipient antibody) in which CDR residues of the recipient antibody are replaced by CDR residues from a non-human species immunoglobulin (the donor antibody) such as a mouse, rat or rabbit having the desired specificity, affinity and capacity. As such, at least a portion of the framework sequence of said antibody or fragment thereof may be a human consensus framework sequence. In some instances, Fv framework residues of the human immunoglobulin need

to be replaced by the corresponding non-human residues to increase specificity or affinity. Furthermore, humanised antibodies can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximise antibody performance. In general, the humanised antibody will comprise substantially all of at least one, and typically at least two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanised antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, which (eg human) immunoglobulin constant region may be modified (eg by mutations or glycoengineering) to optimise one or more properties of such region and/or to improve the function of the (eg therapeutic) antibody, such as to increase or reduce Fc effector functions or to increase serum half-life. Exemplary such Fc modification (for example, Fc engineering or Fc enhancement) are described elsewhere herein.

The human constant region will most likely be derived from a Mu chain sequence, however, any variant thereof, such as Fc region binding attenuated for example gamma chain constant sequences might be used as an IgM variant according to the present invention.

The term “chimeric antibody” according to the present invention refers to an antibody whose light and/or heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant regions which are identical to, or homologous to, corresponding sequences of different species, such as mouse and human. Alternatively, variable region genes derive from a particular antibody class or subclass while the remainder of the chain derives from another antibody class or subclass of the same or a different species. It covers also fragments of such antibodies. For example, a typical therapeutic chimeric antibody is a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody, although other mammalian species may be used.

In particular of such embodiments, a monospecific IgM-type antibody, or variant thereof, of the invention comprises an antigen binding domain of an antibody wherein the antigen binding domain is of a human antibody. Preferably, a monospecific oligomeric antibody, or variant thereof, comprises an antigen binding domain of an antibody or an antigen binding fragment thereof, which is a human antigen binding domain; (ii) the antibody is a

monoclonal antibody, or wherein the antigen binding fragment is a fragment of a monoclonal antibody; and (iii) the antibody is a human antibody or a humanised antibody, or wherein the antigen binding fragment is a fragment of a human antibody, a humanised antibody or a chimeric-human antibody.

Light chains of human antibodies generally are classified as kappa and lambda light chains, and each of these contains one variable region and one constant domain. Heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon chains, and these define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively, as described above. Human IgG has several subtypes, including, but not limited to, IgG1, IgG2, IgG3, and IgG4. Human IgM subtypes include IgM. Human IgA subtypes include IgA1 and IgA2. In humans, the IgA isotypes contain four heavy chains and four light chains; the IgG and IgE isotypes contain two heavy chains and two light chains; and the IgM isotype contains ten or twelve heavy chains and ten or twelve light chains. Antibodies according to the invention may be IgG, IgE, IgD, IgA, or IgM immunoglobulins.

In some embodiments, a monospecific oligomeric antibody, or variant thereof, of the invention is an IgM antibody or fragment thereof. Preferably the antibody of the invention is, comprises or is derived from an IgG immunoglobulin or fragment thereof; such as a human, human-derived IgM immunoglobulin, or a rabbit- or rat-derived IgM.

A monospecific oligomeric antibody, or variant thereof, of the invention, where comprising at least a portion of an immunoglobulin constant region (typically that of a human immunoglobulin) may have such (e.g. human) immunoglobulin constant region modified – for example e.g. by glycoengineering or mutations - to optimise one or more properties of such region and/or to improve the function of the (e.g. therapeutic) antibody, such as to increase or reduce Fc effector functions or to increase serum half-life.

Accordingly, any of the ABPs of the invention described above can be produced with different antibody isotypes or mutant isotypes to control the extent of binding to different Fc-gamma receptors. Antibodies lacking an Fc region (e.g., Fab fragments) lack binding to different Fc-gamma receptors. Selection of isotype also affects binding to different Fc-gamma receptors. The respective affinities of various human IgG isotypes for the three different Fc-gamma receptors, Fc-gamma-RI, Fc- gamma-RII, and Fc- gamma-RIII, have been determined. (See Ravetch & Kinet, Annu. Rev. Immunol. 9, 457 (1991)). Fc-gamma-RI is a high affinity receptor that binds to IgGs in monomeric form, and the latter two are low affinity receptors that bind IgGs only in multimeric form. In general, both IgG1 and IgG3 have significant binding activity to all three receptors, IgG4 to Fc-gamma-RI,

and IgG2 to only one type of Fc-gamma-RII called IIaLR (see Parren et al., J. Immunol. 148, 695 (1992). Therefore, human isotype IgG1 is usually selected for stronger binding to Fc-gamma receptors, and IgG2 or IgG4 is usually selected for weaker binding. Preferred embodiments of the invention provide such antibodies where the Fc receptor binding is reduced or eliminated.

A correlation between increased Fc-gamma-R binding with mutated Fc has been demonstrated using targeted cytoxicity cell-based assays (Shields et ah, 2001, J. Biol. Chem. 276:6591-6604; Presta et ah, 2002, Biochem Soc. Trans. 30:487-490). Methods for increasing ADCC activity through specific Fc region mutations include the Fc variants comprising at least one amino acid substitution at a position selected from the group consisting of: 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264, 265, 266, 267, 269, 296, 297, 298, 299, 313, 325, 327, 328, 329, 330 and 332, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat (Kabat et ah, Sequences of Proteins of Immunological Interest (National Institute of Health, Bethesda, Md. 1987).

In certain specific embodiments, said Fc variants comprise at least one substitution selected from the group consisting of L234D, L234E, L234N, L234Q, L234T, L234H, L234Y, L234I, L234V, L234F, L235D, L235S, L235N, L235Q, L235T, L235H, L235Y, L235I, L235V, L235F, S239D, S239E, S239N, S239Q, S239F, S239T, S239H, S239Y, V240I, V240A, V240T, V240M, F241W, F241L, F241Y, F241E, F241R, F243W, F243L, F243Y, F243R, F243Q, P244H, P245A, P247V, P247G, V262I, V262A, V262T, V262E, V263I, V263A, V263T, V263M, V264L, V264I, V264W, V264T, V264R, V264F, V264M, V264Y, V264E, D265G, D265N, D265Q, D265Y, D265F, D265V, D265I, D265L, D265H, D265T, V266I, V266A, V266T, V266M, S267Q, S267L, E269H, E269Y, E269F, E269R, Y296E, Y296Q, Y296D, Y296N, Y296S, Y296T, Y296L, Y296I, Y296H, N297S, N297D, N297E, A298H, T299I, T299L, T299A, T299S, T299V, T299H, T299F, T299E, W313F, N325Q, N325L, N325I, N325D, N325E, N325A, N325T, N325V, N325H, A327N, A327L, L328M, L328D, L328E, L328N, L328Q, L328F, L328I, L328V, L328T, L328H, L328A, P329F, A330L, A330Y, A330V, A330I, A330F, A330R, A330H, I332D, I332E, I332N, I332Q, I332T, I332H, I332Y and I332A, wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat.

Fc variants can also be selected from the group consisting of V264L, V264I, F241W, F241L, F243W, F243L, F241L/F243L/V262I/V264I, F241W/F243W, F241W/F243W/V262A/V264A, F241L/V262I, F243L/V264I, F243L/V262I/V264W,

F241Y/F243Y/V262T/V264T, F241E/F243R/V262E/V264R, F241E/F243Q/V262T/V264E, F241R/F243Q/V262T/V264R, F241E/F243Y/V262T/V264R, L328M, L328E, L328F, I332E, L3238M/I332E, P244H, P245A, P247V, W313F, P244H/P245A/P247V, P247G, V264I/I332E, F241E/F243R/V262E/V264R/I332E, F241E/F243Q/V262T/264E/I332E, F241R/F243Q/V262T/V264R/I332E, F241E/F243Y/V262T/V264R/I332E, S298A/I332E, S239E/I332E, S239Q/I332E, S239E, D265G, D265N, S239E/D265G, S239E/D265N, S239E/D265Q, Y296E, Y296Q, T299I, A327N, S267Q/A327S, S267L/A327S, A327L, P329F, A330L, A330Y, I332D, N297S, N297D, N297S/I332E, N297D/I332E, N297E/I332E, D265Y/N297D/I332E, D265Y/N297D/T299L/I332E, D265F/N297E/I332E, L328I/I332E, L328Q/I332E, I332N, I332Q, V264T, V264F, V240I, V263I, V266I, T299A, T299S, T299V, N325Q, N325L, N325I, S239D, S239N, S239F, S239D/I332D, S239D/I332E, S239D/I332N, S239D/I332Q, S239E/I332D, S239E/I332N, S239E/I332Q, S239N/I332D, S239N/I332E, S239N/I332N, S239N/I332Q, S239Q/I332D, S239Q/I332N, S239Q/I332Q, Y296D, Y296N, F241Y/F243Y/V262T/V264T/N297D/I332E, A330Y/I332E, V264I/A330Y/I332E, A330L/I332E, V264I/A330L/I332E, L234D, L234E, L234N, L234Q, L234T, L234H, L234Y, L234I, L234V, L234F, L235D, L235S, L235N, L235Q, L235T, L235H, L235Y, L235I, L235V, L235F, S239T, S239H, S239Y, V240A, V240T, V240M, V263A, V263T, V263M, V264M, V264Y, V266A, V266T, V266M, E269H, E269Y, E269F, E269R, Y296S, Y296T, Y296L, Y296I, A298H, T299H, A330V, A330I, A330F, A330R, A330H, N325D, N325E, N325A, N325T, N325V, N325H, L328D/I332E, L328E/I332E, L328N/I332E, L328Q/I332E, L328V/I332E, L328T/I332E, L328H/I332E, L328I/I332E, L328A, I332T, I332H, I332Y, I332A, S239E/V264I/I332E, S239Q/V264I/I332E, S239E/V264I/A330Y/I332E, S239E/V264I/S298A/A330Y/I332E, S239D/N297D/I332E, S239E/N297D/I332E, S239D/D265V/N297D/I332E, S239D/D265I/N297D/I332E, S239D/D265L/N297D/I332E, S239D/D265F/N297D/I332E, S239D/D265Y/N297D/I332E, S239D/D265H/N297D/I332E, S239D/D265T/N297D/I332E, V264E/N297D/I332E, Y296D/N297D/I332E, Y296E/N297D/I332E, Y296N/N297D/I332E, Y296Q/N297D/I332E, Y296H/N297D/I332E, Y296T/N297D/I332E, N297D/T299V/I332E, N297D/T299I/I332E, N297D/T299L/I332E, N297D/T299F/I332E, N297D/T299H/I332E, N297D/T299E/I332E, N297D/A330Y/I332E, N297D/S298A/A330Y/I332E, S239D/A330Y/I332E, S239N/A330Y/I332E, S239D/A330L/I332E, S239N/A330L/I332E, V264I/S298A/I332E, S239D/S298A/I332E, S239N/S298A/I332E, S239D/V264I/I332E, S239D/V264I/S298A/I332E, and S239D/264I/A330L/I332E, wherein the numbering of

the residues in the Fc region is that of the EU index as in Kabat. See also WO2004029207, incorporated by reference herein.

In particular embodiments, mutations on, adjacent, or close to sites in the hinge link region (e.g., replacing residues 234, 235, 236 and/or 237 with another residue) can be made, in all of the isotypes, to reduce affinity for Fc-gamma receptors, particularly Fc-gamma-RI receptor (see, eg US6624821). Optionally, positions 234, 236 and/or 237 are substituted with alanine and position 235 with glutamate. (See, eg US5624821.) Position 236 is missing in the human IgG2 isotype. Exemplary segments of amino acids for positions 234, 235 and 237 for human IgG2 are Ala Ala Gly, Val Ala Ala, Ala Ala Ala, Val Glu Ala, and Ala Glu Ala. A preferred combination of mutants is L234A, L235E and G237A, or is L234A, L235A, and G237A for human isotype IgG1. A particular preferred variant of a monospecific IgM-type antibody of the invention is an antibody having human isotype IgG1 and one of these three mutations of the Fc region. Other substitutions that decrease binding to Fc-gamma receptors are an E233P mutation (particularly in mouse IgG1) and D265A (particularly in mouse IgG2a). Other examples of mutations and combinations of mutations reducing Fc and/or C1q binding are E318A/K320A/R322A (particularly in mouse IgG1), L235A/E318A/K320A/K322A (particularly in mouse IgG2a). Similarly, residue 241 (Ser) in human IgG4 can be replaced, e.g. with proline to disrupt Fc binding.

Additional mutations can be made to a constant region to modulate effector activity. For example, mutations can be made to the IgG1 or IgG2 constant region at A330S, P331S, or both. For IgG4, mutations can be made at E233P, F234V and L235A, with G236 deleted, or any combination thereof. IgG4 can also have one or both of the following mutations S228P and L235E. The use of disrupted constant region sequences to modulate effector function is further described, eg in WO2006118,959 and WO2006036291.

Additional mutations can be made to the constant region of human IgG to modulate effector activity (see, e.g., WO200603291). These include the following substitutions: (i) A327G, A330S, P331S; (ii) E233P, L234V, L235A, G236 deleted; (iii) E233P, L234V, L235A; (iv) E233P, L234V, L235A, G236 deleted, A327G, A330S, P331S; and (v) E233P, L234V, L235A, A327G, A330S, P331S to human IgG1; or in particular, (vi) L234A, L235E, G237A, A330S and P331S (eg, to human IgG1), wherein the numbering of the residues in the Fc region is that of the EU index as in Kabat. See also WO2004029207, incorporated by reference herein.

The affinity of an antibody for the Fc-gamma-R can be altered by mutating certain

residues of the heavy chain constant region. For example, disruption of the glycosylation site of human IgG1 can reduce Fc-gamma-R binding, and thus effector function, of the antibody (see, e.g. WO2006036291). The tripeptide sequences NXS and NXT, where X is any amino acid other than proline, are the enzymatic recognition sites for glycosylation of the N residue. Disruption of any of the tripeptide amino acids, particularly in the CH2 region of IgG, will prevent glycosylation at that site. For example, mutation of N297 of human IgG1 prevents glycosylation and reduces Fc-gamma-R binding to the antibody.

Although activation of ADCC and CDC is often desirable for therapeutic antibodies, there are circumstances in which a monospecific IgM-type antibody, or variant thereof, of the invention is unable to activate effector functions is preferential (eg, an antibodies of the invention that is an agnostic modulator). For these purposes IgG4 has commonly been used but this has fallen out of favour in recent years due the unique ability of this sub-class to undergo Fab-arm exchange, where heavy chains can be swapped between IgG4 in vivo as well as residual ADCC activity. Accordingly, Fc engineering approaches can also be used to determine the key interaction sites for the Fc domain with Fc-gamma receptors and C1q and then mutate these positions, such as in an Fc of a monospecific IgM-type antibody, or variant thereof, of the invention, to reduce or abolish binding. Through alanine scanning Duncan and Winter (1998; Nature 332:738) first isolated the binding site of C1q to a region covering the hinge and upper CH2 of the Fc domain. Researchers at Genmab identified mutants K322A, L234A and L235A, which in combination are sufficient to almost completely abolish Fc-gamma-R and C1q binding (Hezareh et al, 2001; J Virol 75:12161). In a similar manner MedImmune later identified a set of three mutations, L234F/L235E/P331S (dubbed TM), which have a very similar effect (Oganesyan et al, 2008; Acta Crystallographica 64:700). An alternative approach is modification of the glycosylation on asparagine 297 of the Fc domain, which is known to be required for optimal FcR interaction. A loss of binding to Fc-gammaRs has been observed in N297 point mutations (Tao et al, 1989; J Immunol 143:2595), enzymatically degylcosylated Fc domains (Mimura et al, 2001; J Biol Chem 276:45539), recombinantly expressed antibodies in the presence of a glycosylation inhibitor (Walker et al, 1989; Biochem J 259:347) and the expression of Fc domains in bacteria (Mazor et al 2007; Nat Biotechnol 25:563). Accordingly, the invention also includes embodiments of the monospecific oligomeric antibody, or variant thereof, in which such technologies or mutations have been used to reduce effector functions.

IgG naturally persists for a prolonged period in (e.g. human) serum due to FcRn-mediated recycling, giving it a typical half-life of approximately 21 days. Despite this there have

been a number of efforts to engineer the pH dependant interaction of the Fc domain with FcRn to increase affinity at pH 6.0 while retaining minimal binding at pH 7.4. Researchers at PDL BioPharma identified the mutations T250Q/M428L, which resulted in an approximate 2-fold increase in IgG half-life in rhesus monkeys (Hinto et al, 2004; J Biol Chem 279:6213), and researchers at MedImmune have identified mutations M252Y/S254T/T256E (dubbed YTE), which resulted in an approximate 4-fold increase in IgG half-life in cynomolgus monkeys (Dall'Acqua, et al 2006; J Biol Chem 281:23514). A combination of the M252Y/S254T/T256E mutations with point mutations H433K/N434F lead to similar effects (Vaccaro et al., 2005, Nat Biotechnol. Oct;23(10):1283-8). ABPs of the invention may also be PEGylated. PEGylation, ie chemical coupling with the synthetic polymer poly-ethylene glycol (PEG), has emerged as an accepted technology for the development of biologics that exercise prolonged action, with around 10 clinically approved protein and peptide drugs to date (Jevsevar et al., 2010; Biotechnol J 5:113). A monospecific oligomeric antibody, or variant thereof, of the invention may also be subjected to PASylation, a biological alternative to PEGylation for extending the plasma half-life of pharmaceutically active proteins (Schlapschy et al, 2013; Protein Eng Des Sel 26:489; XL-protein GmbH, Germany). Similarily, the XTEN half-life extension technology from Amunix provides another biological alternative to PEGylation (Schellenberger, 2009, Nat Biotechnol.;27(12):1186-90. doi: 10.1038/nbt.1588). Accordingly, the invention also includes embodiments of the antibody in which such technologies or mutations have been used to prolong serum half-life, especially in human serum.

Antibody fragments include "Fab fragments", which are composed of one constant and one variable domain of each of the heavy and the light chains, held together by the adjacent constant region of the light chain and the first constant domain (CH1) of the heavy chain. These may be formed by protease digestion, e.g. with papain, from conventional antibodies, but similar Fab fragments may also be produced by genetic engineering. Fab fragments include Fab', Fab and "Fab-SH" (which are Fab fragments containing at least one free sulfhydryl group).

Fab' fragments differ from Fab fragments in that they contain additional residues at the carboxy terminus of the first constant domain of the heavy chain including one or more cysteines from the antibody hinge region. Fab' fragments include "Fab'-SH" (which are Fab' fragments containing at least one free sulfhydryl group).

Further, antibody fragments include F(ab')2 fragments, which contain two light chains and two heavy chains containing a portion of the constant region between the CH1 and CH2

domains ("hinge region"), such that an interchain disulphide bond is formed between the two heavy chains. A F(ab')2 fragment thus is composed of two Fab' fragments that are held together by a disulphide bond between the two heavy chains. F(ab')2 fragments may be prepared from conventional antibodies by proteolytic cleavage with an enzyme that cleaves below the hinge region, e.g. with pepsin, or by genetic engineering.

An "Fv region" comprises the variable regions from both the heavy and light chains, but lacks the constant regions. "Single-chain antibodies" or "scFv" are Fv molecules in which the heavy and light chain variable regions have been connected by a flexible linker to form a single polypeptide chain, which forms an antigen binding region.

An "Fc region" comprises two heavy chain fragments comprising the CH2 and CH3 domains of an antibody. The two heavy chain fragments are held together by two or more disulphide bonds and by hydrophobic interactions of the CH3 domains.

Accordingly, in some embodiments, the antibodies of the invention is an antibody fragment selected from the list consisting of: Fab', Fab, Fab'-SH, Fab-SH, Fv, scFv and F(ab')2.

In a preferred embodiment, an antibody of the invention is an antibody wherein at least a portion of the framework sequence of said antibody or fragment thereof is a human consensus framework sequence, for example, comprises a human germline-encoded framework sequence.

In other certain embodiments, the monospecific oligomeric antibody, or variant thereof, of the invention is modified to prolong serum half-life, especially in human serum. For example, an antibody of the invention may be PEGylated and/or PASylated, or has an Fc region with a T250Q/M428L, H433K/N434F/Y436 or M252Y/S254T/T256E/H433K/N434F modification.

In preferred embodiments, an antibody of the invention can comprise at least one antibody constant domain, in particular wherein at least one antibody constant domain is a CH1, CH2, or CH3 domain, or a combination thereof.

In further of such embodiments, an antibody of the invention having antibody constant domain comprises a mutated Fc region, for example for decreasing interaction of the Fc region with a Fc receptor (Fc receptor on an immune effector cell (eg Saxena & Wu, 2016; Front Immunol 7:580). Examples and embodiments thereof are described elsewhere herein.

In other embodiments, a monospecific oligomeric antibody, or variant thereof, of the

invention may comprises an effector group and/or a labelling group. The term “effector group” means any group, in particular one coupled to another molecule such as an antigen binding protein, that acts as a cytotoxic agent. Examples for suitable effector groups are radioisotopes or radionuclides. Other suitable effector groups include toxins, therapeutic groups, or chemotherapeutic groups. Examples of suitable effector groups include calicheamicins, auristatins, geldanamycins, alpha-amanitine, pyrrolobenzodiazepines and maytansines.

The term “label” or “labelling group” refers to any detectable label. In general, labels fall into a variety of classes, depending on the assay in which they are to be detected: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic labels (e.g., magnetic particles); c) redox active moieties; d) optical dyes; enzymatic groups (e.g. horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase); e) biotinylated groups; and f) predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags, etc.).

The binding of the protective-regulative antibody, variant or fragment of the invention can restore, protect, maintain and/or prolong the biological function of the molecule comprising the target antigen (see e.g. Fig. 16) in that the binding of the protective-regulative antibody, variant or fragment of the invention is in competition with function limiting binding partners and/or prevents degradation of the molecule comprising the target antigen. In some embodiments, the protective-regulative antibody, variant or fragment of the invention binds reversibly to the target antigen.

Accordingly, the invention is at least in part based on the surprising finding that the protective-regulative antibody, variant or fragment of the invention protects and/or regulates the function of the antigen by competing with the binding of antigen-function limiting antigen-binding agents.

In certain embodiments, the invention relates to the protective-regulative antibody, variant or fragment of the invention, wherein the protective-regulative antibody, variant or fragment comprises: a) a CDR3 as defined in SEQ ID NO: 4 and a variable light (VL) chain comprising a CDR3 as defined in SEQ ID NO: 7; b) a variable heavy (VH) chain comprising a CDR3 as defined in SEQ ID NO: 11 and a variable light (VL) chain comprising a CDR3 as defined in SEQ ID NO: 14; or c) a variable heavy (VH) chain

comprising a CDR3 as defined in SEQ ID NO: 18 and a variable light (VL) chain comprising a CDR3 as defined in SEQ ID NO: 21.

In certain embodiments, the invention relates to the protective-regulative antibody, variant or fragment of the invention, wherein the protective-regulative antibody, variant or fragment comprises a) a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO: 2, CDR2 as defined in SEQ ID NO: 3 and CDR3 as defined in SEQ ID NO: 4 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO: 6, CDR2 as defined by the sequence DAS and CDR3 as defined in SEQ ID NO: 7;b) a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO: 9, CDR2 as defined in SEQ ID NO: 10 and CDR3 as defined in SEQ ID NO: 11 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO: 13, CDR2 as defined by the sequence GAS and CDR3 as defined in SEQ ID NO: 14; or c) a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO: 16, CDR2 as defined in SEQ ID NO: 17 and CDR3 as defined in SEQ ID NO: 18 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO: 20, CDR2 as defined by the sequence DAS and CDR3 as defined in SEQ ID NO: 21.

In certain embodiments, the invention relates to the protective-regulative antibody, variant or fragment of the invention, wherein the protective-regulative antibody, variant or fragment comprises a a) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 1 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 1 and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 4 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 4;b) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 8 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 8 and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 12 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 12; or c) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 15 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 15 and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 19 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 19.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention, the pharmaceutical composition for use of the invention or the method of treatment of the invention , wherein the IgM antibody comprises: a variable heavy (VH) chain comprising CDR1 sequence as encoded by SEQ ID NO: 60, CDR2 sequence as encoded by SEQ ID NO: 61 and CDR3 sequence as encoded by SEQ ID NO: 62 and a variable light (VL) chain comprising CDR1 sequence as encoded by SEQ ID NO: 57, CDR2 sequence as encoded by GGTGCATCC and CDR3 sequence as encoded by SEQ ID NO: 58.

In certain embodiments, the invention relates to the pharmaceutical composition of the invention, the pharmaceutical composition for use of the invention or the method of treatment of the invention, wherein the IgM antibody comprises: a variable heavy (VH) chain sequence comprising the amino acid sequence encoded by the sequence as defined by SEQ ID NO: 59 or by a sequence having at least 90% sequence identity to SEQ ID NO: 59, preferably at least 95% sequence identity to SEQ ID NO: 59; and a variable light (VL) chain sequence comprising the amino acid sequence encoded by the sequence as defined by SEQ ID NO: 56 or by a sequence having at least 90% sequence identity to SEQ ID NO: 56, preferably at least 95% sequence identity to SEQ ID NO: 56.

In certain embodiments, the invention relates to a host cell comprising a polynucleotide having a) a sequence as defined by SEQ ID NO: 59 or a sequence having at least 90% sequence identity to SEQ ID NO: 59, preferably at least 95% sequence identity to SEQ ID NO: 59; and/or b) a sequence as defined by SEQ ID NO: 56 or a sequence having at least 90% sequence identity to SEQ ID NO: 56, preferably at least 95% sequence identity to SEQ ID NO: 56; wherein the polynucleotide further encodes an IgM constant region and/or wherein the host cell comprises a further polynucleotide encoding an IgM constant region.

In certain embodiments, the invention relates to a method for producing an IgM antibody, the method comprising the steps of: a) culturing the host cell according to the invention, b) isolating an IgM antibody.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid

sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity, the specificity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved target antigen binding, decreased immunogenicity, or altered ADCC or CDC.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, increased specificity, increased protective properties, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity-matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity or specificity).

Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity. Such alterations may be made in CDR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, 2008, Methods Mol. Biol. 207:179-196), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by

constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al., 2002 in Methods in Molecular Biology 178:1-37. In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (e.g., 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR- H3 and CDR-L3 in particular are often targeted. In another embodiment look-through mutagenesis is used to optimize antibody affinity with a multidimensional mutagenesis method that simultaneously assesses and optimizes combinatorial mutations of selected amino acids (Rajpal, Arvind et al., 2005, Proceedings of the National Academy of Sciences of the United States of America vol. 102,24:8466-71).

In certain embodiments, substitutions, insertions, or deletions may occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions) that do not substantially reduce binding affinity and/or specificity may be made in CDRs. Such alterations may be outside of CDR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells, 1989, Science, 244: 1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex is used to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites

to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al., 1997, TIBTECH 15:26-32. The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g., complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fe region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have an altered influence on inflammation (Irvine, Edward B, and Galit Alter., 2020, Glycobiology vol. 30,4: 241-253). See, e.g., US 2003/0157108; US 2004/0093621. Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO 2005/053742; WO 2002/031140; Okazaki et al. 2004 J. Mol. Biol. 336:1239-1249; Yamane-Ohnuki et al., 2004, Biotech. Bioeng. 87: 614. Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al., 1986, Arch. Biochem. Biophys. 249:533-545; US 2003/0157108; and WO 2004/056312, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al.,

2004, Biotech. Bioeng. 87: 614; Kanda, Y. et al., 2006, Biotechnol. Bioeng., 94(4):680-688; and WO 2003/085107).

Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have altered fucosylation and/or altered influence on inflammation (Irvine, Edward B, and Galit Alter., 2020, Glycobiology vol. 30,4: 241-253). Examples of such antibody variants are described, e.g., in WO 2003/011878; US Patent No. 6,602,684; and US 2005/0123546. Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., 1976, J. Immunol. 117:587 and Kirn et al., 1994 J. Immunol. 24:249), are described in US2005/0014934. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US 2006/0194291).

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in US 7521541.

In certain embodiments, an antibody provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In certain embodiments, the invention relates to an antibody, or antigen-binding fragment thereof, comprising at least one of the sequences described above, wherein the antigen-binding fragment is a Fab fragment, an F(ab') fragment or an Fv fragment.

The binding of the protective-regulative antibody comprising the sequences described herein can restore, protect, maintain and/or prolong the biological function of insulin or a variant or fragment thereof in that the binding of the protective-regulative antibody, variant or fragment of the invention is in competition with function limiting binding partners and/or prevents degradation of insulin or the variant or fragment thereof. In some embodiments, the protective-regulative antibody, variant or fragment of the invention binds reversibly to insulin or the variant or fragment thereof.

Accordingly, the invention is at least in part based on the surprising finding that the protective-regulative antibody, variant or fragment of the invention comprising the sequence(s) described herein protects and/or regulates the function of the antigen, in particular of insulin by competing with the binding of antigen-function limiting antigen-binding agents.

In certain embodiments, the invention relates to a polynucleotide that encodes the protective-regulative antibody, variant or fragment, of the invention.

The term “polynucleotide”, as used herein, refers to a nucleic acid sequence. The nucleic acid sequence may be a DNA or a RNA sequence, preferably the nucleic acid sequence is a DNA sequence. The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well. The polynucleotides of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. isolated from its natural context) or in genetically modified form. An isolated polynucleotide as referred to herein also encompasses polynucleotides which are present in cellular context other than their natural cellular context, i.e. heterologous polynucleotides. The term polynucleotide encompasses single as well as double stranded polynucleotides. Moreover, comprised are also chemically modified polynucleotides including naturally occurring modified polynucleotides such as glycosylated or methylated polynucleotides or artificial modified one such as biotinylated polynucleotides.

In an embodiment, the polynucleotide of the invention encodes at least one of a variable heavy (VH) chain sequence and/or a variable light (VL) chain sequence of a protective-regulative antibody according to the invention.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a variable heavy (VH) chain sequence comprising the nucleotide sequence of SEQ ID NO: 22 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 22, preferably comprising the sequence SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a variable light (VL) chain sequence comprising the nucleotide sequence of SEQ ID NO: 26 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 26, preferably comprising the sequence SEQ ID NO: 27, GATGCATCC and SEQ ID NO: 28.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a) a variable heavy (VH) chain sequence comprising the nucleotide sequence of SEQ ID

NO: 22 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 22, preferably comprising the sequence SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25; and b) a variable light (VL) chain sequence comprising the nucleotide sequence of SEQ ID NO: SEQ ID NO: 26 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 26, preferably comprising the sequence SEQ ID NO: 27, GATGCATCC and SEQ ID NO: 28.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a variable heavy (VH) chain sequence comprising the nucleotide sequence of SEQ ID NO: 29 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 29, preferably comprising the sequence SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a variable light (VL) chain sequence comprising the nucleotide sequence of SEQ ID NO: 33 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 33, preferably comprising the sequence SEQ ID NO: 34, GGTGCATCC and SEQ ID NO: 35.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a) a variable heavy (VH) chain sequence comprising the nucleotide sequence of SEQ ID NO: 29 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 29, preferably comprising the sequence SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32; and b) a variable light (VL) chain sequence comprising the nucleotide sequence of SEQ ID NO: 33 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 33, preferably comprising the sequence SEQ ID NO: 34, GGTGCATCC and SEQ ID NO: 35.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a variable heavy (VH) chain sequence comprising the nucleotide sequence of SEQ ID NO: 36 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 36, preferably comprising the sequence SEQ ID NO: 37, SEQ ID NO: 38 and SEQ ID NO: 39.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a variable light (VL) chain sequence comprising the nucleotide sequence of SEQ ID NO: 40 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 40, preferably comprising the sequence SEQ ID NO: 41, GATGCATCC and SEQ ID NO: 42.

In certain embodiments, the invention relates to a polynucleotide sequence encoding a) a variable heavy (VH) chain sequence comprising the nucleotide sequence of SEQ ID NO: 36 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 36, preferably comprising the sequence SEQ ID NO: 37, SEQ ID NO: 38 and SEQ ID NO: 39; and b) a variable light (VL) chain sequence comprising the nucleotide sequence of SEQ ID NO: 40 or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 40, preferably comprising the sequence SEQ ID NO: 41, GATGCATCC and SEQ ID NO: 42.

In some embodiments, the polynucleotide of the invention is operably linked with another nucleic acid sequence. For instance, a transcription regulatory sequence is operably linked to the polynucleotide of the invention.

In certain embodiments, the invention relates to a vector comprising the polynucleotide of the invention.

The term “vector”, as used herein, refers to a nucleic acid molecule, capable transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, i.e., inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. Useful vectors include, for example, plasmids (e.g., DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors.

In some embodiments, the vector of the invention is transfected with the support of a transfection enhancer, e.g., a transfection enhancer selected from the group of oligonucleotides, lipoplexes, polymersomes, polyplexes, dendrimers, inorganic

nanoparticles and cell-penetrating peptides.

Accordingly, the invention is at least in part based on the surprising finding that the vector of the invention enables the expression of an antibody, variant or fragment that protects and/or regulates the function of a target antigen, in particular of insulin, by competing with the binding of antigen-function limiting antigen-binding agents.

In certain embodiments, the invention relates to a host cell comprising the polynucleotide of the invention.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

In certain embodiments the host cell is directly or indirectly used in therapy (e.g., cell therapy). In certain embodiments a method for cell therapy comprises the steps of (i) obtaining a cell from a subject; (ii) transform the cell using a tool (e.g. a vector) comprising the polynucleotide of the invention and/or transform the cell to produce the antibody of the invention; and (iii) administering the transformed cell to a subject. In certain embodiments, the subject in step (i) and step (iii) of the method for cell therapy are the same subject. In certain embodiments, the subject in step (i) and step (iii) of the method for cell therapy are different subjects. In certain embodiments, the subject in step (i) and step (iii) of the method for cell therapy are different subjects that belong to different species. In certain embodiments, the subject in step (i) of the method for cell therapy is a subject from the genus Sus and the subject in step (iii) of the method for cell therapy is a subject from the species Homo Sapiens.

In certain embodiments, the host cell is a stem cell. In other embodiments, the host cell is a differentiated cell.

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, *see, e.g.,*

U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Val. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli.)*

Accordingly, the invention is at least in part based on the surprising finding that the host cell of the invention enables the production of an antibody, variant or fragment that protects and/or regulates the function of a target antigen, in particular of insulin, by competing with the binding of antigen-function limiting antigen-binding agents.

In certain embodiments, the invention relates to a method for producing an antibody comprising culturing the host cell of the invention.

In a certain embodiment the invention relates to a method for producing an antibody comprising culturing the host cell of the invention, wherein the host cell comprises the polynucleotide of the invention.

In a particular embodiment, the method of producing an antibody comprises culturing the host cell of the invention under conditions suitable to allow efficient production of the antibody of the invention.

In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody of the invention, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody of the invention. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., YO, NSO, Sp20). In one embodiment, a method of making an antibody, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an antibody according to the invention (e.g. a protective-regulative antibody), nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., US 5648237, US 5789199, and US 5840523; Charlton, 2003, Methods in Molecular Biology, Vol. 248; BKC Lo, 2003, Humana Press, pp. 245-254. After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, 2004, Nat. Biotech. 22:1409-1414, and Li et al., 2006, Nat. Biotech. 24:210-215.

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of Spodoptera frugiperda cells.

Plant cell cultures can also be utilized as hosts. See, e.g., US 5959177; US 6040498, US 6420548, US 7125978, and US 6417429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are macaque kidney CVI line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., 1997, J. Gen Viral. 36:59); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, 1980, Biol. Reprod. 23:243-251); macaque kidney cells (CV l); African green macaque kidney cells (VER0-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (Wl38); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., 1982, Annals N. Y Aead. Sei. 383:44-68; MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR CHO cells (Urlaub et al., 1980, Proc. Natl. Acad. Sc. USA 77:4216); and myeloma cell lines such as YO, NSO and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, Methods in Molecular Biology,

Vol. 248 BKC Lo, 2003., Humana Press, pp. 255-268.

The amount of obtained specific antibody can be quantified using an ELISA, which is also described herein below. Further methods for the production of antibodies are well known in the art, see, e.g. Harlow and Lane, 1988, CSH Press, Cold Spring Harbor.

Accordingly, the invention is at least in part based on the surprising finding that the method for production of the invention enables the production of an antibody, variant or fragment that protects and/or regulates the function of a target antigen, in particular of insulin, by competing with the binding of antigen-function limiting antigen-binding agents.

In certain embodiments, the invention relates to the composition of the invention further comprising the protective-regulative antibody, variant or fragment of the invention and/or the vector of the invention.

The addition of the protective-regulative antibody, variant or fragment of the invention and/or the vector of the invention may enhance the effect of the composition of the invention, reduce the onset time of the effect of the composition of the invention and/or render the effect of the composition of the invention less dependent on the endogenous protective-regulative antibody production.

Accordingly, the invention is at least in part based on the surprising finding that the protective-regulative antibody, variant or fragment of the invention can support the effect of the composition of the invention.

In certain embodiments, the invention relates to a pharmaceutical product comprising a therapeutic agent and a) the composition of the invention; b) the protective-regulative antibody, variant or fragment of the invention; c) the vector of the invention; and/or d) a monovalent antigen particle, wherein the monovalent antigen particle is composed of an antigenic portion comprising not more than one antigenic structure capable of inducing an antibody mediated immune response against a target antigen, wherein the therapeutic agent is the target antigen.

In certain embodiments, the invention relates to a pharmaceutical product comprising a therapeutic agent and a) the composition of the invention; b) the protective-regulative antibody, variant or fragment of the invention; and/or c) the vector of the invention, wherein the therapeutic agent is the target antigen.

The term “therapeutic agent”, as used herein, refers to a compound that upon administration to a subject in a therapeutically effective amount, provides a therapeutic benefit to the subject. A therapeutic agent may be any type of drug, medicine, pharmaceutical, hormone, antibiotic, protein, gene, growth factor, bioactive material, used for treating, controlling, or preventing diseases or medical conditions. Those skilled in the art will appreciate that the term "therapeutic agent" is not limited to drugs that have received regulatory approval.

In some embodiments, the therapeutic agent may be selected from the group of a small molecule drug, a protein/polypeptide, an antibody, molecule drug with antibiotic activity, phage-based therapy, a nucleic acid molecule and an siRNA.

The protective-regulative effect of the binding of the antibody, fragment or variant comprised in the pharmaceutical product or of the antibody, fragment or variant induced by the ingredients of the pharmaceutical product can improve the pharmacokinetic and pharmacodynamic properties of the therapeutic agent. In some embodiments, the pharmaceutical product comprises insulin or a variant or fragment thereof and a protective-regulative antibody comprising a) a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO: 2, CDR2 as defined in SEQ ID NO: 3 and CDR3 as defined in SEQ ID NO: 4 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO: 6, CDR2 as defined by the sequence DAS and CDR3 as defined in SEQ ID NO: 7;

b) a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO: 9, CDR2 as defined in SEQ ID NO: 10 and CDR3 as defined in SEQ ID NO: 11 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO: 13, CDR2 as defined by the sequence GAS and CDR3 as defined in SEQ ID NO: 14; or

c) a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO: 16, CDR2 as defined in SEQ ID NO: 17 and CDR3 as defined in SEQ ID NO: 18 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO: 20, CDR2 as defined by the sequence DAS and CDR3 as defined in SEQ ID NO: 21., more preferably comprising d) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 1 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 1 and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 4 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 4; e) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 8 or a sequence having at least 90%, preferably

at least 95% sequence identity to SEQ ID NO: 8 and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 12 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 12; or f) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 15 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 15 and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 19 or a sequence having at least 90%, preferably at least 95% sequence identity to SEQ ID NO: 19., or a variant or fragment thereof (of a),b),c),d),e), and/or f)) or a host cell or vector for expression thereof (of a),b),c),d),e), and/or f)). Upon administration to a subject, the binding of the protective-regulative antibody can protect the insulin, insulin variant or insulin fragment against an immune response of the subject.

Accordingly, the invention is at least in part based on the surprising finding that a therapeutic agent can be protected and/or regulated as described herein.

In certain embodiments, the invention relates to the pharmaceutical product according to the invention, wherein the therapeutic agent is a therapeutic antibody.

The term “therapeutic antibody”, as used herein, refers to a therapeutic agent as described herein that is an antibody.

In some embodiments the therapeutic antibody is at least one antibody selected from the group of Abagovomab, Abciximab, Abituzumab, Abrezekimab, Abrilumab, Actoxumab, Adalimumab, Adecatumumab, Aducanumab, Afasevikumab, Afelimomab, Alacizumab pegol, Alemtuzumab, Alirocumab, Altumomab, Amatuximab, Amivantamab, Anatumomab mafenatox, Andecaliximab, Anetumab ravtansine, Anifrolumab, Ansuvimab, Anrukinzumab, Apolizumab, Aprutumab ixadotin, Arcitumomab, Ascrinvacumab, Aselizumab, Atezolizumab, Atidortoxumab, Atinumab, Atoltivimab, Atorolimumab, Avelumab, Azintuxizumab vedotin, Bamlanivimab, Bapineuzumab, Basiliximab, Bavituximab, BCD-100, Bectumomab, Begelomab, Belantamab mafodotin, Belimumab, Bemarituzumab, Benralizumab, Berlimatoxumab, Bermekimab, Bersanlimab, Bertilimumab, Besilesomab, Bevacizumab, Bezlotoxumab, Biciromab, Bimagrumab, Bimekizumab, Birtamimab, Bivatuzumab, Bleselumab, Blinatumomab, Blontuvetmab, Blosozumab, Bococizumab, Brazikumab, Brentuximab vedotin, Briakinumab, Brodalumab, Brolucizumab, Brontictuzumab, Burosumab, Cabiralizumab, Camidanlumab tesirine, Camrelizumab, Canakinumab, Cantuzumab mertansine, Cantuzumab ravtansine, Caplacizumab, Casirivimab, Capromab, Carlumab,

Carotuximab, Catumaxomab, cBR96-doxorubicin immunoconjugate, Cedelizumab, Cemiplimab, Cergutuzumab amunaleukin, Certolizumab pegol, Cetrelimab, Cetuximab, Cibisatamab, Cirmtuzumab, Citatuzumab bogatox, Cixutumumab, Clazakizumab, Clenoliximab, Clivatuzumab tetraxetan, Codrituzumab, Cofetuzumab pelidotin, Coltuximab ravtansine, Conatumumab, Concizumab, Cosfroviximab, Crenezumab, Crizanlizumab, Crotedumab, CR6261, Cusatuzumab, Dacetuzumab, Daclizumab, Dalotuzumab, Dapirolizumab pegol, Daratumumab, Dectrekumab, Demcizumab, Denintuzumab mafodotin, Denosumab, Depatuxizumab mafodotin, Derlotuximab biotin, Detumomab, Dezamizumab, Dinutuximab, Dinutuximab beta, Diridavumab, Domagrozumab, Dorlimomab aritox, Dostarlimab, Drozitumab, DS-8201, Duligotuzumab, Dupilumab, Durvalumab, Dusigitumab, Duvortuxizumab, Ecromeximab, Eculizumab, Edobacomab, Edrecolomab, Efalizumab, Efungumab, Eldelumab, Elezanumab, Elgemtumab, Elotuzumab, Elsilimomab, Emactuzumab, Emapalumab, Emibetuzumab, Emicizumab, Enapotamab vedotin, Enavatuzumab, Enfortumab vedotin, Enlimomab pegol, Enoblituzumab, Enokizumab, Enoticumab, Ensituximab, Epcoritamab, Epitumomab cituxetan, Epratuzumab, Eptinezumab, Erenumab, Erlizumab, Ertumaxomab, Etaracizumab, Etesevimab, Etigilimab, Etrolizumab, Evinacumab, Evolocumab, Exbivirumab, Fanolesomab, Faralimomab, Faricimab, Farletuzumab, Fasinumab, FBTA05, Felvizumab, Fezakinumab, Fibatuzumab, Ficlatuzumab, Figitumumab, Firivumab, Flanvotumab, Fletikumab, Flotetuzumab, Fontolizumab, Foralumab, Foravirumab, Fremanezumab, Fresolimumab, Frovocimab, Frunevetmab, Fulranumab, Futuximab, Galcanezumab, Galiximab, Gancotamab, Ganitumab, Gantenerumab, Gatipotuzumab, Gavilimomab, Gedivumab, Gemtuzumab ozogamicin, Gevokizumab, Gilvetmab, Gimsilumab, Girentuximab, Glembatumumab vedotin, Golimumab, Gomiliximab, Gosuranemab, Guselkumab, Ianalumab, Ibalizumab, Sintilimab, Ibritumomab tiuxetan, Icrucumab, Idarucizumab, Ifabotuzumab, Igovomab, Iladatuzumab vedotin, Imalumab, Imaprelimab, Imciromab, Imdevimab, Imgatuzumab, Inclacumab, Indatuximab ravtansine, Indusatumab vedotin, Inebilizumab, Infliximab, Intetumumab, Inolimomab, Inotuzumab ozogamicin, Ipilimumab, Iomab-B, Iratumumab, Isatuximab, Iscalimab, Istiratumab, Itolizumab, Ixekizumab, Keliximab, Labetuzumab, Lacnotuzumab, Ladiratuzumab vedotin, Lampalizumab, Lanadelumab, Landogrozumab, Laprituximab emtansine, Larcaviximab, Lebrikizumab, Lemalesomab, Lendalizumab, Lenvervimab, Lenzilumab, Lerdelimumab, Leronlimab, Lesofavumab, Letolizumab, Lexatumumab, Libivirumab, Lifastuzumab vedotin, Ligelizumab, Loncastuximab tesirine, Losatuxizumab vedotin, Lilotomab satetraxetan, Lintuzumab, Lirilumab, Lodelcizumab,

Lokivetmab, Lorvotuzumab mertansine, Lucatumumab, Lulizumab pegol, Lumiliximab, Lumretuzumab, Lupartumab, Lupartumab amadotin, Lutikizumab, Maftivimab, Mapatumumab, Margetuximab, Marstacimab, Maslimomab, Mavrilimumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Minretumomab, Mirikizumab, Mirvetuximab soravtansine, Mitumomab, Modotuximab, Mogamulizumab, Monalizumab, Morolimumab, Mosunetuzumab, Motavizumab, Moxetumomab pasudotox, Muromonab-CD3, Nacolomab tafenatox, Namilumab, Naptumomab estafenatox, Naratuximab emtansine, Narnatumab, Natalizumab, Navicixizumab, Navivumab, Naxitamab, Nebacumab, Necitumumab, Nemolizumab, NEOD001, Nerelimomab, Nesvacumab, Netakimab, Nimotuzumab, Nirsevimab, Nivolumab, Nofetumomab merpentan, Obiltoxaximab, Obinutuzumab, Ocaratuzumab, Ocrelizumab, Odesivimab, Odulimomab, Ofatumumab, Olaratumab, Oleclumab, Olendalizumab, Olokizumab, Omalizumab, Omburtamab, OMS721, Onartuzumab, Ontuxizumab, Onvatilimab, Opicinumab, Oportuzumab monatox, Oregovomab, Orticumab, Otelixizumab, Otilimab, Otlertuzumab, Oxelumab, Ozanezumab, Ozoralizumab, Pagibaximab, Palivizumab, Pamrevlumab, Panitumumab, Pankomab, Panobacumab, Parsatuzumab, Pascolizumab, Pasotuxizumab, Pateclizumab, Patritumab, PDR001, Pembrolizumab, Pemtumomab, Perakizumab, Pertuzumab, Pexelizumab, Pidilizumab, Pinatuzumab vedotin, Pintumomab, Placulumab, Prezalumab, Plozalizumab, Pogalizumab, Polatuzumab vedotin, Ponezumab, Porgaviximab, Prasinezumab, Prezalizumab, Priliximab, Pritoxaximab, Pritumumab, PRO 140, Quilizumab, Racotumomab, Radretumab, Rafivirumab, Ralpancizumab, Ramucirumab, Ranevetmab, Ranibizumab, Raxibacumab, Ravagalimab, Ravulizumab, Refanezumab, Regavirumab, Regdanvimab, Relatlimab, Remtolumab, Reslizumab, Rilotumumab, Rinucumab, Risankizumab, Rituximab, Rivabazumab pegol, Robatumumab, Rmab, Roledumab, Romilkimab, Romosozumab, Rontalizumab, Rosmantuzumab, Rovalpituzumab tesirine, Rovelizumab, Rozanolixizumab, Ruplizumab, SA237, Sacituzumab govitecan, Samalizumab, Samrotamab vedotin, Sarilumab, Satralizumab, Satumomab pendetide, Secukinumab, Selicrelumab, Seribantumab, Setoxaximab, Setrusumab, Sevirumab, Sibrotuzumab, SGN-CD19A, SHP647, Sifalimumab, Siltuximab, Simtuzumab, Siplizumab, Sirtratumab vedotin, Sirukumab, Sofituzumab vedotin, Solanezumab, Solitomab, Sonepcizumab, Sontuzumab, Spartalizumab, Stamulumab, Sulesomab, Suptavumab, Sutimlimab, Suvizumab, Suvratoxumab, Tabalumab, Tacatuzumab tetraxetan, Tadocizumab, Tafasitamab, Talacotuzumab, Talizumab, Talquetamab, Tamtuvetmab, Tanezumab, Taplitumomab paptox, Tarextumab, Tavolimab, Teclistamab, Tefibazumab, Telimomab

aritox, Telisotuzumab, Telisotuzumab vedotin, Tenatumomab, Teneliximab, Teplizumab, Tepoditamab, Teprotumumab, Tesidolumab, Tetulomab, Tezepelumab, TGN1412, Tibulizumab, Tildrakizumab, Tigatuzumab, Timigutuzumab, Timolumab, Tiragolumab, Tiragotumab, Tislelizumab, Tisotumab vedotin, TNX-650, Tocilizumab, Tomuzotuximab, Toralizumab, Tosatoxumab, Tositumomab, Tovetumab, Tralokinumab, Trastuzumab, Trastuzumab duocarmazine, Trastuzumab emtansine, TRBS07, Tregalizumab, Tremelimumab, Trevogrumab, Tucotuzumab celmoleukin, Tuvirumab, Ublituximab, Ulocuplumab, Urelumab, Urtoxazumab, Ustekinumab, Utomilumab, Vadastuximab talirine, Vanalimab, Vandortuzumab vedotin, Vantictumab, Vanucizumab, Vapaliximab, Varisacumab, Varlilumab, Vatelizumab, Vedolizumab, Veltuzumab, Vepalimomab, Vesencumab, Visilizumab, Vobarilizumab, Volociximab, Vonlerolizumab, Vopratelimab, Vorsetuzumab mafodotin, Votumumab, Vunakizumab, Xentuzumab, XMAB-5574, Zalutumumab, Zanolimumab, Zatuximab, Zenocutuzumab, Ziralimumab, Zolbetuximab and Zolimomab.

Therapeutic antibodies can induce immune responses in subjects. The protective-regulative effect of the binding of the antibody, fragment or variant comprised in the pharmaceutical product or of the antibody, fragment or variant induced by the ingredients of the pharmaceutical product can improve the pharmacokinetic and pharmacodynamic properties of the therapeutic antibody, by protecting against the immune response.

Accordingly, the invention is at least in part based on the surprising finding that a therapeutic antibody can be protected and/or regulated as described herein.

In certain embodiments, the invention relates to the composition of the invention, the protective-regulative antibody, variant or fragment of the invention, the vector of the invention or the pharmaceutical product of to the invention, further comprising a pharmaceutically acceptable carrier.

Compositions or pharmaceutical products comprising the antibody, variant or fragment thereof, the vector, the host cell as described herein can be prepared by mixing such antibody/variant/fragment/polynucleotide/host cell having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Osol et al., 1980 Remington's Pharmaceutical Sciences 16th edition), in certain examples, in the form of lyophilized formulations or aqueous solutions.

Exemplary lyophilized antibody compositions are described in US 6267958. Aqueous antibody compositions include those described in US 6171586 and WO 2006/044908, the latter formulations including a histidine-acetate buffer.

Active ingredients of the compositions/pharmaceutical products described herein and/or the antibody/variant/fragment/vector/host cell described herein may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Osol et al., 1980, Remington's Pharmaceutical Sciences 16th edition.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the composition, pharmaceutical product, antibody, variant, fragment, vector, host cell of the invention and/or the polynucleotide of the invention, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

In some embodiments, at least one ingredient of the composition of the invention or of the pharmaceutical product of the invention are in a different modified-release formulation than another ingredient. For example, the polyvalent antigen particle but not the monovalent antigen particle is bound to a release extender or vice versa.

In some embodiments, the invention relates to a composition for use in eliciting and/or modulating a cell-mediated target antigen-specific immune response in a subject, wherein the composition is used by contacting one or more immune-cells of the subject with the composition.

In some embodiments, the composition described herein is used in the treatment or prevention (vaccination) of a disease in a subject or patient comprises the administration of the composition or of at least one monovalent antigen particle or one polyvalent antigen particle of the composition to the subject or patient in a therapeutically or preventively effective amount.

A therapeutically effective amount in context of the present invention is an amount that induces or suppresses a certain B-cell mediated immune response such as an IgG- or IgM-type (or an IgA) immune response.

In some embodiments, the invention pertains a method for treating or preventing a disease by vaccination in a subject, the method comprising administering an effective amount of a vaccination composition comprising:

(i) a monovalent antigen particle which is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against a disease-associated antigen, and

(ii) a polyvalent antigen particle which is composed of an antigenic portion comprising more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen and wherein the more than one of an antigenic structure are covalently or non-covalently cross-linked.

In this embodiment it may be preferred to administer the treatment to the subject in a vaccination scheme that comprises a priming/boosting scheme as disclosed herein elsewhere.

In some embodiments, the invention pertains to vaccination composition for use in treating or preventing a disease in a subject, the vaccination composition comprising:

(iii) a monovalent antigen particle which is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against a disease-associated antigen, and

(iv) a polyvalent antigen particle which is composed of an antigenic portion comprising more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen and wherein the more than one of an antigenic structure are covalently or non-covalently cross-linked.

In some embodiments, the invention pertains to an immunogenic composition, comprising:

(v) a monovalent antigen particle which is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against an antigen, and

(vi) a polyvalent antigen particle which is composed of an antigenic portion comprising more than one of an antigenic structure capable of inducing an antibody mediated immune response against the antigen and wherein the more than one of an antigenic structure are covalently or non-covalently cross-linked.

In a further embodiment, the composition described herein is for use in the treatment or prevention (vaccination) of a disease in a subject or patient comprises the administration of the composition or of at least (i) or (ii) of the composition to the subject or patient in a therapeutically or preventively effective amount. In some embodiments a therapeutically effective amount is an amount that induces or suppresses a certain B-cell mediated immune response such as an IgG- or IgM-type (or an IgA) immune response.

In certain embodiments, the invention relates to the composition of the invention, the protective-regulative antibody, variant or fragment of the invention, the vector of the invention or the pharmaceutical product of the invention, for use as a medicament.

In some embodiments, the disease or condition to be treated by the medicament is selected from a disease or condition which is characterized in that an increased or reduced cell-mediated immune response is beneficial for a treatment. Hence, the invention offers the herein described modulation of the immune system according to the herein described methods as a treatment of diseases such as a disease or condition selected from an inflammatory disorder, an autoimmune disease, a proliferative disorder, or an infectious disease.

In some embodiments, the composition of the invention, the protective-regulative antibody, variant or fragment of the invention, the vector of the invention or the pharmaceutical product of the invention, is formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular subject being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. An the composition of the invention, the protective-regulative antibody, variant or fragment of the invention, the vector of the invention or the pharmaceutical product of the invention, need not be, but is optionally formulated with one or more further therapeutic agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount the composition of the invention, the protective-regulative antibody, variant or fragment of the

invention, the vector of the invention or the pharmaceutical product of the invention, the type of disorder or treatment, and other factors for consideration discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of the composition of the invention, the protective-regulative antibody, variant or fragment of the invention, the vector of the invention or the pharmaceutical product of the invention, (when used alone or in combination with one or more other further therapeutic agents) will depend on the type of disease to be treated, the type of composition/antibody/variant/fragment/vector/pharmaceutical product, the severity and course of the disease, whether the administration is for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the composition/antibody/variant/fragment/vector/pharmaceutical product and the discretion of the attending physician.

The protective-regulative antibody, variant or fragment of the invention and/or the antibody used as a further therapeutic agent are/is suitably administered to the patient at one time or over a series of treatments. In some embodiments, depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg-10 mg/kg) of antibody variant or fragment can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors for consideration mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody or antigen-binding fragment would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody variant or fragment). An initial higher loading dose followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

In some embodiments, the invention pertains to a monospecific oligomeric antibody, or a variant thereof, for use in the treatment of an autoimmune disorder, wherein the monoclonal IgM-type antibody is specific and has a high affinity for an antigen associated with the autoimmune disorder.

In some embodiments, the invention relates to the composition, the pharmaceutical product or the vector of the invention for use in treatment, wherein the vector is administered in doses in the range from at least 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15}, 10^{16}, or more vector genomes per kilogram (vg/kg) of the weight of the subject, to achieve a therapeutic effect.

In certain embodiments, the invention relates to the composition, the pharmaceutical product or the host cell of the invention for use in treatment, wherein a clinically relevant number or population of host cells, e.g., at least 10^4, 10^5,10^6, 10^7, 10^8, 10^9, typically more than 10^9 or at least 10^{10} cells per dose are administered. The number of cells will depend upon the use for which the composition, the pharmaceutical product or the host cell of the invention is intended as will the type of cell. For uses provided herein, the cells are typically in a volume of a liter or less, can be 500 ml or less, even 250 ml or 100 ml or less. Hence the density of the desired cells is typically be greater than 10^6 cells/ml and generally is greater than 10^7 cells/ml. The clinically relevant number of host cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^9, 10^{10} or 10^{11} cells.

The total dose of the host cell of the invention for one therapy cycle is typically about 1×10^4 cells/kg to 1×10^{10} cells/kg host cells or more, depending on the factors for consideration mentioned above.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention can be used to therapeutically modulate an immune response to a target antigen as described herein.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention, the vector for use of the invention, or the protective-regulative antibody, variant or fragment for use of the invention, for use in the treatment and/or prevention of a humoral and/or B-cell-mediated target antigen-specific disease or disorder.

The term “B cell-mediated inflammatory disease”, as used herein, refers to a disease or

disorder, wherein the pathogenesis and/or progression of the disease is primarily dependent upon the activity of B cells and/or macromolecules of the immune system such as antibodies and complement proteins.

The invention provides the means and methods to modulate humoral and/or B-cell-mediated immune response e.g. by protective-regulative binding.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention can be used to therapeutically modulate a B-cell mediated immune response to a target antigen and/or to therapeutically modulate a humoral antigen, as described herein.

In certain embodiments, the invention relates to the composition for use of the invention the pharmaceutical product for use of the invention, the vector for use of the invention, or the protective-regulative antibody, variant or fragment for use of the invention, wherein the humoral and/or B-cell-mediated target antigen-specific disease or disorder is an autoimmune disease or disorder or an alloimmune disease or disorder, preferably wherein the target antigen is an autoantigen.

The term “autoantigen”, as used herein, refers to an antigen or epitope which is native to the subject and which is immunogenic in an autoimmune disease or disorder or an alloimmune disease or disorder.

The term "autoimmune disease" refers to a disease or disorder arising from immune reactions directed against an individual's own tissues, organs or manifestation thereof or resulting condition therefrom. In some embodiments, the autoimmune disease or disorder described herein is a condition that results from, or is aggravated by, the production of autoantibodies by B cells of antibodies that are reactive with normal body tissues and/or antigens. In other embodiments, the autoimmune disease is one that involves secretion of an autoantibody that is specific for an epitope from an autoantigen.

In some embodiments, the autoimmune disease refers to at least one disease or disorder selected from the group of myocarditis, post myocardial infarction syndrome, post pericardiotomy syndrome, subacute bacterial endocarditis, anti-glomerular basement membrane nephritis, lupus nephritis, interstitial cystitis, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, antisynthetase syndrome, alopecia areata, autoimmune angioedema, autoimmune progesterone dermatitis, autoimmune urticaria, bullous pemphigoid, cicatricial pemphigoid, dermatitis herpetiformis, discoid lupus erythematosus, epidermolysis bullosa acquisita, erythema nodosum, gestational pemphigoid, hidradenitis suppurativa, lichen planus, lichen sclerosus, linear IgA disease,

morphea, pemphigus vulgaris, pityriasis lichenoides et varioliformis acuta, mucha–habermann disease, psoriasis, systemic scleroderma, vitiligo, Addison's disease, autoimmune polyendocrine syndrome (type 1, 2 or 3), autoimmune pancreatitis, diabetes, autoimmune thyroiditis, ord's thyroiditis, graves' disease, autoimmune oophoritis, endometriosis, autoimmune orchitis, Sjögren syndrome, autoimmune enteropathy, coeliac disease, Crohn's disease, esophageal achalasia, microscopic colitis, ulcerative colitis, antiphospholipid syndrome, aplastic anemia, autoimmune hemolytic anemia, autoimmune lymphoproliferative syndrome, autoimmune neutropenia, autoimmune thrombocytopenic purpura, cold agglutinin disease, essential mixed cryoglobulinemia, Evans syndrome, pernicious anemia, pure red cell aplasia, thrombocytopenia, adiposis dolorosa, adult-onset still's disease, ankylosing spondylitis, crest syndrome, drug-induced lupus, enthesitis-related arthritis, eosinophilic fasciitis, felty syndrome, IgG4-related disease, juvenile arthritis, lyme disease (chronic), mixed connective tissue disease, palindromic rheumatism, Parry–Romberg syndrome, parsonage–turner syndrome, psoriatic arthritis, reactive arthritis, relapsing polychondritis, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schnitzler syndrome, systemic lupus erythematosus, undifferentiated connective tissue disease, dermatomyositis, fibromyalgia, inclusion body myositis, myositis, myasthenia gravis, neuromyotonia, paraneoplastic cerebellar degeneration, polymyositis, acute disseminated encephalomyelitis, acute motor axonal neuropathy, anti-n-methyl-d-aspartate (anti-nmda) receptor encephalitis, Balo concentric sclerosis, Bickerstaff's encephalitis, chronic inflammatory demyelinating polyneuropathy, Guillain–Barré syndrome, Hashimoto's encephalopathy, idiopathic inflammatory demyelinating diseases, Lambert–Eaton myasthenic syndrome, multiple sclerosis, Oshtoran syndrome, pediatric autoimmune neuropsychiatric disorder associated with streptococcus, progressive inflammatory neuropathy, restless legs syndrome, stiff-person syndrome, Sydenham's chorea, transverse myelitis, autoimmune retinopathy, autoimmune uveitis, Cogan syndrome, graves' ophthalmopathy, intermediate uveitis, ligneous conjunctivitis, Mooren's ulcer, neuromyelitis optica, opsoclonus myoclonus syndrome, optic neuritis, scleritis, Susac's syndrome, sympathetic ophthalmia, Tolosa–hunt syndrome, autoimmune inner ear disease, Ménière's disease, Behçet's disease, eosinophilic granulomatosis with polyangiitis, giant cell arteritis, granulomatosis with polyangiitis, IgA vasculitis, Kawasaki disease, leukocytoclastic vasculitis, lupus vasculitis, rheumatoid vasculitis, microscopic polyangiitis, polyarteritis nodosa, polymyalgia rheumatica, urticarial vasculitis, vasculitis and primary immunodeficiency.

The term “alloimmune disease or disorder”, as used herein, refers to an immune response to nonself antigens from members of the same species. In some embodiments, the alloimmune disease or disorder is a disease or disorder selected from the group of transfusion reaction, hemolytic disease of the fetus and/or newborn and transplant rejection.

The invention provides the means and methods to modulate auto- or alloimmune responses e.g. by protective-regulative binding.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention can be used to therapeutically modulate the autoimmune response and/or alloimmune response to a target antigen.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention, the vector for use of the invention or the protective-regulative antibody, variant or fragment for use of the invention, wherein the humoral and/or B-cell-mediated target antigen-specific disease or disorder or the autoimmune disease or disorder or an alloimmune disease or disorder is an antibody-mediated disease or disorder.

The term “antibody-mediated disease or disorder”, refers to an autoimmune disease or disorder or an alloimmune disease or disorder that is characterized by the presence of antibodies. In some embodiments, the antibodies present in the antibody-mediated disease or disorder are disease specific antibodies.

In some embodiments, the antibody-mediated disease or disorder described herein is at least one disease or disorder selected from the group of Addison's disease, Ankylosing spondylitis, Behcet's syndrome, Celiac disease, congenital adrenal hyperplasia, dermatitis herpetiformis, Goodpasture syndrome, Graves' disease, Hashimoto's disease, hereditary hemochromatosis, insulin-dependent diabetes mellitus, idiopathic membranous glomerulonephritis, multiple sclerosis, myasthenia gravis, narcolepsy, psoriasis vulgaris, pemphigus vulgaris, rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis

The invention provides the means and methods to modulate antibody expression and/or the binding of antibodies of the immune system e.g. by protective-regulative binding.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention can be used to therapeutically modulate the antibody-

mediated autoimmune response and/or antibody-mediated alloimmune response to a target antigen.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention, the vector for use of the invention, or the protective-regulative antibody, variant or fragment for use of the invention, wherein the target antigen is insulin for use in treatment of an insulin- associated disease or disorder.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention, the vector for use of the invention, or the protective-regulative antibody, variant or fragment for use of the invention, wherein the target antigen is insulin and the antibody-mediated disease or disorder is an insulin-associated disease or disorder.

The term “insulin- associated disease or disorder”, as used herein, refers to any disease or disorder wherein the insulin production, insulin effect, insulin signalling, insulin distribution, insulin metabolism and/or insulin elimination is dysregulated.
In some embodiments, the insulin- associated disease or disorder is at least one disease or disorder selected from the group of polycystic ovary syndrome, metabolic syndrome and diabetes.
In some embodiments, the insulin- associated disease or disorder is at least one disease or disorder associated with increased levels of at least one agent selected from the group adrenaline, glucagon, cortisol, somatostatin.
In some embodiments, the insulin- associated disease or disorder is at least one side effect of a treatment of an insulin modulating agent. In some embodiments, the insulin modulation agent is selected from the group adrenaline, glucagon, steroid and somatostatin.

The means and methods provided by the invention enable modulation of the immune response against insulin. An immune response against insulin can occur in healthy subjects and/or patients and/or during insulin treatment. The inventors show that a broad range of insulin associated symptoms can be influence by the means and methods of the invention (See e.g. Fig 11, 12, 16, 20B, 20D, 20F, 22). Therefore, the means and methods can improve the effect of administered and/or endogenous insulin and reduce any insulin-associated disease or disorder.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention can be used to protect and/or regulate insulin function.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention or the protective-regulative antibody, variant or fragment for use of the invention, wherein the protective-regulative antibody, variant or fragment for use of the invention binds to insulin with a K_d of less than 10^{-7}, preferably of less than 10^{-8}, more preferably of less than 10^{-9} and most preferably in the range of about 10^{-10} to about 10^{-12}.

The high affinity of the antibody, variant or fragment of the invention to insulin enables efficient binding in competition with other antibodies (e.g. polyspecific IgG- antibodies of the immune system).

Accordingly, the invention is at least in part based on the surprising finding that high-affinity binding enabled by the means and methods of the invention protect and/or regulate insulin function by competing with function-limiting insulin-binding agents.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention, the vector for use of the invention, or the protective-regulative antibody variant or fragment for use of the invention, wherein the insulin-associated disease or disorder is diabetes or a symptom thereof.

The term “diabetes”, as used herein, refers to a disease or disorder characterized by hyperglycemia. In some embodiments, diabetes is diagnosed by a glucose level above 140 mg / dl, 150 mg / dl, 160 mg / dl, 170 mg / dl, 180 mg / dl, 190 mg / dl, 200 mg / dl, 210 mg / dl, or 220 mg / dl 2 hours after glucose intake (typically 75g glucose) during an oral glucose tolerance test. In some embodiments, diabetes is diagnosed by a fasting glucose levels above 100 mg / dl or 110 mg / dl.

Symptoms of diabetes include, without limitation, hyperglycemia, hypoinsulinemia, insulin resistance, polyuria, polydipsia, weight loss, ketoacidosis, glucosuria , fatigue, irritability, blurred vision, slow-healing sores, frequent infections (e.g. gums or skin infections and vaginal infections) and increased inflammation (e.g. chronic-low grade inflammation).

In certain embodiments, the invention relates to the composition of the invention, the pharmaceutical product of the invention, the vector of the invention, or the protective-regulative antibody, variant or fragment of the invention, wherein the target antigen is insulin for use to enhance the insulin effect. The insulin effect can also be enhanced in patients or in healthy subjects, wherein the insulin effect is regulated by antibodies without necessarily inducing a disease or disorder. For example the composition of the invention, the pharmaceutical product of the invention, the vector of the invention, or the protective-regulative antibody, variant or fragment of the invention, wherein the target antigen is insulin can be used to increase weight gain such as muscle gain. In some embodiments, enhancement of the insulin effect includes, without limitation, increase of glucose uptake, increase of DNA replication, increase of protein synthesis, increased fat synthesis, increased esterification of fatty acids, decreased lipolysis, induction of glycogen synthesis, decreased gluconeogenesis and glycogenolysis, decreased proteolysis, decreased autophagy, increased amino acid uptake, increased blood flow, increase of hydrochloric acid secretion in the stomach, increased potassium uptake, decreased renal sodium excretion.

The means and methods provided by the invention enable modulation of the immune response against insulin. An immune response against insulin can occur in all forms of diabetes and in all forms of insulin treatment. Therefore, the means and methods can improve the effect of administered and/or endogenous insulin and reduce any insulin-deficit related symptom e.g. in diabetes.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention protect and/or regulate dysregulated insulin function in diabetes.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention, the vector for use of the invention, or the protective-regulative antibody, variant or fragment for use of the invention, wherein the diabetes is selected from the group of type 1 diabetes, type 2 diabetes and gestational diabetes.

The term “type 1 diabetes”, as used herein, refers to diabetes, primarily characterized by decreased insulin production. Typically type 1 diabetes is characterized by an autoimmune reaction that leads to damage in the insulin producing beta cells of the

pancreas.

The term “type 2 diabetes”, as used herein, refers to diabetes primarily characterized by increased insulin resistance. Type 2 diabetes often occurs when levels of insulin are normal or even elevated and appears to result from the inability of tissues to respond appropriately to insulin. Most of the type 2 diabetics are obese.

The term “gestational diabetes“, as used herein, refers to diabetes during pregnancy. gestational diabetes. Symptoms of gestational diabetes additionally includes pregnancy-related symptoms such as preeclampsia and symptoms for the child from a mother with gestational diabetes including, without limitation, growth abnormalities (e.g. macrosomia), impaired glucose homeostasis, jaundice, polycythemia, hypocalcemia, and hypomagnesemia. In some embodiments, the gestational diabetes is diagnosed during pregnancy. In some embodiments, the gestational diabetes is diagnosed before pregnancy.
The means and methods provided by the invention enable modulation of the immune response against insulin. Antibody types differ in their placental transfer capabilities, therefore, the means and methods of the invention enable selective and/or simultaneous treatment of the mother and the fetus.

An immune response against insulin occurs particularly during chronic insulin treatment. Therefore, the means and methods can improve the effect of treatment of type 1 diabetes, type 2 diabetes and gestational diabetes.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention protect and/or regulate dysregulated insulin function in type 1 diabetes, type 2 diabetes and gestational diabetes.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention, the vector for use of the invention, or the protective-regulative antibody, variant or fragment for use of the invention, wherein the diabetes is type 1 diabetes.

The means and methods provided by the invention enable modulation of the immune response against insulin. The immune response is considered a key factor in the pathology of type 1 diabetes and the treatment of type 1 diabetes is particularly reliant on

insulin. Therefore, the means and methods can improve the effect of treatment of type 1 diabetes.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention protect and/or regulate dysregulated insulin function in type 1 diabetes.

In certain embodiments, the invention relates to the composition for use of the invention the pharmaceutical product for use of the invention, the vector for use of the invention, or the protective-regulative antibody, variant or fragment for use of the invention, wherein the target antigen is a cancer associated antigen, or a pathogen associated antigen.

The term “cancer associated antigen”, as used herein, refers to a protein or polypeptide antigen that is expressed by a cancer cell. In some embodiments, the cancer associated antigen described herein is at least one selected from the group of surface proteins or polypeptides, nuclear proteins or glycoproteins, or fragments thereof, of a cancer cell.
The term “pathogen associated antigen”, as used herein, refers to a protein or polypeptide antigen that is expressed by a pathogen. A pathogen associated antigen of the invention may be any antigen that is expressed in, on or by a pathogen, such as a pathogenic virus or microorganism, preferably wherein the pathogen is selected from a parasite, a monocellular eukaryote, a bacterium, a virus or virion.

Therefore, the means and methods can modulate the immune response to a pathology.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods can be used to increase and/or regulate the immune response against a cancer or a pathogen.

In certain embodiments, the invention relates to the composition for use of the invention, the pharmaceutical product for use of the invention, the vector for use of the invention or the protective-regulative antibody, variant or fragment for use of the invention, wherein the humoral and/or B-cell-mediated target antigen-specific disease or disorder is an infection and wherein the target antigen is a pathogen associated antigen, preferably wherein the pathogen is at least one pathogen selected from the group of parasite, monocellular eukaryote, bacterium, virus and virion.

The term “parasite”, as used herein, refers to an organism that lives in or on a second

organism. In some embodiments, the parasite described herein is a parasite selected from the group of ectoparasites, protozoan organisms and helminths.

The term “virus”, as used herein, refers to an infectious agent that replicates only inside the living cells of an organism. In some embodiments, the virus described herein is a virus selected from the group of adenoviridae, anelloviridae, arenaviridae, astroviridae, bunyaviridae, bunyavirus, caliciviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, herpesviridae, orthomyxoviridae, papillomaviridae, paramyxoviridae, parvoviridae, picornaviridae, pneumoviridae, polyomaviridae, poxviridae, reoviridae, retroviridae, rhabdoviridae, rhabdovirus, and togaviridae.

In some embodiments, the monocellular eukaryote described herein is selected from the group of Plasmodium falciparum, Toxoplasma gondii, Trypanosoma brucei, Giardia duodenalis and Leishmania species.

The term “virion”, as used herein refers to viral nucleic acid core with a protein coat, and optionally an external envelope.

In some embodiments, the bacterium described herein is a bacterium from the genus selected from the group of Bacillus, Bartonella, Bordetella, Borrelia, Brucella, Campylobacter, Chlamydia, 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.

Therefore, the means and methods of the invention can be used to elicit an immune response against an infectious agent (see e.g. Fig. 18).

Accordingly, the invention is at least in part based on the surprising finding that the means and methods can be used to increase and/or regulate the immune response to an infection.

In certain embodiments, the invention relates to the composition for use according to the invention or the pharmaceutical product for use of the invention, wherein treatment comprises administering the monovalent antigen particle before the polyvalent antigen

particle.

The means and methods of the various embodiments of the present invention in certain embodiments can be viewed as immunization methods for the generation of certain desired antibody responses in a vertebrate. In this context, preferred embodiments of the inventive methods comprise a priming/boosting immunization scheme of the subject.

The term “priming” an immune response to an antigen refers to the administration to a subject with an immunogenic composition which induces a higher level of an immune response to the antigen upon subsequent administration with the same or a second composition, than the immune response obtained by administration with a single immunogenic composition.

The term “boosting” an immune response to an antigen refers to the administration to a subject with a second, boosting immunogenic composition after the administration of the priming immunogenic composition. In one embodiment, the boosting administration of the immunogenic composition is given about 2 to 27 weeks, preferably 1 to 10 weeks, more preferably 1 to 5 weeks, and most preferably about 3 weeks, after administration of the priming dose.

In a preferred embodiment of the invention the step of priming is performed with the monovalent antigen particle which is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen, whereas the step of boosting comprises the administration of the polyvalent antigen particle which is composed of an antigenic portion comprising more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen and wherein the more than one of an antigenic structure are covalently or non-covalently cross-linked. In such priming/boosting embodiment of the invention, the antigenic structure used for inducing the immune response in the priming and the boosting step is the same antigenic structure.

In some embodiments of the invention, the step of boosting may be performed with a composition of monovalent and polyvalent antigen particles as it is described herein in the first aspect of the invention.

Accordingly, the invention is at least in part based on the surprising finding that priming with a monovalent antigen particle increases the immune response to the polyvalent antigen particle.

In certain embodiments, the invention relates to the composition for use according to the invention or the pharmaceutical product for use of the invention, wherein treatment and/or prevention comprises at least two administration timepoints.

Therefore, the ingredients of the composition/pharmaceutical product of the invention can be administered at different timepoint to achieve a certain immune modulation (see e.g., Fig. 19) or can be administered repeatedly to boost achieve an enhanced effect (see e.g. Fig. 16a, Fig. 3c).

Accordingly, the invention is at least in part based on the surprising finding that priming and/or boosting modulates the immune response alteration induced by the means and method of the invention.

In certain embodiments, the invention relates to a monovalent antigen particle, wherein the monovalent antigen particle is composed of an antigenic portion comprising not more than one antigenic structure capable of inducing an antibody mediated immune response against a target antigen, the composition for use of the invention the vector for use of the invention the protective-regulative antibody, variant or fragment for use of the invention or the pharmaceutical product for use of the invention, for use in the treatment and/or prevention of a disease characterized by (i) the presence of Immunoglobulin G (IgG) type antibody binding to the target antigen, wherein the binding of the IgG type antibody reduces the function of the target antigen; and/or (ii) the presence of an endogenous polyvalent antigen particle which is composed of an antigenic portion comprising more than one antigenic structures capable of inducing an antibody mediated immune response against the target antigen and wherein the more than one antigenic structures are cross-linked.

In an alternative aspect of the invention there is provided a method for treating or preventing a disease which is characterized by the presence of antibodies other than IgG which specific for a disease-associated antigen in a subject, the method comprising administering a therapeutically effective amount of a monovalent antigen particle to the subject, wherein the monovalent antigen particle is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen. Such disorders of the alternative third aspect can be for example IgE mediated allergies.

A disease which is characterized by the presence of Immunoglobulin G (IgG) type antibodies specific for a disease-associated antigen is preferably a disease characterized

by the presence in a subject's serum of pathological IgG molecules, such as autoimmune and alloimmune IgG antibodies. The term “IgG mediated disease” thus includes autoimmune and alloimmune diseases. As used herein, the term “alloimmune disease” refers to when there is a host immune response to foreign antigens of another individual (for example, major or minor histocompatibility alloantigens), for example when there is a host-versus-graft rejection, or alternatively when there is graft-versus-host disease, wherein engrafted immune cells mediate deleterious effects against the host receiving the graft.

In some embodiments, the invention pertains to a monovalent antigen particle for use in treating or preventing a disease which is characterized by the presence of Immunoglobulin G (IgG) type antibodies specific for a disease-associated antigen in a subject, wherein the monovalent antigen particle is composed of an antigenic portion comprising not more than one of an antigenic structure capable of inducing an antibody mediated immune response against the disease-associated antigen.

In some embodiments, the disease characterized by the presence of Immunoglobulin G (IgG) type antibody is a disease selected from the group of Mikulicz's disease, chronic sclerosing sialadenitis, Küttner's tumour, IgG4-related ophthalmic disease, IgG4-related pharyngitis, IgG4-related thyroid disease, IgG4-related hypophysitis, IgG4-related panhypophysitis, IgG4-related adenohypophysitis, IgG4-related infundibuloneurohypophysitis, IgG4-related pachymeningitis, IgG4-related leptomeningitis, IgG4-related pancreatitis, IgG4-related lung disease, IgG4-related pleuritis, IgG4-related hepatopathy, IgG4-related sclerosing cholangitis, IgG4-related cholecystitis, IgG4-related aortitis, IgG4-related periaortitis, IgG4-related periarteritis, IgG4-related pericarditis, IgG4-related mediastinitis, IgG4-related retroperitoneal fibrosis, IgG4-related mesenteritis, IgG4-related mastitis, IgG4-related kidney disease, IgG4-related prostatitis, IgG4-related perivasal fibrosis, IgG4-related paratesticular pseudotumor, IgG4-related epididymo-orchitis, IgG4-related lymphadenopathy, IgG4-related skin disease and IgG4-related perineural disease.

Therefore, the ingredients of the means and methods described herein can be administered in a disease wherein a part of the composition of the invention is already present (e.g. based on the pathology of the disease) to modulate the immune response according to Figure 18. Therefore, in diseases wherein IgG and/or polyvalent antigen particles are present, the administration of a monovalent particle and/or a protective-regulative antibody, variant or fragment and/or a vector as described herein can be

sufficient. Alternatively, the composition or the pharmaceutical product of the invention comprising a substantial amount of a monovalent particle and/or a protective-regulative antibody, variant or fragment and/or a vector as described herein can be sufficient

Accordingly, the invention is at least in part based on the surprising finding that a monovalent antigen particle can support protective-regulative antibody expression.

In certain embodiments, the invention relates to the monovalent antigen particle for use of the invention, the pharmaceutical product for use of the invention or the composition for use of the invention, wherein the treatment and/or prevention comprises use of the monovalent antigen particle in a dose that results in a (tissue)content ratio of monovalent antigen particle:polyvalent antigen particle is greater than 1, preferably greater than 10^1, more preferably greater than 10^2, more preferably greater than 10^3, more preferably greater than 10^4.

Therefore, the (tissue) content of the polyvalent antigen particle is determined by any method known to the person skilled in the art. The monovalent antigen particle (or the composition/pharmaceutical product comprising the monovalent antigen particle) is the administered in the appropriate dose to achieve the desired (tissue) content ration. The pharmacologic and/or pharmacokinetic properties of the monovalent particle as well as polyvalent antigen particle-related parameters, subject- related parameters and/or disease-related parameters may be considered.

Accordingly, the invention is at least in part based on the surprising finding that a high monovalent antigen particle:polyvalent antigen particle ratio can support protective-regulative antibody expression.

In certain embodiments, the invention relates to a polyvalent antigen particle, wherein the polyvalent antigen particle is composed of an antigenic portion comprising more than one antigenic structures capable of inducing an antibody mediated immune response against a target antigen and wherein the more than one antigenic structure is cross-linked, the pharmaceutical product for use of the invention or the composition for use of the invention, for use in the treatment and/or prevention of a disease characterized by (i) the presence of an oligomeric antibody binding to the target antigen, wherein the binding of the oligomeric antibody protects the function of the target antigen; and/or (ii) the presence of a monovalent antigen particle, wherein the monovalent antigen particle is composed of an antigenic portion comprising not more than one antigenic structure capable of

inducing an antibody mediated immune response against a target antigen.

In certain disease or disorders, the protective-regulatory effect of the endogenous IgM antibodies may be undesired (e.g. protection of cytokines in inflammatory diseases). The polyvalent antigen particle or the means and methods of the invention comprising the polyvalent antigen particle can be used to modulate the immune response to suppress the IgM antibody production or to increase competitively binding antibodies. Immune responses in diseases or disorders characterized by the presence of a monovalent antigen particle can be modulated by the polyvalent antigen particle or the means and methods of the invention comprising the polyvalent antigen particle.

Accordingly, the invention is at least in part based on the surprising finding that a monovalent antigen particle can support protective-regulative antibody expression.

In certain embodiments, the invention relates to the vector for use of the invention, the pharmaceutical product for use of the invention or the protective-regulative antibody, variant or fragment for use of the invention, for use in the treatment and/or prevention of a disease or disorder in a subject having a reduced IgD-type level.

The term “patient” or “subject”, as used herein, refers to all animals classified as mammals and includes, without limitation, domestic and farm animals, primates and humans, e.g., human beings, non-human primates, cows, horses, pigs, sheep, goats, dogs, cats, or rodents suffering from a disorder or disease. Preferably, the patient is a male or female human of any age or race.

The endogenous IgM expression and/or maturation is reduced in subjects with IgD-type antibody expression (see e.g. Example 8-11). Therefore, the effect of the means and methods of the invention are particularly pronounced in this subject population, in particular if the means and methods comprise a protective-regulative antibody, variant or fragment, or induce expression thereof.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention can modulate the immune response in subjects with reduced endogenous protective-regulative antibody production/maturation.

In certain embodiments, the invention relates to the vector for use of the invention, the pharmaceutical product for use of the invention or the protective-regulative antibody, variant or fragment for use of the invention, for use in the treatment and/or prevention of

a disease or disorder in a subject having an IgD-type antibody-associated genetic deficiency.

The term “IgD-type antibody-associated genetic deficiency”, as used herein, refers to any disease or disorder wherein IgD-type antibody expression, production, and/or function is reduced.

The endogenous IgM expression and/or maturation is reduced in subjects with IgD-type antibody-associated genetic deficiency(see e.g. Example 8-11). Therefore, the effect of the means and methods of the invention are particularly pronounced in this subject population, in particular if the means and methods comprise a protective-regulative antibody, variant or fragment, or induce expression thereof.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention can modulate the immune response in subjects with reduced endogenous protective-regulative antibody production/maturation.

In certain embodiments, the invention relates to the vector for use of the invention, the pharmaceutical product for use of the invention or the protective-regulative antibody, variant or fragment for use of the invention, for use in the treatment and/or prevention of a disease or disorder in a pediatric subject, preferably in a pediatric subject below the age of 11.

The term “pediatric subject”, as used herein, refers to a subject below the age of 18, 17, 16, 15, 14, 13, 12, 11 or 10. In some embodiments, the pediatric subject is a subject with a reduced ratio of matured B-cells.

The endogenous IgM expression and/or maturation is reduced in pediatric subjects at least partially due to incomplete development and/or ratio of the required B-cell species. Therefore, the effect of the means and methods of the invention are particularly pronounced in this subject population, in particular if the means and methods comprise a protective-regulative antibody, variant or fragment, or induce expression thereof.

Accordingly, the invention is at least in part based on the surprising finding that the means and methods of the invention can modulate the immune response in subjects with incomplete development the endogenous protective-regulative antibody

production/maturation.

The terms “of the [present] invention”, “in accordance with the invention”, “according to the invention” and the like, as used herein are intended to refer to all embodiments of the invention described and/or claimed herein.

As used herein, the term “comprising” is to be construed as encompassing both “including” and “consisting of”, both meanings being specifically intended, and hence individually disclosed embodiments in accordance with the present invention. Where used herein, “and/or” is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein. In the context of the present invention, the terms “about” and “approximately” denote an interval of accuracy that the person skilled in the art will understand to still ensure the technical effect of the feature in question. The term typically indicates deviation from the indicated numerical value by ±20%, ±15%, ±10%, and for example ±5%. As will be appreciated by the person of ordinary skill, the specific such deviation for a numerical value for a given technical effect will depend on the nature of the technical effect. For example, a natural or biological technical effect may generally have a larger such deviation than one for a man-made or engineering technical effect. As will be appreciated by the person of ordinary skill, the specific such deviation for a numerical value for a given technical effect will depend on the nature of the technical effect. For example, a natural or biological technical effect may generally have a larger such deviation than one for a man-made or engineering technical effect. Where an indefinite or definite article is used when referring to a singular noun, e.g. "a", "an" or "the", this includes a plural of that noun unless something else is specifically stated.

It is to be understood that application of the teachings of the present invention to a specific problem or environment, and the inclusion of variations of the present invention or additional features thereto (such as further aspects and embodiments), will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein.

In particular the individual definitions provided, as well as described specific embodiments in context of one aspect of the invention shall equally apply to the other aspects of the invention.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

The general methods and techniques described herein may be performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990).

All references, patents, and publications cited herein are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: shows soluble hapten inhibits antibody immune responses induced by hapten-carrier complexes. a: Schematic wild type B cell expressing IgM (green) and IgD (yellow) B cell receptors. b: Serum anti-NP-Ig titers of NP-KLH immunized (red and green) and CI mice (grey) measured by ELISA at indicated days. Ratios indicated refer to molar ratios of soluble to complex NP (sNP:cNP). Dots represent mice, mean ± SD. c: Serum anti-KLH-IgG titers measured by ELISA at indicated days. Dots represent mice, mean ± SD. d: ELISpot assay showing NP-specific immunoglobulin producing cells. n = 2/group, mean ± SD. e: Schematic IgD BCR-knock out B cell. f: Serum anti-NP-Ig titers of NP-KLH immunized (red and green) and CI mice measured by ELISA (IgD-/- mice) at indicated days. Dots represent mice, mean ± SD. CI: control immunization.

Figure 2: shows very high ratios of soluble to complex NP suppress antigen-specific IgM responses. a: Scheme showing 4-Hydroxy-3-Nitrophenylacetyl hapten soluble or conjugated to key hole limpet hemocyanin (KLH). b: Scheme showing immunization schedule with soluble/complex NP and CpG-ODN1826. c: Antibody titers of NP-valency injected mice were analysed via ELISA. Sera were applied in duplicates onto NP-BSA coated plates and diluted in a 1:3 series.

Figure 3: shows induction of autoantibodies depends on the self-antigen-valency and is modulated by its ratios. a: Scheme of proinsulin-derived full-length CP coupled to KLH carrier. b: Table comparing human to murine CP and Insulin-A chain amino acid sequences. Sequences used as peptides shown underlined, conserved amino acids in bold. c: Schematic immunization schedule. d - e: Serum anti-CP-Ig titers of CP-SAV immunized (red and green) and CI mice (grey) measured by ELISA at indicated days. Boost on d42 was done without CpG (e). Dots represent mice, mean ± SD. f: ELISpot

assay showing CP-specific immunoglobulin producing spleen-derived cells at d14. Top lane showing representative pictures of wells. n = 4 mice/group, mean ± SD. g: Serum anti-CP-Ig titers of CP-SAV immunized (red and green) and CI IgD-/- mice (grey) measured by ELISA. Dots represent mice, mean ± SD. CP: C-peptide, KLH: key hole limpet hemocyanin, SAV: Streptavidin, CI: control immunization.

Figure 4: shows soluble antigen interferes with plasma cell differentiation. a: Flow cytometric analysis (FACS) of splenocytes derived from C-peptide (CP) immunized mice. Data representative for two independent experiments (n = 4). Ratios on the X-axis refer to molar ratios of monovalent (sCP) to polyvalent (cCP) CP. CD138+ and B220- cells were identified as plasma cells. Top panel showing 0:1 and bottom panel showing 20:1 injected mice. b: Statistical analysis of presented FACS data. Mean +- SD. c: Flow cytometric (FACS) analysis of splenocytes derived from C-peptide (CP) immunized mice. Data representative for two independent experiments (n = 4). Ratios on the X-axis refer to molar ratios of monovalent (sCP) to polyvalent (cCP) CP. Top panel showing 0:1 and bottom panel showing 20:1 injected mice. Right panel: quantification. d: Western blot of pancreas lysate with C-peptide (CP) mice sera as primary antibody. Proinsulin (15 kD). e: Streptavidin(carrier)-specific IgG titers of C-peptide (CP) immunized mice were measured via ELISA. Sera of CP:SAV immunized mice were applied onto CP-coated ELISA plates in duplicates and diluted in 1:3 series.

Figure 5: shows complex native insulin (InsNat) provokes autoreactive IgG responses inducing autoimmune diabetes symptoms in wildtype mice. a: Serum anti-Insulin-Ig titers of InsNat immunized and CI mice measured by ELISA at indicated days. Dots represent mice, mean ± SD. b: Flow cytometric analysis of blood showing B cells (CD19+ Thy1.2-) and T cells (Thy1.2+ CD19-) of wildtype (left) and B cell-deficient (right) mice. Cells were pre-gated on lymphocytes. Representative for three independent experiments. c: Blood glucose levels of InsNat immunized (red: WT, yellow: B cell-deficient) and CI mice (grey) were assessed at indicated days post immunization. Dots represent mice, mean ± SD. d: Urine glucose levels of InsNat immunized (red) and CI mice (grey) were monitored at indicated days post immunization. Left panel showing visualization of glucose standard (top lane) and representative pictures of tested animals (middle and bottom lanes). Right panel showing quantification. Dots represent mice, mean ± SD. e: Water intake of CI and InsNat immunized mice monitored from d21 to d26. f: Flow cytometric analysis of the pancreas of InsNat immunized (red) and CI mice (grey) at day 27. Left panel showing pancreatic macrophages (CD11b+ Ly6G-), neutrophils (Ly6G+ CD11b+) and B cells (CD19+) pre-gated on living cells. Right Panel showing histograms for insulin-binding

(top) and streptavidin (SAV)-binding (bottom). Representative for two independent experiments with n = 5/group. g: ELISpot of InsNat immunized (red) and CI mice (grey) showing insulin-specific IgG-producing spleen-derived cells (d27). Representative wells are shown (top lane). n = 3/group, mean ± SD. h: Quantification of total (red) and insulin-specific (salmon) IgG after serum IgG purification of InsNat immunized mice. i: Coomassie stained SDS-page showing purified serum IgG of InsNat immunized (red) and CI mice (grey) under reducing (β□-ME), left lanes, and non-reducing conditions, right lanes. HC: heavy chain, LC: light chain. Representative for two independent experiments. j: Blood glucose levels of intravenously (i.v.) injected WT mice. 20 µg of purified serum IgG from InsNat immunized mice (red) or CI mice (grey) at indicated hours post injection. Dots represent mice, mean ± SD. CI: control immunization, InsNat: complexed native insulin, β-ME: β -Mercaptoethanol.

Figure 6: shows an immunization with self-antigen does not alter splenic B cell compartments. a: Flow cytometric analysis of splenocytes derived from InsNat immunized and CI mice. Top panel gating strategy for lymphocytes and single cells single cells. Middle panel showing B cells pre-gated on lymphocytes. Lower panel showing IgM and IgD expression on B cells. Left: Control immunization (CI), right: InsNat immunization (complex native Insulin). n = 3/group.

Figure 7: shows ratios of self-antigen-specific IgM to IgG control the harmfulness of autoimmune reactions and induce protective IgM. a: Serum anti-Insulin-Ig titers of InsA peptide immunized (red and green) and CI mice (grey) measured by ELISA at indicated days. Dots represent mice, mean ± SD. b: Blood glucose levels of InsA peptide immunized (red and green) and CI mice (grey) were assessed at indicated days. Dots represent mice, mean ± SD. c: Urine glucose levels of InsA peptide immunized (red and green) and CI mice (grey) were monitored at indicated days post immunization. Dots represent mice, mean ± SD. d: Ratios of IgG to IgM derived from ELISA values plotted against molar ratios of antigens. n = 5/group, mean ± SD. e: Western blot analysis of insulin-specific serum IgG derived from InsA peptide immunized mice. Top panel (green): 100:1 serum, lower panel (red): 0:1 serum (sInsA:cInsA). Black filled arrow: Proinsulin (12 kD), Black non-filled arrow: Insulin (6 kD), β -actin (42 kD, loading control). Representative for two independent experiments. f: ELISpot of InsA peptide immunized (red) and CI mice (grey) on d14 showing insulin-specific IgG-producing spleen-derived cells. Representative wells are shown (top lane). n = 4/group, mean ± SD. g: Ratios of IgG to IgM derived from ELISA values plotted on a two-dimensional graph against blood glucose levels (left panel) and urine glucose levels (right panel). n = 5/group, mean ± SD.

h: Serum anti-Insulin-Ig titers of InsA peptide immunized mice with a γ/μ ratio < 0.1 (black) and CI mice (grey) measured by ELISA at indicated days. Dots represent mice, mean ± SD. i: Blood glucose levels of InsA peptide immunized mice (γ /μ < 0.1; black) and CI mice (grey) were assessed at indicated days post immunization. Dots represent mice, mean ± SD. j: Insulin-specific IgM affinity maturation of InsA-peptide immunized mice (left panel) and virus-peptide immunized mice (right panel) at indicated days was measured by ELISA. k: Blood and urine glucose levels of mice immunized with cInsA (red) and cInsA plus pIgM i.v. (salmon). Dots represent mice, mean ± SD. CI: control immunization, cInsA: complex Insulin-A peptide.

Figure 8: shows monovalent soluble virus-derived peptide antigen modulates the IgG versus IgM antibody response induced by corresponding complex antigen. a: Determination of virus-peptide specific serum immunoglobulin titres. Sera of virus-peptide immunized mice were applied onto virus-peptide-bio:Streptavidin (SAV) coated plates in duplicates with 1:3 serial dilution. Mean +- SD. b – c: Determination of KLH(carrier)-specific serum IgG titers. Indicated ratios on the X-axis refers to molecular ratios of soluble to complex virus-peptide. Mean +- SD.

Figure 9: shows Increased IgMhigh/IgDlow positive compartment upon immunization with autoantigen but not with foreign antigen and pancreatic macrophages bindng InsA peptides via IgG. a - b: Flow cytometric analysis of splenocytes derived from virus- or insulin-peptide immunized mice. Top panel (a) showing B cells (CD19+ B220+) pre-gated on lymphocytes. Lower panel (b) showing B cell subsets: mature B cells (IgDhi IgMlo), transitional/marginal zone B cells (IgDlo IgMhi). Cells were pre-gated on B cells. Left: PBS (grey), middle: Virus-peptide (grape), right: Insulin-peptide (teal). Outer right shows quantification, mean +- SD. c: Flow cytometric analysis of pancreatic cells. Left panel showing gating strategy for cells (top) and Macrophages (bottom). Right panel showing histograms for InsA-peptide and peptide control binding as indicated.

Figure 10: shows splenic macrophages bind insulin-specific IgG in cInsA-peptide immunized mice. a: Flow cytometric analysis (FACS) of splenocytes of cInsA—peptide immunized mice. Left panel showing gating strategy for macrophages (CD11b+ CD19-). Top panel showing IgG binding histograms of control immunization (black) and cInsA-immunized (red) mice. Lower panel showing InsA-peptide binding of macrophages. Representative data for three independent experiments.

Figure 11: shows dysregulated glucose metabolism is prevented by increasing IgM upon repeated re-challenge with cInsA complexes. a: Determination of Insulin-specific serum

immunoglobulin titres. Sera of InsA-peptide immunized mice were applied in duplicates onto native Insulin coated ELISA plates in 1:3 serial dilution. Left panel showing anti-Insulin IgM on d49, right panel showing anti-Insulin IgG in arbitrary units (AU). Indicated ratios on the X-axis refers to molecular ratios of soluble to complex InsA-peptide. Mean +- SD. b: Urine glucose levels were monitored by test stripes. Mean +- SD.

Figure 12: shows polyreactive IgM induced by InsA peptide immunization leads to diabetes symptoms depending on the antigen valence and day. a: Blood glucose levels were monitored by AccuCheck system (Roche). Freshly drawled blood from the tail vein was applied onto test stripes and blood glucose was measured in mmol/L. Mean +- SD. b: Urine glucose levels were monitored by Combur M stripes (Roche). Freshly obtained urine was applied onto the glucose fields of test stripes and analysed according to manufacturer's standard. Green bars indicate 100:1 (soluble:complex) InsA-peptides. Mean +- SD. Dots represent mice used in this study.

Figure 13: shows generation of autoreactive IgM by increased ratio of monovalent antigen (100:1, sInsA:cInsA) protects from dysregulated glucose metabolism induced by complex antigen (0:1, sInsA:cInsA). a: Blood glucose levels were monitored by AccuCheck system (Roche). Freshly drawled blood from the tail vein was applied onto test stripes and blood glucose was measured in mmol/L. Mean +- SD. b: Urine glucose levels were monitored by Combur M stripes (Roche). Freshly obtained urine was applied onto the glucose fields of test stripes and analysed according to manufacturer's standard. Green bars indicate 100:1 (soluble:complex) InsA-peptides. Mean +- SD. Dots represent mice. c: Determination of Insulin-specific serum immunoglobulin titers. Sera of InsA-peptide immunized mice were applied in duplicates onto native Insulin coated ELISA plates in 1:3 serial dilution. (a) showing anti-Insulin IgM on d59, whereas (b) showing anti-Insulin IgG in arbitrary units (AU). Indicated ratios on the X-axis refer to molecular ratios of soluble to complex InsA-peptide. Mean +- SD.

Figure 14: shows repeated re-challenge with cInsA complexes results in accumulation of insulin-specific IgM+ B cells. a: Flow cytometric analysis (FACS) of splenocytes (d79) of cInsA immunized (d71) WT mice. Left panel showing forward and sideward scatter with lymphocyte gating. Middle panel pre-gated on lymphocytes shows B cells (CD19+ B220+). Right panel pre-gated on B cells shows histogram of InsA-peptide binding. Red: g/µ< 0.1; black: g/µ< 0.1 SAV only control.

Figure 15: shows Intravenous administration of purified serum pIgM does not lead to autoimmune dysglycemia. a: Coomassie stained SDS-page showing purified serum IgM

of InsA peptide (d49) immunized (red) and CI mice (grey) under reducing (b-ME), left lanes, and non-reducing conditions, right lanes. HC: heavy chain, LC: light chain. Representative for two independent experiments. b – c: Blood glucose levels of intravenously injected mice with either 20 µg CI IgM (grey) or InsA IgM (black). Dots represent mice, mean ± SD. CI: control immunization, pIgM: protective IgM. d: anti-KLH-IgM serum titers measured by ELISA.

Figure 16: shows differences in the affinity and specificity of primary versus memory IgM control autoimmune responses. a: Schematic illustration of immunization schedule with complex Ins-A-peptides (cInsA) intraperitoneally and insulin-specific protective IgM (PR-IgM) in 48 hours cycles intravenously (i.v.). *monitoring: diabetes symptoms were only observed within cInsA only group. b: Blood and urine glucose levels of wild-type mice on day 7 immunized with complex InsA-peptides (cInsA) (red, n=5) and cInsA plus intravenously injected (i.v.) pIgM (salmon, n=5). Dots represent individual mice, mean ± SD. c: Serum anti-dsDNA-IgM titers of Insulin-A-peptide immunized mice on day 7 (n=8) and day 85 (n=4) measured by ELISA. Dots represent individual mice, mean ± SD. d, f: Serum anti-nuclear-IgM (ANA) of control-immunized (CI, n=3), Insulin-A-peptide immunized mice on day 7 (n=3) and day 85 (n=3) with total serum or Insulin-specific IgM (Isotype control: n=3, day 7: n=3, day 85: n=3) analyzed via HEp-2 slides. Scale bar: 10 µm. Green fluorescence indicates IgM bound to nuclear structures e: Coomassie stained SDS-page showing primary (cInsA d7) and memory (cInsA d85) Insulin-specific IgM after incubation with Insulin/ DNA and size exclusion with a cut-off at 10.000 kD (referring to >/< 104 kD). IgM heavy chain: 69 kD, IgM light chain: 25 kD, J-segment: 15 kD. Data presented are representative of three independent experiments. g: Blood glucose levels of wild-type mice intravenously injected with either IgM isotype ctrl (grey, n=6), memory PR-IgM (black, protective Insulin-IgM d85, n=5), or primary Insulin-IgM (red, d7, n=4) after Insulin-pulldown.

Figure 17: shows insulin-specific pulldown of sera of cInsA immunized mice contains Insulin-reactive IgM. a: Western blot analysis of Insulin-specific pulldown of cInsA immunized mice sera. CI: control immunization. Top panel (green) shows IgM heavy chain (IgM HC, 69 kD) and bottom panel shows IgG heavy chain (IgG HC, 55 kD). b: Serum IgM of control immunized mice against DNA (left) and Insulin (right) measured via ELISA. Mean +- SD. Dots represent individual mice.

Figure 18: shows a graphical summary in the case of insulin. Responsiveness of insulin-specific B cells is controlled by antigen-valences leading to inducible protective

autoreactive IgM under physiological conditions. pIgM: protective IgM, sInsulin: soluble (monovalent), cInsulin: complex (multivalent).

Figure 19: Antibody responses after immunization with SARS-CoV-2-derived RBD. Mice were pre-treated as indicated two weeks before immunization. Subsequently, the mice were immunized at day 1 and day 21. Serum was collected at day 28 after immunization concentrations and used in ELISA to determine Ig concentration.

Figure 20: Immunization of mice with cInsulin induces acute inflammatory pancreatitis.

A) FACS measurement showing germinal center B cells that bind native Insulin

B) ELISA measurement showing serum pancreatic lipase which was used as marker for pancreas damage. In agreement with the autoimmune reaction induced by polyvalent Insulin, a remarkable increase in serum pancreatic lipase was detected as a clear sign for organ damage.

C) Competition assay for insulin binding to IgM. Serum of wild-type mice immunized with cInsA was preincubated either with BSA (untreated control, UT) or with 50 µg/mLcalf-thymus dsDNA (+ DNA). Data show the relative reduction in insulin binding to primary IgM (d7) after preincubation with dsDNA suggesting that dsDNA competes with insulin for binding to primary IgM, which is, in contrast to PR-IgM, poly-specific

D) Quantitative data for the affinity measurements Interferometric assay for direct Insulin:IgM interactions showing differences in the affinities of primary IgM compared with PR-IgM.

E) Flow cytometry-based bead array of pancreas supernatant of mice immunized with cInsulin (n=3) or control immunization (n=3). Representative histograms of cytokine beads (left) and cytokine detection (right).

F) Quantification of the FACS bead array for the indicated cytokines. Dots represent individual mice.

Figure 21: schematic illustration of Insulin-4mer (cInsulin) and CP-4mer (cCP)

Figure 22: CpG adjuvant is not required for initiation of autoantibody responses against InsA peptides. a: Serum anti-Insulin-IgM titers of mice injected with complex Insulin-A peptides (cInsA, n=5) or control injection (PBS, n=3) measured by ELISA (coating: Insulin). Dots represent individual mice. Mean ± SD, statistical significance was calculated by using Mann-Whitney-U test. b: Blood glucose levels of mice injected with complex Insulin-A peptides (cInsA, n=5) or control injection (PBS, n=3) were monitored

with a commercial blood glucose monitor device. Dots represent individual mice. Mean ± SD, statistical significance was calculated by using Mann-Whitney-U test.

Figure 23: IgD-deficient mice mount robust polyreactive IgM responses one day after immunization.

A - B: Serum immunoglobulin titers of NP-KLH (IgD-deficient n=5, WT n=4) and CpG ODN1826 (control immunization: CI, IgD-deficient n=4, WT n=2) injected mice measured by ELISA. NP-reactive IgM of day 1 and 3 (A) and day 7 (B). Mean, ± SD.

C: Serum immunoglobulins reactive to self-molecules (DNA/RNA) of NP-KLH and CI injected IgD-deficient and WT mice tested via HEp2 slides. Fluorescence microscopy images are representative for three independent experiments. Scale bar: 10 pm.

D: Serum immunoglobulin titers of NP-KLH (IgD-deficient n=5, WT n=4) and CI injected (IgD-deficient n=4, WT n=2) mice measured by ELISA. dsDNA-reactive IgM of day 7 post immunization. Students t-tests with Welch's correction were used to compare two groups within one experiment. Mean, ± SD.

Figure 24: The IgD-class BCR is required to prevent rapid immune response to autoantigens and induces affinity maturation.

A: Schematic illustration of insulin-A- chain-derived peptide (InsA) polyvalent complex together with keyhole limpet hemocyanin (KLH). Amino acid sequence of InsA is stated in the illustration. B: Immunization schedule of IgD-deficient and WT mice injected with InsA-KLH + CpG ODN1826 on day 0 and InsA-KLH on days 21 and 42. C: Serum immunoglobulin titers reactive to native insulin of IgD-deficient (n=4) and WT (n=10) mice immunized with InsA-KLH or CI (n=3) measured by ELISA. Days are indicated in the figure. Mean, ± SD. D: Serum immunoglobulins reactive to self¬molecules (DNA/RNA) of InsA-KLH and CI injected IgDko (n=5/day) and WT (n=5/day) mice tested via HEp2 slides. Fluorescence microscopy images are representative for three independent experiments. Scale bar: 10 pm. Students t-tests with Welch's correction were used to compare two groups within one experiment.

Figure 25: The IgD-class BCR is required for affinity maturation of insulin-IgM to be protective and prevent autoimmune pathology.

A: Affinity of IgM to InsA peptides of clnsA (InsA-KLH + CpG ODN1826) immunized IgDko (n=4), WT (n=5) and CI (n=3) mice measured by peptide-ratio ELISA .Plates were coated with Streptavidin bearing one (InsA(1)) or four (InsA(4)) biotin binding sites. Mean, ± SD. B: Urine glucose values (mmol/L) measured by commercial urine stripes (Roche) of IgD-

deficient (n=4) and WT (n=5) mice immunized with InsA-KLH or CI (n=3). Mean, ± SD. C: Blood glucose values (mmol/L) of IgD-deficient (n=4) and WT (n=5) mice immunized with InsA. Injections were done on day 0 (InsA-KLH + CpG ODN1826), day 21 (InsA-KLH), day 42 (InsA-KLH). D: Coomassie-stained SDS-page showing reduced (+ ß-ME) and non-reduced (- ß-ME) IgM of clnsA and control immunized mice. Total serum IgM was isolated via HiTrap IgM columns (clnsA d85 refers to PR-IgM). IgM monomer: 150 kD, IgM HC: 70 kD, IgM LC: 25 kD. E: Blood glucose values (mmol/L) of IgD-deficient immunized with InsA-KLH (n=9), WT control immunized mice (n=4) and IgD-deficient mice immunized with InsA-KLH and injected with PR-IgM i.v. (n=5). Mean, ± SD. F: Serum immunoglobulins reactive to self¬molecules (DNA/RNA) of IgM (PR-IgM and day 7 primary IgM) isolated from InsA-KLH immunized mice tested via HEp2 slides. Fluorescence microscopy images are representative for three independent experiments. Scale bar: 10 pm. G: Interferometric assay to determine the affinity of IgM to insulin. Insulin-specific isolated IgM of IgD-deficient (top panel) and WT (bottom panel) mice immunized with clnsA. Affinity of IgM of different days is shown in pm. Graphs are representative of three independent experiments.

Figure 26: The IgD-class BCR controls a rapidly responding CD2TCD23´ B cell population that is able to secrete autoantibodies 24 hours after immunization.

A - E: Flow cytometric analysis of IgD-deficient (n=4/group) and WT (n=4/group) mice immunized with InsA-KLH + CpG ODN1826 or CpG ODN1826 (CI). All panels show representative plots pre-gated on lymphocytes (FSC/SSC), single cells (SSC-H/SSC- W) and viable cells (FVD). A: Left panel shows histogram of CD19 expression used to gate B cells (CD19+) within lymphocyte gate. Right panel shows enlarged (activated) IgM+ B cells (FSChi B220+) within B cell gate. B: Histogram showing activated B cells by CD69 expression pre-gated on IgM+ B cells. C: Representative plot showing Marginal zone B cells (CD21hi CD23'°), Follicular B cells (CD21'° CD23hi) and CD2T CD23'' (double negative) B cell population. D: Left panel showing histograms of CD2T CD23'' B cells IgM expression. Right panel showing histograms of CD2T CD23'' B cells IgD expression. E: Histogram showing CD23 expression of IgM+ splenic B cells.

Figure 27: The CD21/CD23 negative B cell population is the major source of IgM secreting cells under the control of the IgD-class BCR.

A - D: ELISpot analyses showing IgM secreting splenic cells of InsA-KLH + CpG ODN1826 or control (CpG ODN1826) immunized (CI) mice 24 hours after injection. (A) IgM secreting total splenic cells, (B) IgM secreting CD21/CD23 negative sorted B cells,

(C) IgM secreting CD23+ Follicular B cells, (D) Representative images of ELISpot wells of indicated cells and genotypes. Two independent experiments with n=3/group for CI and n=6/group for InsA-KLH were performed. Mean, ± SD. Students t-tests with Welch's correction were used to compare two groups within one experiment.

Figure 28: Primary IgM is antigen-specific and polyreactive but not cross-reactive.

A, C: Blood glucose levels (mmol/L) of IgD-deficient and WT mice immunized with NP-KLH + CpG and controls (CpG-ODN1826) (A), or immunized with InsA-KLH + CpG and controls (C). Mean, ± SD. B, D: Serum immunoglobulin titers of IgD-deficient and WT mice immunized with either NP-KLH + CpG and controls (B) or InsA-KLH + CpG and controls (D) reactive to dsDNA measured by ELISA. Mean, ± SD. E, F: Serum immunoglobulin titers of IgD-deficient and WT mice immunized with either NP-KLH + CpG or InsA-KLH + CpG and controls reactive to Insulin (top panel) or InsA-KLH + CpG and controls immunized mice reactive to NP (bottom) (E) and reactive to NP or Insulin (F) measured by ELISA. Mean, ± SD.

Figure 29: Graphical abstract: IgD is required for IgM maturation

Figure 30: IgD-deficient mice require multiple boosts for controlling autoreactivity

a) Immunization schedule of IgD-/- (n=5) and WT (n=5) mice injected with cInsA (KLH + CpG ODN1826). Days of injections and boosts are indicated in the scheme.

b) blood glucose titers of IgDko and WT mice immunized with cIsA (InsA-KLH + CpG ODN1826) and control (CI, CpG ODN1826)

Figure 31: IgD-deficient mice require multiple boosts for affinity maturation of insulin-specific IgM. Affinity of IgM to InsA peptides of cInsA (InsA-KLH + CpG ODN1826) immunized IgD-/- (n=4), WT (n=5) and CI (n=3) mice measured by peptide-ratio ELISA (Shimizu et al. 2004) Plates were coated with Streptavidin bearing one (InsA(1)) or four (InsA(4)) biotin binding sites. Mean ±SD

Figure 32: IgD-deficient mice show activated B cells within the $CD21^{-}CD23^{-}$ B cell population one day after immunization.

A: General gating strategy used in this study. Top panel showing total splenic cells with gating of lymphocytes. Middle panel showing lymphocytes with gating of single cells. Bottom panel showing single cells with gating of viable cells (Fixable viability dye (FVD) negative). B - C: Flow cytometric analysis of splenic B cells of InsA (InsA- KLH + CpG ODN1826) immunized WT and IgD-/- mice. Histograms showing FSC (cell size) were

pre-gated on $CD21^{-}CD23^{-}$ B cells (C) and CD23+ FO (follicular) B cells (C). Data shown is representative for two independent experiments.

Figure 33: The IgD-class BCR controls plasma cell differentiation in the peritoneal cavitiy.

A: Flow cytometric analysis of peritoneal cavity cells of clnsA (InsA-KLH + CpG ODN1826) and control (ctrl) immunized mice. Panel shows CD138+ plasma blasts and plasma cells. Data shown are representative for two independent experiments with n=3/group.

Figure 34 Schematic illustration of A) 1,2,-phenylene-bis-maleimide RBD dimer B) activated RBD monomers C) reaction with cysteine D) linking with IgG E) polymerization with IgG F) complexation with endogenous proteins

Figure 35 Mimicking immune complexes by chemical crosslinking of RBD results in robust antibody responses

A. Concentration of RBD-specific IgM (left), IgG (middle) and total Ig (right) determined by ELISA in samples used for neutralization assay.

B – C. The neutralizing potential measured in sera from mice immunized with cRBD*MM. Results were compared to neutralizing capacities determined in mice immunized with cRBD-SAV after RBD-pre-treatment.

IgM is not exclusively required to achieve virus neutralization -> can also be achieved by samples that contain mainly IgG. Higher concentrations of RBD-specific total Ig correlates with potent neutralization capacity.

cRBD MM: complexed RBD with maleimide(MM)

Figure 36 Activated antigen forms IgG complexes that boost immune responses

A. Schematic illustration of the SARS-CoV-2 spike protein with localization of the recptor binding domain (RBD).

B. Reaction scheme of chemical cross-linking. At pH 6.5 - 7.5 reactive groups of 1,2-phenylene-bis-maleimide undergo oxidation with sulfhydryl-groups on cysteine residues of proteins to form a stable thioether linkage.

C. Coomassie staining for RBD complexed by 1,2-phenylene-bis-maleimide (bismale). RBD indicates native RBD without crosslinking.

D. & E. Immunization with RBD

Figure 37 Autoantibodies are required to balance homeostasis in mice.

A: Insulin-specific IgG concentrations of different IgG pulldowns measured via ELISA (coating: native Insulin). Total: total IgG pulldown via protein G (n=5), Insulin-specific: IgG pulldown via Insulin bait column (n=5), control IgG (n=3). B: Coomassie stained SDS

page showing total IgG (pulldown from serum) and IgG control (total IgG depleted for anti-Insulin-IgG). Presented image is representative of three independent experiments. Marker on the left is shown in kilodaltons (kD). C: Anti-Insulin-IgG secreting splenocytes of naïve wildtype and B cell-deficient (B cell-def) mice measured by ELISpot (coating: native Insulin). Cells were seeded at 300.000 cells/well and incubated for 48 hours. D: Blood glucose levels of naïve wildtype and B cell deficient mice measured with a commercial blood glucose monitor (mmol/L). E: Blood glucose levels of wildtype and B cell deficient mice intravenously injected with 200 µg total IgG, IgG depleted for anti-Insulin-IgG measured at indicated hours. F: Motor function of wildtype (WT) and B cell-deficient (B cell-def) mice as measured by wire hanging test (in on-wire seconds). Grey: WT untreated, blue: B cell-def untreated, green: B cell-def injected with 200 µg total IgG. G: Insulin titers of B cell-deficient (B cell-def) mice injected with 100 µg commercial human IVIg as measured by ELISA at indicated time points. H: Blood glucose levels of wildtype mice injected with 200 µg commercial human IVIg (black) and commercial human IVIg depleted for anti-Insulin-IgG (grey) measured by a commercial blood glucose monitor (mmol/L) at indicated hours. I: Serum glucose levels of immunodeficiency patients (common variable immune deficiency, CVID) that received (500 mg/kg) IVIg before (pre) and after (post) treatment compared to healthy donor (HD) controls.

J: Insulin-binding affinity of human anti-insulin-IgG determined by bio-layer interferometry (BLI). The Kd (dissociation constant) was calculated by using the Ka (association constant): 1/Ka. Shown data are representative for three independent experiments.

Figure 38 Neutralizing and PR-IgM exists in humans

A: Serum anti-Insulin-IgM concentrations of young (< 30 years) and old (> 65 years) individuals measured via ELISA (coating: native Insulin). Women (young): n=25, women (old): n=11, men (young): n=15, men (old): n=12. Mean, ± SD, statistical significance was calculated using Kruskal-Wallis-test. B: Scheme showing column-based purification of insulin-specific IgM fractionated into low and high affinity fractions. C: Coomassie stained SDS page showing low-affinity anti-Insulin IgM (red) and high-affinity anti-Insulin-IgM (green) after purification. Presented image is representative of three independent experiments. Marker on the left is shown in kilodaltons (kD), HC (heavy chain): 70 kD, LC (light chain): 25 kD, J (J-segment): 15 kD. D: HEp2 slides showing anti-DNA-reactive IgM of insulin-specific IgM pulldowns. Black: monoclonal IgM control (n=6), red: low-affinity anti-Insulin IgM (n=6), green: high-affinity anti-Insulin IgM (n=6). Scale bar: 10 µm. Green fluorescence indicates HEp2 cell binding. Images representative of three independent experiments. E: Anti-dsDNA-IgM concentration of insulin-specific IgM pulldowns as

measured by ELISA (coating: calf-thymus DNA). IgM control (ctrl, n=3), IgMlow (n=3), IgMhigh (n=3). Mean, ± SD, statistical significance was calculated using Kruskal-Wallis-test. F: Insulin-binding affinity of human anti-insulin-IgM pulldowns determined by bio-layer interferometry (BLI). The Kd (dissociation constant) was calculated by using the Ka (association constant): 1/Ka. Shown data are representative for three independent experiments. Uppercase letter refers to affinity fractions. G: Blood glucose levels of wildtype mice intravenously injected with 100 µg human insulin-specific IgM (uppercase refers to affinity fraction) and human IgM control. H, I: Blood glucose levels of wildtype mice intravenously injected with 100 µg human insulin-specific IgM (uppercase refers to affinity fraction) and human IgM control together with 500 ng native Insulin (H) and together with 100 µg human anti-Insulin-IgG (I). J: Ratio of insulin-specific IgM of young (< 30 years) and old (> 65 years) individuals as determined by ELISA. Insulin-specific IgM was isolated via insulin-bait columns before experiments.

Figure 39 Endogenous Insulin complexes induce robust autoimmunity in mice

A: Schematic illustration of insulin tetramers (cInsulin) generated by thiol group mediated disulfide crosslinking via 1,2-phenylene-bis-maleimide. Black lines: endogenous disulfide bonds, gray lines: induced disulfide bonds. B: Coomassie stained SDS page showing Insulin (left lane) and crosslinked insulin (right lane; left panel) and cInsulin complexes after purification with a 10 kD size exclusion column (right panel). Presented images are representative of three independent experiments. Marker on the left is shown in kilodaltons (kD). C: Blood glucose levels of wildtype mice intraperitoneally injected with PBS (control injection; CI, n=5), cInsulin (n=5), Insulin:SAV (n=5) on day 0. Mean, ± SD, statistical significance was calculated using repeated measure ANOVA test. D: Serum anti-Insulin-IgM concentrations of wildtype mice intraperitoneally injected with PBS (control injection; CI, n=5) and cInsulin (n=3) on day 0 measured by ELISA at indicated days (coating: native Insulin). Mean, ± SD, statistical significance was calculated using Kruskal-Wallis-test. E: Blood glucose levels of wildtype mice intraperitoneally injected with PBS (control injection; CI, n=5) and cInsulin (n=5) on day 0 and day 21 followed by intravenous injections of 100 µg anti-Insulin IgM (high affinity) or 100 µg IgM ctrl on day 22. F: Flow cytometric analysis of mice intraperitoneally injected with PBS (n=5) and cInsulin (n=5/group) together with intravenous 100 µg anti-Insulin-IgM (high-affinity) or 100 µg IgM control. Panels show pancreatic macrophages (CD11b+) and neutrophils (Ly6G+) pre-gated on viable cells. Images are representative of three independent experiments. G: Serum pancreatic lipase levels of wildtype mice intraperitoneally injected with PBS (n=5) and cInsulin (n=5/group) together with intravenous 100 µg anti-Insulin-

IgM (high-affinity) or 100 µg IgM control. H: Schematic illustration of the macrophage assay used to assess phagocytosis activity. I: Flow cytometric analysis of bead-based phagocytosis assay performed with high or low affinity murine anti-Insulin-IgM. Left panel shows representative FACS plots for the percentage of phagocytosing macrophages in the presence of low or high affinity IgM. Right panel show quantitative analysis for the percentage of phagocytosing macrophages.

Figure 40 Monoclonal human insulin-IgM is able to protect Insulin in vivo.

A: Coomassie stained SDS page showing monoclonal anti-Insulin-IgM and IgG after purification. Presented image is representative of three independent experiments. Marker on the left is shown in kilodaltons (kD). B: Insulin-binding affinity of monoclonal human anti-insulin-Ig determined by bio-layer interferometry (BLI). The Kd (dissociation constant) was calculated by using the Ka (association constant): 1/Ka. Shown data are representative for three independent experiments. C: Anti-dsDNA-IgM concentration of insulin-specific IgM pulldowns as measured by ELISA (coating: calf-thymus DNA). IgM control (ctrl, n=4), IgMMY (n=4), IgGMY (n=4). D: HEp2 slides showing anti-DNA-reactive monoclonal IgMMY (n=6) and IgGMY (n=6).. Scale bar: 10 µm. Green fluorescence indicates HEp2 cell binding. Images representative of three independent experiments. E: Blood glucose levels of wildtype mice intraperitoneally injected with PBS (control injection; CI, n=5) and cInsulin (n=5) on day 0 and day 21 followed by intravenous injections of 100 µg anti-Insulin IgM (high affinity) or 100 µg IgM ctrl on day 22. F: Blood glucose levels of wildtype mice intraperitoneally injected with PBS (control injection; CI, n=5) and cInsulin (n=5) on day 0 and day 21 followed by intravenous injections of 100 µg anti-Insulin IgM (high affinity) or 100 µg IgM ctrl on day 22. G: Urine glucose levels of wildtype mice intraperitoneally injected with PBS (control injection; CI, n=5) and cInsulin (n=5) on day 0 and day 21 followed by intravenous injections of 100 µg anti-Insulin IgM (high affinity) or 100 µg IgM ctrl on day 22.

Figure 41 No antibody secreting cells in mb1-deficient mice.

A: Flow cytometric analysis of blood of wild-type and B cell-deficient mice. Left panel showing cells in forward and sideward scatter. Middle and right panel showing cells pre-gated on lymphocytes.

B: IgG secreting splenocytes of wild-type and B cell-deficient mice measured by ELISpot. 50.000 splenocytes were seeded per well.

C, D: Serum total IgG (C) and total IgM (D) titers of wild-type and B cell deficient mice as measured by ELISA

Figure 42 Recombinant low affinity anti-insulin IgM destructs insulin *in vivo*

A) Schematic representation of the recombinant in-house purified anti-insulin IGHV highlighting the two mutations in the CDR2 which were reverted to the germline version of the IGHV3-74*01 allele. Bright gray: α-insulin IgM^{high} (WT-IGHV); medium gray: α-insulin IgM^{low} (gl-IGHV). B) Coomassie-stained SDS-PAGE showing purified α-insulin IgM^{high} and α-insulin IgM^{low} under reducing conditions (with β-mercaptoethanol). The image is representative of three independent experiments. C) Insulin-binding affinity of α-insulin IgM^{high} and α-insulin IgM^{low} measured by bio-layer interferometry. K_D (dissociation constant) was calculated by the software. The experiment shown is representative of 3 independent experiments. D) Blood glucose concentrations of WT mice intravenously (i.v.) injected with 100 µg α-insulin IgM^{high} (n=4) or α-insulin IgM^{low} (n=4) measured at indicated time points. Mean ± SD, statistical significance was calculated using two-way ANOVA with Tukey's multiple comparison test. *p<0,05

Figure 43 High affinity RF enhances the effect of autoreactive IgG

A) Blood glucose concentrations of WT mice intravenously injected with 100 µg anti-insulin IgG alone (black bar, n=4) or in combination with 20 µg RF concentrate from Rheumatoid Arthritis patients ($RF^{high,}$ green bar, n=4) or monoclonal IgM control (mIgM, blue bar, n=4) measured at indicated time points. Mean ± SD, statistical significance was calculated using two-way ANOVA with Tukey's multiple comparison test. ** p<0,01 B) Scheme depicting the procedure for isolation of total IgM from healthy donors (HD) sera. C) Coomassie-stained SDS-PAGE showing total IgM isolation from n=2 healthy donors (IgM^{HD}) under reducing conditions (with β-mercaptoethanol). The image is representative of three independent experiments. D) IgG-binding affinity of IgM isolated from healthy donors (dark red line), RF^{high} (green line) and mIgM (blue line) measured by bio-layer interferometry. K_D (dissociation constant) was calculated by the software. The experiment shown is representative of 3 independent experiments. E) Hep-2 slides showing anti-nuclear structure-reactive IgM (ANA) for total IgM isolation ($IgM^{HD,}$ dark red square), RF^{high} (green square) and monoclonal IgM control (blue square). Scale bar 65 µm. Green fluorescence indicates IgM binding to Hep-2 cells. Images are representative of three independent experiments. F) Blood glucose concentrations of WT mice intravenously (i.v.) injected with 100 µg anti-insulin IgG combination with 20 µg total IgM purified from healthy donors (dark red line, n=4) or with monoclonal IgM control (blue line, n=4) measured at indicated time points. Mean ± SD, statistical significance was calculated using two-way ANOVA with Sidak's multiple comparison test. * p<0,01

Figure 44 Recombinant low-affinity RF is polyreactive and binds DNA

A) Schematic representation of the immunoglobulin heavy and light variable genes (IGHV and IGLV, respectively) of the recombinant purified low-affinity RF as compared to the closest germline respective alleles. Mutations are bold.

IGHM: immunoglobulin heavy constant mu; IGVK: immunoglobulin variable kappa

B) Coomassie-stained SDS-PAGE showing recombinant monoclonal (in-house purified) low affinity RF (RF^{low}), commercial RF from Rheumatoid Arthritis patients (RF^{high}) and monoclonal control IgM (mIgM) under reducing conditions (with β-mercaptoethanol). The image is representative of three independent experiments. C) IgG-binding affinity of RF^{low}, RF^{high} and monoclonal IgM (blue line) measured by bio-layer interferometry. K_D (dissociation constant) was calculated by the software. The experiment shown is representative of 3 independent experiments. D) Anti-IgG IgM concentrations detected in purified RF^{low} (n=3), RF^{high} (n=3) and mIgM control (n=3) measured by ELISA (coating: human IgG) Mean ± SD, statistical significance was calculated using ordinary one-way ANOVA with Tukey's multiple comparisons test. ** p<0,01 E) Anti-dsDNA-IgM concentrations of RF^{low} (n=3), RF^{high} (n=3) and IgM control (n=3) measured by ELISA (coating: calf-thymus dsDNA). Mean ± SD. Results are representative of three independent measurements. F) Hep-2 slides showing anti-nuclear structure-reactive IgM (ANA). Scale bar 65 µm. Green fluorescence indicates IgM binding to Hep-2 cells. Images are representative of three independent experiments. G) Schematic summary of the characteristics of RF^{high} and RF^{low}.

Figure 45 RF^{low} controls IgG *in vivo* function by enhanced degradation

A Blood glucose concentrations of WT mice intravenously (i.v.) injected with 100 µg anti-insulin IgG alone (n=4) or in combination with 20 µg RF^{low} (n=4) or mIgM control (n=4) measured at indicated time points. Mean ± SD, statistical significance was calculated using two-way ANOVA with Tukey's multiple comparison test. ** p<0,01

B Serum human IgG concentrations of WT mice at day 0 and day 1 after a single i.v. injection of 20 µg of α-CD20 human IgG (Rituximab) alone (n=4) or in combination with RF^{high} (n=4) or mIgM control (n=4) as measured by ELISA. Mean ± SD, statistical significance was calculated using two-way ANOVA with Tukey's multiple comparison test. **** p<0,0001

C Serum human IgG concentrations of WT mice at day 0 and day 1 after a single i.v. injection of 20 µg α-CD20 human IgG (Rituximab) in combination with RF^{high} (n=4), with RF^{low} (n=4) or IgM ctrl (n=5) as measured by ELISA. Mean ± SD, statistical significance was calculated using two-way ANOVA with Tukey's multiple comparison test. * p<0,05; **** p<0,0001

Figure 46 Deregulated ratios of high affinity and low affinity RFs in autoimmune diseases

A Total IgM amount detected in serum from young (n=20) and aged (n=17) healthy donors (HD), Rheumatoid Arthritis (RA) patients (n=15) and Multiple Sclerosis (MS) patients (n=28) measured by ELISA. Bars depict mean ± SD, individual values are represented by single dots. Statistical significance was calculated using Kruskal Wallis test. * p<0,05; ** p<0,01

Mean values of IgM (µg/ml) as follows: Young HD 1517,55; Aged HD 1258,02; MS patients 2143,72; RA patients 2361,29.

B Total IgG amount detected in serum from young (n=20) and aged (n=17) healthy donors (HD), Rheumatoid Arthritis (RA) patients (n=15) and Multiple Sclerosis (MS) patients (n=28) measured by ELISA. Bars depict mean ± SD, individual values are represented by single dots. Statistical significance was calculated using Kruskal Wallis test. ** p<0,01

Mean values of IgG (µg/ml) as follows: Young HD 7733,22; Aged HD 6856,48; MS patients 10419,28; RA patients 10345,23.

C Total RF-IgM detected in serum from young (n=20) and aged (n=17) healthy donors (HD), Rheumatoid Arthritis (RA) patients (n=15) and Multiple Sclerosis (MS) patients (n=28) measured by ELISA (coating: human IgG). Bars depict mean ± SD, individual values are represented by single dots. Values from RA patients plotted separately for simplified visualization. Statistical significance was calculated using Kruskal Wallis test. * p<0,05; ** p<0,01; **** p<0,0001

Mean values of RF-IgM (AU) as follows: Young HD 4,71; Aged HD 2,31; MS patients 1,72; RA patients 737,58.

Figure 47

Anti-Insulin-IgM concentrations detected in recombinant in-house purified anti-Insulin IgM^{high} (WT, n=3) and anti-Insulin IgM^{low} (gl, n=3) as measured by ELISA (coating: human Insulin). Mean ± SD depicted. Data are representative of three independent measurements.

Figure 48

A Kinetic plot showing mean ± SD of blood glucose levels after injection of 100 µg anti-Insulin IgG (n=5) or IgG isotype control (n=5). Statistical significance was calculated using two-way ANOVA with Sidak's multiple comparison test. ** p<0,01

B IgG-binding IgM concentrations in RF-IgM elution from healthy donors ($RF\text{-}IgM^{HD}$, n=3) and from RA patients ($RF\text{-}IgM^{RA}$, n=3) as measured by ELISA (coating: human IgG). Mean ± SD, statistical significance was calculated using unpaired t test. * p<0,05.

C IgG-binding affinity of RF-IgM isolated from healthy donors and from RA patients

measured by bio-layer interferometry. K_D (dissociation constant) was calculated by the software. The experiment shown is representative of 3 independent experiments.

D IgG-binding IgM concentrations in total IgM isolated from healthy donors (n=3) compared with IgG-binding IgM amount detected in RF^{high} (n=3) and in monoclonal IgM (n=3) as measured by ELISA (coating: human IgG). Mean ± SD, statistical significance was calculated using ordinary one-way ANOVA with Tukey's multiple comparisons test. *** p<0,001

Figure 49

A Serum human IgG concentrations of WT mice after a single i.v injection of 20 µg α-CD20 IgG alone (n=4), 20 µg RF^{high} alone (n=5) or 20 µg α-CD20 IgG+ RF^{high} (n=4). Mean ± SD, statistical significance was calculated using two-way ANOVA with Sidak's multiple comparison test. *** p<0,001, **** p<0,0001

B Serum human IgG concentrations of WT mice after a single i.v injection (day 0) of 20 µg α-CD20 IgG (Rituximab) alone (n=4) or in combination with 20 µg RF^{high} (n=4) or 20 µg mIgM ctrl (n=4) at indicated time points. Mean ± SD, statistical significance was calculated using two-way ANOVA with Tukey's multiple comparison test ** p<0,01; **** p< 0,0001.

EXAMPLES

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the description, figures and tables set out herein. Such examples of the methods, uses and other aspects of the present invention are representative only, and should not be taken to limit the scope of the present invention to only such representative examples.

The examples show:

Example 1: Immunization experiments and antibody response

<u>The presence of soluble hapten suppresses IgG production:</u> To test the concept of relative responsiveness of B cells in vivo, immunization experiments were performed using NP (4-hydroxy-3-nitrophenylacetyl) as hapten coupled to KLH (Keyhole Limpet Hemocyanin) as carrier (Fig. 2a and b). To this end, groups of wild-type mice were injected with either NP as soluble compound (sNP) or NP-KLH, referred to as multivalent complex antigen (cNP), at equal molar ratios for NP (Fig. 1a). Antibody responses were determined at day 7 (IgM) and day 14 (IgG) post immunization (Fig. 1b). Similar to control immunization (CI) lacking the studied antigen (CI), injection of only soluble hapten (sNP:cNP, 1:0) failed to

induce clear IgM or IgG antibody responses, while injection of cNP as multivalent antigen (sNP:cNP, 0:1) was able to induce both. Adding sNP to cNP at different molar ratios interfered with antibody responses. Interestingly, the IgG response was significantly impeded at already 100:1 ratio for sNP to cNP. Using higher ratios of sNP to cNP (>10.000:1) was also able to significantly repress the IgM antibody response to NP hapten (Fig 2c). Importantly, the IgG response to the carrier (KLH) was similar regardless of the amount of soluble hapten (Fig. 1c).

To further confirm these findings, ELISpot assays were performed to directly assess the ratio of antibody secreting cells. In agreement with the serum immunoglobulin data, the ELISpot results showed that combining the soluble hapten with hapten-coupled carrier at 100:1 ratio reduces the number of IgG secreting cells while IgM secreting cells are unaffected (Fig. 1d). These data are in agreement with the inventors' concept that soluble monovalent antigen inhibits immune response to complex forms of the same antigen. In contrast to IgM, the inhibitory effect on IgG immune responses is observed at lower concentrations of the soluble monovalent antigen.

An important part, it was suggested that the presence of IgD-type BCR is important for this regulation. Thus, tested the role of IgD was tested by conducting the NP immunization experiments in IgD knockout mice lacking IgD-type BCR. The IgD knockout mice showed no inhibitory effects when soluble NP was added to cNP immunization (Fig. 1e, f; Fig 2c).

Together, these data suggest that mature B cells are able to fine-tune their immune response according to the density of antigenic determinants thereby leading to distinct IgM and IgG responses to different epitopes of the same antigen.

Presence of soluble peptides enhances IgM antibody responses: After testing hapten-specific antibody responses, it was tested whether the concept is valid for autoantigens and might thus provide a different scenario for the selection of B cells and the control of self-destructive immune responses. To avoid the usage of transgenic mice that artificially harbor mono-specific B cells expressing a defined BCR that recognizes either a transgene product or endogenous structure, insulin-related autoantigens were selected as a physiologically relevant system for autoimmune diseases. During biosynthesis in the pancreas, proinsulin is cleaved into the well-known hormone insulin and the so-called C-peptide (CP) and both are secreted into the blood stream. While insulin is found in nanomolar amounts in the blood and plays pivotal role in the regulation of blood glucose levels and diabetes, C-peptide is barely detectable and is present at low picomolar quantities in the blood and seems to have no homeostatic function [30]. Using full length

C-peptide or insulin-derived peptides, the autoreactive antibody responses towards an abundant and functionally important (insulin) should be investigated as compared to a barely detectable autoantigen without physiological function (C-peptide) (Fig. 3a). Moreover, in contrast to insulin C-peptide is not conserved (Fig. 3b).

Either biotinylated C-peptides that were complexed by incubation were used with streptavidin (SAV). Alternatively, KLH was used as carrier coupled to the C-peptides to generate a multivalent complex antigen (cCP). The non-complexed form of the C-peptide (sCP) was used as soluble antigen. As with the NP hapten, wildtype mice were injected with sCP, cCP or combinations thereof to test their potential to induce autoreactive antibody responses (Fig. 3c). As expected, sCP induced no detectable IgM or IgG immune responses, while the multivalent form cCP induced both IgM and IgG as measured at d7 and 14, respectively (Fig. 3d). In addition to ELISA experiments, the serum from immunized mice was used to determine the specificity of the generated antibody responses. Western blot analysis using mouse serum revealed that mice immunized with cCP were positive for IgG antibodies recognizing pancreatic C-peptide (Fig 4a). This is in full agreement with the hapten immunization and shows that soluble peptide, which is alone unable to induce a detectable immune response, prevents the production of IgG memory B cells. In fact, later challenge with the same antigen at d21 resulted in weak IgG response in mice immunized with sCP:cCP ratio of 20:1 as compared to mice immunized only with cCP, sCP:cCP ratio of 0:1 (Fig. 3d, d14 and d28 IgG). To confirm the memory response against C-peptide as autoantigen, a recall immunization at d42 was performed using cCP without the adjuvant CpG and detected a robust IgG response against C-peptide in the mice immunized only with sCP:cCP ratio of 0:1 (Fig. 3e).

In contrast to IgG, a C-peptide-specific IgM antibody response was induced upon recall immunization of sCP:cCP at 20:1 ratio (Fig 3d, d28 IgM). FACS analysis of splenic B cells revealed no significant differences in the different groups of mice (Suppl. Fig. 3b, c). Moreover, no difference was detected in the IgG response against the carrier for the C-peptide (Fig 4d).

These data suggest that soluble monovalent antigen modulates the immune response and determines the IgG:IgM ratio of antibody secreting cells during immune responses. This conclusion was confirmed by performing an ELISpot analysis to determine the number of IgG or IgM secreting cells in the different mouse groups. In full agreement with the serum Ig results, the ELISpot experiments showed that mice immunized with ratio

20:1 of sCP:cCP possess increased numbers of IgM secreting cells whilst the numbers of IgG secreting cells are decreased as compared to mice immunized with cCP, sCP:cCP ratio of 0:1 (Fig 3f).

To test whether similar to NP immunization experiments, IgD is required for the regulation of B cell responsiveness by sCP:cCP ratios, the C-peptide immunization was performed in IgD knockout mice. The IgD knockout mice showed generally reduced IgG responses and no regulatory effect of the soluble peptide on the IgG antibody response observed in the mice immunized with sCP:cCP at 0:1 ratio (Fig. 3g).

Together, these data show that antibody responses can be directed against an autoantigen suggesting that the respective autoreactive B cells were neither clonally deleted by central tolerance nor functionally silenced by anergy. Most importantly, regardless of self or non-self-antigen, the results show that B cell responses are induced by multivalent antigen and modulated by soluble counterparts thereby regulating B cell responsiveness and the isotype of generated antibody. This results in a dynamic and pivotal B cell function that is completely different from the current view.

Example 2: Autoantibody responses against insulin

Multivalent native insulin induces harmful anti-insulin IgG responses: Since C-peptide can be hardly detected in the blood and has no known physiological relevance, it is not excluded that autoantibody responses might be feasible against autoantigens present at such extremely low concentrations. Therefore, the autoantibody responses against insulin were tested. First, the fundamental postulate was tested that autoreactive B cells are naturally present in the periphery and not deleted by central tolerance or turned unresponsive by anergy as proposed by the current view. According to this concept, the formation of autoantigen complexes triggers the secretion of autoreactive antibodies from naturally existing autoreactive peripheral B cells. To test this, autoantigen were generated complexes by incubating biotinylated native murine insulin with streptavidin (Ins^{Nat}). Importantly, the biotinylated murine insulin is biologically active as it regulates glucose metabolism similarly to its unbiotinylated endogenous counterpart when injected in soluble form (data not shown). Wild-type mice were injected with 10 µg of Ins^{Nat} complexes and monitored over time for the presence of anti-insulin antibodies in serum. In parallel, it was tested whether the immunized mice developed a diabetes-like dysregulation of glucose metabolism by monitoring glucose levels in blood and urine. Considerable amounts of anti-insulin IgM at day 7 were detected, while anti-insulin IgG was detected at d14 post injection of complexed insulin (Fig. 5a). Both isotypes were

detected after boost immunization (d21) at d28. Importantly, the mice showed clear signs of diabetes as measured by increased concentrations of blood glucse starting by d7 (data no shown), continuing through d14 and further increasing after boost (d21) at d26 (Fig. 5c). To show that the elevated blood glucose levels depended on autoantibody production, 10 µg Ins^{Nat} complexes into B cell-deficient mice (mb-1 knockout mice lacking the BCR component Igα also known as CD79A) were injected and monitored blood glucose (Fig. 5b, c). Interestingly, no increase in blood glucose was observed in the B cell-deficient mice suggesting that the presence of B cells and autoantibody secretion are crucial for the development of diabetes symptoms observed in wild-type mice (Fig. 5c). Moreover, the increase in blood glucose was accompanied by detectable glucose in the urine of wildtype mice injected with complex Ins^{Nat} (Fig. 5d). In agreement with diabetes development, water consumption of wildtype mice injected with complex Ins^{Nat} dramatically increased (Fig. 5e). Due to the unexpected severity of diabetes symptoms the mice were sacrificed at day 27 and analyzed the pancreas and spleen.

In contrast to control mice, complex Ins^{Nat} immunized mice showed highly increased recruitment of macrophages, neutrophils and B cells to the pancreas (Fig. 5f). Further, IgG+ macrophages of Ins^{Nat} complex immunized mice showed binding of native insulin (Fig. 5f). Thus, suggesting autoantibody-mediated acute inflammatory processes at the pancreas. While FACS analysis showed no difference of splenic B cells between control mice and those immunized with complex Ins^{Nat} (Fig. 6), however, ELISpot analysis revealed a significantly increased number of splenic B cells secreting anti-insulin IgG in mice injected with complex Ins^{Nat} (Fig. 5g).

To test whether the secreted IgG was responsible for the diabetes symptoms, IgG pulldown experiments using serum from mice injected with complex Ins^{Nat} and control immunization (Fig 5h, i) were performed. Since the IgG purification is expected to result in dissociation of endogenous insulin from serum insulin-specific IgG (see methods section), we determined the anti-insulin IgG within total IgG after purification. It was found that up to 40% (0.4 mg/mg) of the IgG isolated from Ins^{Nat} mice was reactive to insulin suggesting that direct serum IgG measurements fail to detect the entire insulin-specific IgG due to binding to endogenous insulin (compare Fig. 5a and 5h). To test the pathogenicity of isolated anti-insulin IgG, equal amounts of IgG from control immunization were intravenously injected or mice injected with complex insulin into wildtype animals and monitored blood glucose. It was found that injecting total IgG containing 2 µg anti-insulin IgG was sufficient to induce increased blood glucose in recipient mice suggesting that IgG from mice injected with complex insulin causes diabetes symptoms (Fig. 5j).

These data demonstrate that autoreactive B cells recognizing a pivotal metabolic hormone are neither deleted nor functionally silenced, but are present in the periphery and can induce severe autoimmunity when the balance of autoantigen is shifted towards multivalent forms.

Insulin-derived epitope induces harmful anti-insulin IgG response: To further confirm the above findings, immunization experiments using an insulin-A chain-derived peptide sequence were performed, referred to as InsA (Fig. 3 b) which is a frequently reported epitope in autoantibody responses against insulin [32]. A virus-derived peptide from HIV gp12033 was included as a nonrelated foreign peptide (virus-peptide). As for C-peptide, the selected peptide was coupled to the carrier KLH to generate a complex polyvalent antigen (cInsA) which was then used in immunization experiments either alone or in combination with the soluble peptide (sInsA). Subsequently, the antibody responses against the immunogen was measured, InsA peptide, or native insulin to confirm the induction of harmful autoantibody responses. It was found that InsA induced IgM and IgG autoantibody responses recognizing native insulin (Fig. 7a). One week after boost (d21) at day 28, the multivalent insulin-derived peptide alone (sInsA:cInsA ratio of 0:1) readily induced the production of anti-insulin IgG, while addition of soluble peptide (sInsA:cInsA ratio of 100:1) resulted in profound reduction of this autoreactive IgG at day 28 (Fig. 7a). Importantly, the amount of autoreactive anti-insulin IgG is most likely higher than detected in direct serum ELISA as anti-insulin IgG bound to endogenous insulin escapes detection as described above (Fig. 5a, i).

Notably, the presence of soluble InsA resulted in robust insulin-specific IgM production at d28, which was slightly reduced in the mice immunized with multivalent peptide alone (sInsA:cInsA ratio of 0:1) showing detectable anti-insulin IgM at d28 (Fig. 7a). This was not observed in mice immunized with the virus-peptide (Fig. 8a, b). In contrast to control peptides, insulin is present in relatively high amounts in the organism, suggesting that the presence of endogenous soluble insulin might modulate that immune response of the multivalent InsA thereby leading to increased autoreactive booster IgM responses. Taken together, the data indicate that the ratio of multivalent to monovalent antigen is mirrored by the ratio of antigen-specific IgG to IgM (γ/μ ratio) antibody responses at day 28 after booster immunization (Fig. 7b).

In contrast to the serum IgG of mice immunized in the presence of soluble peptide (sInsA:cInsA ratio of 100:1), serum IgG of mice immunized with multivalent peptide only (sInsA:cInsA ratio of 0:1) readily detected native insulin in western blot analysis (Fig. 7c).

Moreover, ELISpot analysis using splenic B cells from mice immunized with cInsA confirmed the increased presence of autoreactive IgG secreting cells in respective mice (Fig. 7d).

To confirm that the increased anti-insulin IgG is associated with harmful autoimmune responses, it was tested whether mice immunized with cInsA (sInsA:cInsA ratio of 0:1) show signs of diabetes. It was found that about one week after booster immunization (d21) at day 28, this group of mice showed increased blood glucose and water intake by d27 to d33 (Fig. 7e & Fig. 10). In addition, it was tested whether the glucose concentration was also increased in the urine of mice immunized with multivalent insulin peptide (sInsA:cInsA, 0:1). In full agreement, the increased autoreactive anti-insulin IgG led to increased urine glucose concentrations (Fig 7f). In contrast to autoreactive IgG, no detectable signs of autoimmune diabetes were observed in mice possessing increased amounts of autoreactive anti-insulin IgM in the booster immunization (Fig. 7e & f).

The presence of antigen-specific B cells at d28 after immunization was confirmed by FACS analysis (Fig. 9a & b). Compared with controls, mice immunized with complex peptide only (sInsA:cInsA ratio 0:1) show increased proportion of macrophages in the pancreas which bound autoreactive IgG as determined by the increased InsA peptide binding (Fig. 9c). Similar results were observed in the spleen (Fig. 10).

Together, the data suggest that increased ratio of complex multivalent auto-antigen leads to increased amount of autoreactive IgG and subsequent self-destructive autoimmune responses in wild-type animals.

Example 3: Protective anti-insulin-IgM expression after InsA-peptide immunization

Monovalent autoantigen induces immune tolerance by protective IgM: Apart from the self-destructive role of autoreactive IgG, the data mentioned previously point towards a protective role of autoreactive IgM in diabetes. In fact, the results suggest that high anti-insulin IgM in comparison to corresponding anti-insulin IgG protects from deregulation of glucose metabolism and diabetes in the mice immunized with InsA (Fig. 7a-f). In full agreement, mice showing low ratio of insulin-reactive IgG to IgM ($\gamma/\mu<0.1$) were protected from diabetes at d28 (Fig. 7g). A second InsA booster immunization at d42 resulted in anti-insulin IgM but no IgG when monovalent peptide was included (sInsA:cInsA ratio 100:1) and the corresponding mice showed no signs of diabetes between d42 and d49 (Fig. 11a & b).

To directly test whether increased ratio of autoreactive anti-insulin IgM counters the negative effects on glucose metabolism induced by autoreactive anti-insulin IgG, the mice immunized initially in the presence of monovalent InsA peptide (sInsA:cInsA ratio 100:1) was challenged with only multivalent antigen (sInsA:cInsA, 0:1) at d51. Interestingly, the treatment that induced autoimmune diabetes from d14 to 28 (Fig. 12, d7 vs. d14), generated only autoreactive anti-insulin IgM response but neither anti-insulin IgG nor deregulation of glucose metabolism at d51 to 59 (Fig. 13 a-c).

These data suggest that primary immunization with the presence of monovalent InsA peptide (sInsA:cInsA ratio 100:1) induced tolerance against the pathogenic immunization with multivalent InsA (sInsA:cInsA ratio 0:1). Moreover, the findings indicate that this unique tolerance mechanism creates a novel class of memory responses by eliciting and maintaining the production of protective autoreactive IgM (pIgM). To further test this, the decline of the anti-insulin IgM concentration over time was monitored followed by anti-insulin recall responses (Fig. 7h). The inventors show that anti-insulin IgM persists for weeks and that booster cInsA immunization at day 71 induces only IgM, but no IgG without any signs of deregulated glucose metabolism (Fig 7h, i & Fig. 14). Since the increase of antibody affinity towards antigen is usually associated with memory responses, ELISA experiments were performed to compare the affinity of the insulin-specific antibodies at different time points. It was found that IgM generated after booster InsA immunizations show higher anti-insulin affinity compared to the primary IgM collected at day 7 (Fig. 7j). Further, to examine the protective role of pIgM, mice were immunized with cInsA or cInsA together with intravenous injections of 50 µg purified IgM containing 5 µg of pIgM (Fig. 15a, b) every 48 hours starting from d0. Interestingly, the presence of insulin-specific pIgM mitigated autoimmune dysglycemia and completely prevented glycosuria as observed in the mice immunized with cInsA only (Fig. 7k). To exclude that pIgM i.v. injections neutralized the immunogen (cInsA, i.p.), anti-carrier-ELISA was performed. As expected, no difference in anti-KLH-IgM levels were observed at day 7 (Fig. 15c).

Since insulin and the InsA peptide in particular are highly conserved between mouse and man (Fig. 3b), the data not only present a novel and dynamic concept for B cell tolerance, but also introduces a fundamental animal model for understanding autoimmune diabetes triggered by anti-insulin antibodies in humans.

Example 4: Protective memory anti-Insulin-IgM is monospecific

The results presented above point towards an unexpected fundamental difference between autoreactive primary IgM and PR-IgM. In fact, primary anti-insulin-IgM induced diabetes symptoms although produced at much lower quantity as compared to memory PR-IgM which possesses a higher insulin affinity but did not induce pathology. To directly test the protective function of autoreactive memory PR-IgM against destructive autoimmunity, mice were immunized with cInsA alone or cInsA together with intravenous injections of 50 µg total IgM containing 5 µg of anti-insulin memory PR-IgM every 48 hours starting from d0 (Fig. 16a and b). Interestingly, the presence of insulin-specific PR-IgM mitigated autoimmune dysglycemia and completely prevented glycosuria on day 7 as compared to mice immunized with cInsA alone (Fig. 16b). To exclude that PR-IgM injections neutralized injected cInsA, we performed anti-carrier (KLH) ELISA and found no difference in anti-KLH-IgM levels between the two groups at day 7 (Figure 15 C). These data suggest that memory anti-insulin PR-IgM prevents the depletion of insulin by primary anti-insulin IgM thereby preventing the initiation of diabetes. One explanation for the differences between the autoreactive primary and memory PR-IgM might be that primary IgM is polyreactive and might be produced by B1 B cells as a first line of immune protection. Presumably, this polyreactivity results in joint immune complexes with a high molecular weight containing multiple autoantigens allowing elimination by phagocytes thereby depleting the bound insulin. In contrast, autoreactive memory PR-IgM might be mono-specific for autoantigen and may therefore release the autoantigen after binding without formation of immune complexes. To test this, the polyreactive potential of primary IgM as compared to memory PR-IgM was analyzed. Anti-DNA ELISA (Fig. 16c) and indirect immune fluorescence using HEp-2 slides (Fig. 16d) showed that in contrast to primary IgM, memory PR-IgM is not polyreactive but specifically binds to insulin (Fig. 16c and d).

To show that anti-insulin IgM is specifically responsible for the observed effects, the inventors performed insulin-specific pulldown assays using sera from InsA-immunized mice. The pulldown resulted in pure insulin-specific IgM as revealed by western blot analysis against insulin (Fig. 17). We performed anti-DNA ELISA (Fig. 16e) and indirect immune fluorescence on HEp-2 slides (Fig. 16f) using purified primary anti-insulin IgM or memory anti-insulin PR-IgM. The results confirm the finding that in contrast to primary IgM, purified anti-insulin PR-IgM is not polyreactive and specifically binds to insulin (Fig. 16e and f). To directly test the hypothesis that primary anti-insulin IgM forms large immune complexes whereas PR-IgM does not, we incubated anti-insulin primary IgM or PR-IgM with insulin and DNA and determined the formation of immune complexes using size

exclusion spin columns. In contrast to PR-IgM, we found that primary anti-insulin IgM forms mainly large complexes of >104 kD (Fig. 16g). To show that the purified primary anti-insulin IgM is responsible for the dysregulation of glucose metabolism, we intravenously injected 5 µg of purified anti-insulin primary IgM or PR-IgM and monitored blood glucose. In contrast to PR-IgM, we observed a vigorous increase in blood glucose after injection of purified primary anti-insulin IgM (Fig. 16h). Interestingly, the increase in blood glucose emerged faster after injection of purified anti-insulin primary IgM as compared to total primary IgM (Fig. 16h).

In summary, these data suggest that increased specificity to autoantigen is important for autoreactive memory PR-IgM to be protective during immune responses (Figure 18). Moreover, the induced generation of autoreactive PR-IgM is most likely a critical step in B cell tolerance.

Example 5: Immunization Scheme

The impact of the immunization concept of the invention with regard to vaccine design was tested using pathogen-specific antigens derived from SARS-CoV-2 coronavirus causing Covid-19. During infection, SARS-CoV-2 coronavirus binds via the receptor-binding domain (RBD) to angiotensin-converting enzyme 2 (ACE2) on the host cell surface. Thus, triggering antibody responses blocking the RBD/ACE2 interaction is considered to be key for preventing coronavirus infection. Thus, the inventors used RBD from SARS-CoV-2 to the role of antigen form in immune responses during immunization.

It was found that immunization with complex RBD (cRBD) (For complexation with streptavidin and biotinylated RBD were used at a ratio of 4:1. For complexation with 1,2-phenylen-bis-maleimide with a minimum of 20 µg 1,2-PBM per 100 µg RBD) induces a stronger IgG immune response as compared with soluble RBD (sRBD). For production of RBD, an expression vector encoding hexahistidine-tagged version of RBD was transiently transfected into HEK293-6E cells (Amanat, F., et al., 2020, Nature medicine, 26(7), 1033–1036). Soluble RBD was purified from the supernatant 5 days after transfection by nickel-based immobilized metal affinity chromatography (TaKaRa)). However, the antibody concentration was not sufficient to allow virus neutralization using in-vitro infection experiments. Hence, it was tested whether pretreating the mice with sRBD prior to immunization boosts immune responses. In fact, pre-treatment of the mice with soluble RBD two weeks prior to immunizations resulted in greatly augmented immune response (Figure 19). Importantly, the serum of the pretreated mice showed an enormously high capacity to prevent SARS-CoV-2 infection *in vitro*.

Moreover, it was found that different ratios of sRBD to cRBD in the composition of the immunization cocktail result in different ratios of immunoglobulin isotypes (i.e. IgG to IgM) which allow refined control of immune responses after immunization.

Example 6: Accelerated primary immune response in IgD-deficient mice

To further investigate the dynamics of immune responses in IgD-deficient mice, we monitored early antibody production. Compared to WT mice, IgD-deficient mice showed a NP-reactive IgM response already at day 1 after immunization (Fig. 23A). This response was further amplified at day 3 and peaked at day 7 (Fig. 23B). In contrast, no antibody response was observed at day 1 in WT mice. By day 3, WT mice showed a slight NP-reactive IgM response which peaked at day 7, however, remained slightly weaker than the day 7 NP-reactive IgM response in IgD-deficient mice (Fig. 1 A, B). To further characterize the specificity of the induced antibody response we performed classical autoreactivity assays including indirect immunofluorescence (IF) on HEp-2 slides and ELISA for anti-double stranded DNA (dsDNA). These experiments show that primary IgM antibodies, detected at day 1 throughout day 7 of IgD-deficient mice or by day 7 of WT mice, are autoreactive determined by recognition of nuclear structures (Fig. 23C, D). Importantly, the control immunizations using only the adjuvant CpG showed no induction of anti-dsDNA antibodies as compared with unimmunized mice (Fig. 23C, data not shown). Further, to exclude a role of CpG in the production of autoreactive IgM, we performed HEp-2 slides with sera of control immunizations. Neither IgD-deficient mice nor WT mice show elevated levels of autoreactive IgM in control immunizations with PBS or CpG alone.

In summary, these results show that primary IgM responses are autoreactive and that IgD-deficiency allows rapid primary immune responses.

Example 7: Sustained primary immune response to autoantigen in IgD-deficient mice

After testing hapten-specific antibody responses, we investigated whether the production of autoreactive primary antibodies in IgD-deficient mice differ when using autoantigens. To avoid the usage of transgenic mice that artificially harbor mono- specific B cells expressing a defined BCR that recognizes either a transgene product or endogenous structure, we selected insulin-related autoantigens as a physiologically relevant system for autoimmune diseases (Amendt and Jumaa, under revision).

To this end, we performed immunization experiments using an Insulin-A chain-derived peptide, referred to as InsA which is the most abundant epitope in autoantibody

responses against insulin. The selected peptide was covalently coupled to the carrier KLH to generate a complex polyvalent antigen (InsA-KLH) which was then used in immunization experiments (Fig. 24A). Subsequently, we monitored the antibody responses against the InsA peptide and native insulin to confirm the induction of harmful autoantibody responses. We found that InsA-KLH induced IgM antibody responses recognizing native insulin already at day 1 after immunization (Fig. 24B). WT mice showed no insulin-reactive IgM at day 1. By day 7, WT mice showed anti-insulin IgM which, however, was reduced as compared to IgD-deficient mice (Fig. 24B). By day 28 comparable amounts of insulin-reactive IgM was detected in both IgD-deficient and WT mice. However, only WT mice showed a considerable increase of insulin- specific IgG (Fig. 24B, right panel). This IgG is responsible for the increased blood glucose detected in these mice.

To evaluate the polyreactive potential of the elicited anti-insulin IgM, we performed HEp-2 slides. The data show that anti-insulin IgM remained polyreactive throughout day 28 (boost on day 21), whereas the anti-insulin IgM of WT mice was no longer polyreactive (Fig. 24C). Importantly, immunization with mixture of InsA-KLH and soluble InsA peptide at 1:100, respectively, resulted in highly elevated blood glucose at day 1 and were therefore discontinued.

Together, our data suggest that IgD-deficient B cells elicit a rapid and strong primary immune response that persists for longer periods compared to WT mice. Thus, the shift into secondary, mature immune responses is delayed in IgD-deficient mice. Interestingly, IgD, which is a marker for mature B cells, is required for the maturation of the immune response from primary to secondary phases.

Example 8: Delayed affinity maturation is associated with sustained autoimmunity in IgD-deficient mice

We performed ELISA to determine the binding efficiency of the insulin-specific antibodies to high-valence or low-valence antigen. To this end, we used monovalent and polyvalent streptavidin and biotinylated InsA peptides to generate monovalent InsA(1) and polyvalent InsA(4) streptavidin complexes. Our experiments revealed no significant increase in the affinity of insulin-reactive IgM antibodies between primary (d7) and secondary (d28) immunization for IgD-deficient mice. However, insulin-reactive IgM of WT mice showed a clear increase in InsA(1)/InsA(4) ratio which is characteristic for affinity maturation (Fig. 25A). We confirmed these results by examining the direct binding affinity of insulin-specific IgM of different days from WT and IgD-deficient mice. Interferometric

assays revealed that WT mice already reach high affinity insulin-IgM at day 52, whereas IgD-deficient mice reach that level of affinity later on day 72 (Fig. 25G). Interestingly, IgD-deficient mice and WT mice showed clear signs of diabetes as detected by glucose amount in the urine and increased blood glucose. However, the symptoms of diabetes were more severe and evident already at day 1 in IgD-deficient mice as compared to WT mice (Fig. 25B, C). Importantly, the increase in blood glucose in WT mice gradually declined after repeated boost immunizations and shortly after the second boost at day 42 the WT mice became resistant for InsA-induced diabetes (Fig. 25C). IgD-deficient mice in contrast showed sustained diabetes symptoms throughout d60 after immunization (Fig. 25C). In fact, IgD-deficient required a third boost by day 70 to develop resistance to InsA-induced diabetes (Fig. 30).

To show that high-affinity IgM generated during secondary booster immunizations is responsible for the control of diabetes induced by InsA immunization, we isolated insulin-specific IgM from WT mice after secondary immunization and injected it into IgD-deficient mice shortly after immunization with InsA-KLH. The results show that the isolated high-affinity IgM protect the IgD-deficient mice from developing diabetes at day 1 and therefore we refer to this IgM as protective IgM (PR-IgM) (Fig. 25E). We performed indirect IF to confirm that affinity maturation resulted in PR-IgM which is highly specific for insulin without binding other autoantigens (Fig. 25F).

These data show that defective in affinity maturation in IgD-deficient mice (Fig. 31) leads to insufficient production of protective highly specific IgM thereby resulting in sustained autoimmune disorder.

Example 9: Rapid activation of IgD-deficient B cells after immunization

To examine the early changes on B cells after immunization, we performed FACS experiments to analyze lymphoid organs analysis after immunization with InsA-KLH. This analysis revealed that immunization induced a substantial expansion of splenic B cells in IgD-deficient mice as compared to WT mice at day 1 (Fig. 26A). Notably, the expansion of splenic B cells in IgD-deficient mice was associated with increased cell size, as shown by forward-scatter (FSC), in the IgD-deficient mice (Fig. 26A). In full agreement, a considerable fraction (>21%) of B cells expressed the activation marker CD69 after immunization of IgD-deficient mice with InsA-KLH. Similar to control immunization of IgD-deficient mice with CpG alone, WT mice immunized with InsA- KLH or CpG alone showed a limited fraction (about 2-5%) of CD69 expressing cells (Fig. 5B).

Further characterization revealed that a population of CD23-/CD21- cells was increased in the IgD-deficient mice immunized with InsA-KLH compared to immunized WT counterpart or IgD-deficient mice from control immunization (Fig. 26C). The CD23-/CD21- correspond to the activated B cells (Fig. 32) which predominantly express IgM BCR and intermediate amounts of IgD in the WT (Fig. 26D). Especially, CD23 expression on B cells is greatly down-regulated in InsA-KLH immunized IgD- deficient mice on day 1 as compared to controls (Fig. 26E). This is consistent with available data showing that CD23 is downregulated upon B cell activation .

Thus, our results suggest that IgD-deficient B cells are rapidly activated after immunization and that the responsive cells are CD23-/CD21- with elevated levels of IgM BCR.

Example 10: Antibody secretion by CD23-/CD21- cells

Our recent data showed that CD23-/CD21- cells are antibody secreting cells. Therefore, we investigated antibody secretion by splenocytes at day 1 after immunization. ELISpot analysis showed that the proportion of antibody secreting cells is slightly increased in IgD-deficient mice when total splenic cells were used (Fig. 27A). However, when ELISpot analysis was performed after FACS sorting for CD23-/CD21- cells or CD23+ follicular B cells, we found that antibody secretion was predominantly associated with CD23-/CD21- cells (Fig. 27B). The increased proportion of the CD23- /CD21- in InsA-KLH immunized IgD-deficient mice was associated with increased proportion of antibody secreting cells (Fig. 27B). Interestingly, only a small fraction of follicular CD23+ B cells from WT mice developed into antibody secreting cells and no effect of the immunization was observed at day 1 while IgD-deficient follicular CD23+ B cells showed an increase in antibody secreting cells (Fig. 27C, D).

Interestingly, IgD-deficient B cells in peritoneal cavity further showed an increase of CD138+ cells at day 1 after immunization, whereas WT counterparts remained unchanged (Fig. 33).

In summary, these data suggest that immunization results in increased CD23-/CD21- antibody secreting cells that develop within one day in IgD-deficient mice.

Example 11: Primary immune responses have restricted poly-reactivity

The results presented above indicate that primary immune responses are autoreactive regardless of the utilized antigen. In fact, primary anti-NP as well as primary anti-insulin IgM showed nuclear staining in HEp-2 slides indicative of polyreactive behavior. In full

agreement, the primary anti-NP IgM also showed anti-dsDNA binding in ELISA experiments (Fig. 23D). Subsequently, we tested the hypothesis whether primary IgM immune responses might always induce the same class of autoreactive B cells that might be omnipotent with regard to autoantigen binding. To this end, we tested whether the anti-NP primary immune response induces diabetes symptoms due to binding to insulin. However, despite the increased polyreactivity, neither IgD- deficient nor WT mice showed any changes in blood glucose in the course of NP immunization (Fig. 28A, B). Further, InsA-KLH immunized WT and IgD-/- mice showed an expected increase in blood glucose (Fig. 28C). The primary IgM of these mice also showed significant polyreactivity determined by anti-dsDNA ELISA (Fig. 28D). Nevertheless, no increased anti-insulin binding was observed in the sera of IgD-/- deficient or WT mice after NP immunization (Fig. 28E, top). Vice versa, InsA-KLH immunized mice did not show increased NP binding (Fig. 28E, bottom).

Together, these data suggest that, despite their increased autoreactivity to nuclear structures and dsDNA, primary IgM immune responses in IgD-deficient as well as in WT mice have no infinite poly-reactivity. In conclusion, primary IgM responses appear to be broadly poly- and self-reactive, but still remain somehow specific for their cognate antigen.

Example 12 Antibodies elicited by chemically crosslinked RBD possess high neutralization capacity

The chemical crosslinking of RBD might provide a practical method for the production of SARS-CoV 2 vaccines, as recombinant RBD can easily be produced and used for primary and secondary immunization in typical vaccination. Hence, we tested whether the resulting antibodies can prevent virus infection (Method is described in Hoffmann, M., et al., 2021, Cell, 184(9), 2384-2393). The results show that mice immunized with the chemically crosslinked RBD possess a high capacity in neutralization assays using pseudo-virus preparations (Fig. 35).

These data suggest that chemical crosslinking of RBD allows the simple design of efficient vaccines against SARS-CoV 2.

Example 13 Activated antigen forms IgG complexes that boost immune responses

We analyzed the sequence of RBD and identified a single SH group which is not engaged in intramolecular disulfide bonds. We proposed that bismale treatment of RBD or other proteins may result in saturated binding of bismale so that no additional proteins can be

crosslinked by a bismale molecule (Fig. 36B, middle). It is possible, however, that bismale treatment results in a monomeric RBD bound by bismale, in which a free maleimide group is still available (Fig. 36B, bottom).

RBD* was complexed with 20µg bismale per 100µg of RBD, while RBD** indicates complexation with 100µg per 100 µg of RBD (Fig. 36C).

Immunization was performed in WT C57BL6/J mice using 50 µg of non-complexed native RBD (nRBD, n = 3), 50 µg of RBD complexed with 10µg bismale (RBD*, n = 3) or 50µg of RBD complexed with 10µg bismale in the presence of 25µg polyclonal murine IgG (RBD*IgG). 50 µg CpG-ODN #1826 was used as adjuvant in all conditions. IgM or IgA isotype was used instead of IgG for immunization with RBD*IgM and RBD*IgA. Mice were boosted with the identical immunization mixture 21 days after primary immunization. Serum was collected on day 28 for analysis. (Fig. 36D).

WT mice were immunized either with 50 µg of non-complexed native RBD + CpG-ODN (nRBD, n = 3), 50 µg of RBD complexed with 10µg bismale + CpG-ODN (RBD*, n = 3) or 50 µg of RBD complexed with 10µg bismale in the presence of 25µg murine IgG but in absence of CpG-ODN (RBD*IgG, n = 2).

This results in activated RBD that can undergo bioconjugation with other proteins in vitro or in vivo. Importantly, increasing amount of bismale results in a decrease of the monomeric RBD suggesting that more bismale leads to more protein complexes (Fig 36C). To test the potential of forming heterocomplexes and at the same time to investigate the role of immunoglobulins in randomly formed complexes, we included IgM, IgA and IgG in the crosslinking reaction.

Interestingly, the results showed that, while IgM and IgA failed to boost the immune response, the crosslinking of RBD and IgG led to a dramatic increase of the RBD-specific immune response (Fig 36D). Importantly, adding IgG after terminating the bismale mediated crosslinking did not boost the immune response suggesting that bismale mediated crosslinking is important for the IgG-mediated enhancement.

The enhancement observed by IgG prompted us to test whether IgG may act as adjuvant replacing conventional adjuvants such as alum or CpG. To this end, we compared the immune response generated by complex RBD injected in the presence of CpG or IgG as adjuvant. The results show that IgG containing immune complexes are capable of inducing robust antibody responses in the absence of conventional adjuvants such CpG or alum that activate TLRs. In conclusion, the data suggest that the generation of IgG containing immune complexes by crosslinking IgG and a particular antigen in vitro, or in vivo by injecting the antigen after incubation with bifunctional crosslinkers containing two

reactive groups in vitro. Such activated antigens represent a simple and efficient way for the development and production of effective vaccines.

Example 14

Treating the bismaleimide crosslinked immune complexes with cysteine in vitro results in quenching of still available reactive maleimide groups and reversion of antigen activation thereby reducing antibody production (Figure 34). For quenching, 1 µl of freshly prepared 2 M L-Cysteine solution (Sigma - L-Cysteine BioUltra, ⩾98.5% 30089-25G) were added to 100µg (in 150µl volume) activated RBD and incubated over night at RT. To remove unbound cysteine and maleimide, the sample was cleared by dialysis against 1x PBS at 4°C under constant agitation (Thermo Fisher Scientific Slide-A-Lyzer™10K MWCO 66381).

The data show that increased maleimide (RBD**) results in increased antibody responses and that quenching the maleimide-treated antigen with cysteine (RBD**C) reduces the antibody responses dramatically. This suggests that maleimide treatment led to the generation of activated antigen, which is capable of generating complexes in vivo and this capacity is important for the immune response.

Thus activating the antigen, by making it reactive with SH groups on autoantigens, amplifies the immune response. Including total IgG in the antigen activation leads to the generation of protein complexes that mimic immune complexes thereby inducing efficient antibody responses.

Example 15

Antigen (Ag) complexes were generated by biotinylation and subsequent incubation with streptavidin (SAV). The complex antigen induces antibody responses. Multivalency depends on the number of biotins per molecule. Multiple biotin groups allow multiple SAV binding and higher molecular complexes. Crosslinking with SAV leads to higher molecular complexes and efficient immune responses (Fig. 34).

Example 16: Anti-insulin IgG regulates blood glucose concentration

We noticed that a considerable amount of total IgG isolated from wildtype (WT) mice was reactive to insulin (Fig. 37A & 37B). To confirm these data, we performed ELISpot assays and found that anti-insulin IgG secreting B cells are present in the spleen of WT mice (Fig. 37C). When we measured the blood glucose concentrations in WT and B cell-

deficient mice, which cannot produce antibodies, we detected a surprising difference. Unexpectedly, the B cell-deficient mice showed abnormally reduced blood glucose levels as compared to WT controls (Fig. 37D).

To test whether this abnormal decrease is caused by antibody deficiency, we injected total IgG from WT mice, or an anti-insulin IgG depleted control of the same total IgG, intravenously into B cell-deficient mice. We found that blood glucose concentration increased with the total murine IgG, but not with the anti-insulin IgG depleted control (Fig. 37E). In order to test the consequence of reduced steady-state blood glucose on the fitness, we performed wire hanging tests to assess motor function and found that B cell deficient mice have significantly reduced wire hanging times as compared to WT controls. Importantly, this deficit in wire hang times was restored after intravenous injection of total murine IgG (Fig. 37F). In addition, B cell-deficient mice also showed dysregulated blood glucose levels after rotarod exercise.

Since total IgG preparations from healthy donors are often used as intravenous immunoglobulin (IVIg) injection in the treatment of immunodeficiency we tested the presence of anti-insulin IgG in these preparations. All preparations contained substantial amounts of anti-insulin IgG. However, the anti-insulin IgG concentration seemed to be increased if the USA was the country of origin. Since insulin is highly conserved between man and mouse, we injected human IVIg into the B cell deficient mice and detected a decrease in insulin concentration (Fig. 37G). Moreover, injecting 50 µg of human IVIg into WT mice led to increased blood glucose and this effect required anti-insulin IgG because depletion of the anti-insulin IgG from human IVIg prevented the IVIg-induced increase in blood glucose (Fig. 37H).

To test whether the IVIg injection shows similar results in human patients suffering from antibody deficiency, we monitored blood glucose before and after IVIg injection. Similar to B cell deficient mice, antibody deficient patients showed reduced blood glucose concentrations as compared to healthy donors. Importantly, the concentration of blood glucose increased and reached normal levels after IVIg injection (Fig. 37I). Further, immunodeficiency patients that received IVIg showed decreased serum insulin levels.

To show that the anti-insulin IgG present in IVIg is specific for insulin, we determined the affinity via bio-layer interferometry (BLI). A dissociation constant of 10-11 suggests that the anti-IgG is highly specific for insulin (Fig. 37J).

These data suggest that anti-insulin IgG is present in healthy individuals and might be required for the regulation of blood glucose concentration.

Example 17: Regulation of blood glucose by anti-insulin IgM

To further confirm our finding about the presence of anti-insulin antibodies in healthy individuals, we assessed the anti-insulin IgG and IgM in the blood of different age groups. We found that anti-insulin IgG was similar in young and aged humans, while anti-insulin IgM seemed to decline with age in males and females (Fig. 38A). Interestingly, the human anti-insulin IgM recognizes multiple epitopes on insulin.

In agreement with the high specificity, the anti-insulin IgG showed no binding to any cellular structure in indirect immunofluorescence assay (IIFA) on HEp-2 cells, which is a commonly used method for detection of anti-nuclear antibodies. The anti-insulin IgM however, consisted of two fractions that can be biochemically separated according to their affinity to insulin. Low-affinity anti-insulin IgM is eluted from the insulin column at higher pH (5) as compared to high-affinity anti-insulin IgM which requires acidic conditions (pH= 2.8) for elution (Fig. 38B, 38C). The low affinity IgM shows polyreactivity as detected by binding to nuclear structures in IIFA and dsDNA binding in ELISA, whereas the high affinity IgM is virtually negative in these assays (Fig. 38D, 38E). Furthermore, we confirmed the difference in affinity by performing BLI assays and found that high affinity and low affinity IgM to possess a dissociation constant of 10-10 and 10-7, respectively (Fig. 38F). To test the effect of the different IgM fractions on glucose metabolism, we injected identical amounts of insulin-reactive IgMhigh and IgMlow into WT mice. Increased blood glucose was observed within two hours after injection in the mice that received IgMlow, whereas IgMhigh did not significantly alter blood glucose levels (Fig. 38G). Moreover, we tested whether IgMhigh plays a regulatory role under conditions of abnormally increased insulin concentrations that may cause hypoglycemia. To this end, we injected 0.1 µg insulin in combination with IgMhigh or unspecific IgM isotype control. Strikingly, the presence of anti-insulin IgMhigh, but not the IgM isotype control, prevented the drastic decrease in blood glucose that occurred immediately after insulin injection (Fig. 38H). To further test the regulatory role of IgMhigh in protecting insulin from IgG-mediated degradation, we combined the anti-insulin IgMhigh with anti-insulin IgG purified from IVIg preparations. The data show that the anti-insulin IgMhigh acts as PR-IgM as prevents the IgG-mediated neutralization of insulin which results in increased blood glucose levels (Fig. 38I). These data suggest that anti-insulin IgMhigh is important for regulating glucose metabolism by protecting insulin from IgG-mediated neutralization and by binding excessive insulin thereby preventing drastic declines in insulin concentrations. The decrease in insulin-reactive IgM with age (Fig. 37A) prompted us to test whether the anti-insulin IgMhigh or IgMlow is affected by this decrease. We determined the amount of

anti-insulin IgMhigh or IgMlow in young and old healthy donors and found that the ratio of anti-insulin IgMhigh increases with age (Fig. 38J).

Together, these data suggest that glucose metabolism is regulated by different classes of antibodies and that anti-insulin IgMhigh acts as PR-IgM that regulates glucose metabolism by regulating insulin homeostasis which seems to be particularly important with age.

Example 18: Induction of anti-insulin antibodies by insulin complexes

To investigate whether complexed autoantigen is capable of inducing autoreactive antibody responses independent of any adjuvants, we incubated insulin with a typical homobifunctional crosslinker, 1,2-Phenylene-bis-maleimide, which covalently binds to free sulfhydryl groups in proteins thereby crosslinking the protein of interest (Fig. 39A). Importantly, sulfhydryl group-containing drugs were reported to induce anti-insulin autoantibodies. Moreover, increased pancreas activity and elevated insulin production result in abnormal formation of disulfide bonds between the insulin peptides which may generate abnormal insulin forms that are more susceptible for sulfhydryl group-mediated crosslinking, and thus complex formation, under conditions of oxidative stress. The homobifunctional crosslinking of insulin with 1,2-Phenylene-bis-maleimide was tested in SDS page and the crosslinked insulin was purified using size exclusion spin columns excluding monomeric and dimeric insulin (Fig. 39B). The insulin complexes were dialyzed and injected into WT mice, 5 µg per mouse, without any additional adjuvants. As control, we performed a typical immunization using CpG as adjuvants and streptavidin as a foreign carrier. We found that the insulin complexes lead to increased blood glucose and anti-insulin IgM at d7 of treatment similar to the immunization (Fig. 39C, 39D). In addition, insulin-reactive IgG was detectable by ELISA on d14 and d26. Repeated injection of insulin complexes at d37 resulted in further deregulation of glucose metabolism (Fig 39E). Thus, we injected anti-insulin IgMhigh at d38, one day after injection of the insulin complexes. We found that anti-insulin IgMhigh was able to prevent the blood glucose deregulation induced by the injection of insulin complexes (Fig. 39E).

Further, we found that anti-insulin IgMhigh prevents pancreas inflammation and damage as shown by the decrease of macrophage (CD11b+/LY6G+) and neutrophil (LY6G+) infiltration in the pancreas and the decrease of serum pancreatic lipase in blood (Fig. 39F, 39G).

As a mechanism for the protective role of anti-insulin IgMhigh as compared to anti-insulin IgMlow we proposed that the polyreactivity of the latter, which also binds dsDNA, induces

the formation of immune complexes that can be phagocytosed by macrophages, while anti-insulin IgMhigh is highly specific for insulin and thus do not form large immune complexes that are easily phagocytosed by macrophages. To test this, we incubated anti-insulin IgMhigh or anti-insulin IgMlow with insulin in the presence of genomic dsDNA, (Fig. 39H). We found an increased binding/phagocytosis of anti-insulin IgMlow as compared with anti-insulin IgMhigh (Fig. 39). In addition, IgMhigh was able to protect insulin from degradation, as the decline of insulin was greater in the supernatants containing anti-insulin IgMlow as compared with anti-insulin IgMhigh antibodies.

These data show that anti-insulin antibodies can be generated under conditions activating the formation of insulin complexes, which results in deregulated glucose metabolism that can be counteracted by anti-insulin IgMhigh that acts as PR-IgM.

Example 19 Recombinant anti-insulin IgM is able to regulate blood glucose

The above results suggest that insulin-specific PR-IgM might be of great therapeutic interest, as it regulates insulin homeostasis and might prevent pancreas malfunction, both of which essential for normal physiology and prevention of diabetes. According to our data, an anti-insulin IgM can act as PR-IgM if it possesses high affinity to insulin and is not reactive to autoantigens such as dsDNA or nuclear structure in IIFA. We hypothesized that a human insulin-specific IgG antibody can be converted into insulin-specific PR-IgM by exchanging the constant region.

Hence, we cloned and expressed a published human insulin-specific antibody (Ikematsu, H., et al., 1994, J. Immunol. 152, 1430–1441) as IgG1 (anti-insulin IgGrec) and IgM (anti-insulin IgMrec) (Fig. 40A). To test the quality of our in vitro produced antibodies, we assessed their glycosylation by PNGaseF treatment, which resulted in reduced molecular weight as compared to untreated controls suggesting a functional glycosylation. We determined the affinity of both IgG and IgM to be 10^{-9} (Fig. 40B). Almost no dsDNA binding was observed in ELISA and no nuclear staining was observed in IIFA as compared to total human serum IgM (Fig 40C, 40D). Moreover, we tested if the monomeric anti-Insulin-IgM is capable of protecting insulin from degradation. Anti-Insulin IgG led to blood glucose increase which was abolished when monomeric anti-Insulin IgM was present (Fig. 40E).

To test whether the resulting recombinant human anti-insulin IgMrec possesses protective regulatory functions, we co-injected it with insulin and found that anti-insulin IgMrec prevents a drastic drop in glucose concentration induced by excess of insulin (Fig. 40F). Moreover, anti-insulin IgMrec protects insulin from anti-insulin IgGrec mediated neutralization, as it prevents the increase in blood glucose induced by anti-insulin IgGrec

(Fig. 40G). In addition, anti-insulin IgMrec counteracts the leak of glucose into urine (Fig. 40H).

These data suggest that expressing a high affinity insulin-specific antibody as IgM regulates insulin homeostasis, prevents a deregulation of blood glucose concentration and grants novel strategies for treatment of insulin-associated disease and disorders.

Example 20: High affinity RF enhances the effect of autoreactive IgG

Injection of anti-insulin IgG isolated from intravenous immunoglobulin (IVIg) preparations in WT mice resulted in a significant increase in blood glucose, while the addition of high affinity anti-insulin IgM protected insulin from IgG-mediated degradation (Example 19). The inventors provide further evidence for the hypothesis that high affinity IgM protects its target antigen, by testing whether the protecting effect of high affinity IgM observed with the insulin-specific antibody also applies to other autoantibody:autoantigen combinations. To this end, the inventors tested whether commercially available RF preparations isolated from RA patients act as protective IgM. As rheumatoid factor autoantibodies from RA patients typically bind with high affinity the Fc portion of IgG, this RF is herein referred to as RF^{high}. A high affinity PR-IgM autoantibody protects its target in vivo and therefore, we expected that RF^{high} protect and hence intensify the effect of pathogenic IgG. To test this hypothesis, we co-injected anti-insulin IgG (from IVIg) together with RF^{high} or with a non-specific monoclonal control IgM (mIgM) into WT mice (Figure 43A). We observed increased blood glucose levels in animals that received the insulin-specific IgG which was comparable with the increase shown by mice that received the α-insulin IgG along with mIgM. While the control mIgM showed no effect on the IgG-mediated change in blood glucose, animals co-injected with insulin-specific IgG and RF^{high} showed significantly higher blood glucose levels, suggesting that the RF^{high} binds to IgG and enhances its effect in vivo.

Example 21: High affinity RF enhances the effect of therapeutic antibodies

Next, the protective effect of RF was demonstrated with other IgGs such as therapeutic antibodies. Rituximab was used as a well-known therapeutic IgG antibody targeting CD20. This monoclonal anti-CD20 antibody, which consists of human constant regions and murine variable domains, is approved for treatment of B cell malignancies as well as autoimmune diseases such as RA and Systemic Lupus erythematosus (SLE). We

intravenously injected into WT mice equal molar amounts of anti-CD20 IgG either alone or combined with RF^{high} or with mIgM and we monitored human IgG (hIgG) concentrations over time. Our data showed that mice injected with Rituximab together with RF^{high} exhibited significantly higher levels of hIgG as compared to mice that received anti-CD20 IgG alone or in combination with mIgM (Figure 45B). Importantly, barely detectable traces of IgG in the commercial RF^{high} preparation are unlikely to affect the experimental set-up as shown by the significantly lower IgG levels detected when RF^{high} was injected alone (Figure 49A). Interestingly, the presence of RF^{high} stabilized hIgG in sera up to 7 days post injection, while the addition of mIgM to α-CD20 IgG did not show any significant change in hIgG concentration as compared to the hIgG levels of mice that received anti-CD20 IgG only (Figure 49B). Together with the data above, these results led us to the hypothesis that if the higher hIgG titer was due to the presence of RF^{high}, the co-injection of RF^{low} with anti-CD20 IgG should show opposite effect, i.e. hIgG level reduction over time. Therefore, we injected anti-CD20 IgG combined with equal molar amounts of the recombinant RF^{low} or of the control monoclonal mIgM. We observed a significant difference in the hIgG level between the two groups already one day after injection. In fact, the animals injected with RF^{low} along with anti-CD20 IgG showed significantly lower concentration of hIgG in respect to the mice which received RF^{high} (Figure 45C).

Our results show that a high affinity RF is capable of stabilizing IgG *in vivo* thereby impressively extending its half-life, while a low affinity RF exhibit the opposite destructive effect *in vivo.* Together, these data indicate that RFs have different impact on the half-life of IgG depending on their affinity to their target. Interestingly, this is not only valid for autoreactive antibodies but also for therapeutic antibodies.

Example 22: Recombinant low affinity anti-insulin IgM destructs insulin *in vivo*

To confirm our hypothesis that IgM affinity and specificity determines the outcome of the interaction with the recognized cognate antigen, we used recombinant anti-insulin antibodies as model. Since we proposed that affinity to the target and mono-specificity are the main requirements for determining the effector function of autoreactive antibodies, we expect that reversion of the variable region of the anti-insulin IgM into its respective germline (gl) version would result in reduced affinity to its target. To this end, we reverted the heavy chain (HL) and the light chain (LC) sequences to germline and tested combinations of the reverted HC/LC for their insulin binding affinity. While most combinations lost insulin binding, the recombinant insulin-specific antibody (αnti-insulin IgM^{low}) consisting of the original LC and the germline-reverted HC version of the anti-insulin antibody showed reduced affinity to insulin as compared with the original antibody

(Figure 42A). In fact, the K_D of the germline-reverted anti-insulin IgM^{low} was in the range of 10^{-7} (Figure 42C) and thus, considerably lower than the affinity of the affinity of the original anti-insulin IgM^{high}. In addition, decreased insulin binding was observed for anti-insulin IgM^{low} by ELISA. In order to test whether the two antibodies, namely anti-insulin IgM^{low} and its high affinity counterpart IgM^{high}, had different effects on glucose metabolism, we injected identical molar amounts of anti-insulin IgM^{high} and anti-insulin IgM^{low} into WT mice. Within two hours after injections, higher blood glucose levels (hyperglycemia) were observed in mice that received anti-insulin IgM^{low}, while anti-insulin IgM^{high} did not alter blood glucose and was able to protect insulin from IgG-dependent degradation (Figure 42D).

Interestingly, the reverted version of the anti-insulin IgM differs in only two point mutations in the complementarity-determining region 2 (CDR2) that seem to be responsible for the affinity maturation (Figure 42A). Importantly, the quality of the *in vitro* produced antibodies was assessed and revealed no structural difference between the purified IgM^{high} and the IgM^{low} antibodies (Figure 42B).

These data suggest that a high affinity autoantibody with a protective role can be turned into an autoantibody with a destructive role by reverting the immunoglobulin heavy chain variable region (IGHV) into its germline version (low affinity). This confirms our hypothesis of the regulatory role of IgM antibodies and suggests that mutations acquired during the affinity maturation process can turn destructive IgM antibodies into protective ones.

Example 23: Recombinant low-affinity RF is polyreactive and binds DNA

In order to confirm our findings regarding the role of low affinity RF in the interaction with the target antigen, we reviewed available reports describing the extent of somatic mutations of RFs in RA patients (Randen et al. 1992; Youngblood, Kathy, Lori Fruchter, Guifeng Ding, Javier Lopez, Vincent Bonagura, and Anne Davidson. 1994. Journal of Clinical Investigation 93(2):852–61.). Albeit the majority of RFs isolated from the synovia of RA patients are highly affine for the Fc portion of IgG and not reactive to other tested antigens, we identified one RF isolated from a RA patient that seemed to be polyreactive and bound to other antigens such as tetanus toxoid, DNA and bovine serum albumin (BSA) (Youngblood, Kathy, Lori Fruchter, Guifeng Ding, Javier Lopez, Vincent Bonagura, and Anne Davidson. 1994. “Rheumatoid Factors from the Peripheral Blood of Two Patients with Rheumatoid Arthritis Are Genetically Heterogeneous and Somatically Mutated.” *Journal of Clinical Investigation* 93(2):852–61.). Interestingly, in-depth analysis of the IGHV and IGLV sequences of the selected RF revealed high degree of homology

to the germline gene counterparts. In fact, the selected antibody variable heavy chain shared 96,9% identical residues with the IGHV3-30-3*01 (allele 1) and the light chain had 99,3% identity with the IGKV3-11*01 (Figure 44A). Due to the high degree of identity to germline genes and to the previously published data showing the polyreactivity of this RF, we expected this antibody to be low affinity RF (RF^{low}). Therefore, we cloned and expressed RF^{low} as recombinant IgM (Figure 44B). Bio-layer interferometry assay revealed that the IgG-binding affinity of RF^{low} was in the range of 10^{-7}, while the K_D of RF^{high} was 10^{-9} (Figure 44C).

The ability of RF^{low} to bind IgG was also tested by ELISA revealing that the recombinant RF^{low} binds IgG although to a lesser extent than RF^{high} which is most likely a result of the reduced IgG affinity of RF_{low} (Figure 44D). Additionally, we confirmed the previously published data showing that, in contrast to RF^{high}, recombinant RF^{low} binds double-stranded DNA (Figure 44E) and is reactive in HEp2 slides (Figure 44F).

These data confirm available data suggesting that, in contrast to typical high affinity RFs from RA patients, low affinity RFs are multi-specific/poly-reactive as they bind DNA in addition to IgG (Figure 44G).

Example 24: Deregulated ratios of high affinity and low affinity RFs in autoimmune diseases

The above results suggesting that the effects observed in the presence of a low affinity RF prevails over the effects of a high affinity RF lead us to the hypothesis that a failure in maintaining the balance between the two classes of RFs might contribute to the development of autoimmune diseases. To gain a deeper understanding, we collected sera from young and aged healthy donor and from patients suffering from two well-known autoimmune diseases, namely RA and multiple sclerosis (MS). We characterized these samples for total serum levels of IgM and IgG. Interestingly, total serum IgM levels of MS and RA patients seem to be increased as compared with healthy individuals (Figure 46A). Furthermore, while total serum IgG concentration of young and aged healthy individuals were in similar range, the IgG levels of MS patients were significantly increased as compared to aged healthy individuals and a similar, although not significant, tendency was shown by total IgG levels of RA patients (Figure 46B).

Next, the inventors assessed whether the higher circulating levels of IgG in MS correlate with altered amounts of circulating RF-IgM. Interestingly, MS patients show significantly lower amounts of RF-IgM than the young and aged healthy individuals (Figure 46C). These data suggest that low affinity RF is reduced in MS patients as compared with healthy individuals and, therefore, it is conceivable that the regulation of IgG homeostasis

including autoreactive antibodies is altered.

Altogether, these findings suggest that an increase in IgG-protective RF^{high} in RA patients or a decrease in IgG-destructive RF^{low} in MS patients might be important pathogenic mechanisms associated with the development of autoimmune diseases.

Material and Methods

Mice used for Example 1-15

8 – 30-week-old C57BL/6 mice and B cell-deficient mice were immunized intraperitoneally (i.p.) with a mixture of 13 – 50 µg antigen with 50 µg CpG-ODN1826 (Biomers) in 1x PBS. Control immunization (CI) mice received PBS and CpG-ODN1826 (50 µg/mouse). Native biotinylated murine insulin was purchased from BioEagle.

Mice used for Example 16-19

8 – 15-week-old female C57BL/6 mice and mb1 mice45 were intraperitoneally (i.p.) injected with a mixture of 10 µg antigen (cInsulin or Insulin-bio:SAV) in 1x PBS. Control injections (CI) mice received PBS in a total volume of 100 µL/mouse. Animal experiments were performed in compliance with license 1484 for animal testing at the responsible regional board Tübingen, Germany. All mice used in this study were either bred and housed within the animal facility of the Universiry of Ulm under specific-pathogen-free conditions, or obtained from Jackson company at 6 weeks of age. All animal experiments were done in compliance with the guidelines of the German law and were approved by the Animal Care and Committees of Ulm University and the local government.

Mice used for Example 20-24

8- to 15-week-old female C57BL/6 mice were used in all experiments reported in this study. For antibody stability experiment, 20-50 µg antibodies (as indicated in details in figure legend for each experiment) were injected intravenously (i.v.) into the lateral tail vein and blood was collected at indicated time points to obtain serum.

For blood glucose monitoring experiments 100 µg anti-insulin IgG or anti-insulin IgM were injected i.v. into the lateral tail vein and blood was collected at indicated time points to obtain serum.

Peptides and immunogens

C-Peptide peptides (RoyoBiotech, Shanghai), Insulin and virus-derived peptides (SEQ ID NO: 43; SEQ ID NO: 44) (Peptides&Elephants, Berlin) were dissolved according to their water solubility in pure water, 1% DMSO or 1 % Dimethylformamide (DMF). The virus-derived peptides (SEQ ID NO: 43; SEQ ID NO: 44) were coupled to Biotin or KLH, respectively. An amount of 1 mg was purchased and dissolved in a volume of 1 ml. 10 to 50 µg of KLH-coupled peptide were used for immunization of mice via intraperitoneal injection. For covalent coupling of peptides to key hole limpet hemocyanin (KLH) a N-terminal cysteine was added. Coupling of peptides to Streptavidin (SAV, ThermoScientific) was done by addition of biotin to the N-terminus. The C-terminus was left with an OH-group for better handling. Insulin-A-chain derived peptides (InsA) (Peptides&Elephants, Berlin) were dissolved according to their water solubility in water. 4-hydroxy-3- nitrophenylacetyl coupled to KLH (NP(30)KLH) or BSA (NP(15)BSA) was purchased from Biosearch Technologies.

Crosslinking of native Insulin and InsA peptides

Native human insulin (Merck) was pre-diluted in PBS to 1 mg/mL. Chemical thiol-crosslinking was done using 1,2-Phenylen-bis-maleimide (Santa Cruz, 13118-04-2) at 10 µg/mL and afterwards removed by using a 10 kD cut-off spin column (Abcam, ab93349). Purified insulin complexes (cInsulin) were used for intraperitoneal injections at 10 µg per mouse in 100 µL total volume.

Antibody specificity, host/isotype, conjugate clone, class, supplier catalog number:

Anti-human CD20 (Rituximab, human IgG1, SelleckChem); Rheumatoid Factor Concentrate (Lee Biosolutions), Human IgM (unlabeled, SouthernBiotech, #0158L-01), RF^{low}(human IgM, homemade with a IgM constant region, sequence of heavy chain and light chain from Youngblood, Kathy, Lori Fruchter, Guifeng Ding, Javier Lopez, Vincent Bonagura, and Anne Davidson. 1994. Journal of Clinical Investigation 93(2):852–61.-RC1 having a VH sequence as encoded by the sequence defined by SEQ ID NO: 52 (HDCR1 encoded by the sequence defined by SEQ ID NO: 53, HCDR2 encoded by the sequence defined by SEQ ID NO: 54, HCDR2 encoded by the sequence defined by SEQ ID NO: 55) and a VL sequence as encoded by the sequence defined by SEQ ID NO: 49 (LDCR1 encoded by the sequence defined by SEQ ID NO: 50, LCDR2 encoded by GATGCATCC, LCDR2 encoded by the sequence defined by SEQ ID NO: 51); RF^{high} (human IgM, homemade with a IgM constant region, sequence of heavy chain and light chain from Youngblood, Kathy, Lori Fruchter, Guifeng Ding, Javier Lopez, Vincent Bonagura, and Anne Davidson. 1994. Journal of Clinical Investigation 93(2):852–61.-

RO7 having a VH sequence as encoded by the sequence defined by SEQ ID NO: 59 (HDCR1 encoded by the sequence defined by SEQ ID NO: 60, HCDR2 encoded by the sequence defined by SEQ ID NO: 61, HCDR2 encoded by the sequence defined by SEQ ID NO: 62) and a VL sequence as encoded by the sequence defined by SEQ ID NO: 56 (LDCR1 encoded by the sequence defined by SEQ ID NO: 57, LCDR2 encoded by GGTGCATCC, LCDR2 encoded by the sequence defined by SEQ ID NO: 59), anti-Insulin IgG (purified from IVIg, see below); total serum IgM (isolated from healthy donor serum, see below); anti-insulin IgMhigh and anti-insulin IgMlow (human IgM, homemade, sequence from (Ikematsu et al. 1994), germline reversion was achieved using the online avaible tool IMGT® V-Quest).

Flow cytometry

Cell suspension were Fc-receptor blocked with polyclonal rat IgG-UNLB (2,4G2; BD) and stained according to standard protocols. Biotin-conjugated peptides/antibodies were detected using Streptavidin Qdot605 (Molecular Probes; Invitrogen). Viable cells were distinguished from dead cells by usage of Fixable Viability Dye eFluor780 (eBioscienc). Cells were acquired at a Cato II Flow Cytometer (BD). If not stated otherwise numbers in the plots indicate percentages in the respective gates whilst numbers in histogram plots state the mean fluorescence intensity (MFI).

Enzyme-linked Immunosorbent Assay (ELISA)

96-Well plates (Nunc, Maxisorp) were coated either with, native Insulin (Sigma-Aldrich, Cat. 91077C), Streptavidin (ThermoScientific, Cat. 21125), or calf thymus DNA (ThermoScientific, Cat.15633019), with 10 µg/mL, or anti-IgM, anti-IgG-antibodies (SouthernBiotech). Loading with a biotinylated peptide (2,5 µg/mL) of SAV-plates and blocking was done in 1% BSA blocking buffer (Thermo Fisher). Serial dilutions of 1:3 IgM or IgG antibodies (SouthernBiotech) were used as standard. The relative concentrations, stated as arbitrary unit (AU), were determined via detection by Alkaline Phosphatase (AP)-labeled anti-IgM/anti-IgG (SouthernBiotech), respectively. The p-nitrophenylphosphate (pNPP; Genaxxon) in Diethanolamine buffer was added and data were acquired at 405 nm using a Multiskan FC ELISA plate reader (Thermo Scientific). All samples were measured in duplicates.

For analysis of affinity-maturation, results from plates coated with either peptide(1) or peptide(4) were calculated by dividing peptide(1) by peptide(4). Thus, results were stated as relative units [RU] within the figures.

Antibody specificity, host/isotype, conjugate clone, class, supplier catalog number: anti-human IgM (goat, IgG, unlabeled, polyclonal, SouthernBiotech, #2020-01); anti-human IgG (goat, IgG, unlabeled, polyclonal, SouthernBiotech, #2040-01); human IgM (unlabeled, SouthernBiotech, #0158L-01); human IgG (unlabeled, SouthernBiotech, #0150-01); anti-human IgM (mouse, AP, monoclonal, SouthernBiotech, #9020-04); anti-human IgG (Goat, AP, polyclonal, SouthernBiotech, #2040-04).

Enzyme-linked Immuno-Spot Assay (ELISpot)

Total splenocytes were measured in triplicates with 300.000 cells/well. ELISpot plates were pre-coated with either native Insulin (Sigma-Aldrich, Cat. 91077C), C-peptide (RoyoBiotech). After 12 – 24 h incubation of the cells at 37 °C, antigen-specific IgM or IgG was detected via anti-IgM-bio:SAV-AP or anti-IgG-bio:SAV-AP (Mabtech). Handling of the plates and antibody concentrations was done according to the manufacturer's recommendations.

HEp-2 slides and fluorescence microscopy

HEp-2 slides (EUROIMMUN, F191108VA) were used to asses reactivity of serum IgM to nuclear antigens (ANA). Sera of Insulin-A-peptide immunized mice on days 7 and 85 post immunization were diluted to an equal concentration of IgM (approx. 300 ng/mL anti-Insulin-IgM in both immunized samples) and applied onto the HEp-2 slides. Anti-IgM-FITC (eBioscience, Cat. 11-5790-81 (Examples 1-19); Biolegend, #314506 (Examples 20-24)) was used for detection of ANA-IgM.

Stained HEp-2 slides were analyzed using fluorescence microscope Axioskop 2 (Zeiss) and DMi8 software (Leica).

Glucose level monitoring

Assessment of urine glucose levels was done using Combur 10 M Test stripes (Roche Diagnostics, Mannheim). Sterile stripes were used during daily mouse handling and the displayed color after testing was compared to the manufacturer's standard of glucose levels in mmol/L. AccuCheck (Roche Diagnostics, Mannheim) blood glucose monitor was used to measure blood glucose levels of mice. Blood was taken from the tail vein from ad libitum fed mice and transferred onto sterile test stripes. Glucose levels were measured in mmol/L at days stated in the figures for each mouse per group. Control-immunizations were done with littermates and measured at similar times of the day.

SDS page, Coomassie and western blot

Organs were taken immediately after sacrifice and lysed in RIPA buffer containing protease and phosphatase inhibitors (50 mM TrisHCl, pH 7.4, 1 % NP-40, 0.25 % sodium deoxycholate, 150 mM NaCl, 1 mM EDTA (pH 8), 1 mM sodium orthovanadate, 1 mM NaF, protease inhibitor cocktail (Sigma-Aldrich). Samples were separated on 10 – 20 % SDS-polyacrylamide gels and either blotted onto PVDF membranes (Millipore) or incubated with Coomassie (Coomassie brilliant blue R-250, ThermoFisher) for 45 min and subsequently de-stained. Subsequently, membranes were blocked for one hour at room temperature in 5 % BSA PBS with constant agitation. Primary antibodies were diluted in 5 % BSA PBS (BIOMOL Research Laboratories). Secondary antibodies were diluted in 5 % BSA PBS. Development of the membrane and recording of the data were done with an optical system Fusion SL (Vilber).

Pulldown of total serum immunoglobulins

Sera from immunized mice were taken immediately after euthanasia and either IgM or IgG were purified. Removal of antigen bound to antibodies was achieved by repeated freeze-thaw cycles of the serum and pH-shift during elution52. For IgG protein G sepharose beads (Thermo Fisher) were used according to the manufacturers protocol and dialyzed overnight in 10 times sample volume in 1x PBS. For IgM, HiTrap IgM columns (GE Healthcare, Sigma-Aldrich) were used according to the manufacturers protocol and dialyzed overnight in 10 times sample volume 1 x PBS. Quality check of the isolated immunoglobulins were addressed via SDS page and Coomassie and the amount of insulin-specific immunoglobulins determined via ELISA. Finally, 20 – 50 µg (1 – 10 µg insulin-specific-Ig) were injected intravenously.

Antibody purification and pulldown of total serum IgM

For IgM purification from human serum, IgG depletion was performed by incubating the samples with Protein G Sepharose beads (GE Healthcare, Sigma-Aldrich) according to manufacturer's instructions.

For IgM purification from IgG-depleted human serum and from HEK293-6E cell supernatant, HiTrap® IgM columns (GE Healthcare, Sigma-Aldrich) were used according to the manufacturer's protocol and eluates were dialyzed overnight in 300-fold sample volume 1x PBS. Quality control of the isolated immunoglobulins was addressed via SDS–PAGE stained with Coomassie-brilliant blue R-250 (BIO-RAD) and the quantification of eluted proteins was assessed via ELISA.

Isolation of Insulin-specific serum immunoglobulins

Sera from InsA and control immunized mice were taken immediately after euthanasia and prepared for insulin-specific immunoglobulin isolation. Streptavidin bead columns (Thermo-Scientific, Cat. 21115) were loaded with 10 µg bio-Insulin (BioEagle). The sera were incubated for 90 min at room temperature to ensure binding of insulin-specific antibodies to the beads. Isolation of the insulin-antibodies was done by pH-shift using the manufacturers elution and neutralization solutions. Quality of the isolated immunoglobulins was examined via Coomassie and western blot analysis using anti-IgM heavy chain (Thermo-Scientific, Cat. 62-6820) and anti-IgG heavy chain (Cell Signaling Technologies, Cat. 7076) antibodies. For further in vivo experiments, the isolated antibodies were dialyzed.

Isolation of antigen-specific immunoglobulins from IVIg

Streptavidin bead columns (Thermo Scientific, # 21115) were loaded with 20 µg biotin-insulin (ibt biosystem). IVIg preparation was incubated for 90 min at room temperature to ensure binding of antigen-specific antibodies to the beads. Isolation of the antibodies was performed by acidic pH-shift using the manufacturer's elution and neutralization solutions. Quality of the isolated immunoglobulins was examined via SDS–PAGE stained with Coomassie-brilliant blue R-250 (BIO-RAD) and ELISA. For further in vivo experiments, the isolated antibodies were dialyzed overnight in 300-fold sample volume 1x PBS.

Interferometry

Interferometric assays (BLItz device, ForteBio) were used to determine the affinity of protein-protein interactions. Here, we used insulin-specific IgM (see isolation of insulin-specific immunoglobulins) and insulin-bio (ThermoFisher) as a target. The targets were loaded onto Streptavidin biosensors (ForteBio). Binding affinities of IgM to Insulin were acquired in nm. Subsequently, the calculated affinity value (Ka) was used to determine the dissociation constant (Kd): Kd = 1/Ka. Following protocol was used: 30 sec baseline, 30 sec loading, 30 sec baseline, 240 sec association, 60 sec dissociation. For buffering of samples, targets and probes, the manufacturer's sample buffer (ForteBio) was used.

Flow Cytometric Bead Array for mouse inflammatory cytokines

To determine pancreas supernatant inflammatory cytokine levels of mice immunized with clnsulin or control immunization, we performed a BD Cytometric Bead Array (Mouse Inflammation, BD Biosciences, Cat.: 552364, Lot.: 005197). Samples were diluted according to the manufacturers protocol. IL-12p70, TNF-a, IFN-y, MCP-1, IL- 10 and IL-

6 APC-labeled beads were used together with PE-labeled detector reagent. The assay was measured at a FACS Canto II and analyzed via FlowJoIO software. Relative cytokine levels correlate to the mean fluorescence intensity of each cytokine bead within the PE channel.

Bio-Layer-Interferometry (BLI)

Interferometric assays (BLItz device, ForteBio) were used to determine the affinity of protein-protein interactions (Kumaraswamy, S. & Tobias, R. Label-free kinetic analysis of an antibody-antigen interaction using biolayer interferometry. in Protein-Protein Interactions: Methods and Applications: Second Edition vol. 1278 165–182 (Springer New York, 2015)). Here, we used insulin-specific IgM (see isolation of insulin-specific immunoglobulins) and insulin-bio (ThermoFisher) as target. Targets were loaded onto Streptavidin biosensors (ForteBio). Binding affinities of IgM to Insulin were acquired in nm. Subsequently, the calculated affinity value (K_a) was used to determine the dissociation constant (K_d): $K_d = 1/K_a$. Following protocol was used: 30 sec baseline, 30 sec loading, 30 sec baseline, 240 sec association, 120 sec dissociation. For buffering of samples, targets and probes, the manufacturer's sample buffer (ForteBio) was used.

Wire hanging test

The linear wire hanging test is used to assess motor strength and function of mice. Individual mice were put onto a 36 cm elevated horizontal wire above a cage, subsequently the mice tried to stay on the wire by using their paws and muscle strength. The ability in time (sec) of each mouse to stay on the wire was recorded. A maximum time duration of 240 sec was set. Each mouse went through the test three times in a row. The mean value was calculated from the measured data. Blood glucose values were determined before and after the test.

HEK293-6E cell culturing and antibody production

HEK293-6E cells were cultured in FreeStyle F17 expression media (Invitrogen) supplemented with 0,1 % Kolliphor ® P188 (Sigma-Aldrich) and 4mM L-Glutamine (Gibco Life Technologies). Transfection was performed according to the manufacturer's instructions. Briefly, cells were transfected using Polyethylenimine (Polysciences) with two pTT5 plasmids encoding heavy and light chain of the antibody of interest (total 1 µg DNA/ml of culture). 24-48 hours post transfection cells were fed with Tryptone N1 (TekniScience Inc # 19553) to a final concentration of 0,5%.

Harvesting was performed 120 hours post transfection. Antibodies were purified using HiTrap® IgM columns (GE Healthcare, Sigma-Aldrich) as described below.

Healthy Donors and Patients Samples

Healthy donor blood samples were obtained via the Deutsch Rotes Kreuz Ulm (DRK). Samples were divided into young (18-35 years) and old (above 55 years old) according to their age. Sera was obtained by Pancoll gradient centrifugation.

Sera from Multiple Sclerosis patients were provided by the Biobank of the Rehabilitationskrankenhaus of the University Hospital Ulm (RKU).

Sera from Rheumatoid Arthritis (RA) patients were provided by the Clinic of Rheumatology and clinical Immunology of the University Clinic of Freiburg. RA patients were categorized according to symptoms and positivity for RF.

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Claims

1. A pharmaceutical composition comprising an IgM antibody or a fragment thereof and a therapeutic antibody, wherein the IgM antibody specifically binds to the therapeutic antibody.

2. The pharmaceutical composition of claim 1, wherein the IgM antibody and the therapeutic antibody are comprised in a molar ratio of 5:1 to 1:10, preferably 2:1 to 1:5.

3. A method of treatment of a disease or disorder, the method comprising the steps of:
   a) administering an effective dose of a therapeutic antibody; and
   b) administering a corresponding dose of an IgM antibody or a fragment thereof, wherein the IgM antibody specifically binds to the therapeutic antibody and wherein the corresponding dose of the IgM antibody is between 10% and 400% of the effective dose of the therapeutic antibody, preferably 20% and 200% of the effective dose of the therapeutic antibody.

4. The pharmaceutical composition of claim 1 or 2 or the method of treatment of claim 3, wherein a half-live of the therapeutic antibody is prolonged by the binding of the IgM antibody.

5. The pharmaceutical composition of any one of the claims 1, 2 or 4 or the method of treatment of claim 3 or 4, wherein the IgM antibody binds to the therapeutic antibody with a K_D of at least 10^{-8}, preferably measured with Biolayer Interferometry.

6. The pharmaceutical composition of any one of the claims 1, 2, 4 or 5 or the method of treatment of any one of the claims 3 to 5, wherein the therapeutic antibody is an anti-rheumatoid arthritis antibody.

7. The pharmaceutical composition of any one of the claims 1, 2, 4 to 6 or the method of treatment of any one of the claims 3 to 6, wherein the therapeutic antibody is an anti-CD20 antibody.

8. The pharmaceutical composition of claim 5 or the method of treatment of claim 7, wherein the therapeutic antibody is Rituximab.

9. The pharmaceutical composition of any one of the claims 1 to 7 for use in treatment of an autoimmune disease or disorder.

10. The pharmaceutical composition for use of claim 8, wherein the autoimmune disease or disorder is multiple sclerosis or rheumatoid arthritis.

11. The method of treatment of anyone of the claims 3 to 8, wherein the disease or disorder is an autoimmune disease or disorder.

12. The method of treatment of claim 11, wherein the autoimmune disease or disorder is multiple sclerosis or rheumatoid arthritis.

13. A method for obtaining a protective-regulative antibody comprising the steps of:

(a) providing a blood sample of a subject, wherein the subject experienced elicitation of an IgG and oligomeric antibody response by a target antigen; and

(b) enriching a maturated oligomeric antibody, wherein

(i) the binding of the oligomeric antibody is more specific for the target antigen than the IgG-type antibody, preferably wherein the oligomeric antibody is monospecific for the target antigen; and/or

(ii) the binding affinity of the oligomeric antibody to the target antigen is equal or higher than the IgG-type antibody, preferably wherein the protective-regulative antibody binds to the target antigen with K_d of less than 10^{-7}, preferably of less than 10^{-8}, more preferably of less than 10^{-9} and most preferably in the range of about 10^{-10} to about 10^{-12},

(c) isolating the enriched maturated oligomeric antibody to obtain the protective-regulative antibody that is protective-regulative for the function of the target antigen.

14. The method according to claim 13, wherein the subject experienced elicitation of the IgG and oligomeric antibody response by the target antigen at least 7 days ago, preferably at least 14 days ago, more preferably at least 27 days ago.

15. A method for obtaining a degrading oligomeric antibody comprising the steps of:

(a) providing a blood sample of a subject, wherein the subject experienced elicitation of an IgG and oligomeric antibody response by a target antigen; and

(b) enriching a primary oligomeric antibody, wherein

(i) the binding of the oligomeric antibody is equally or less specific for the target antigen than the IgG-type antibody, preferably wherein the oligomeric antibody is cross-specific for the target antigen and DNA; and/or

(ii) the binding affinity of the oligomeric antibody to the target antigen is lower than the IgG-type antibody, preferably wherein the protective-regulative antibody binds to the target antigen with K_d of more than 10^{-7},

(c) isolating the enriched primary oligomeric antibody to obtain the degrading antibody that can form immune-degradable complexes with the target antigen.

16. The method according to any one of the claims 13 to 15, wherein the blood sample is selected from the group consisting of whole blood, plasma and serum sample, preferably serum sample.

17. The method according to any one of the claims 13 to 16, wherein isolating an oligomeric antibody comprises mass- and/or affinity-related isolation.

18. The method according to any one of the claims 13 to 17, wherein enriching an oligomeric antibody comprises immunoprecipitation of the oligomeric antibody.

19. The method according to any one of the claims 13 to 18, wherein the oligomeric antibody is an IgM antibody.

20. The pharmaceutical composition of any one of the claims 1, 2, 4 to 8, the pharmaceutical composition for use of any one of the claims 9 to 12 or the method of treatment of any one of the claims 3 to 8, wherein the IgM antibody comprises: a variable heavy (VH) chain comprising CDR1 sequence as encoded by SEQ ID NO: 60, CDR2 sequence as encoded by SEQ ID NO: 61 and CDR3 sequence as encoded by SEQ ID NO: 62 and a variable light (VL) chain comprising CDR1 sequence as encoded by SEQ ID NO: 57, CDR2 sequence as encoded by

GGTGCATCC and CDR3 sequence as encoded by SEQ ID NO: 58.

21. The pharmaceutical composition of claim 20, the pharmaceutical composition for use of claim 20 or the method of treatment of claim 20, wherein the IgM antibody comprises:

a variable heavy (VH) chain sequence comprising the amino acid sequence encoded by the sequence as defined by SEQ ID NO: 59 or by a sequence having at least 90% sequence identity to SEQ ID NO: 59, preferably at least 95% sequence identity to SEQ ID NO: 59; and

a variable light (VL) chain sequence comprising the amino acid sequence encoded by the sequence as defined by SEQ ID NO: 56 or by a sequence having at least 90% sequence identity to SEQ ID NO: 56, preferably at least 95% sequence identity to SEQ ID NO: 56.

22. A host cell comprising a polynucleotide having

a) a sequence as defined by SEQ ID NO: 59 or a sequence having at least 90% sequence identity to SEQ ID NO: 59, preferably at least 95% sequence identity to SEQ ID NO: 59; and/or

b) a sequence as defined by SEQ ID NO: 56 or a sequence having at least 90% sequence identity to SEQ ID NO: 56, preferably at least 95% sequence identity to SEQ ID NO: 56;

wherein the polynucleotide further encodes an IgM constant region and/or wherein the host cell comprises a further polynucleotide encoding an IgM constant region.

23. A method for producing an IgM antibody, the method comprising the steps of:

a) culturing the host cell according to claim 22,

b) isolating an IgM antibody.

FIGURE 1

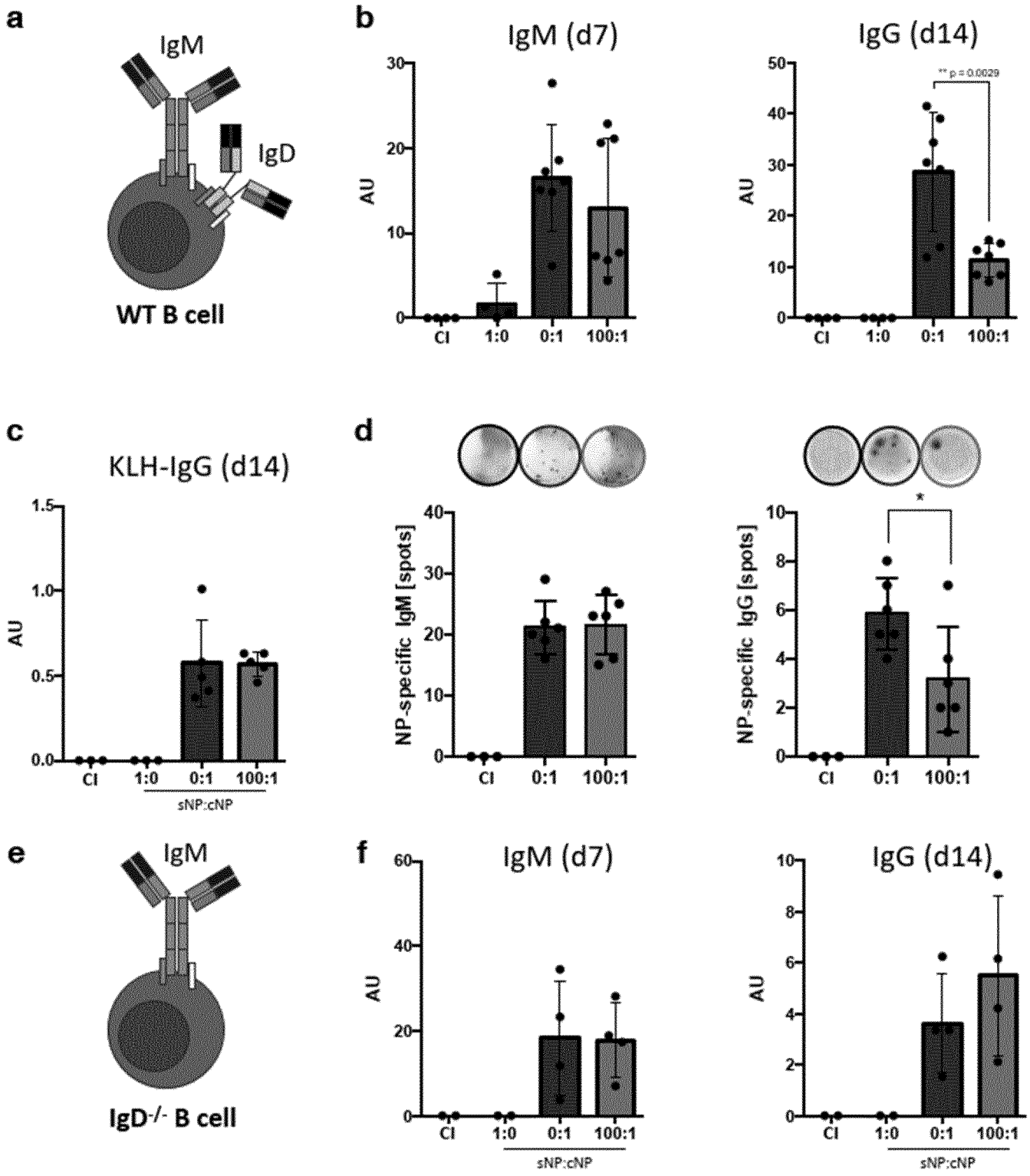

a
IgM
IgD
WT B cell
b
IgM (d7)
AU
Cl
1:0
0:1
100:1
IgG (d14)
** p = 0.0029
c
KLH-IgG (d14)
sNP:cNP
d
NP-specific IgM [spots]
NP-specific IgG [spots]
*
e
IgM
IgD-/- B cell
f
IgM (d7)
IgG (d14)

FIGURE 2

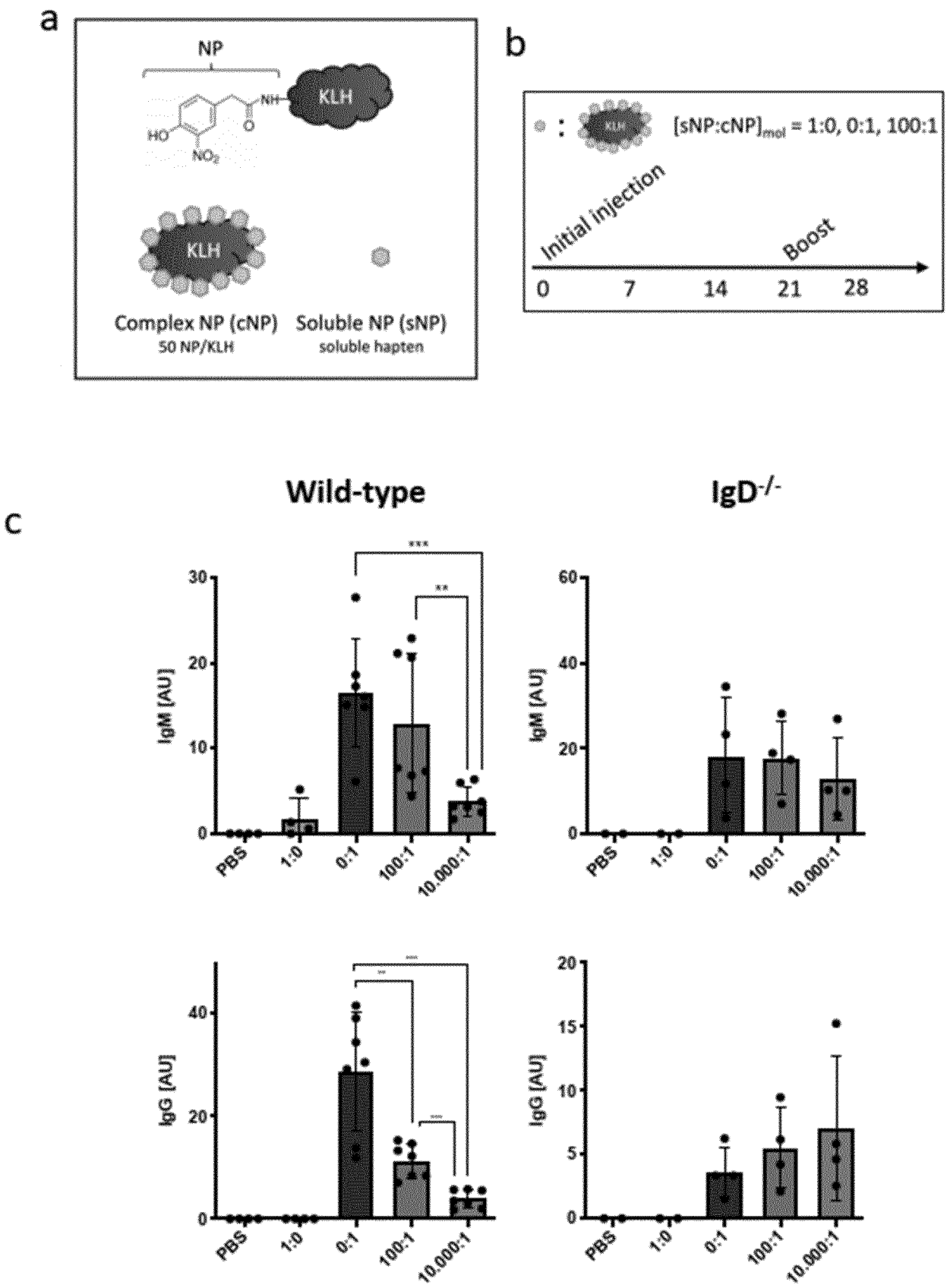

a
NP
KLH
HO
NH
O
NO2
KLH
Complex NP (cNP)
50 NP/KLH
Soluble NP (sNP)
soluble hapten
b
KLH
[sNP:cNP]mol = 1:0, 0:1, 100:1
Initial injection
Boost
0
7
14
21
28
c
Wild-type
IgD-/-
IgM [AU]
IgG [AU]
PBS
1:0
0:1
100:1
10.000:1
***
**

FIGURE 3

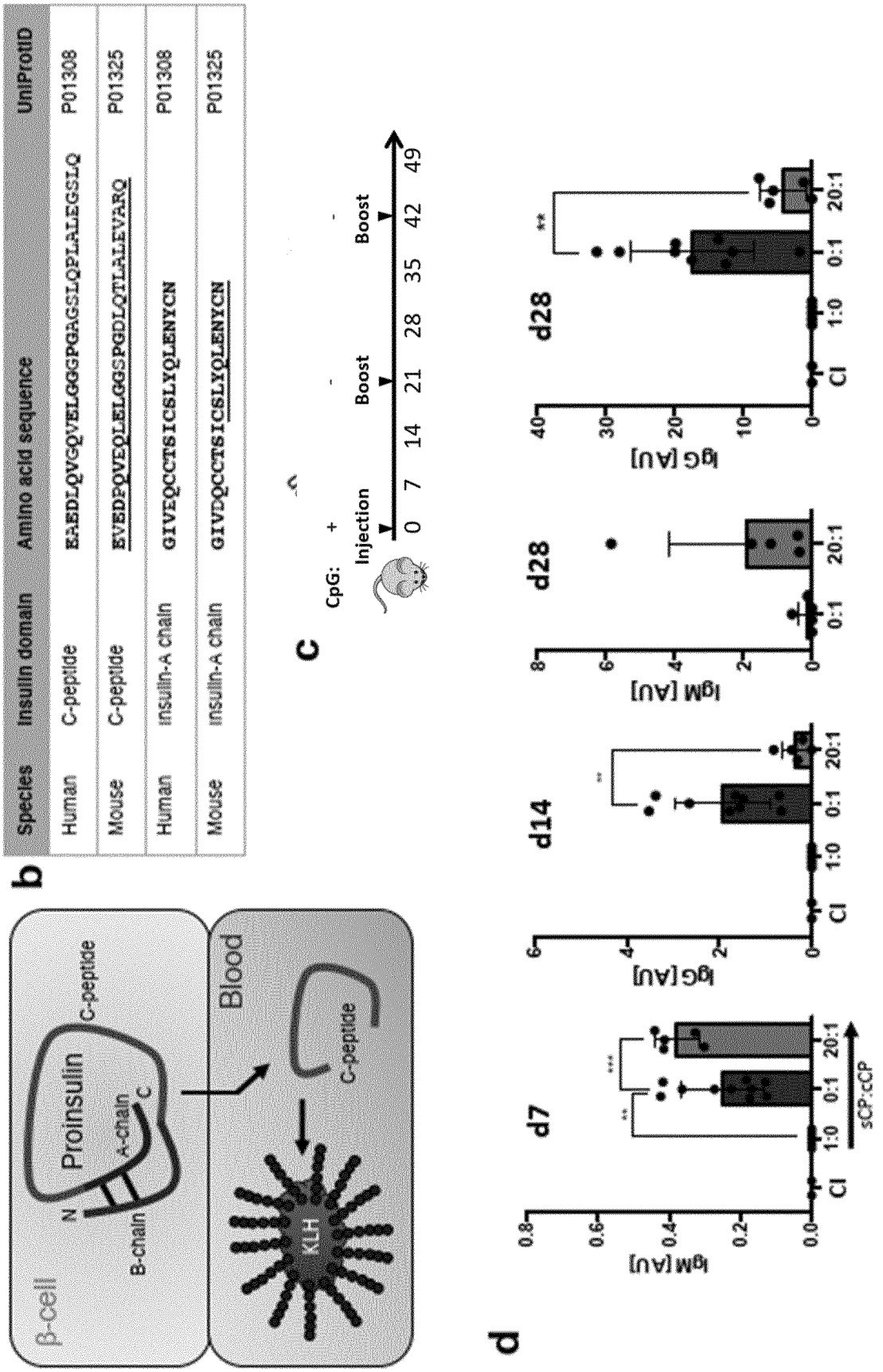

a
β-cell
Proinsulin
N
C-peptide
A-chain
B-chain
C
Blood
KLH
C-peptide
b
Species
Insulin domain
Amino acid sequence
UniProtID
Human
C-peptide
EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ
P01308
Mouse
C-peptide
EVEDPQVEQLELGGSPGDLQTLALEVARQ
P01325
Human
insulin-A chain
GIVEQCCTSICSLYQLENYCN
P01308
Mouse
insulin-A chain
GIVDQCCTSICSLYQLENYCN
P01325
c
CpG:
+
Injection
Boost
Boost
0 7 14 21 28 35 42 49
d
d7
d14
d28
d28
IgM [AU]
IgG [AU]
IgM [AU]
IgG [AU]
Cl
1:0
0:1
20:1
sCP::cCP

FIGURE 3 cont.

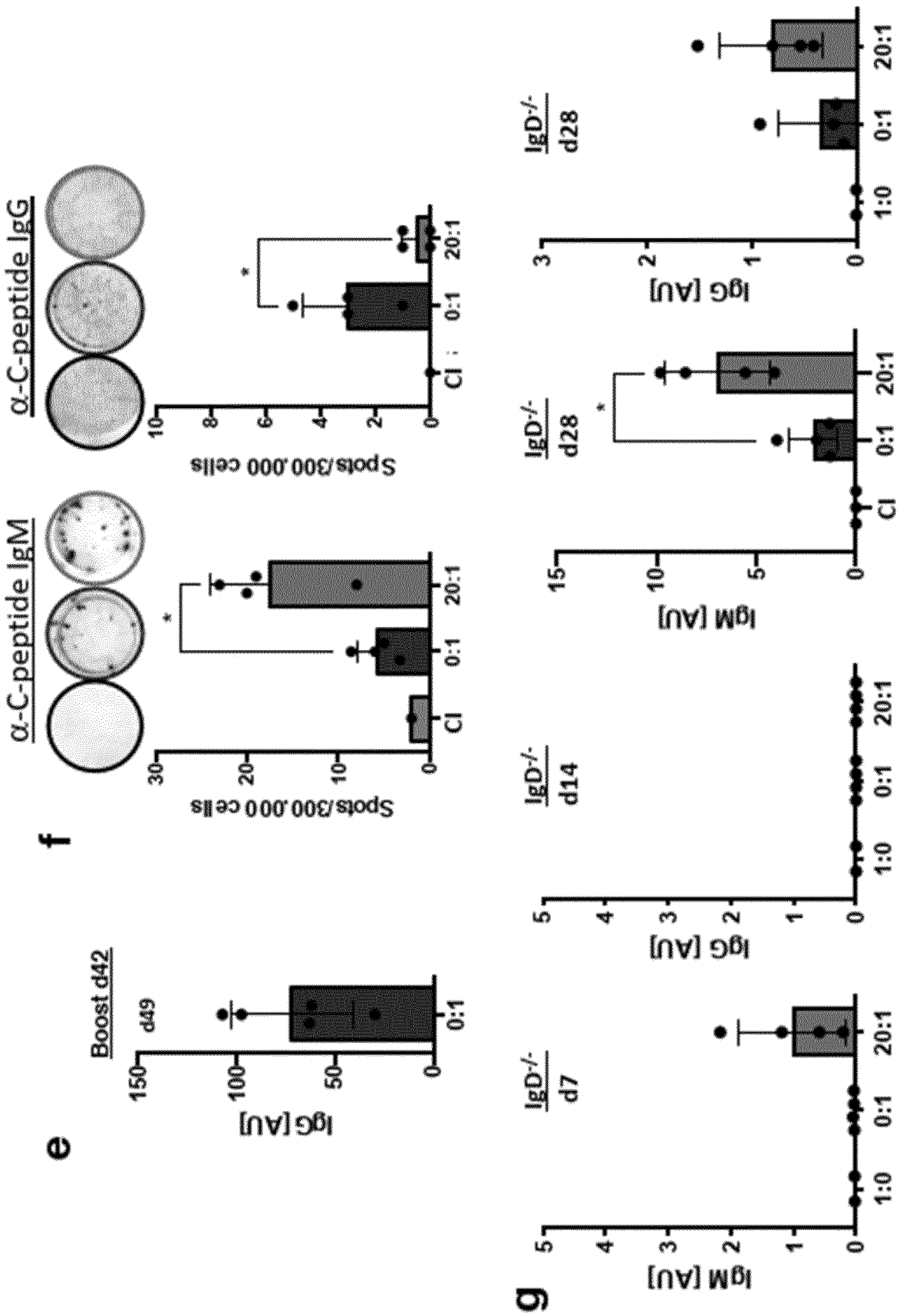


FIGURE 4

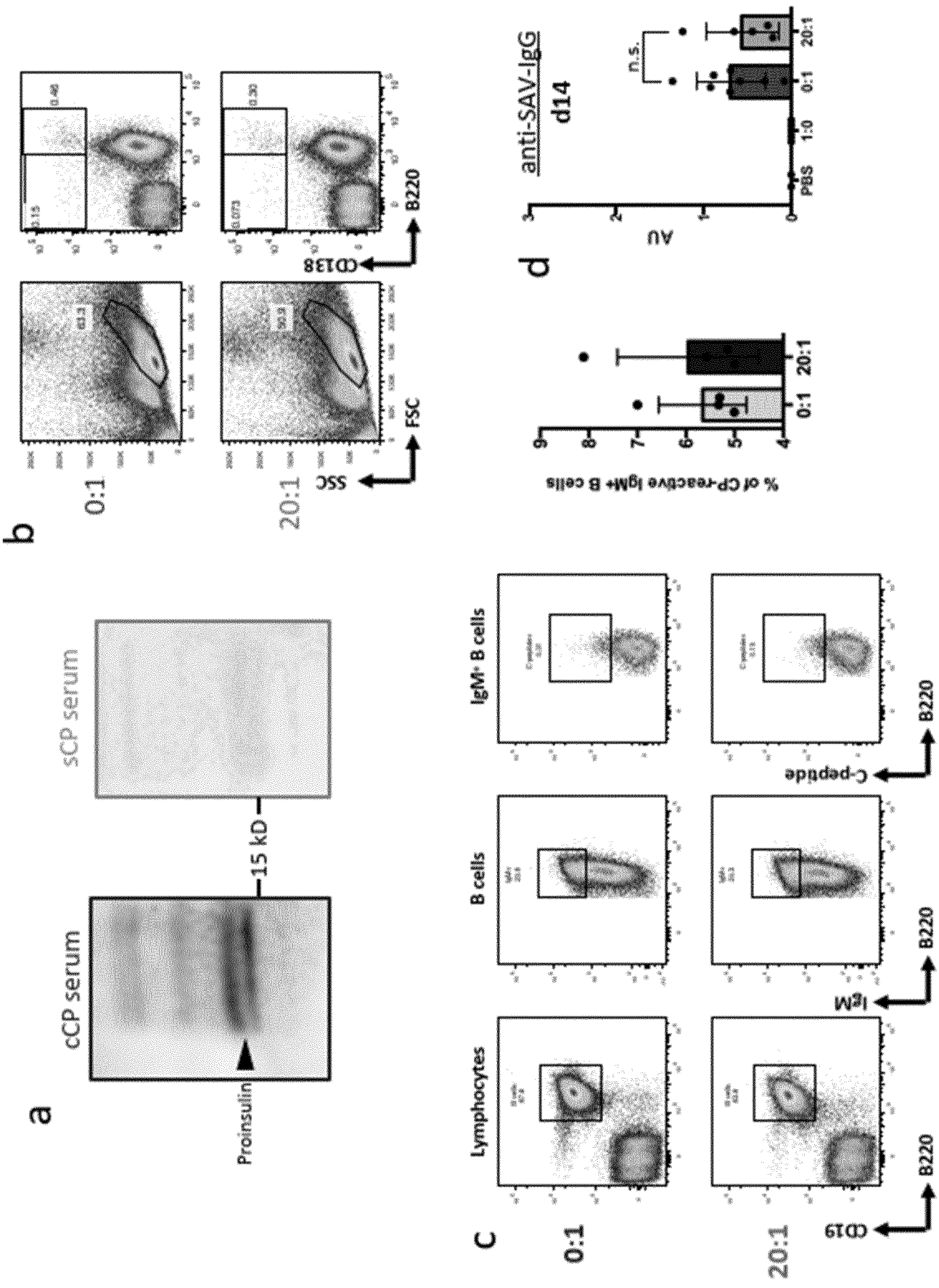

a
cCP serum
sCP serum
15 kD
Proinsulin
b
0:1
20:1
SSC
FSC
CD138
B220
d
anti-SAV-IgG
d14
AU
n.s.
PBS
1:0
0:1
20:1
% of CP-reactive IgM+ B cells
c
Lymphocytes
B cells
IgM+ B cells
0:1
20:1
CD19
IgM
C-peptide
B220

FIGURE 5

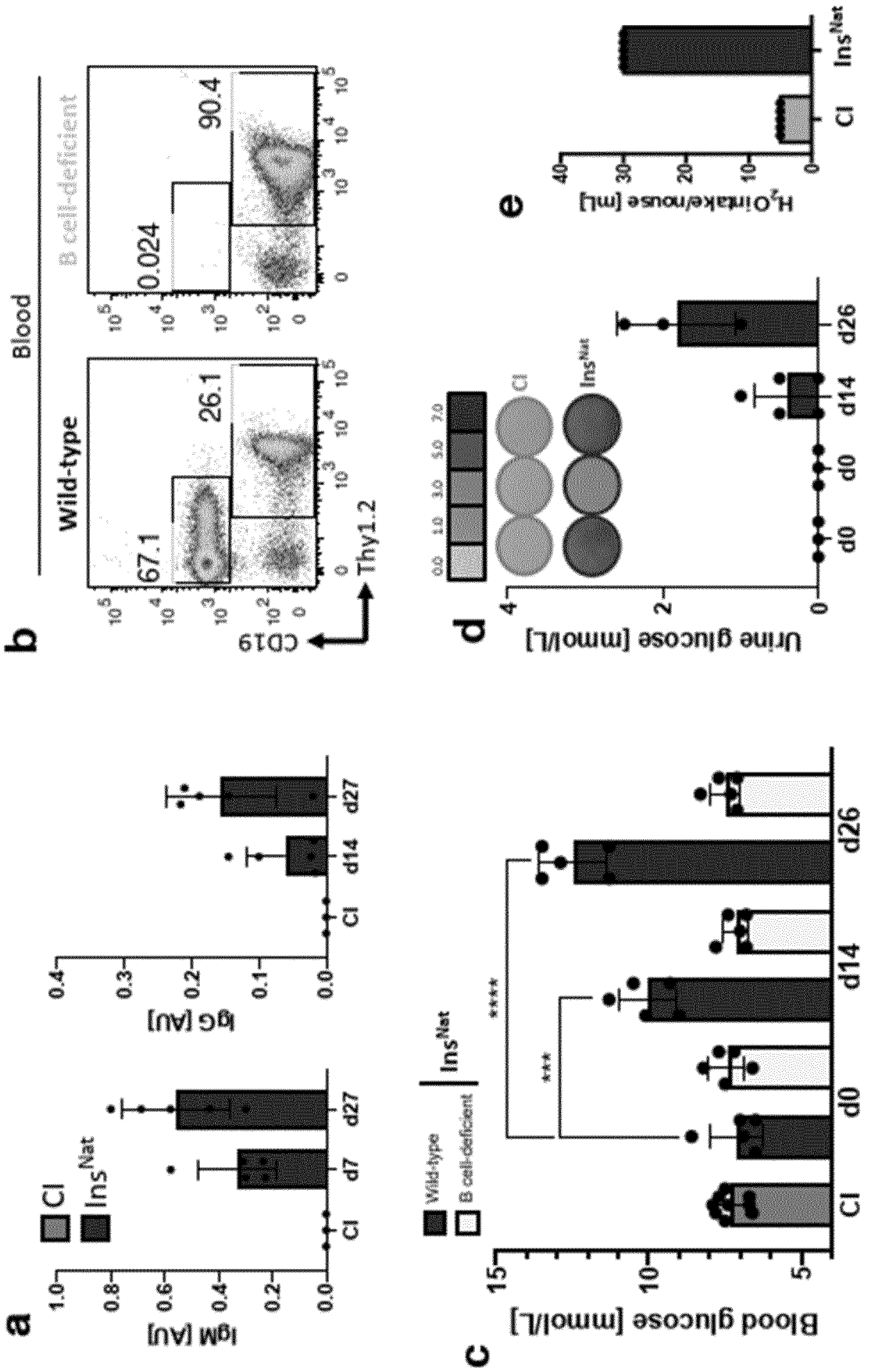

a
Cl
Ins^Nat
IgM [AU]
IgG [AU]
Cl d7 d27
Cl d14 d27
b
Blood
Wild-type
B cell-deficient
67.1
26.1
0.024
90.4
CD19
Thy1.2
c
Wild-type
B cell-deficient
Ins^Nat
Blood glucose [mmol/L]
***
****
Cl d0 d14 d26
d
Cl
Ins^Nat
Urine glucose [mmol/L]
d0 d0 d14 d26
e
H2O intake/mouse [mL]
Cl Ins^Nat

FIGURE 5 cont.

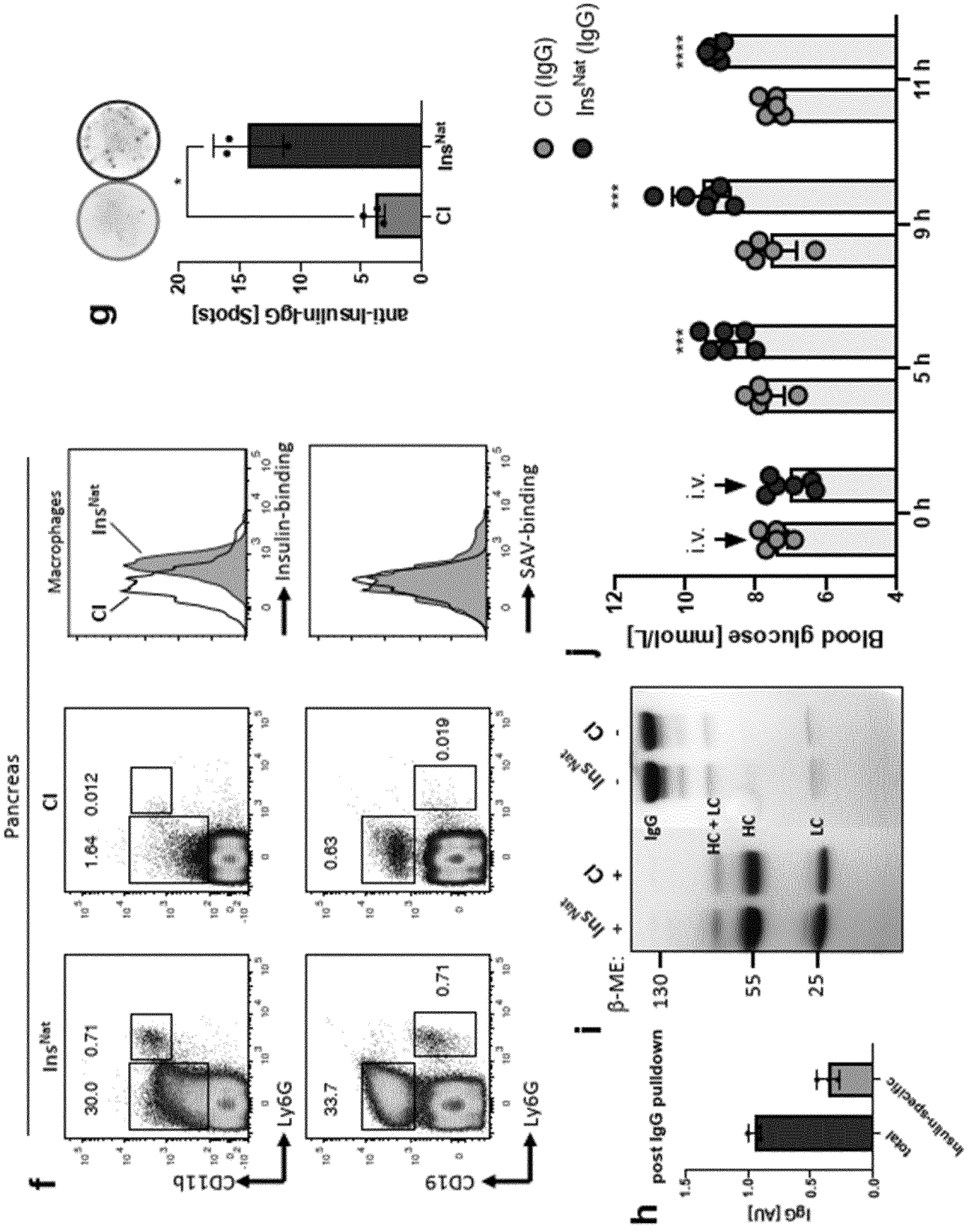


FIGURE 6

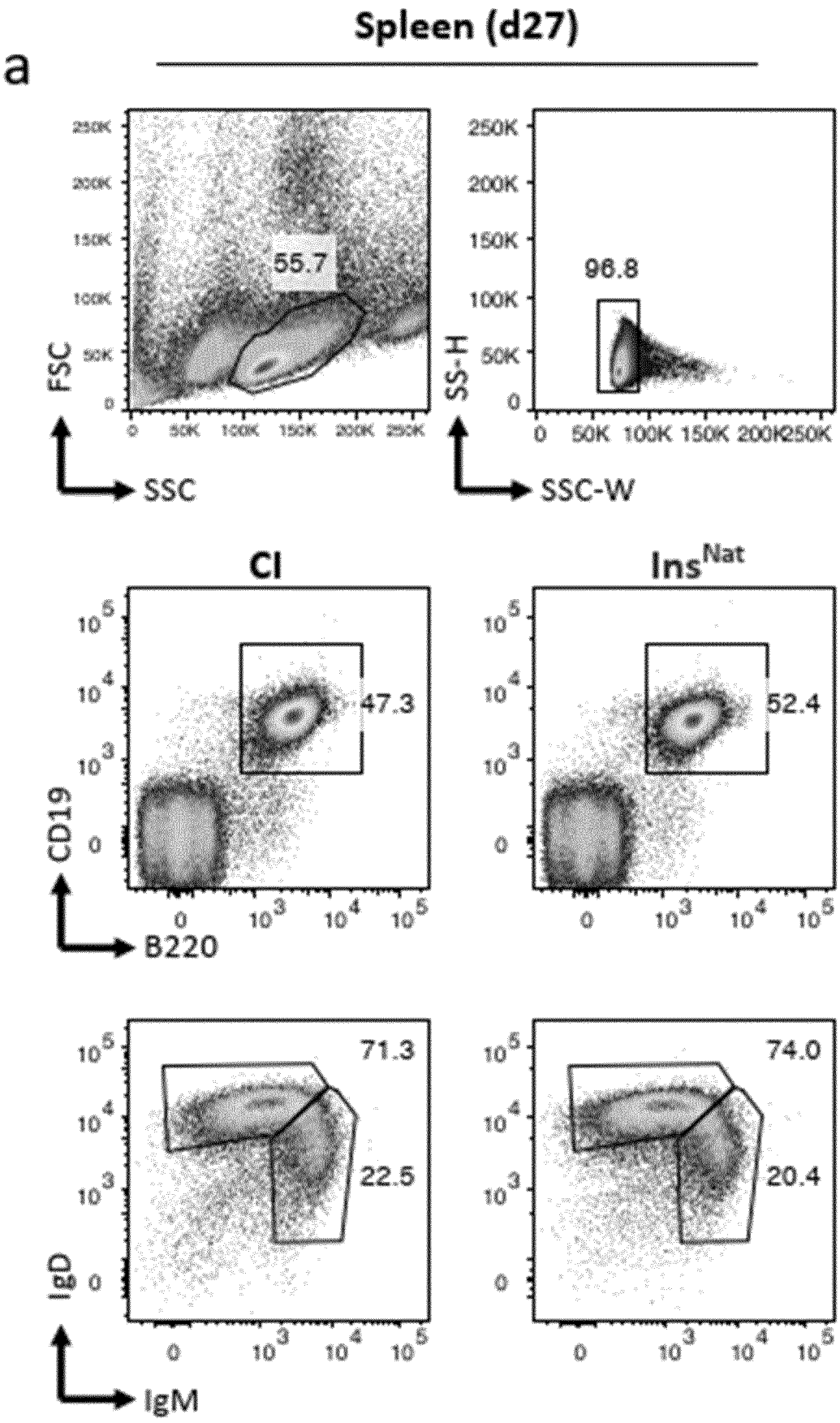

a
Spleen (d27)
55.7
FSC
SSC
96.8
SS-H
SSC-W
CI
47.3
CD19
B220
Ins^Nat
52.4
71.3
22.5
IgD
IgM
74.0
20.4

FIGURE 7

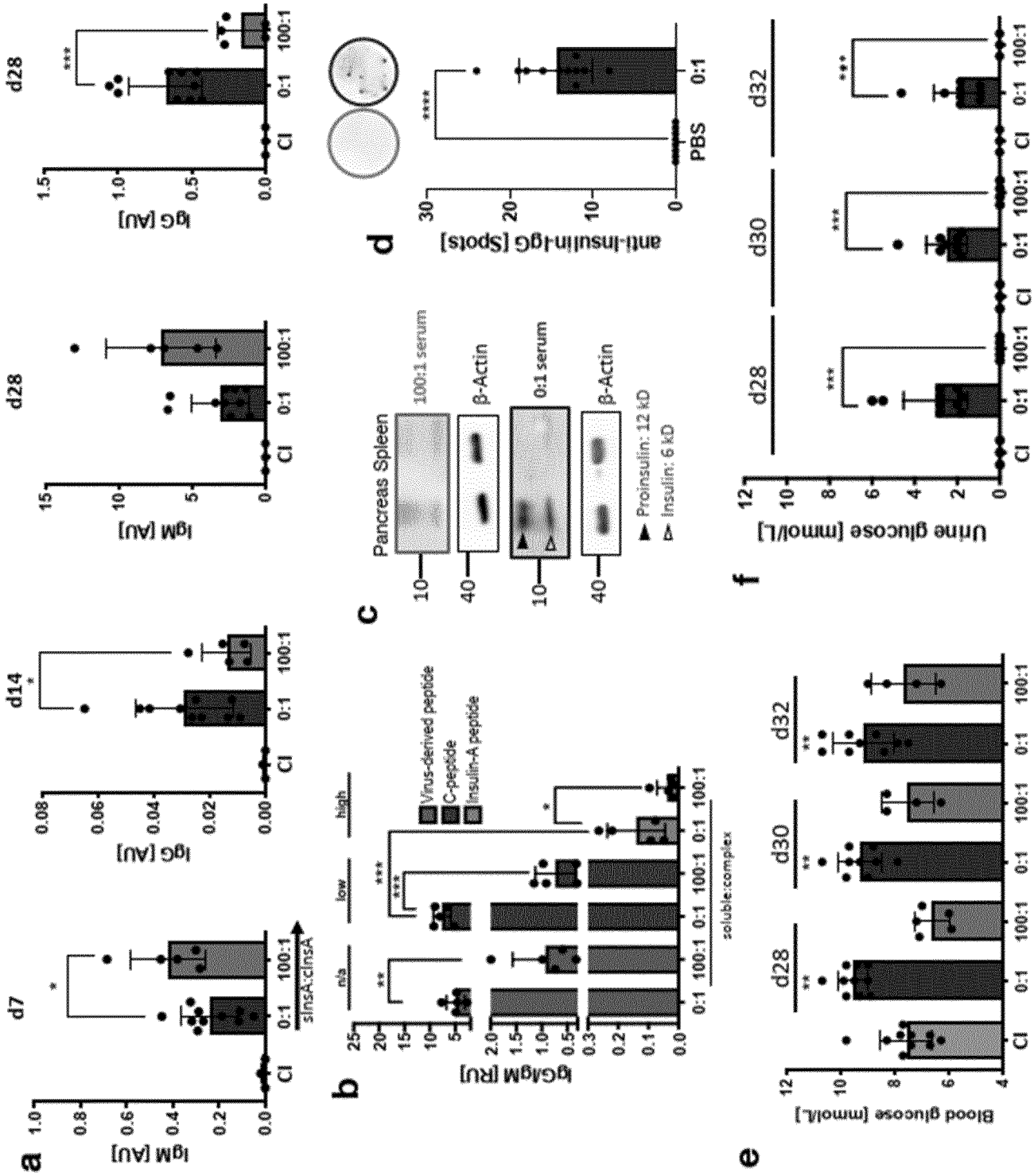

a
IgM [AU]
IgG [AU]
d7
d14
d28
Cl
0:1
100:1
sInsA:clnsA
b
IgG/IgM [RU]
n/a
low
high
Virus-derived peptide
C-peptide
Insulin-A peptide
soluble:complex
c
Pancreas Spleen
100:1 serum
0:1 serum
β-Actin
Proinsulin: 12 kD
Insulin: 6 kD
d
anti-Insulin-IgG [Spots]
PBS
e
Blood glucose [mmol/L]
d28
d30
d32
f
Urine glucose [mmol/L]

FIGURE 7 cont.

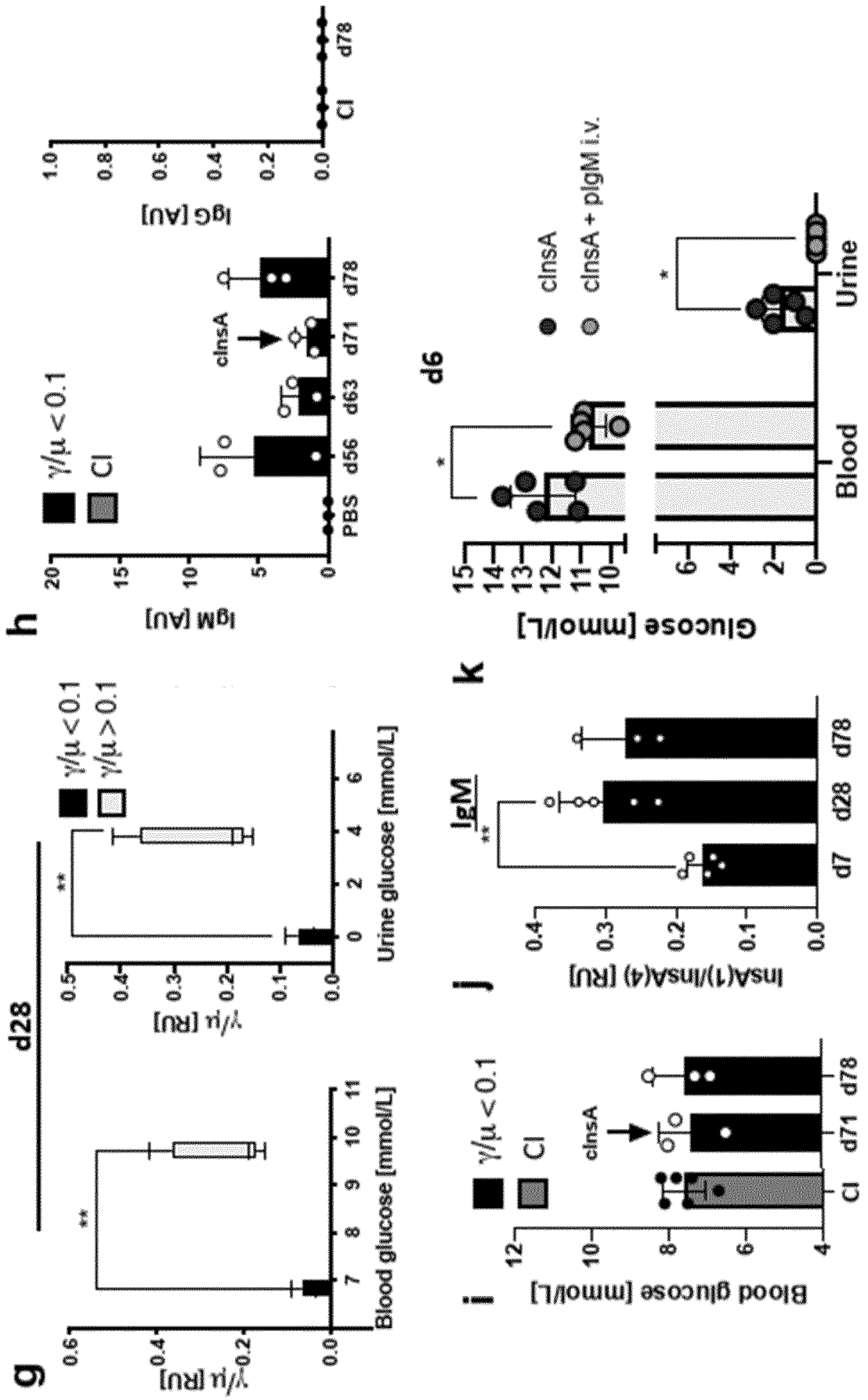


FIGURE 8

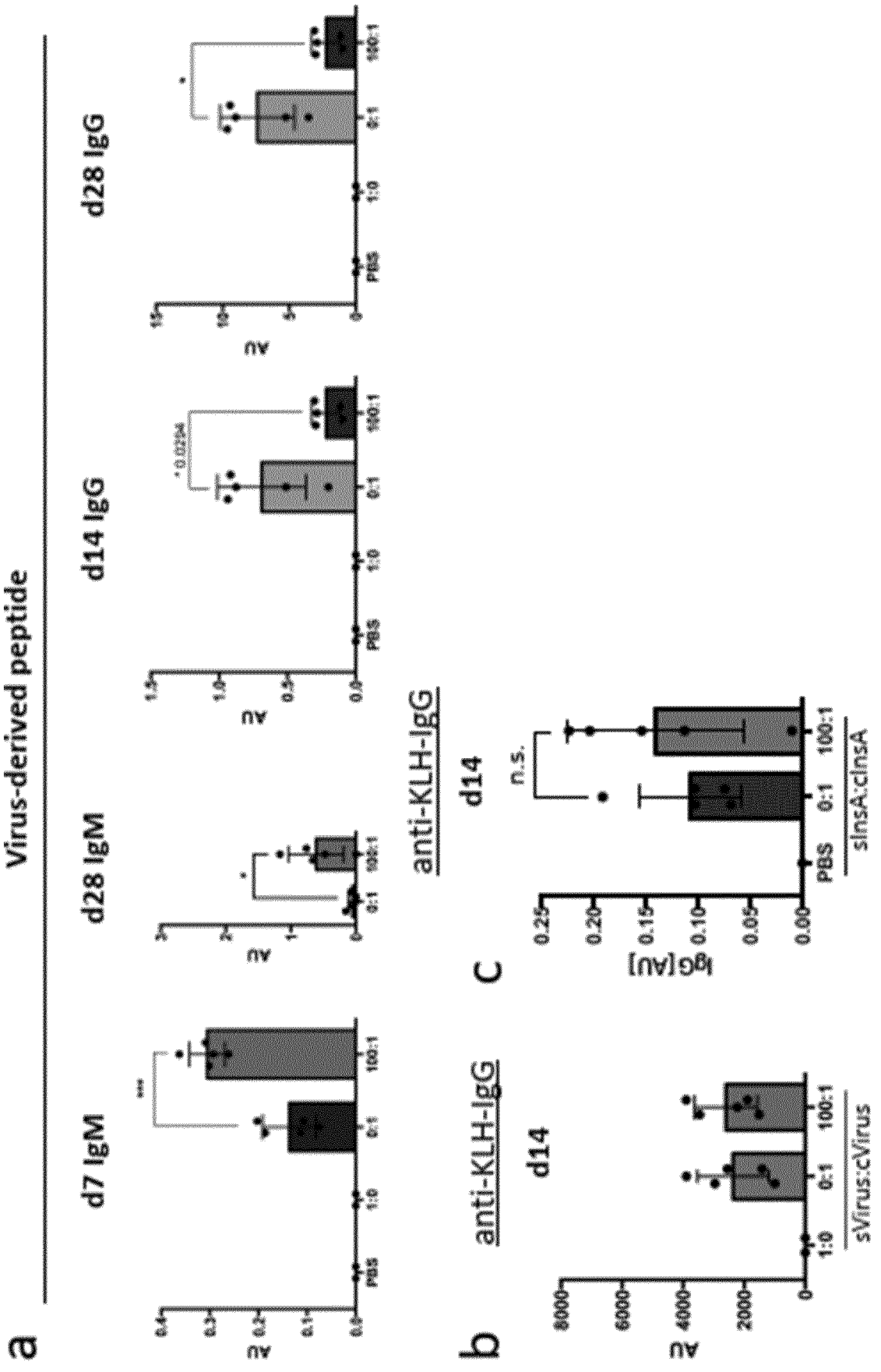

a
Virus-derived peptide
d7 IgM
d28 IgM
d14 IgG
d28 IgG
AU
PBS
1:0
0:1
100:1
b
anti-KLH-IgG
d14
sVirus:cVirus
c
anti-KLH-IgG
d14
n.s.
IgG [AU]
sInsA:cInsA

FIGURE 9

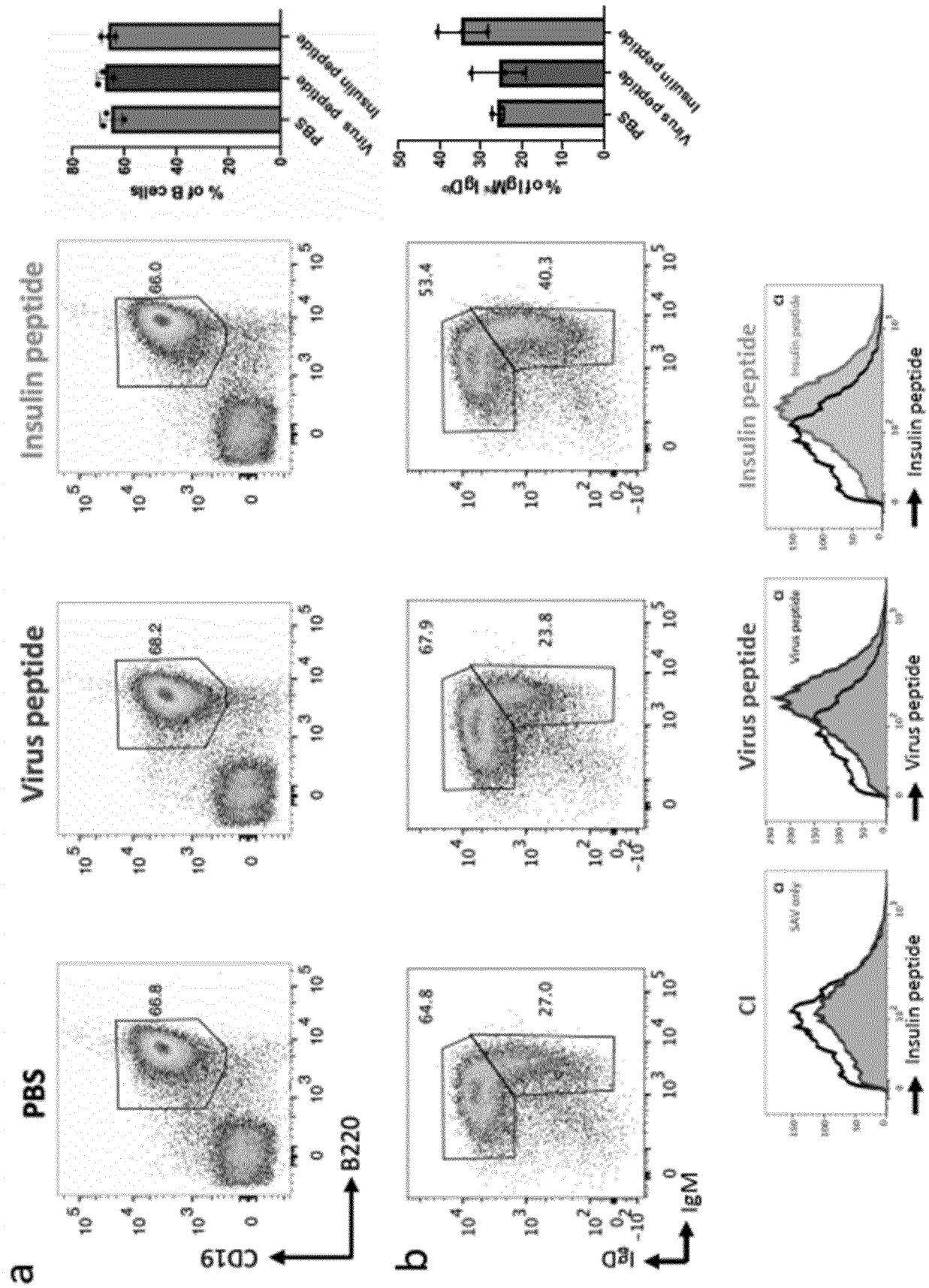

a
PBS
Virus peptide
Insulin peptide
CD19
B220
66.8
68.2
66.0
b
IgD
IgM
64.8
27.0
67.9
23.8
53.4
40.3
% of IgM+ IgD+
% of B cells
PBS
Virus peptide
Insulin peptide
CI
Virus peptide
Insulin peptide

FIGURE 9 cont.

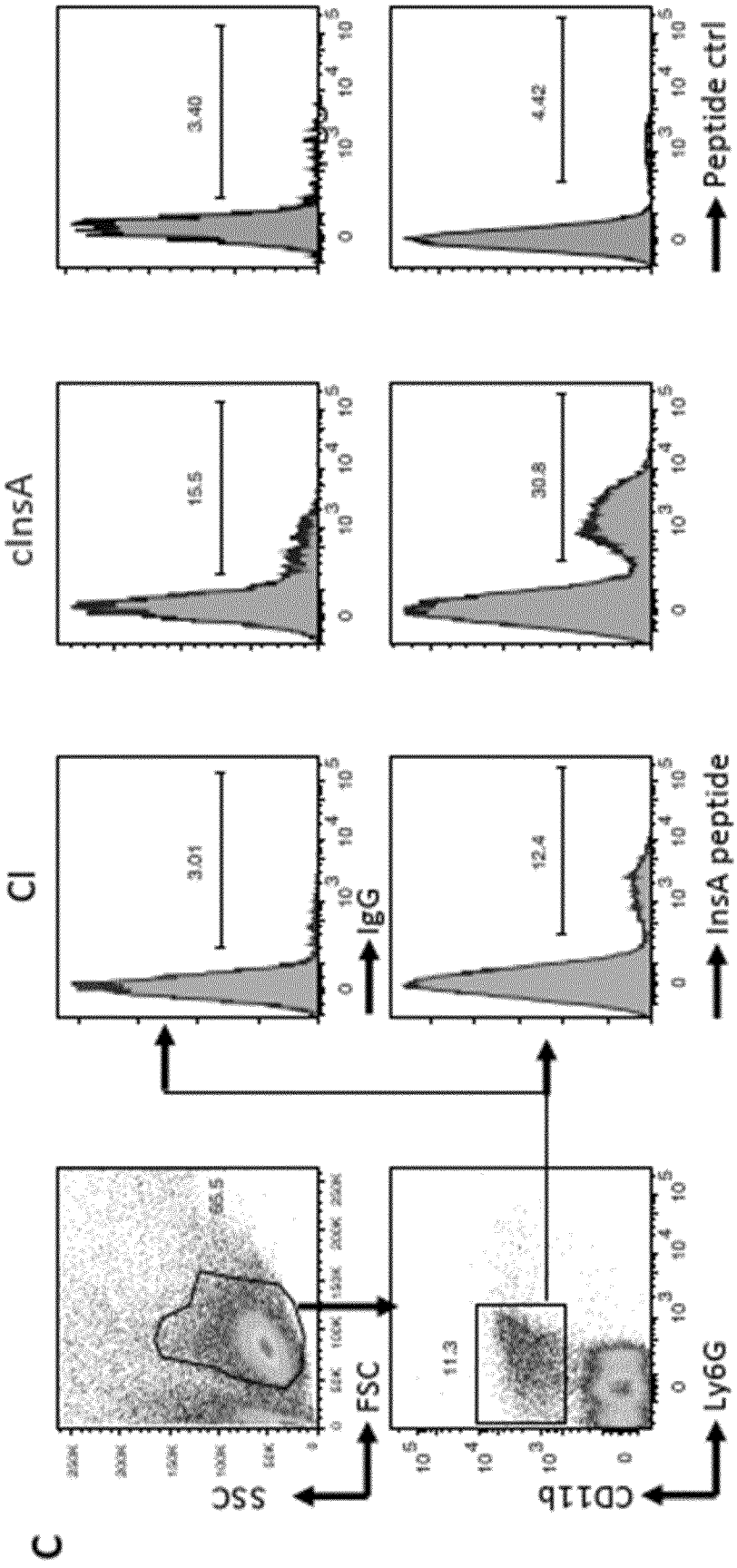


FIGURE 10

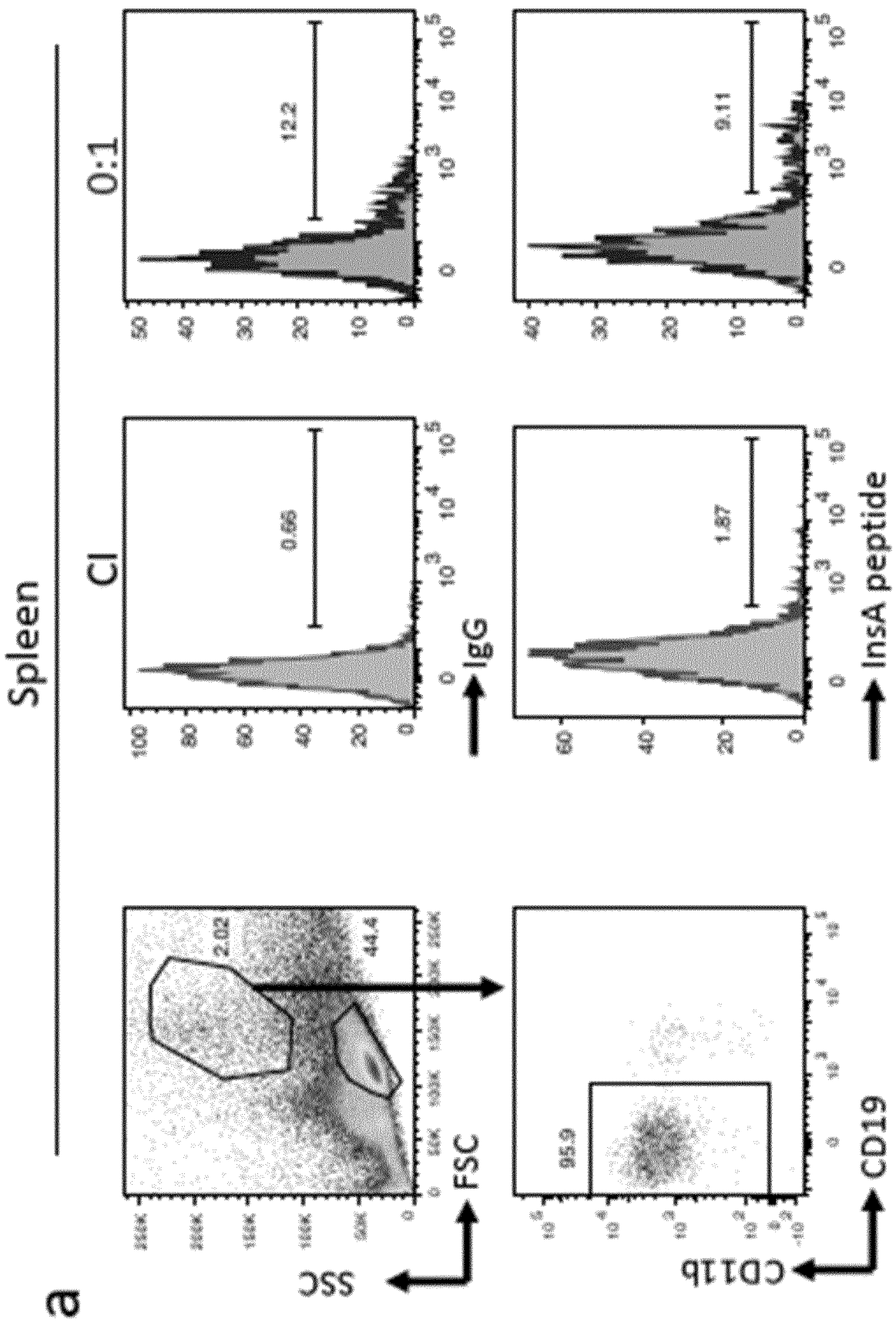

a
Spleen
SSC
FSC
2.02
44.4
CD11b
CD19
95.9
CI
0:1
IgG
InsA peptide
0.65
12.2
1.87
9.11

FIGURE 11

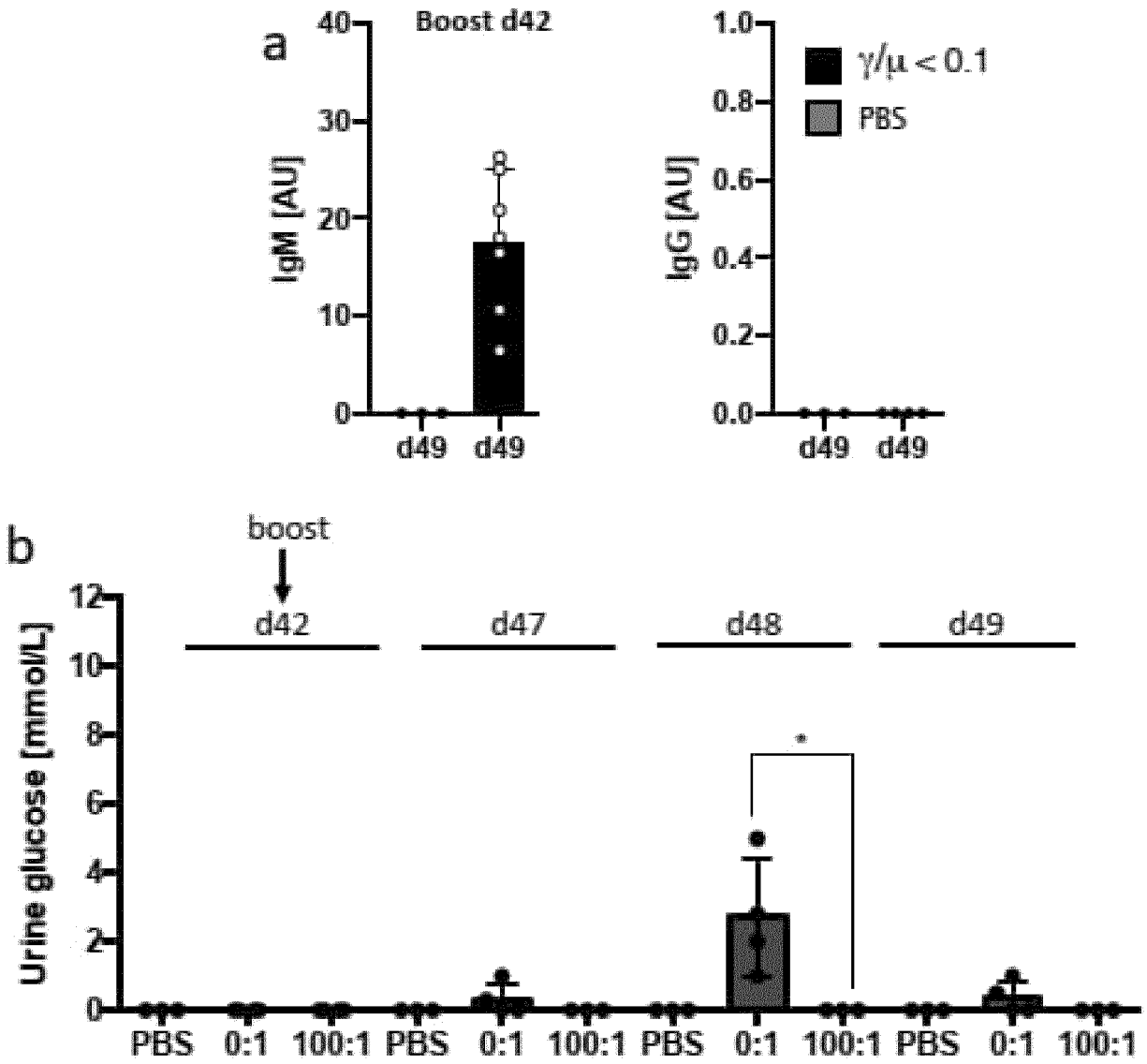

a
Boost d42
IgM [AU]
40
30
20
10
0
d49
d49
IgG [AU]
1.0
0.8
0.6
0.4
0.2
0.0
γ/μ < 0.1
PBS
d49
d49
b
boost
d42
d47
d48
d49
Urine glucose [mmol/L]
12
10
8
6
4
2
0
*
PBS
0:1
100:1
PBS
0:1
100:1
PBS
0:1
100:1
PBS
0:1
100:1

FIGURE 12

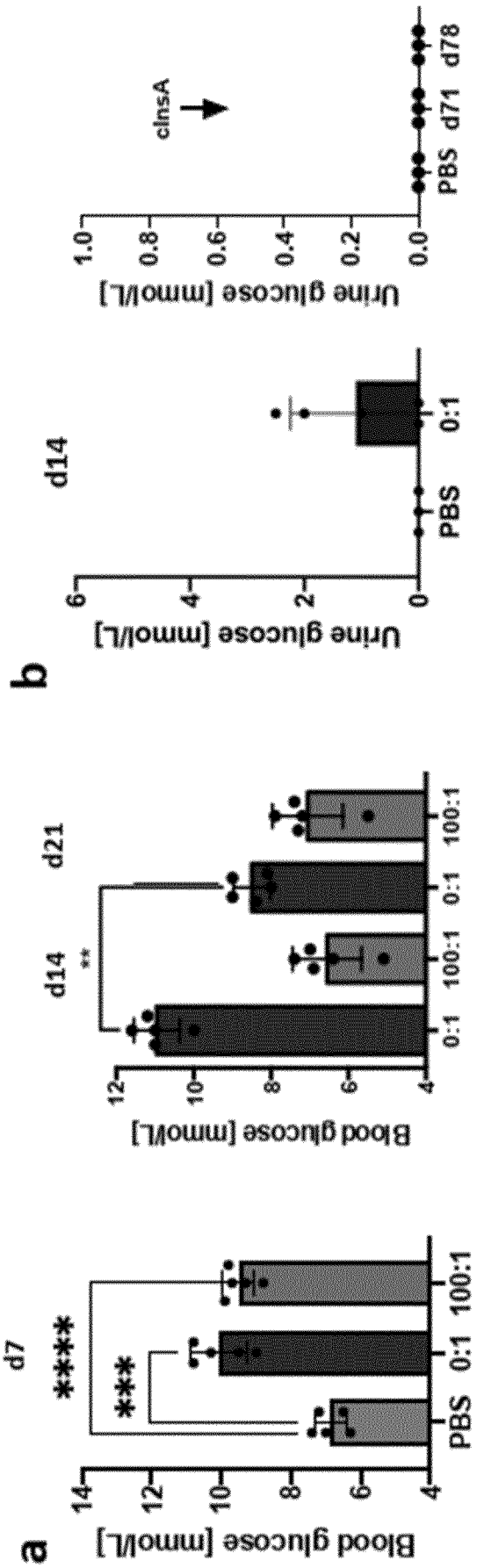

a
Blood glucose [mmol/L]
14
12
10
8
6
4
d7
****
***
PBS
0:1
100:1
Blood glucose [mmol/L]
12
10
8
6
4
d14
d21
**
0:1
100:1
0:1
100:1
b
Urine glucose [mmol/L]
6
4
2
0
d14
PBS
0:1
Urine glucose [mmol/L]
1.0
0.8
0.6
0.4
0.2
0.0
clnsA
PBS
d71
d78

FIGURE 13

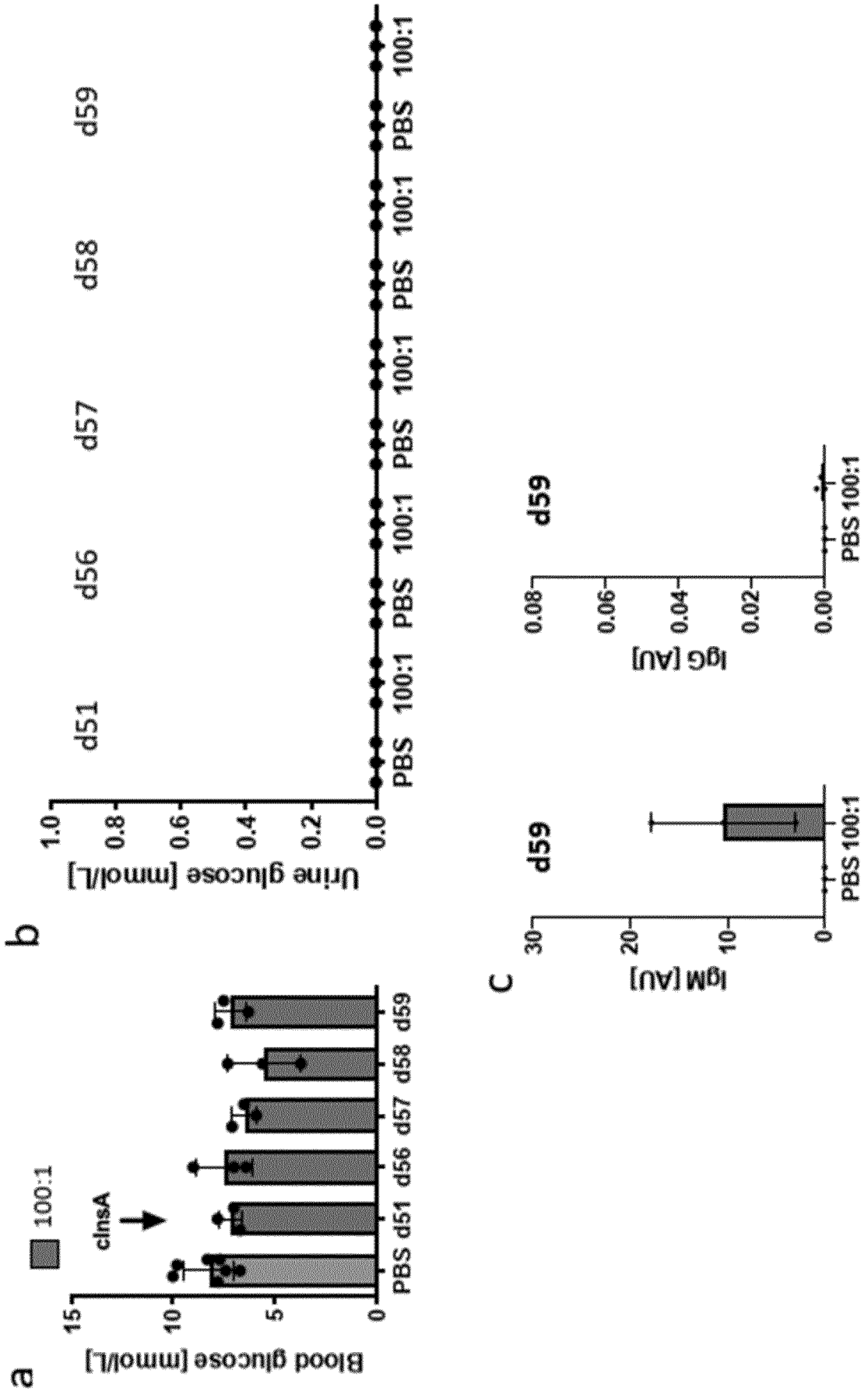

a
Blood glucose [mmol/L]
15
10
5
0
100:1
clnsA
PBS d51 d56 d57 d58 d59
b
Urine glucose [mmol/L]
1.0
0.8
0.6
0.4
0.2
0.0
d51 d56 d57 d58 d59
PBS 100:1 PBS 100:1 PBS 100:1 PBS 100:1 PBS 100:1
C
IgM [AU]
30
20
10
0
d59
PBS 100:1
IgG [AU]
0.08
0.06
0.04
0.02
0.00
d59
PBS 100:1

FIGURE 14

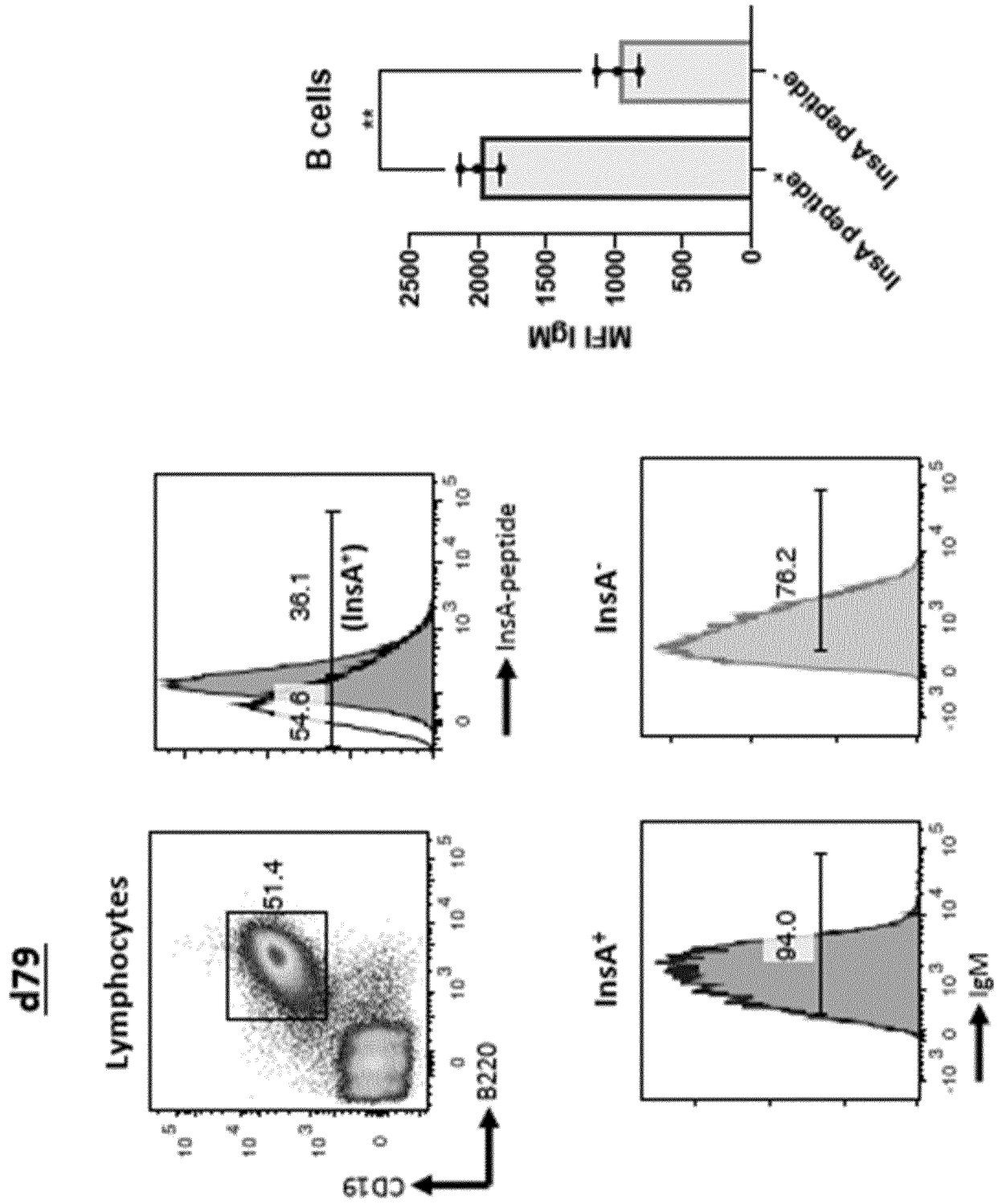

d79
Lymphocytes
51.4
CD19
B220
36.1
(InsA+)
54.6
InsA-peptide
InsA+
94.0
InsA-
76.2
IgM
B cells
**
MFI IgM
2500
2000
1500
1000
500
0
InsA peptide+
InsA peptide-

FIGURE 15

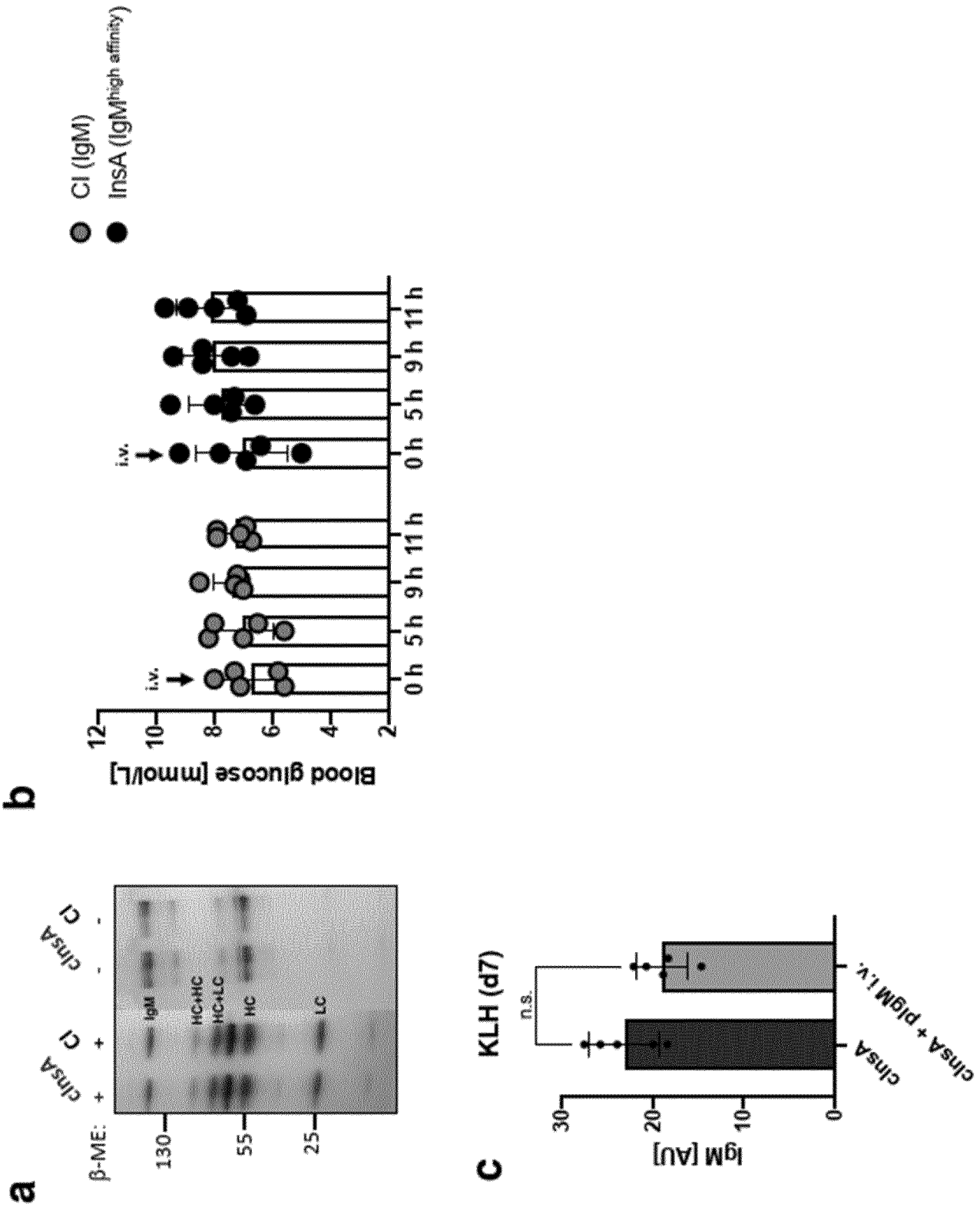

a
β-ME:
clnsA
Cl
clnsA
Cl
IgM
HC+HC
HC+LC
HC
LC
130
55
25
b
Cl (IgM)
InsA (IgM high affinity)
Blood glucose [mmol/L]
i.v.
0 h
5 h
9 h
11 h
c
KLH (d7)
n.s.
IgM [AU]
clnsA
clnsA + pIgM i.v.

FIGURE 16

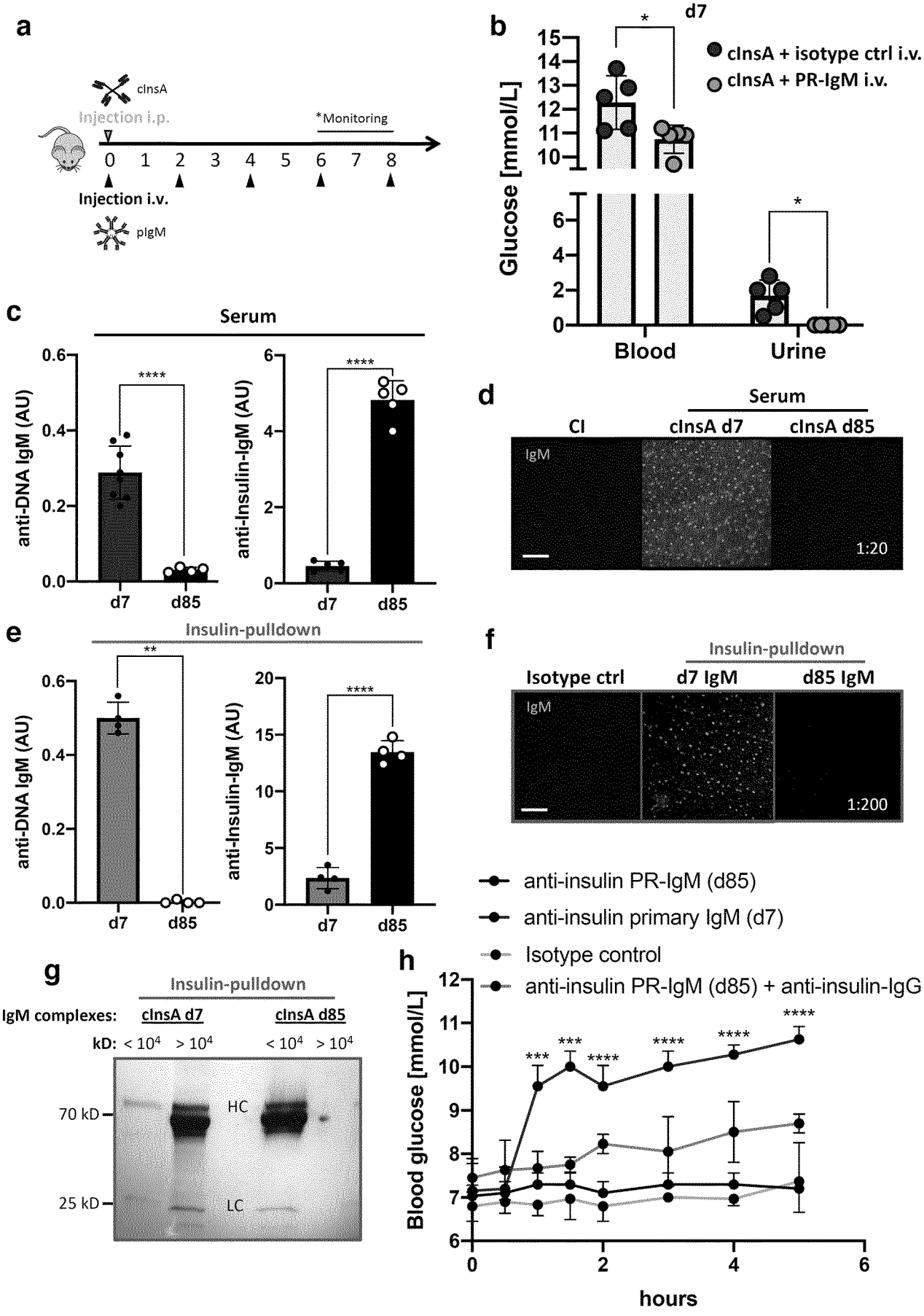


FIGURE 17

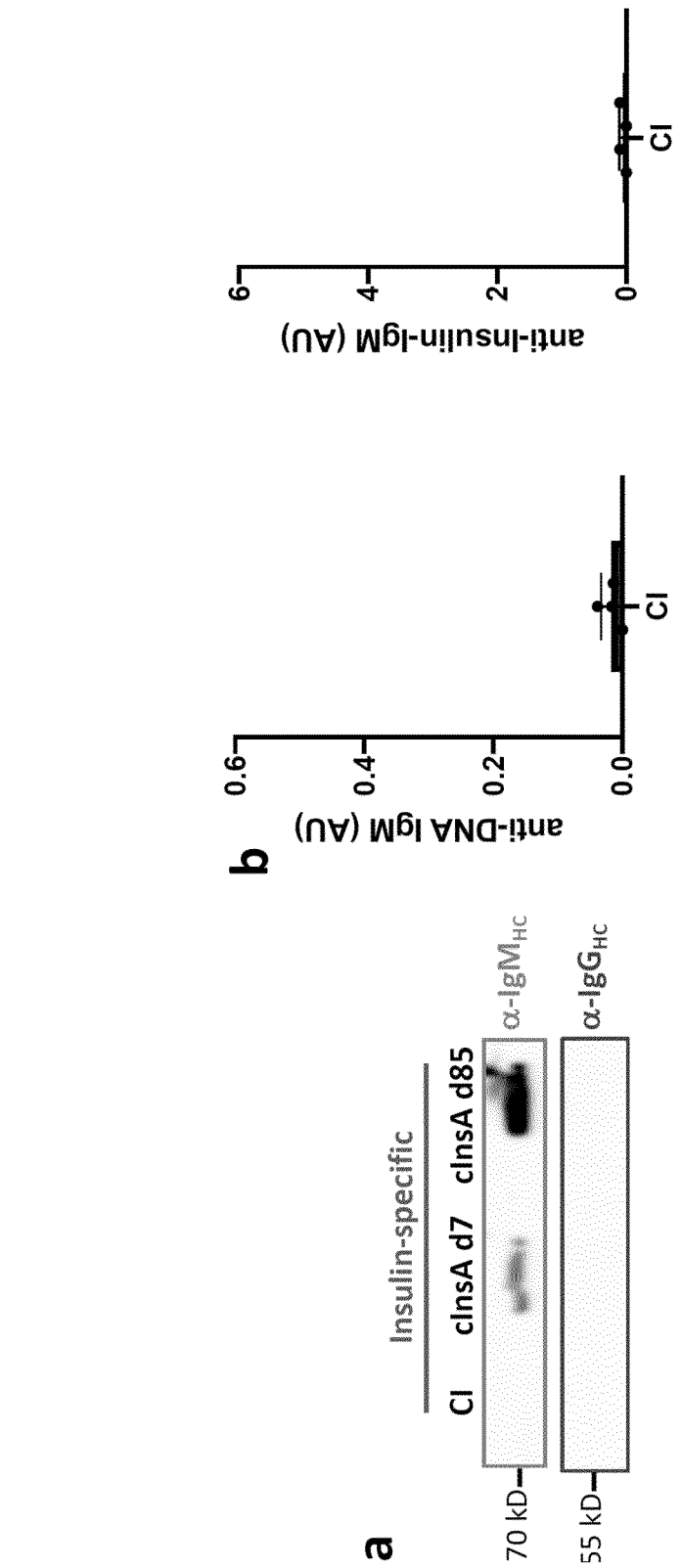
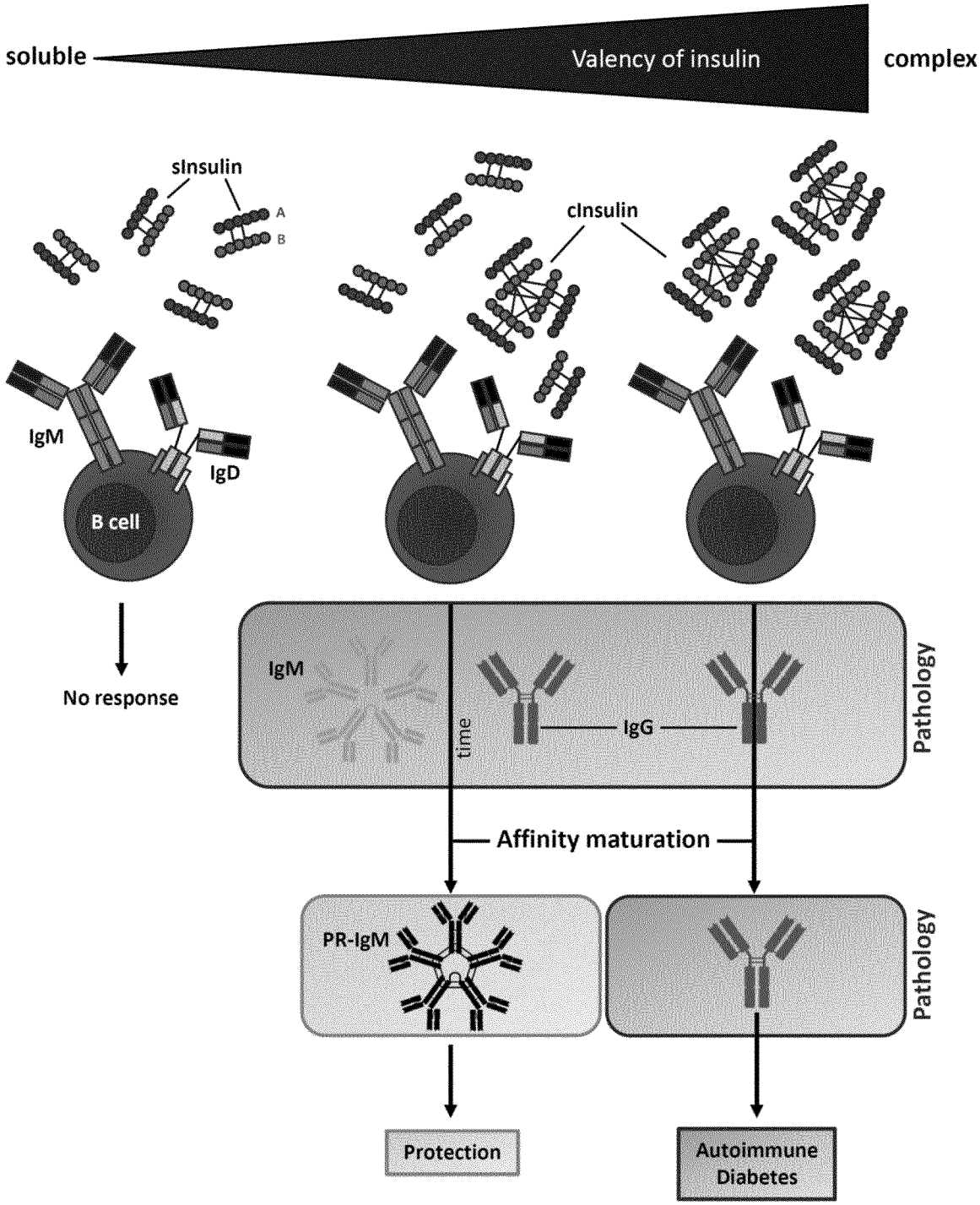

a
Insulin-specific
CI
clnsA d7
clnsA d85
α-IgM$_{HC}$
α-IgG$_{HC}$
70 kD
55 kD
b
anti-DNA IgM (AU)
0.0
0.2
0.4
0.6
CI
anti-Insulin-IgM (AU)
0
2
4
6
CI

FIGURE 18



5

FIGURE 19

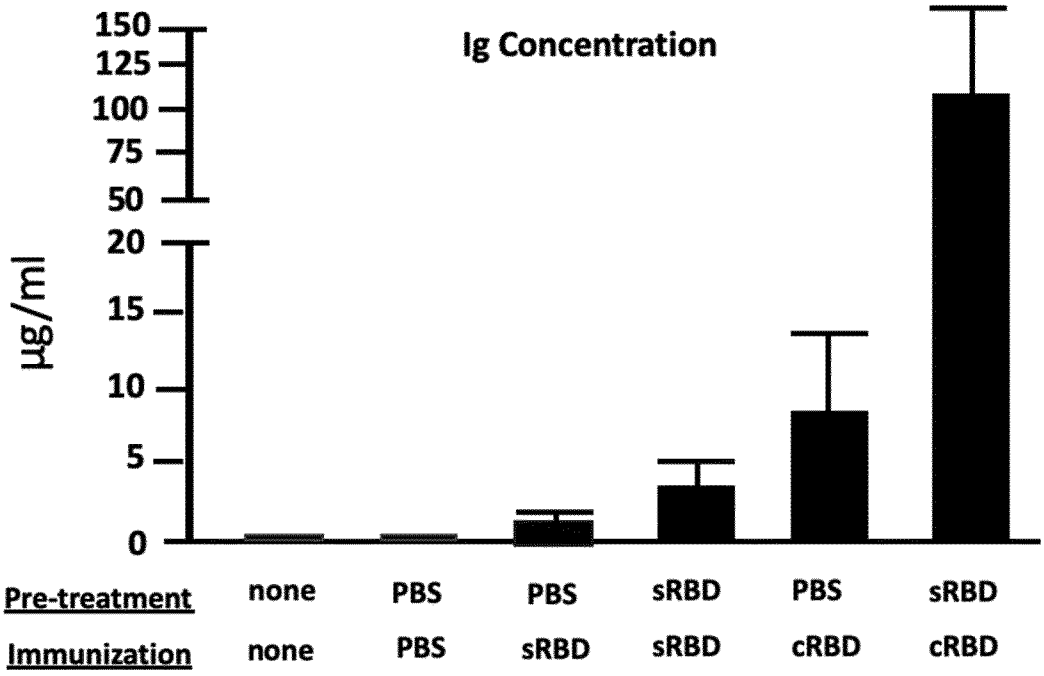

Ig Concentration
µg/ml
150
125
100
75
50
20
15
10
5
0
Pre-treatment none PBS PBS sRBD PBS sRBD
Immunization none PBS sRBD sRBD cRBD cRBD

FIGURE 20

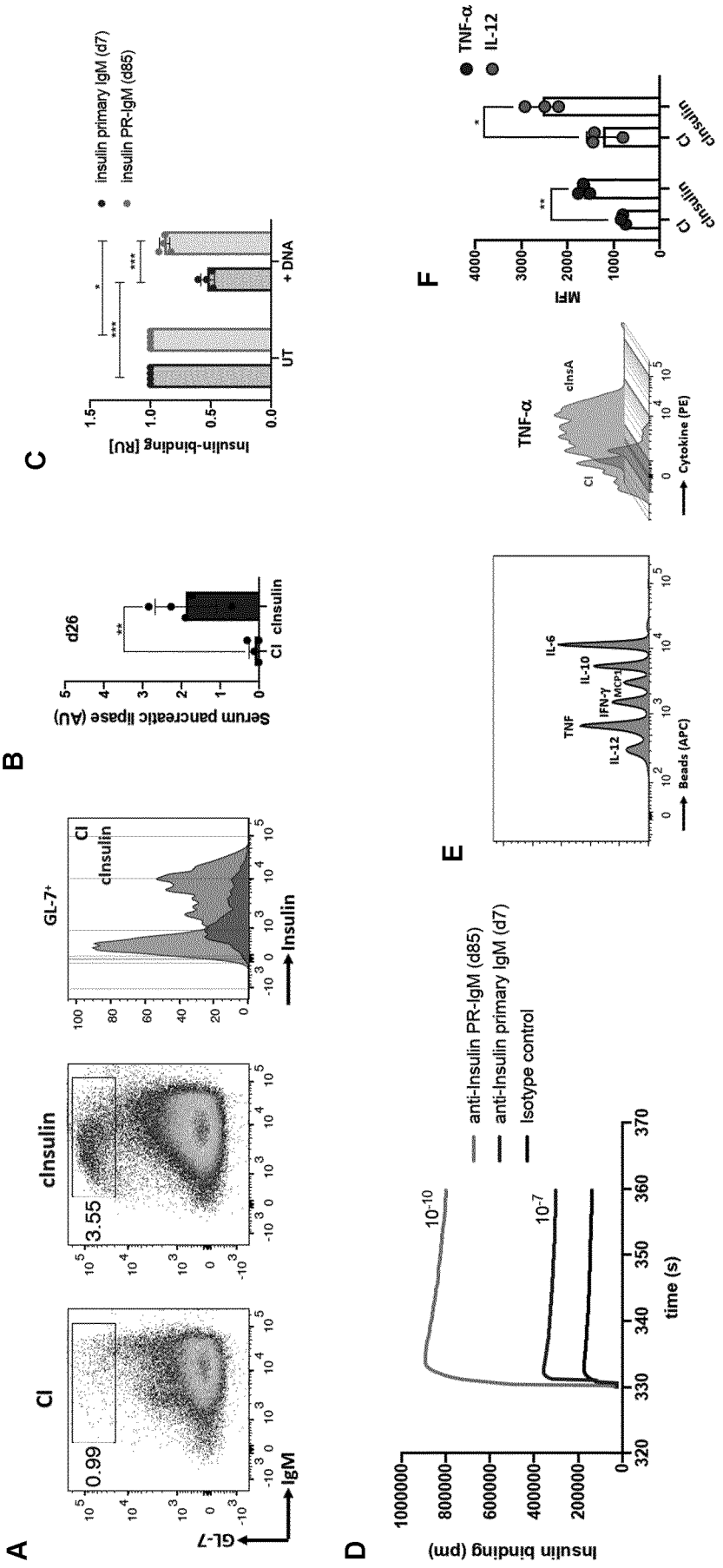


FIGURE 21

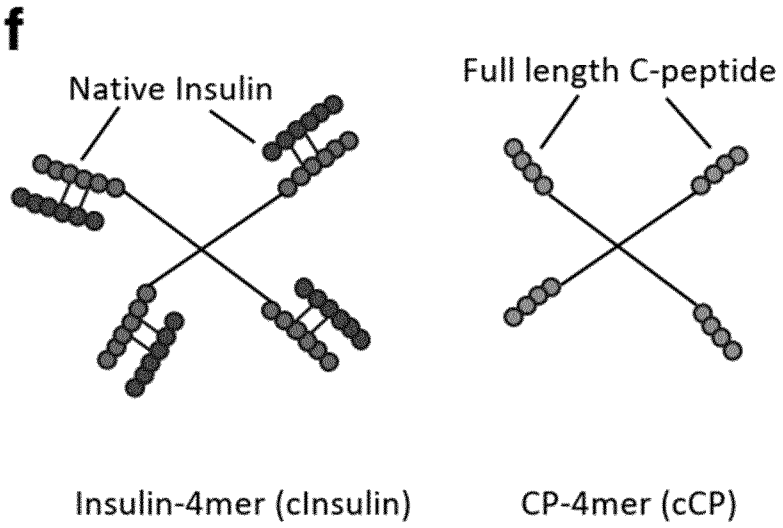
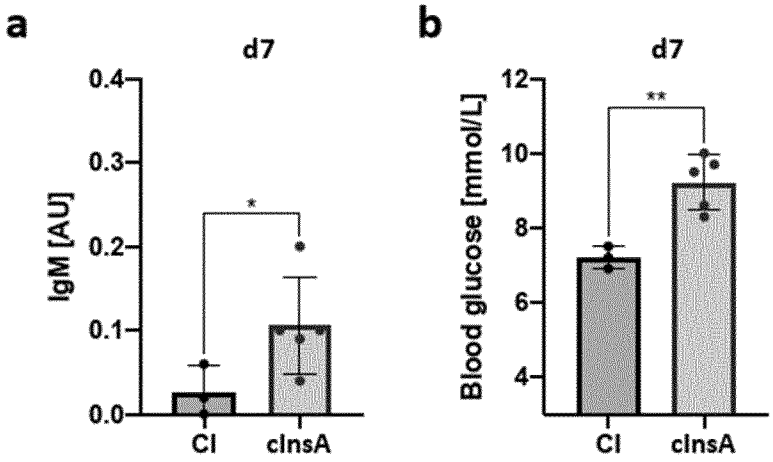

f
Native Insulin
Full length C-peptide
Insulin-4mer (cInsulin)
CP-4mer (cCP)

Figure 22


a
d7
IgM [AU]
0.4
0.3
0.2
0.1
0.0
*
Cl
cInsA
b
d7
Blood glucose [mmol/L]
12
10
8
6
4
**
Cl
cInsA

5

Figure 23

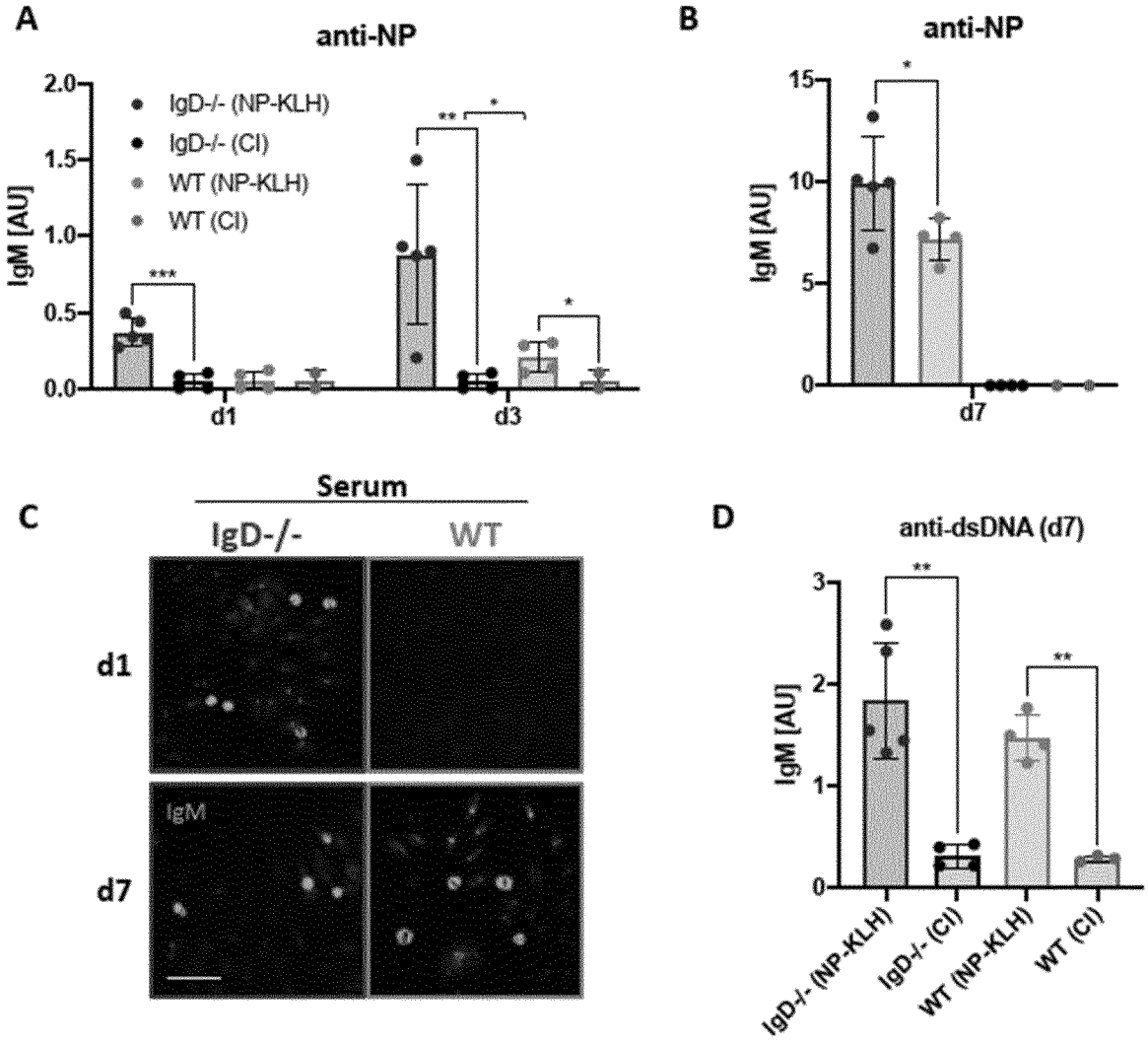

A
anti-NP
IgD-/- (NP-KLH)
IgD-/- (Cl)
WT (NP-KLH)
WT (Cl)
IgM [AU]
2.0
1.5
1.0
0.5
0.0
***
**
*
*
d1
d3
B
anti-NP
IgM [AU]
15
10
5
0
*
d7
C
Serum
IgD-/-
WT
d1
d7
IgM
D
anti-dsDNA (d7)
IgM [AU]
3
2
1
0
**
**
IgD-/- (NP-KLH)
IgD-/- (Cl)
WT (NP-KLH)
WT (Cl)

Figure 24

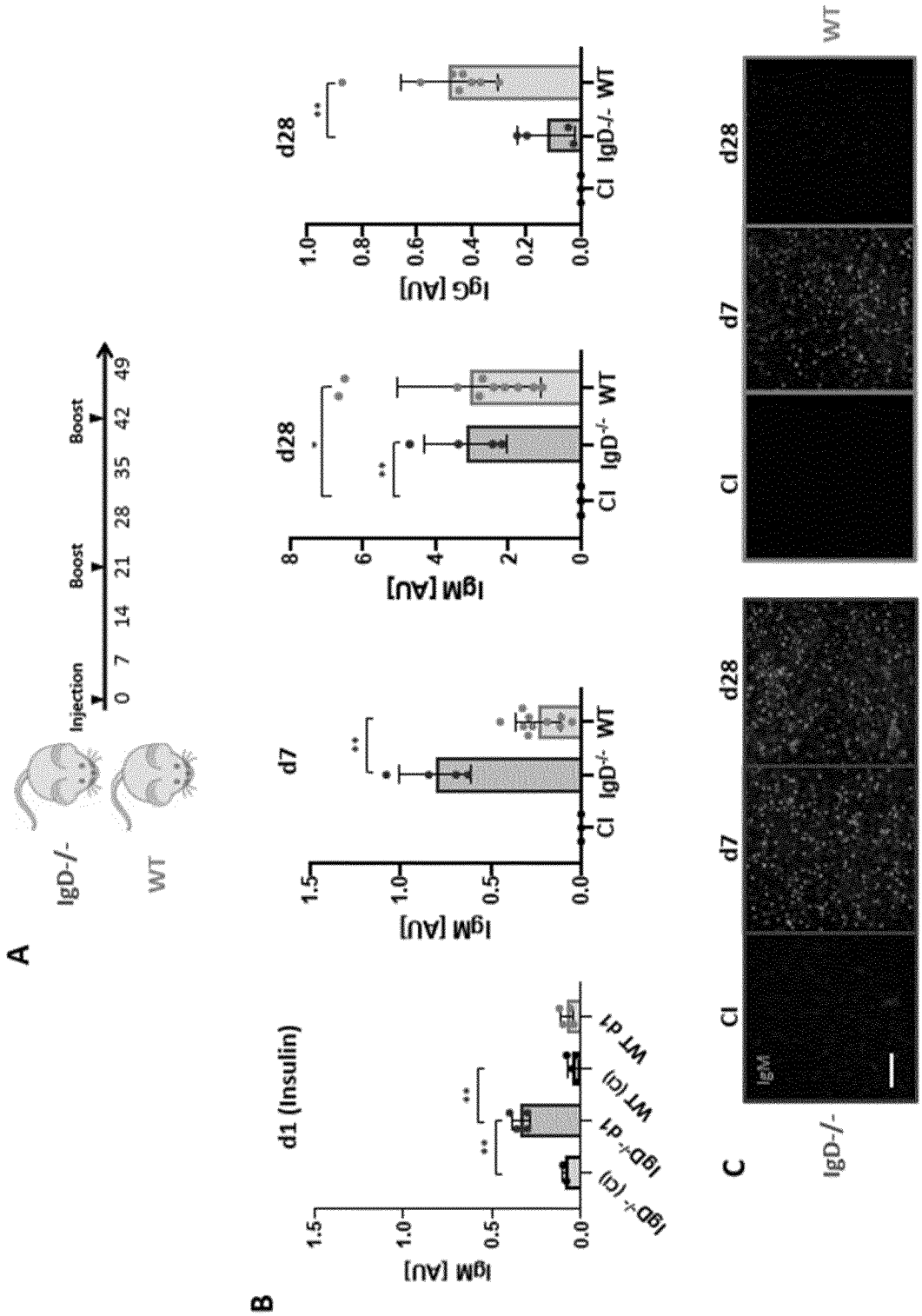

A
IgD-/-
WT
Injection
Boost
Boost
0 7 14 21 28 35 42 49
B
d1 (Insulin)
IgM [AU]
IgD-/- (CI)
IgD-/- d1
WT (CI)
WT d1
d7
IgM [AU]
CI
IgD-/-
WT
d28
IgM [AU]
IgG [AU]
C
CI
d7
d28
IgM
IgD-/-
WT

Figure 25

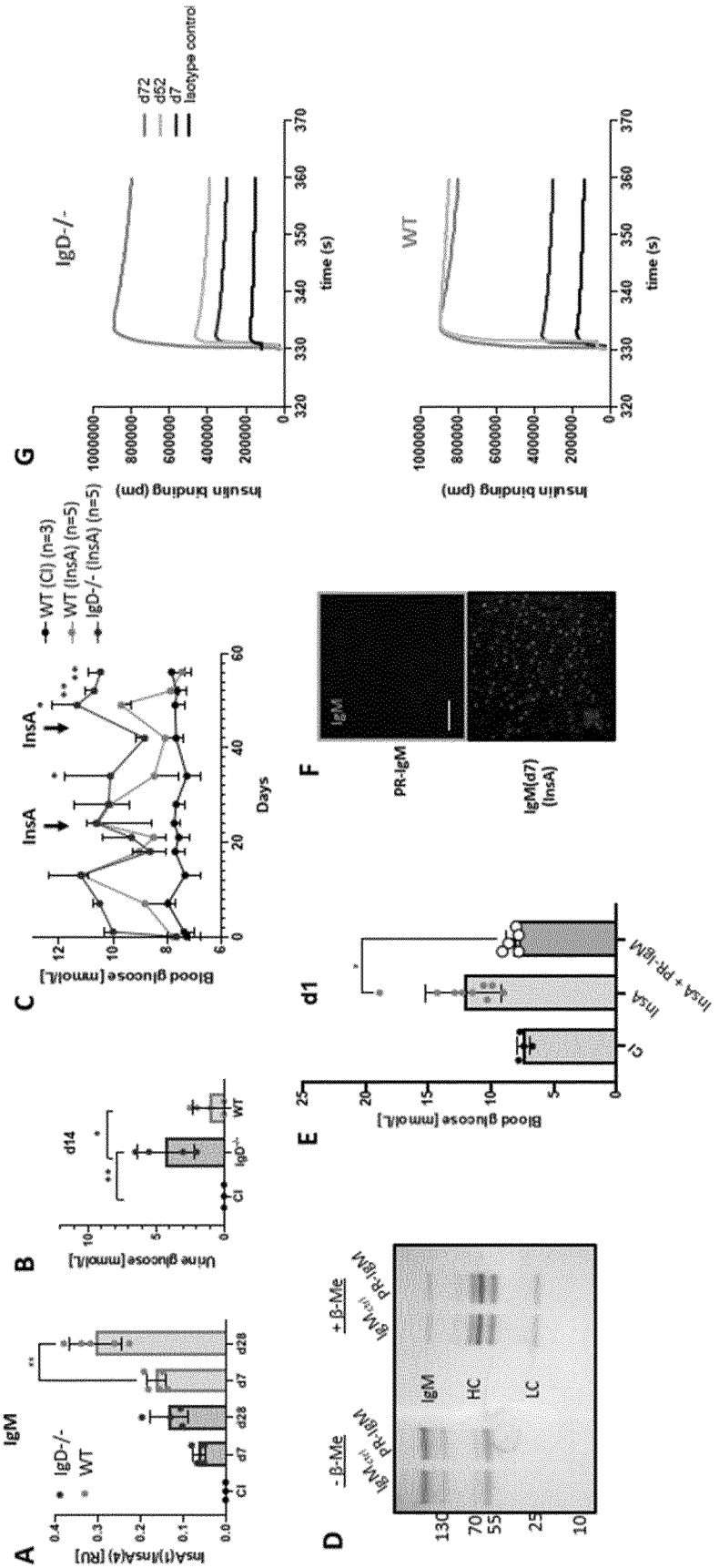

A
IgM
IgD-/-
WT
InsA(1)/InsA(4) [RU]
Cl
d7
d28
d7
d28
B
Urine glucose [mmol/L]
d14
Cl
IgD-/-
WT
C
InsA
InsA
WT (Cl) (n=3)
WT (InsA) (n=5)
IgD-/- (InsA) (n=5)
Blood glucose [mmol/L]
Days
D
-β-Me
+β-Me
IgM
HC
LC
E
d1
Blood glucose [mmol/L]
Cl
InsA
InsA + PR-IgM
F
IgM
PR-IgM
IgM(d7) (InsA)
G
IgD-/-
d72
d52
d7
Isotype control
Insulin binding (pm)
time (s)
WT
Insulin binding (pm)
time (s)

Figure 26

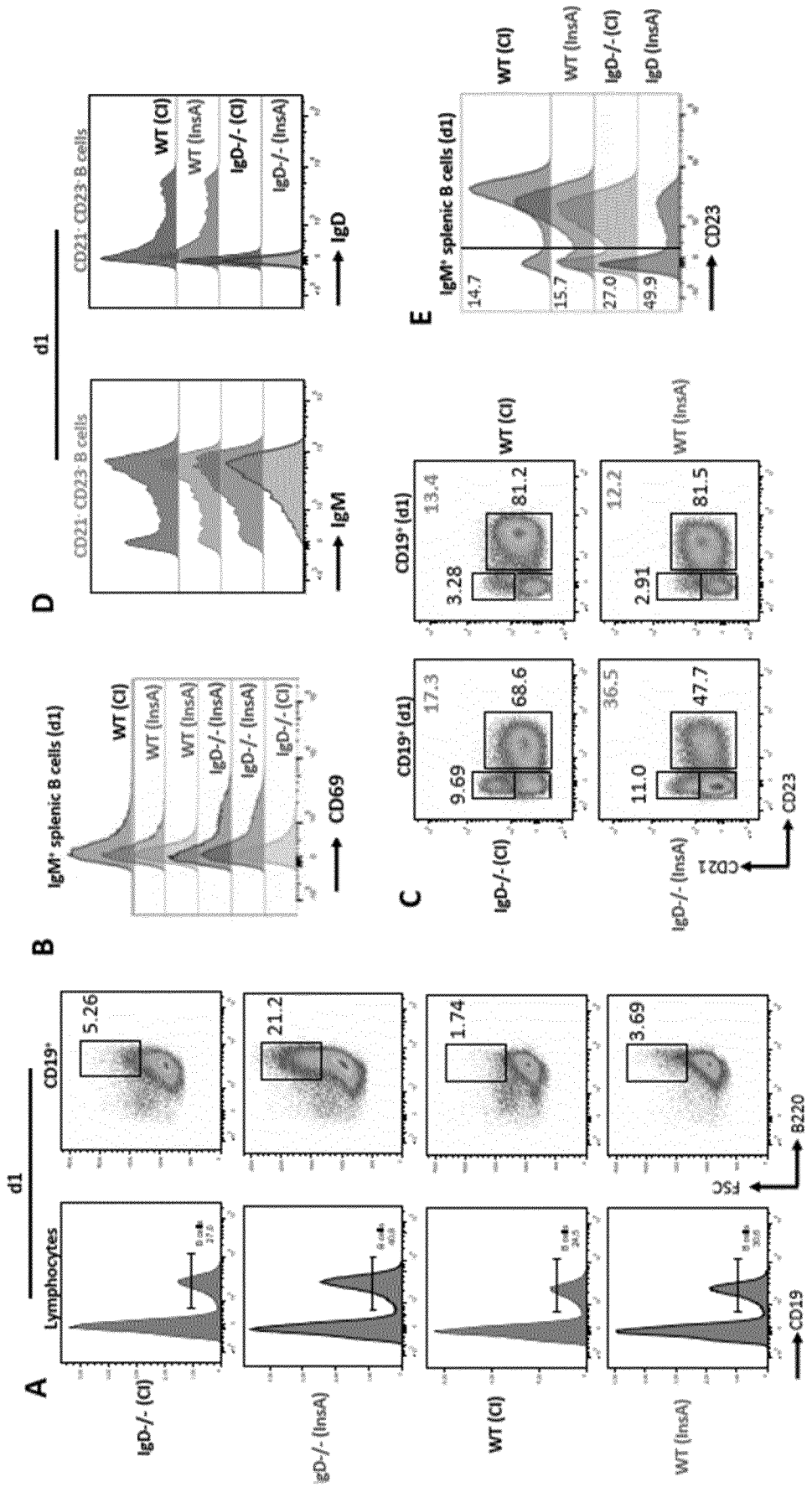

A
d1
Lymphocytes
CD19+
5.26
21.2
1.74
3.69
IgD-/- (CI)
IgD-/- (InsA)
WT (CI)
WT (InsA)
FSC
B220
CD19
B
IgM+ splenic B cells (d1)
WT (CI)
WT (InsA)
IgD-/- (InsA)
IgD-/- (CI)
CD69
C
CD19+ (d1)
17.3
9.69
68.6
36.5
11.0
47.7
IgD-/- (CI)
IgD-/- (InsA)
CD21
CD23
D
d1
CD21- CD23- B cells
WT (CI)
WT (InsA)
IgD-/- (CI)
IgD-/- (InsA)
IgD
IgM
E
IgM+ splenic B cells (d1)
14.7
15.7
27.0
49.9
WT (CI)
WT (InsA)
IgD-/- (CI)
IgD (InsA)
CD23

Figure 27

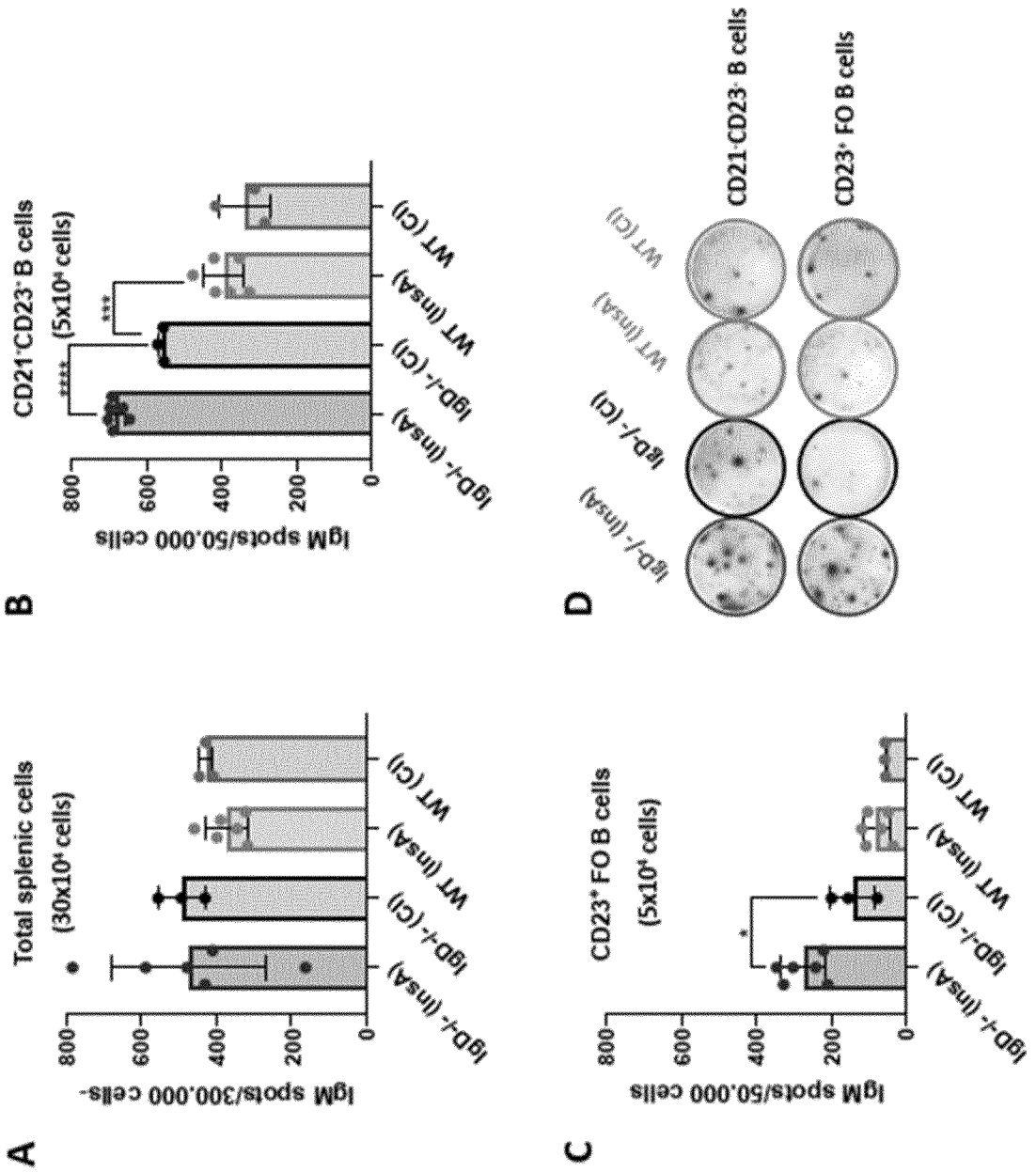

A
Total splenic cells
(30x10⁴ cells)
IgM spots/300,000 cells
800
600
400
200
0
IgD-/- (insA)
IgD-/- (CI)
WT (insA)
WT (CI)
B
CD21-CD23- B cells
(5x10⁴ cells)
IgM spots/50,000 cells
****
***
C
CD23+ FO B cells
(5x10⁴ cells)
IgM spots/50,000 cells
*
D
CD21-CD23- B cells
CD23+ FO B cells
IgD-/- (insA)
IgD-/- (CI)
WT (insA)
WT (CI)

Figure 28

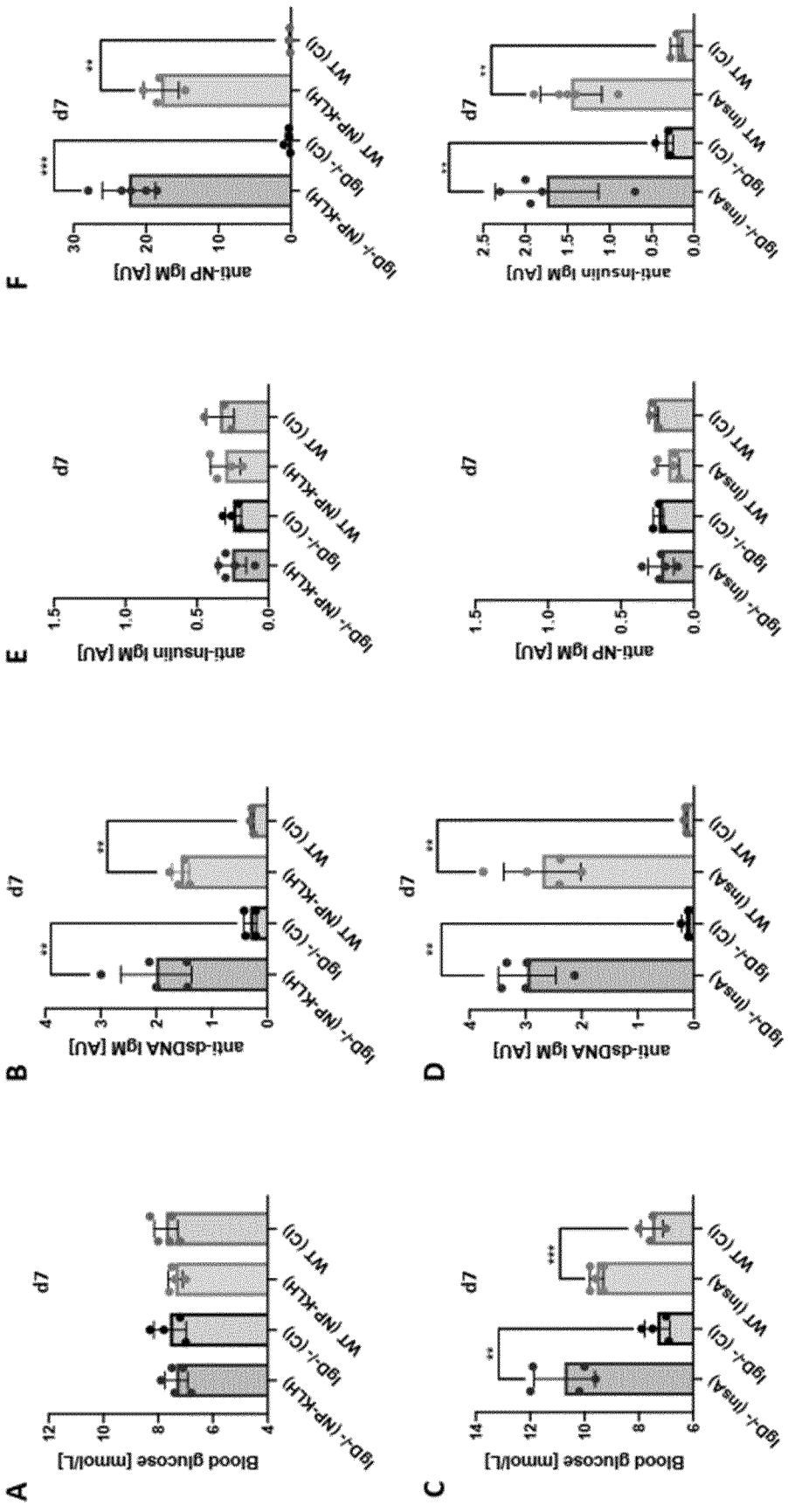

A
Blood glucose [mmol/L]
d7
Igλ-/- (NP-KLH)
Igλ-/- (Ci)
WT (NP-KLH)
WT (Ci)
B
anti-dsDNA IgM [AU]
C
Igλ-/- (insA)
WT (insA)
D
anti-NP IgM [AU]
E
anti-insulin IgM [AU]
F

Figure 29

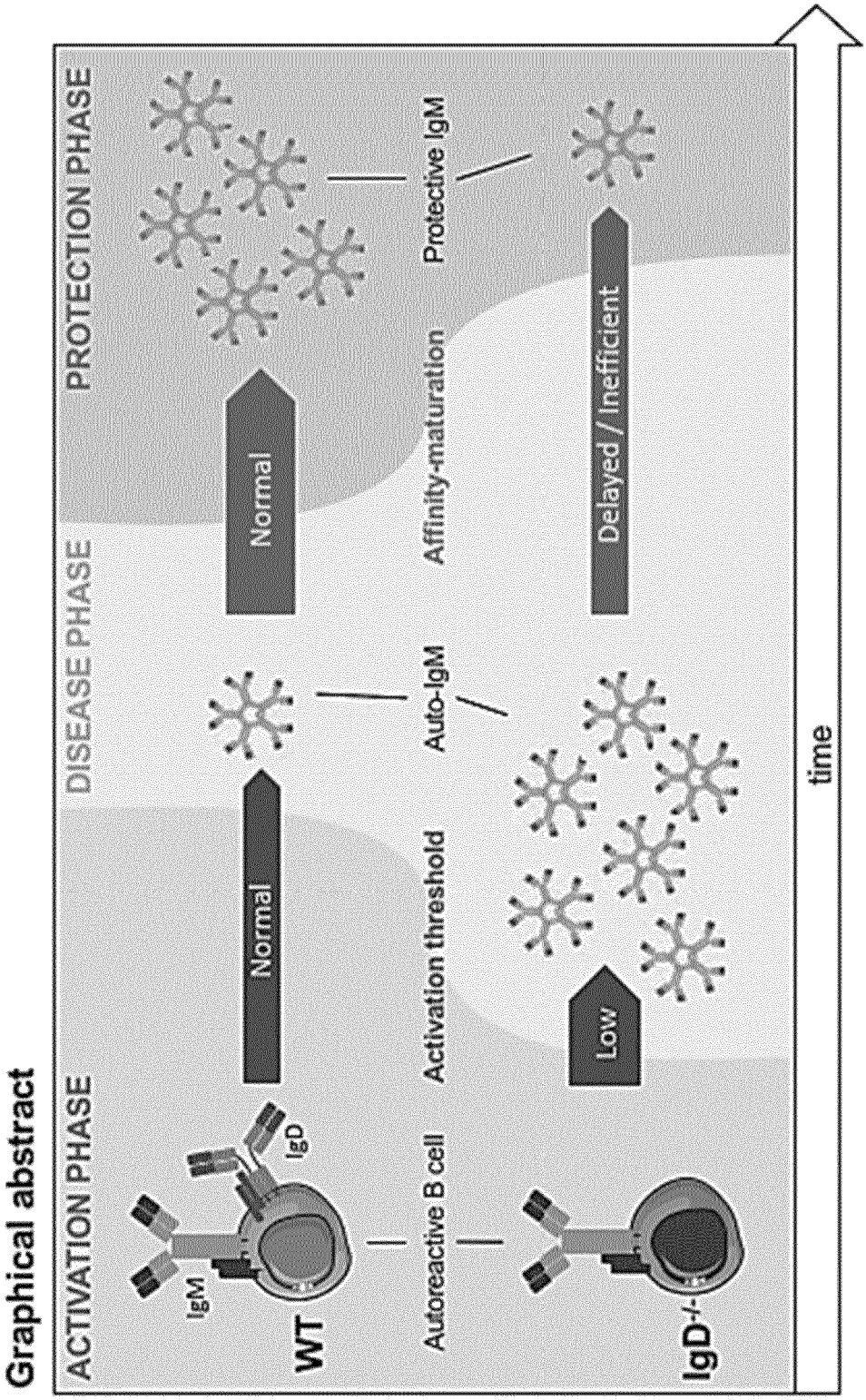
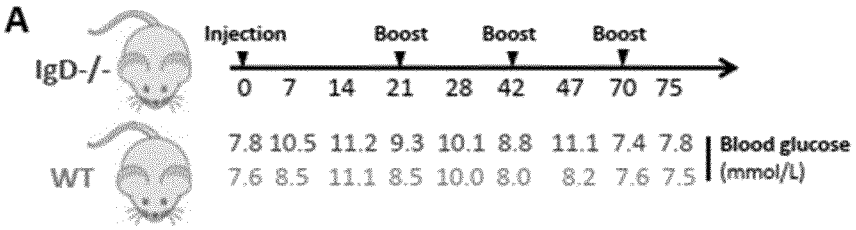

Graphical abstract
ACTIVATION PHASE
DISEASE PHASE
PROTECTION PHASE
IgM
IgD
WT
Autoreactive B cell
Activation threshold
Auto-IgM
Affinity-maturation
Protective IgM
Normal
Normal
Low
Delayed / Inefficient
IgD-/-
time

Figure 30



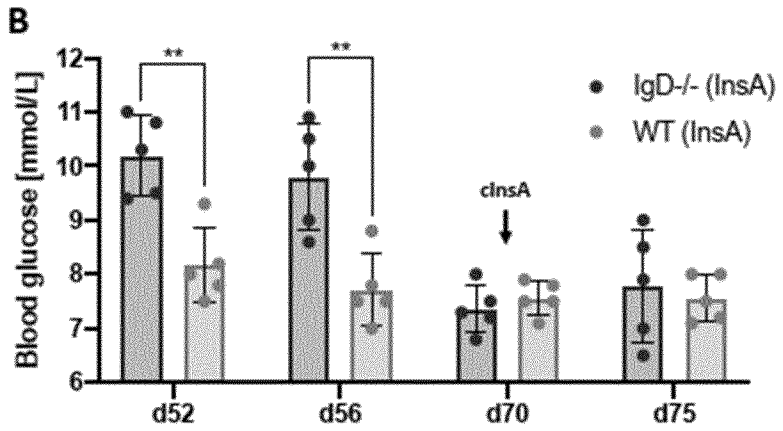


Figure 31

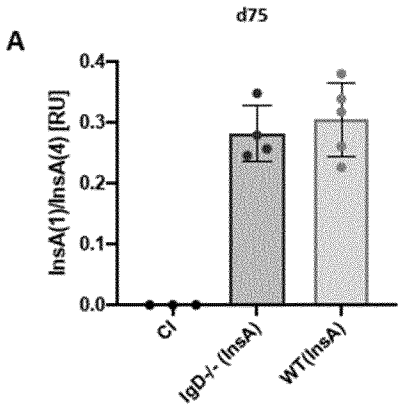


Figure 32

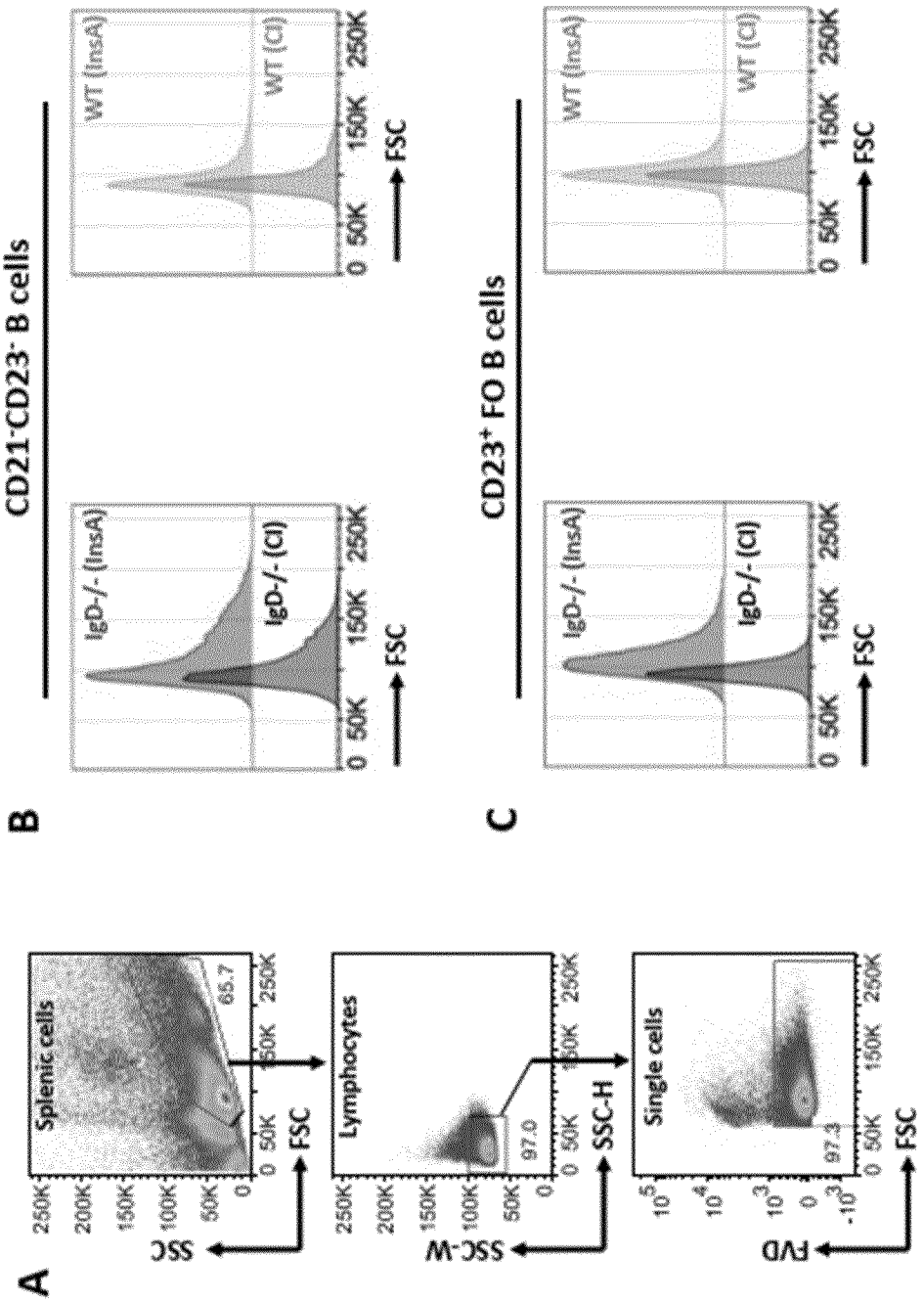

A
Splenic cells
SSC
FSC
65.7
Lymphocytes
SSC-W
SSC-H
97.0
Single cells
FVD
FSC
97.3
B
CD21-CD23- B cells
IgD-/- (InsA)
IgD-/- (Cl)
WT (InsA)
WT (Cl)
FSC
C
CD23+ FO B cells
IgD-/- (InsA)
IgD-/- (Cl)
WT (InsA)
WT (Cl)
FSC

Figure 33

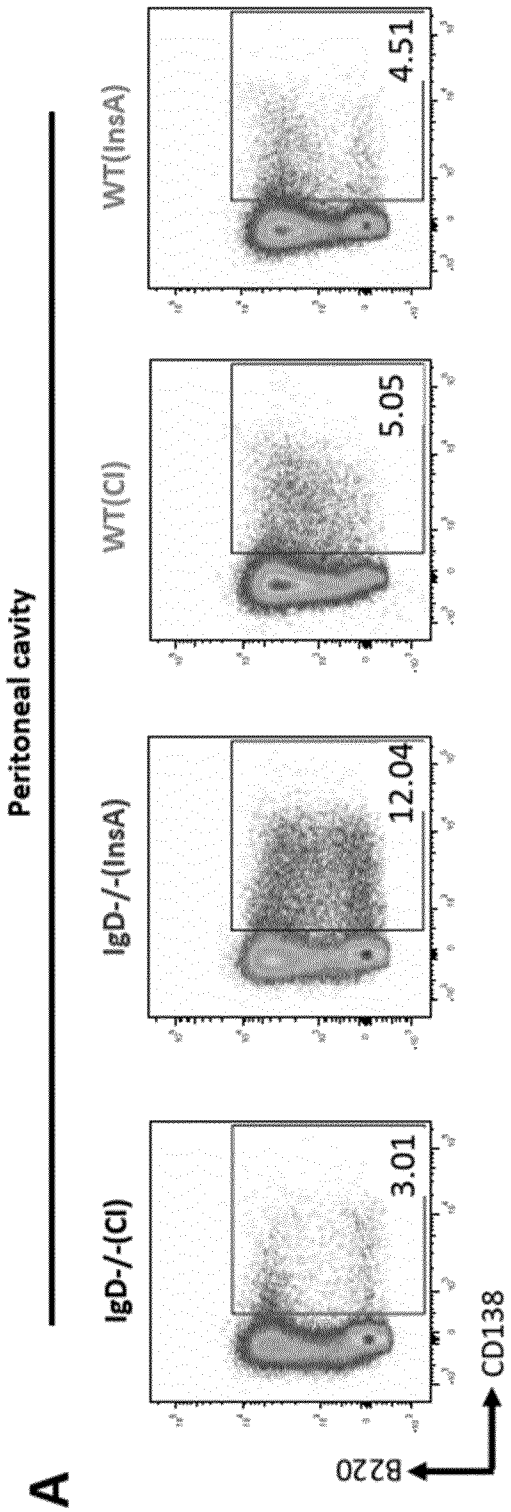

A
Peritoneal cavity
IgD-/-(Cl)
IgD-/-(InsA)
WT(Cl)
WT(InsA)
3.01
12.04
5.05
4.51
B220
CD138

Figure 34

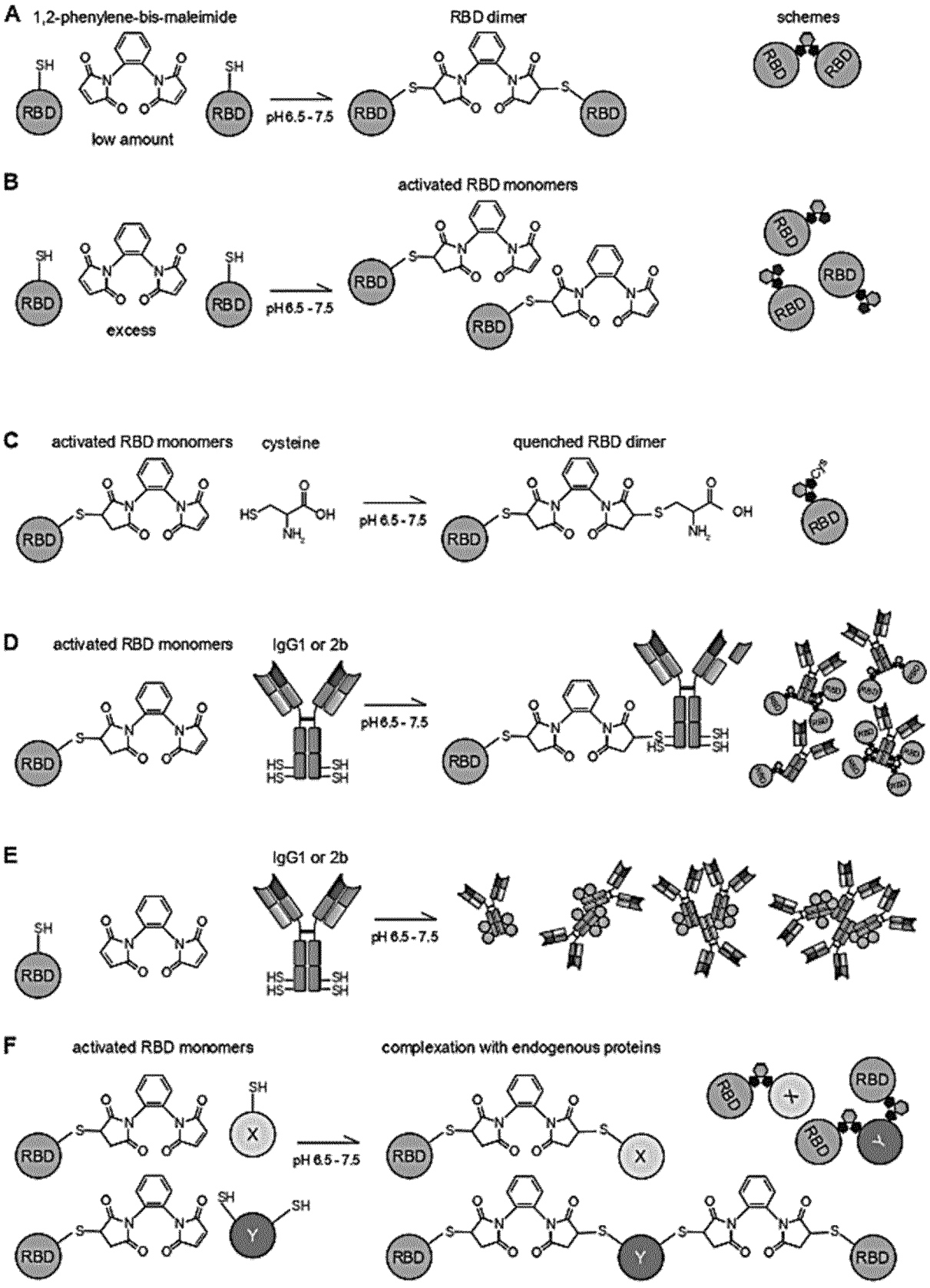

A
1,2-phenylene-bis-maleimide
RBD dimer
schemes
low amount
pH 6.5 - 7.5
B
activated RBD monomers
excess
pH 6.5 - 7.5
C
activated RBD monomers
cysteine
quenched RBD dimer
pH 6.5 - 7.5
D
activated RBD monomers
IgG1 or 2b
pH 6.5 - 7.5
E
IgG1 or 2b
pH 6.5 - 7.5
F
activated RBD monomers
complexation with endogenous proteins
pH 6.5 - 7.5

Figure 35

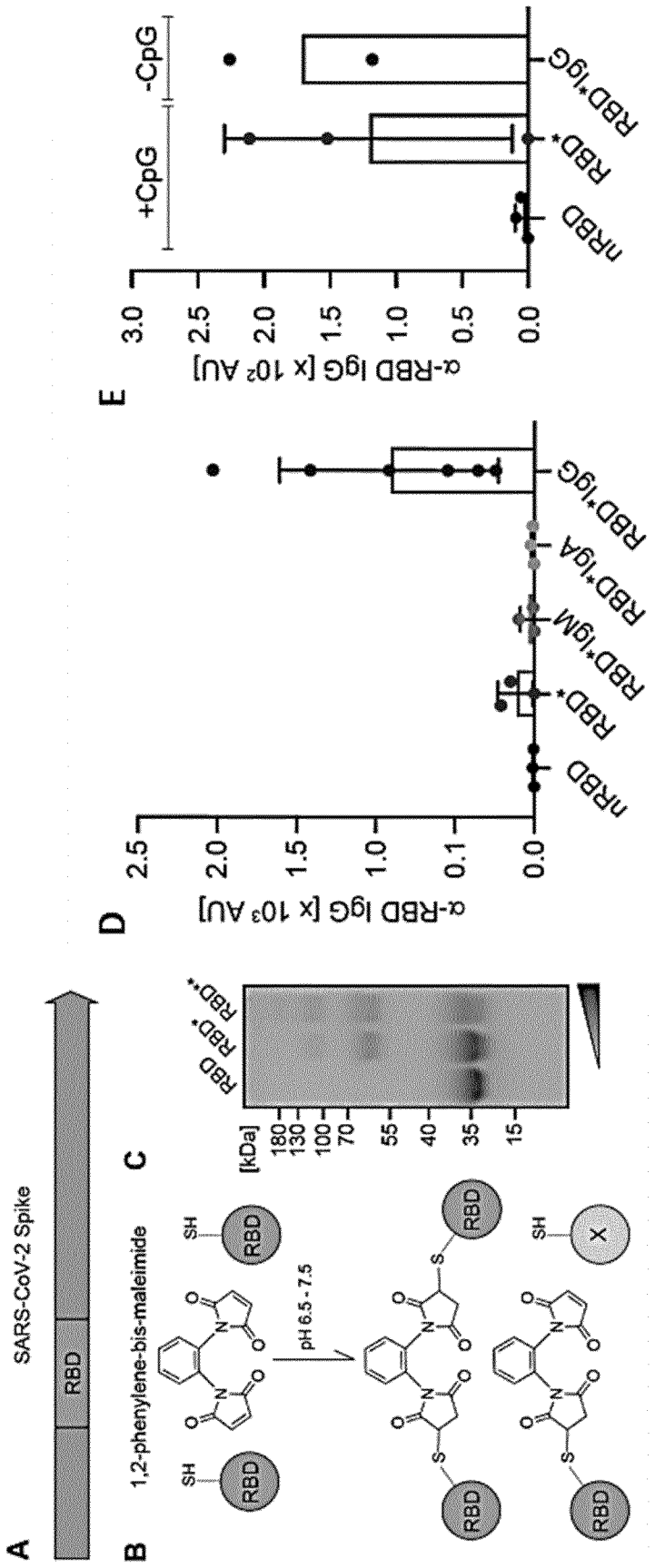

A
SARS-CoV-2 Spike
RBD
B
1,2-phenylene-bis-maleimide
pH 6.5 - 7.5
SH
RBD
X
C
[kDa]
180
130
100
70
55
40
35
15
RBD
RBD*
RBD**
D
α-RBD IgG [x 10³ AU]
2.5
2.0
1.5
1.0
0.1
0.0
nRBD
RBD*
RBD*IgM
RBD*IgA
RBD*IgG
E
α-RBD IgG [x 10² AU]
3.0
2.5
2.0
1.5
1.0
0.5
0.0
+CpG
-CpG
nRBD
RBD*
RBD*IgG

Figure 36

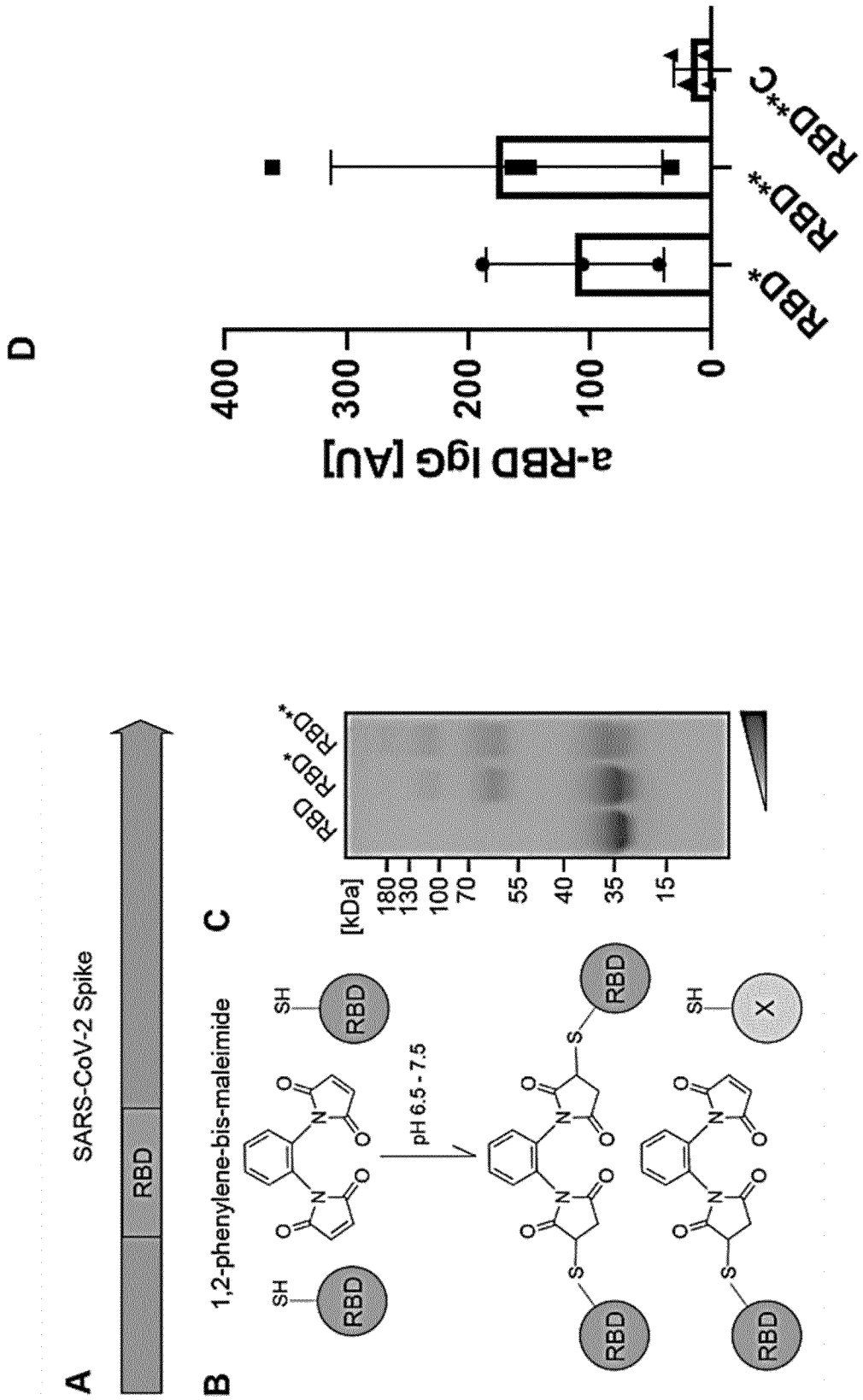

A
SARS-CoV-2 Spike
RBD
B
1,2-phenylene-bis-maleimide
SH
RBD
pH 6.5 - 7.5
X
C
[kDa]
180
130
100
70
55
40
35
15
RBD
RBD*
RBD**
D
a-RBD Ig [AU]
400
300
200
100
0
RBD*
RBD**
RBD**C

Figure 37

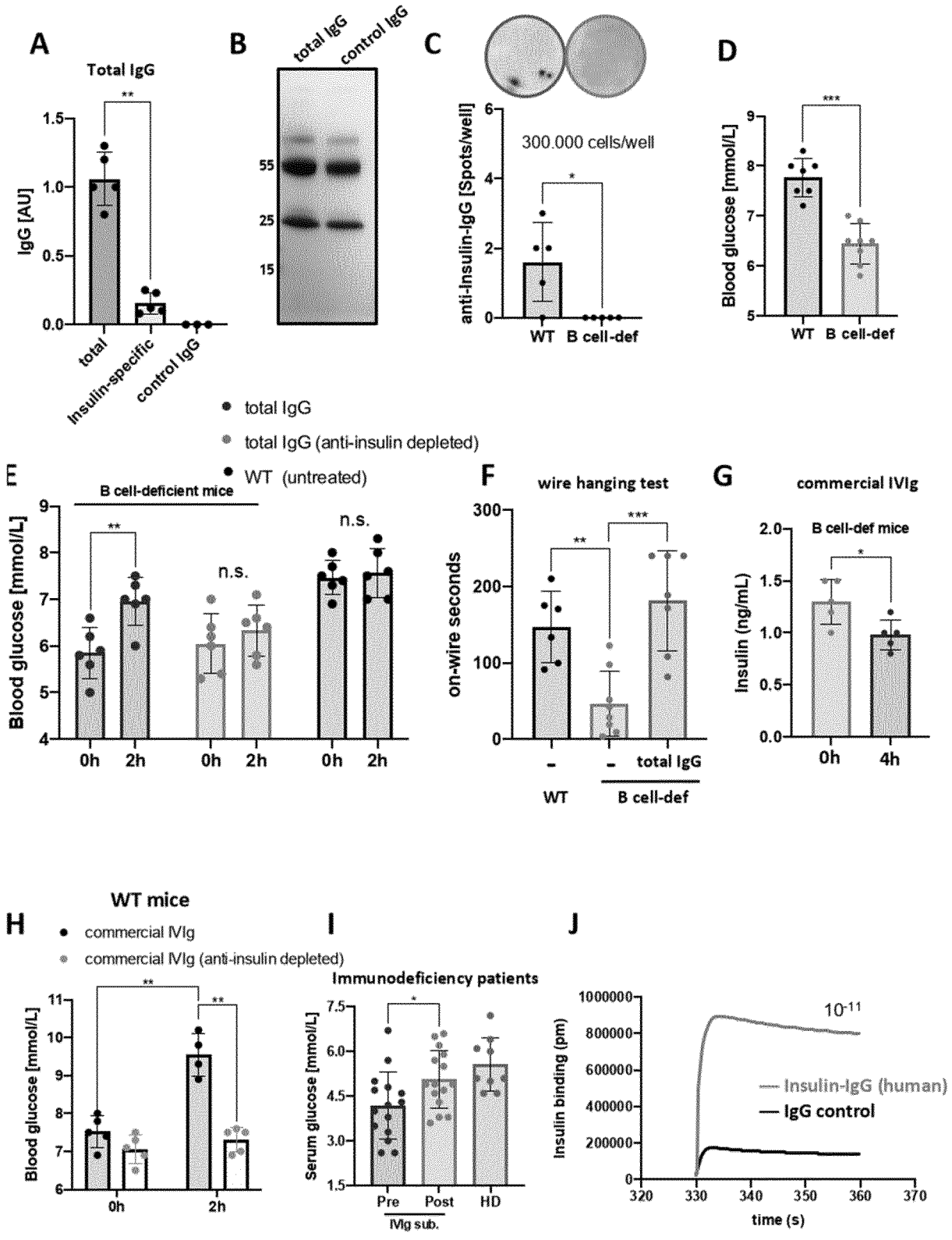

A
Total IgG
IgG [AU]
total
Insulin-specific
control IgG
B
total IgG
control IgG
55
25
15
C
300.000 cells/well
anti-Insulin-IgG [Spots/well]
WT
B cell-def
D
Blood glucose [mmol/L]
WT
B cell-def
total IgG
total IgG (anti-insulin depleted)
WT (untreated)
E
B cell-deficient mice
Blood glucose [mmol/L]
n.s.
0h
2h
F
wire hanging test
on-wire seconds
total IgG
WT
B cell-def
G
commercial IVIg
B cell-def mice
Insulin (ng/mL)
0h
4h
H
WT mice
commercial IVIg
commercial IVIg (anti-insulin depleted)
Blood glucose [mmol/L]
I
Immunodeficiency patients
Serum glucose [mmol/L]
Pre
Post
HD
IVIg sub.
J
Insulin binding (pm)
10-11
Insulin-IgG (human)
IgG control
time (s)

Figure 38

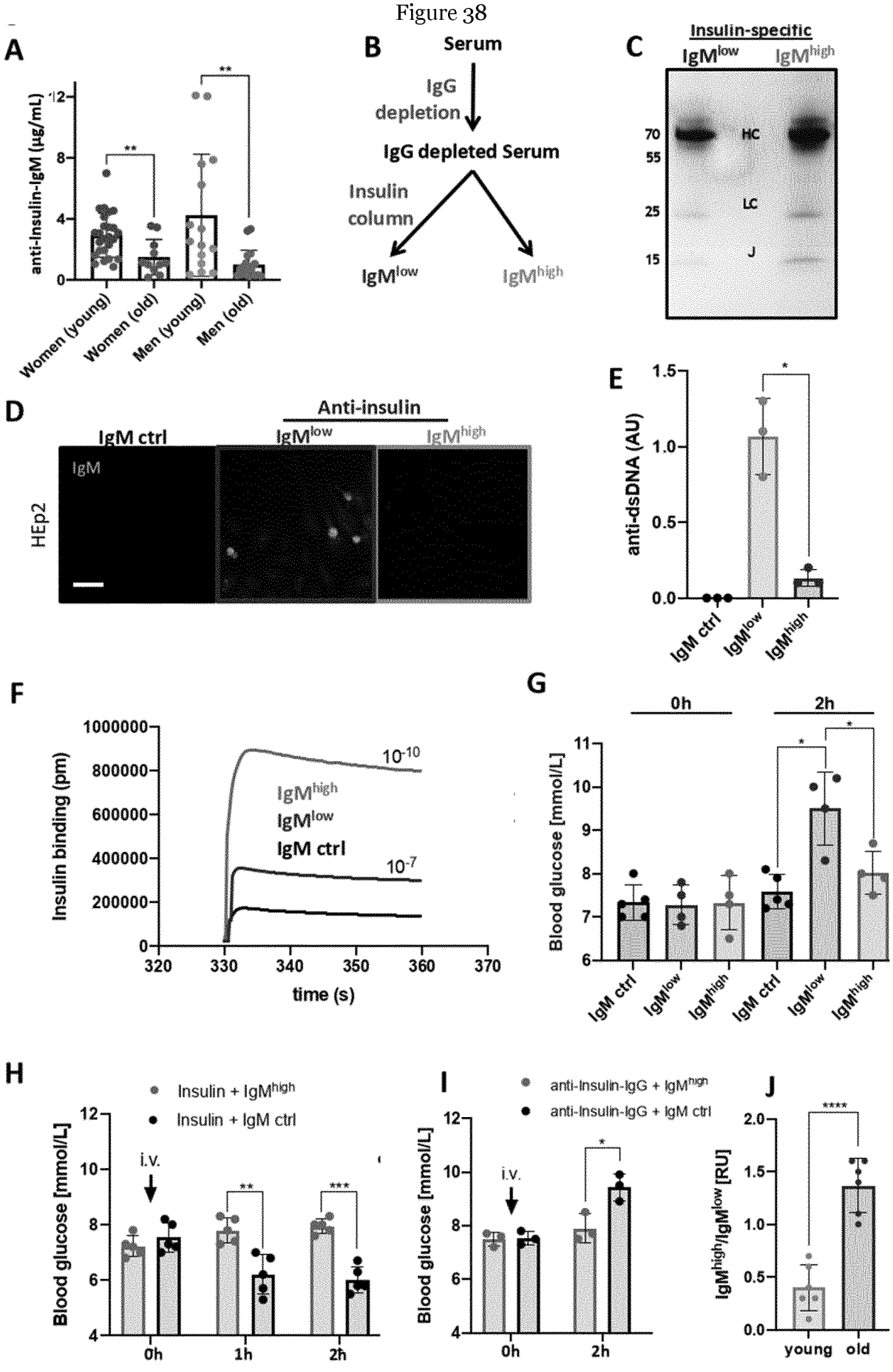

A
anti-insulin-IgM (µg/mL)
Women (young)
Women (old)
Men (young)
Men (old)
B
Serum
IgG depletion
IgG depleted Serum
Insulin column
IgMlow
IgMhigh
C
Insulin-specific
IgMlow
IgMhigh
70
55
25
15
HC
LC
J
D
Anti-insulin
IgM ctrl
IgMlow
IgMhigh
IgM
HEp2
E
anti-dsDNA (AU)
IgM ctrl
IgMlow
IgMhigh
F
Insulin binding (pm)
time (s)
10-10
10-7
IgMhigh
IgMlow
IgM ctrl
G
0h
2h
Blood glucose [mmol/L]
IgM ctrl
IgMlow
IgMhigh
H
Insulin + IgMhigh
Insulin + IgM ctrl
i.v.
Blood glucose [mmol/L]
0h
1h
2h
I
anti-Insulin-IgG + IgMhigh
anti-Insulin-IgG + IgM ctrl
i.v.
Blood glucose [mmol/L]
0h
2h
J
IgMhigh/IgMlow [RU]
young
old

Figure 39

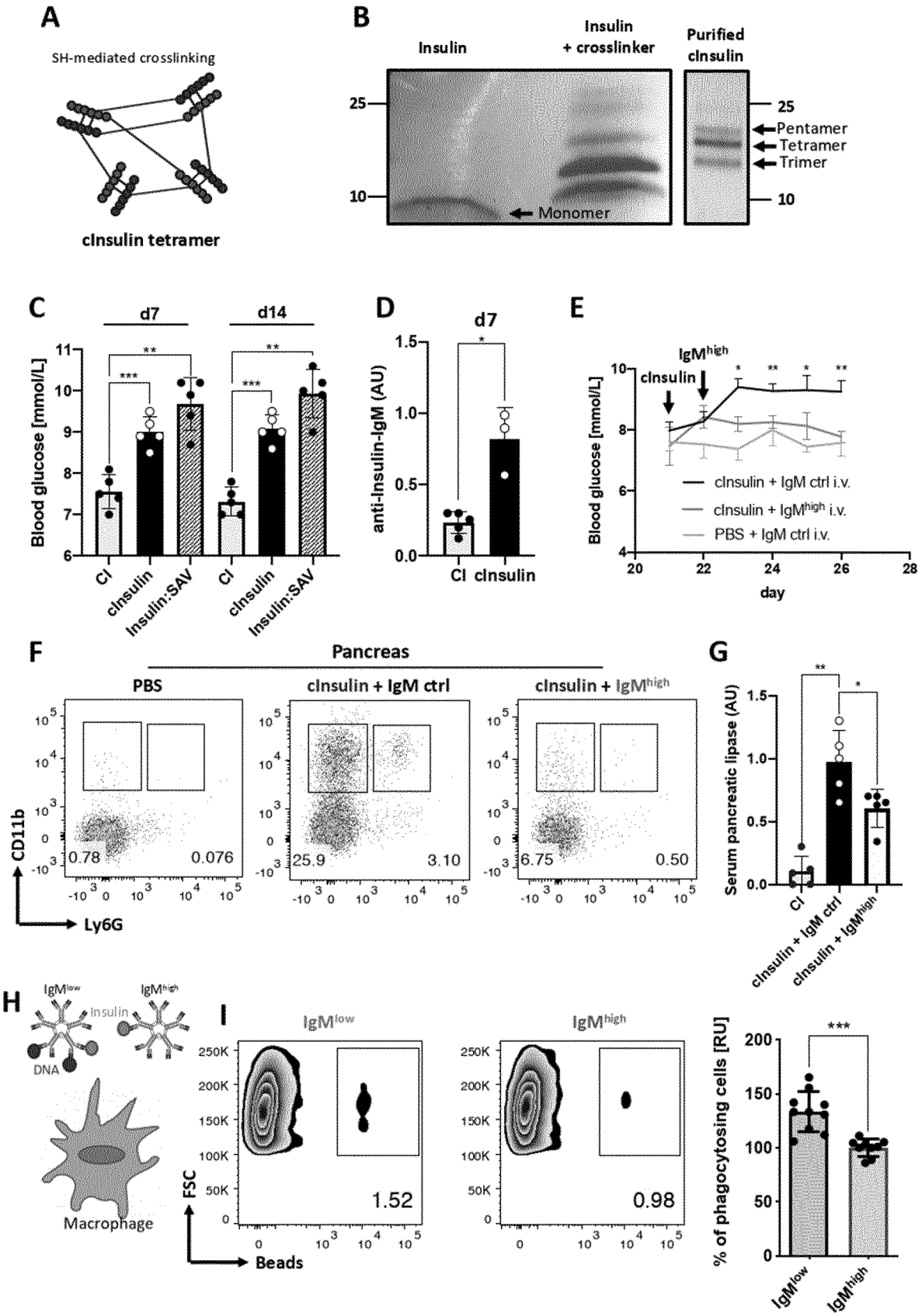

A
SH-mediated crosslinking
clnsulin tetramer
B
Insulin
Insulin + crosslinker
Purified clnsulin
25
10
Monomer
Pentamer
Tetramer
Trimer
C
d7
d14
Blood glucose [mmol/L]
Cl
clnsulin
Insulin:SAV
D
d7
anti-Insulin-IgM (AU)
E
IgM^high
clnsulin
Blood glucose [mmol/L]
clnsulin + IgM ctrl i.v.
clnsulin + IgM^high i.v.
PBS + IgM ctrl i.v.
day
F
Pancreas
PBS
clnsulin + IgM ctrl
clnsulin + IgM^high
CD11b
Ly6G
0.78
0.076
25.9
3.10
6.75
0.50
G
Serum pancreatic lipase (AU)
H
IgM^low
IgM^high
Insulin
DNA
Macrophage
I
IgM^low
IgM^high
FSC
Beads
1.52
0.98
% of phagocytosing cells [RU]

Figure 40

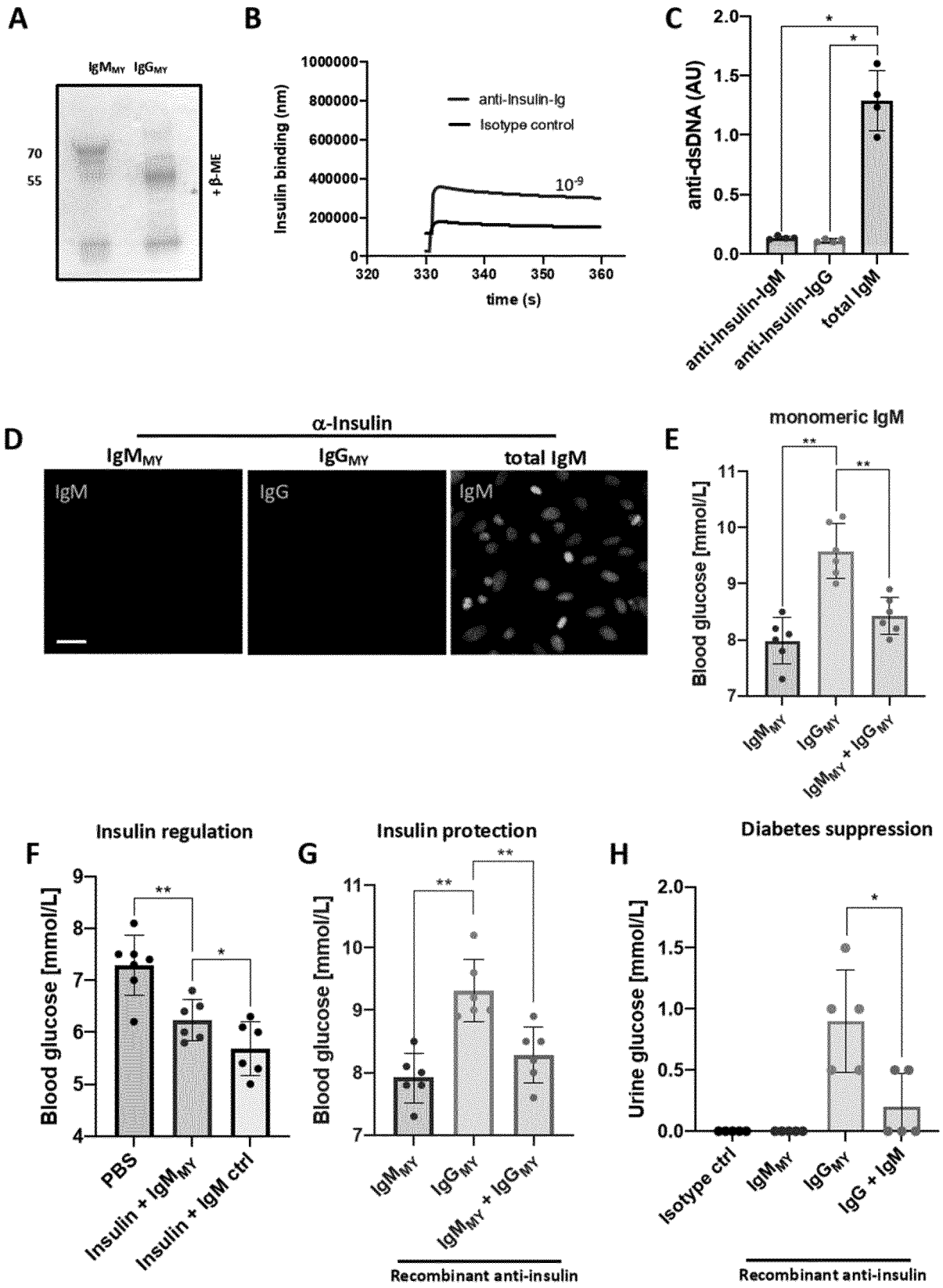

A
IgM$_{MY}$ IgG$_{MY}$
70
55
+ β-ME
B
Insulin binding (nm)
1000000
800000
600000
400000
200000
0
anti-Insulin-Ig
Isotype control
10^{-9}
320
330
340
350
360
time (s)
C
anti-dsDNA (AU)
2.0
1.5
1.0
0.5
0.0
*
*
anti-Insulin-IgM
anti-Insulin-IgG
total IgM
D
α-Insulin
IgM$_{MY}$
IgG$_{MY}$
total IgM
IgM
IgG
IgM
E
monomeric IgM
Blood glucose [mmol/L]
11
10
9
8
7
**
**
IgM$_{MY}$
IgG$_{MY}$
IgM$_{MY}$ + IgG$_{MY}$
F
Insulin regulation
Blood glucose [mmol/L]
9
8
7
6
5
4
**
*
PBS
Insulin + IgM$_{MY}$
Insulin + IgM ctrl
G
Insulin protection
Blood glucose [mmol/L]
11
10
9
8
7
**
**
IgM$_{MY}$
IgG$_{MY}$
IgM$_{MY}$ + IgG$_{MY}$
Recombinant anti-insulin
H
Diabetes suppression
Urine glucose [mmol/L]
2.0
1.5
1.0
0.5
0.0
*
Isotype ctrl
IgM$_{MY}$
IgG$_{MY}$
IgG + IgM
Recombinant anti-insulin

Figure 41

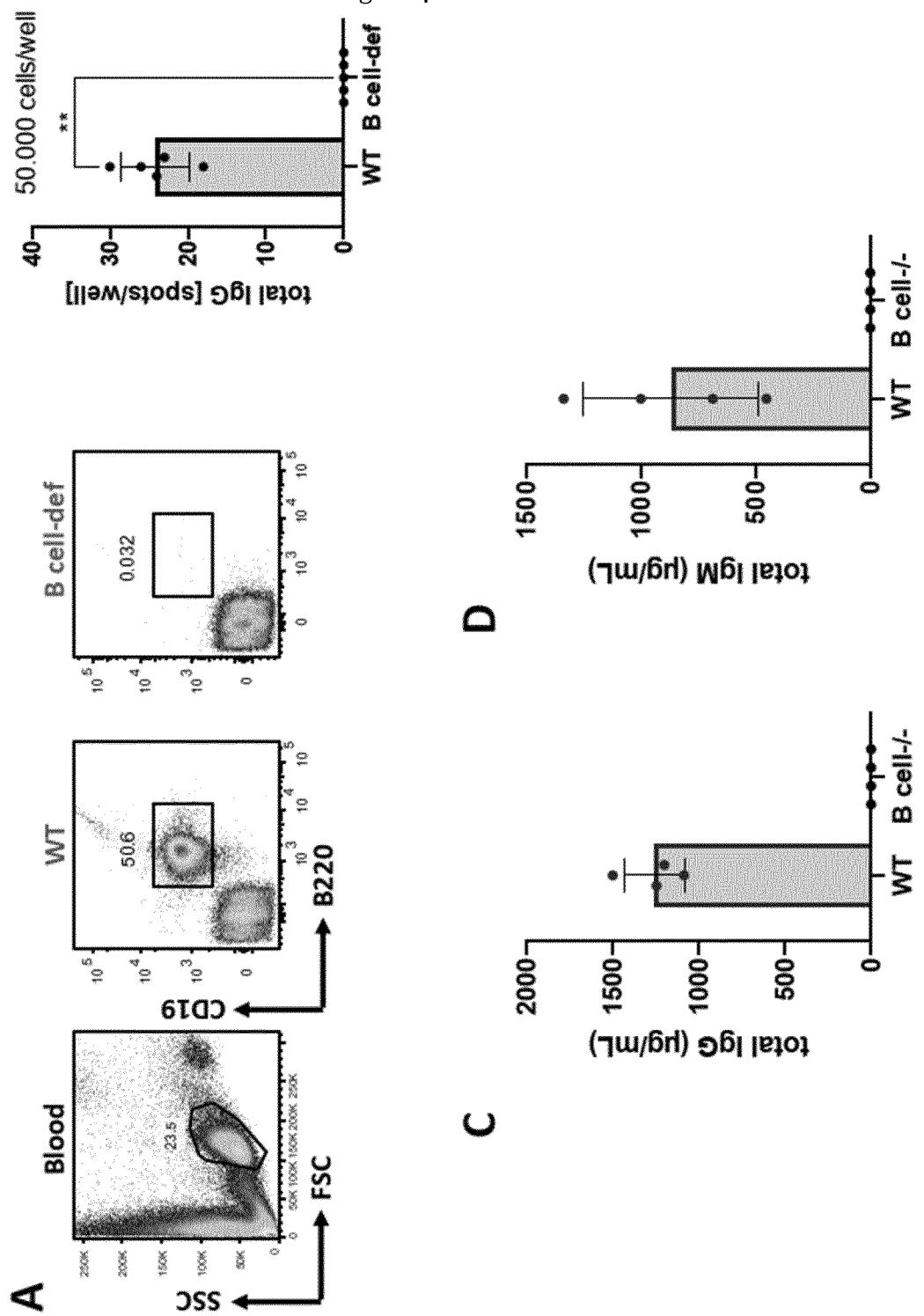

A
Blood
SSC
FSC
23.5
WT
CD19
B220
50.6
B cell-def
0.032
B
50.000 cells/well
total IgG [spots/well]
**
WT
B cell-def
C
total IgG (µg/mL)
WT
B cell-/-
D
total IgM (µg/mL)
WT
B cell-/-

Figure 42

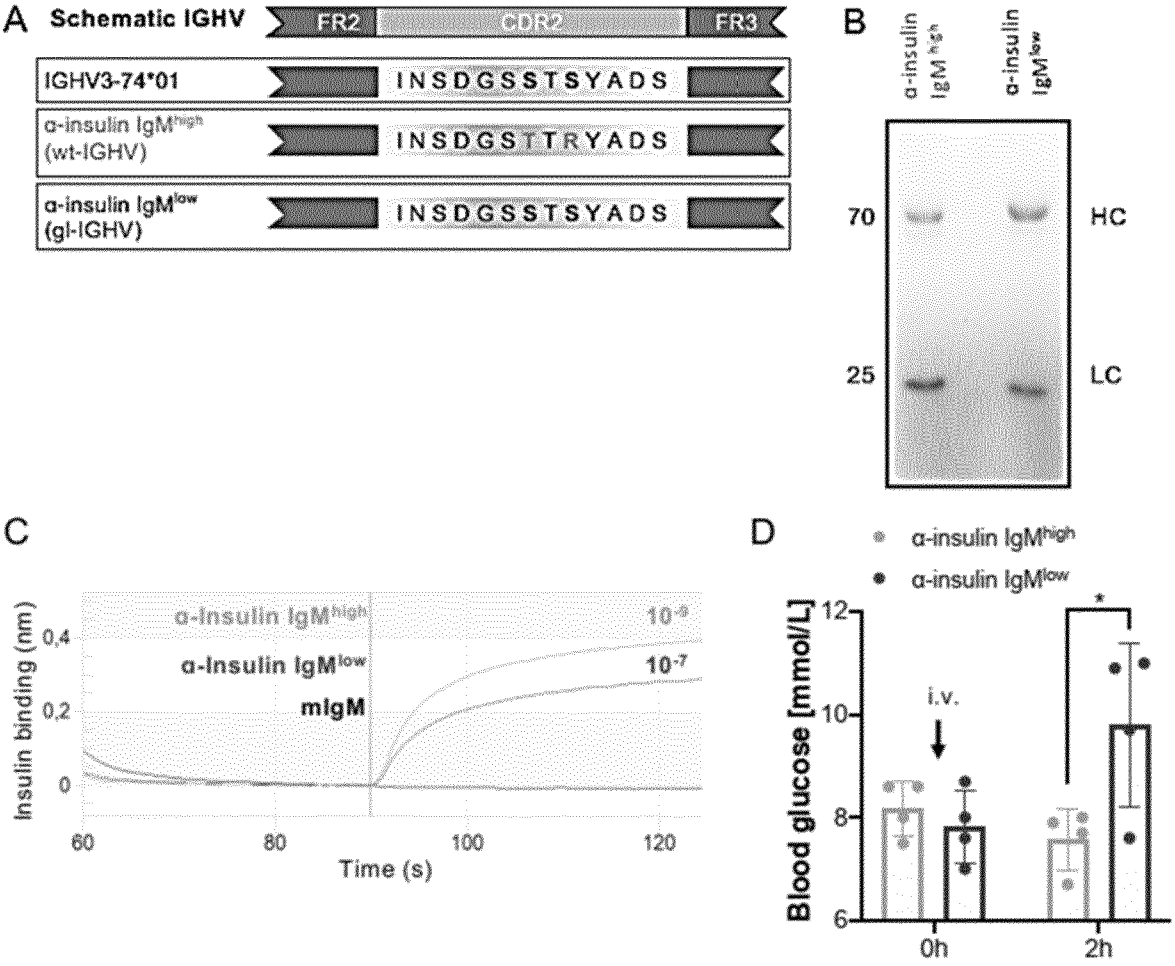

A
Schematic IGHV
FR2
CDR2
FR3
IGHV3-74*01
INSDGSSTSYADS
α-insulin IgM high (wt-IGHV)
INSDGSTTRYADS
α-insulin IgM low (gl-IGHV)
INSDGSSTSYADS
B
α-insulin IgM high
α-insulin IgM low
70
25
HC
LC
C
α-Insulin IgM high
α-Insulin IgM low
mIgM
10-9
10-7
Insulin binding (nm)
0,4
0,2
0
60
80
100
120
Time (s)
D
α-insulin IgM high
α-insulin IgM low
Blood glucose [mmol/L]
12
10
8
6
i.v.
*
0h
2h

Figure 43

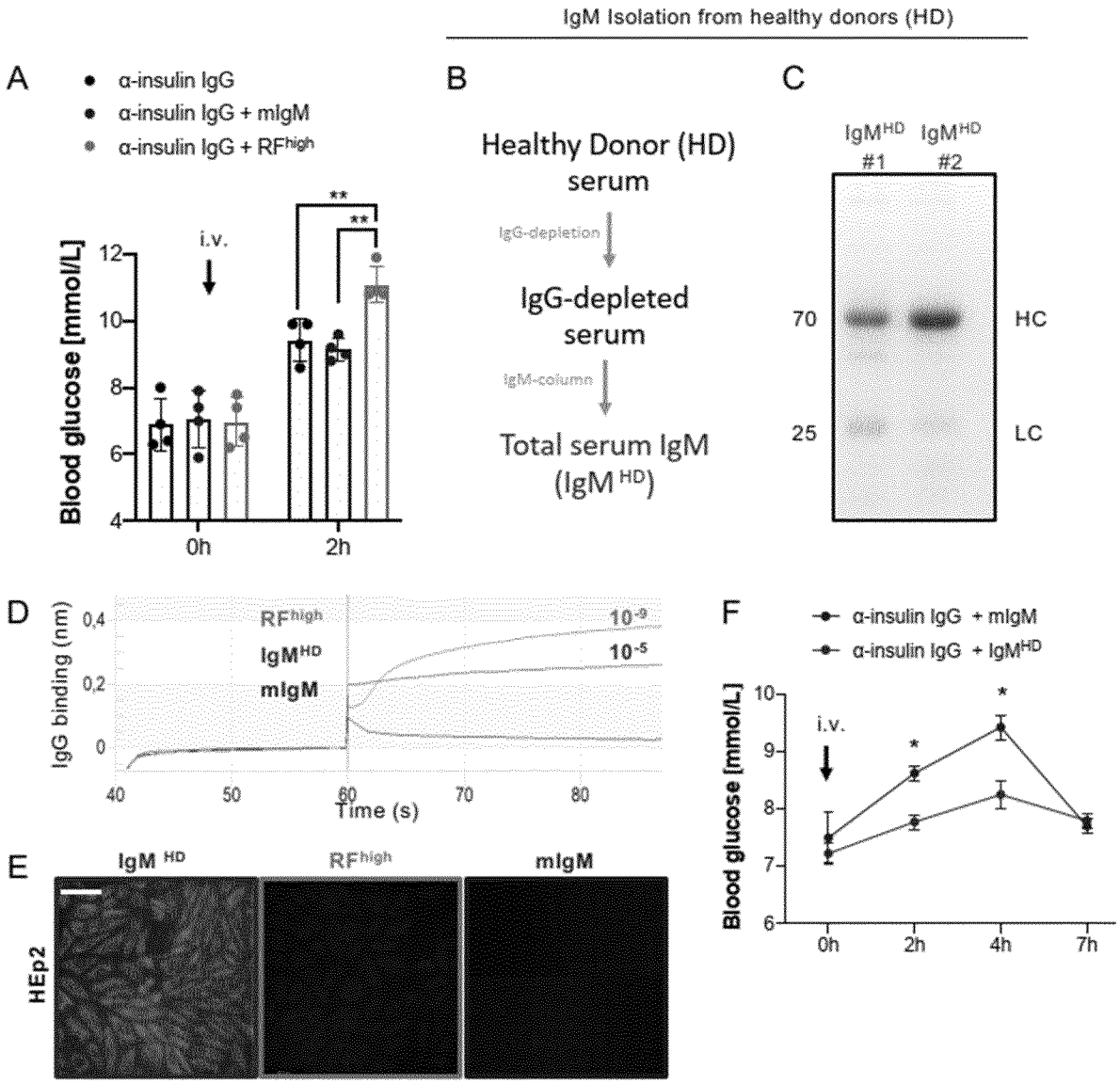

IgM Isolation from healthy donors (HD)
A
α-insulin IgG
α-insulin IgG + mIgM
α-insulin IgG + RF^high
Blood glucose [mmol/L]
i.v.
0h
2h
B
Healthy Donor (HD) serum
IgG-depletion
IgG-depleted serum
IgM-column
Total serum IgM (IgM HD)
C
IgM HD #1
IgM HD #2
70
25
HC
LC
D
IgG binding (nm)
RF high
IgM HD
mIgM
10-9
10-5
Time (s)
E
IgM HD
RF high
mIgM
HEp2
F
α-insulin IgG + mIgM
α-insulin IgG + IgM HD
Blood glucose [mmol/L]
i.v.
0h
2h
4h
7h

Figure 44

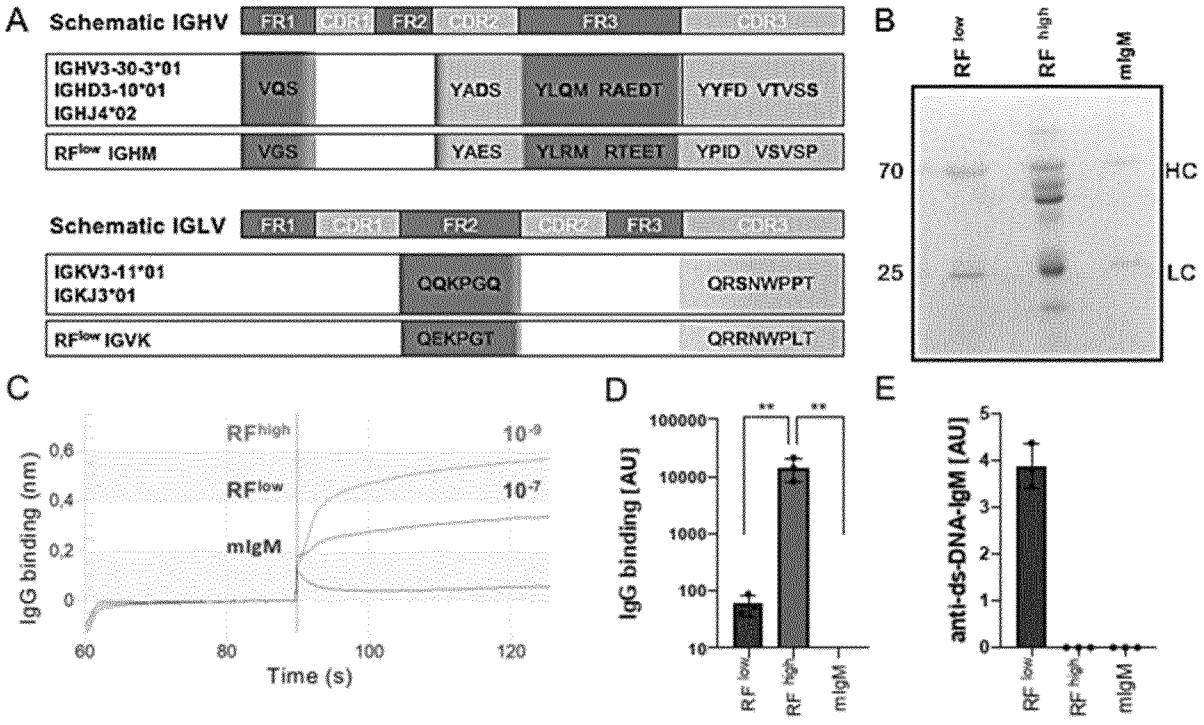


Figure 45

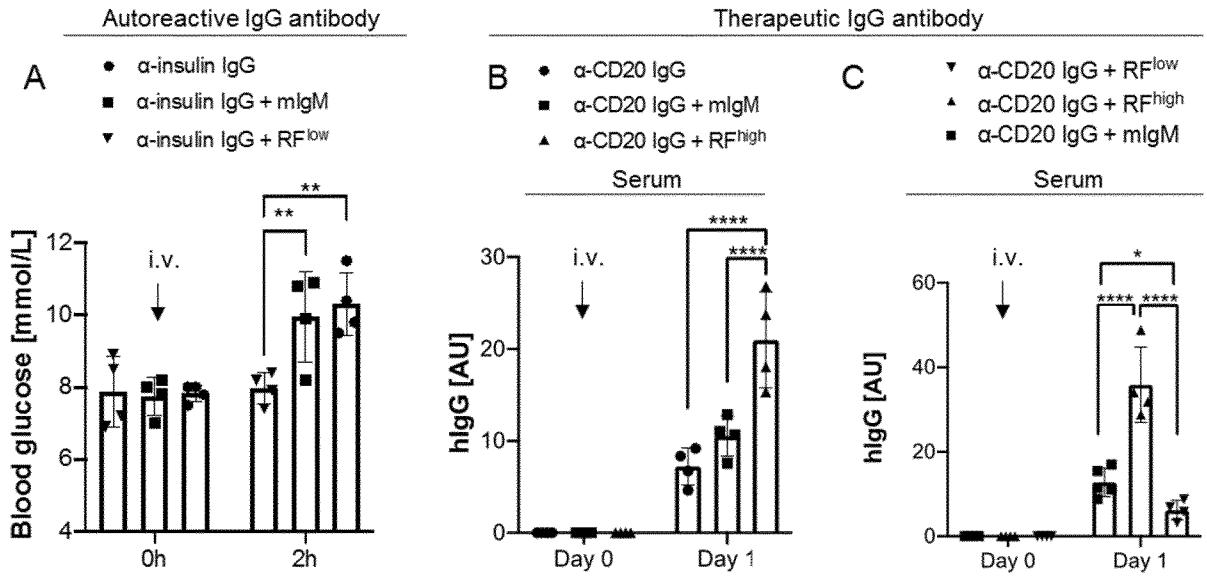


Figure 46

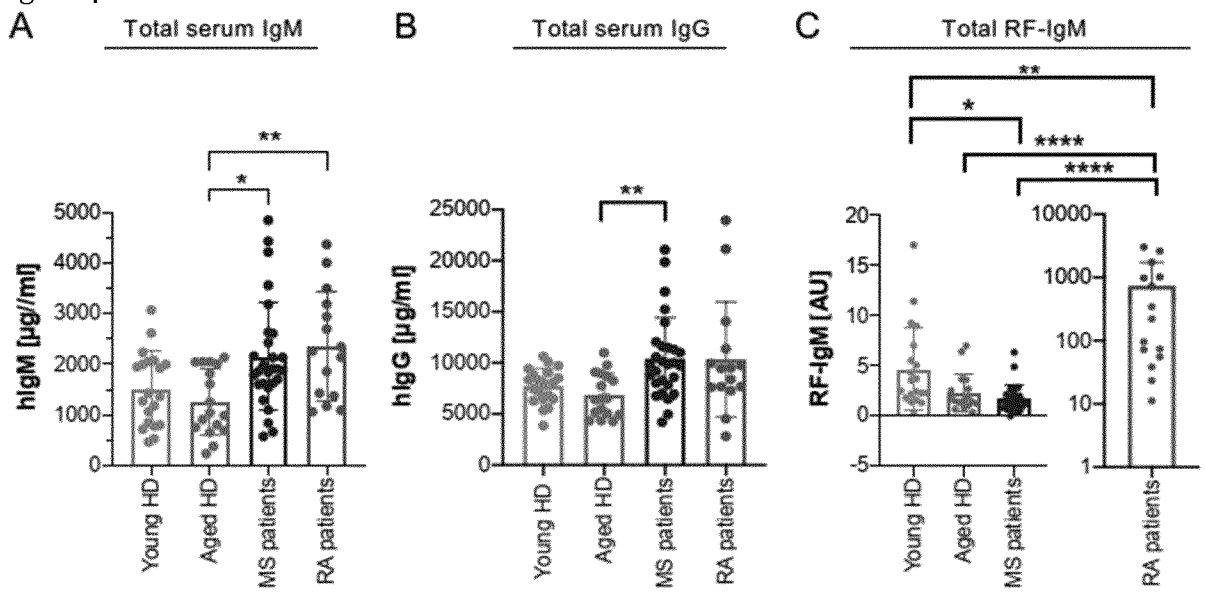


Figure 47

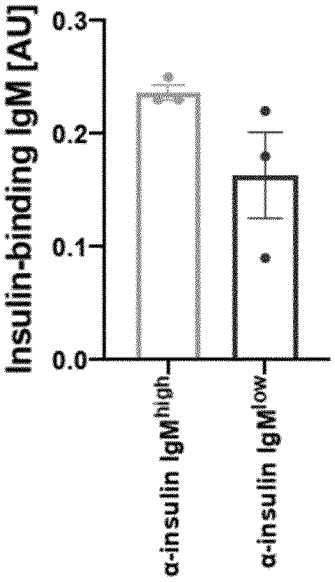


Figure 48

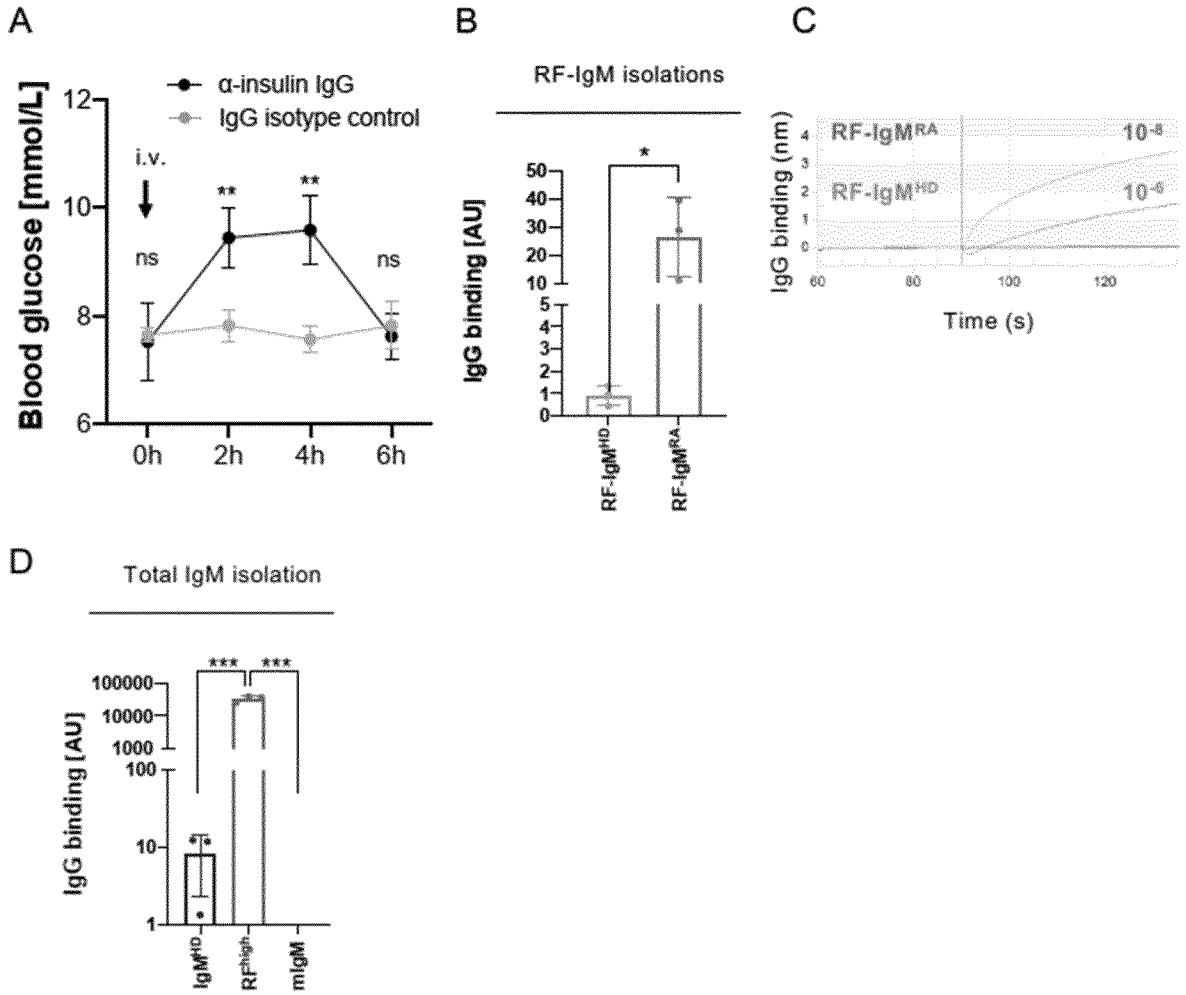


Figure 49