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(54) Title: METHODS FOR THE MODULATION OF LGALS3BP TO TREAT SYSTEMIC LUPUS ERYTHEMATOSUS

(57) Abstract: Embodiments of the present invention describe methods for modulating LGALS3BP and the use of antibodies to the same in the treatment of autoimmune diseases including systemic lupus erythematosus and lupus nephritis.

# METHODS FOR THE MODULATION OF LGALS3BP TO TREAT SYSTEMIC LUPUS ERYTHEMATOSUS

## PRIORITY CLAIM

The instant PCT patent application claims priority to U.S. provisional patent application serial no.: 62/212,163 filed on August 31, 2015, wherein, said provisional application is expressly incorporated by reference, herein, in its entirety.

## SEQUENCE LISTING

The instant application contains a Sequence Listing which has been filed electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 23, 2016, is named P15167WO_SEQ_LISTING.txt and is 5,320 bytes in size.

## FIELD OF THE INVENTION

The invention relates generally to methods for modulating (including, but not limited to, decreasing, reducing, inhibiting, suppressing, limiting or controlling) the activity of LGALS3BP under conditions such that the production of autoantibodies associated with a variety of autoimmune pathologies are reduced or, alternatively augmenting and enhancing natural antibody secretion or vaccine responses to pathogenic infectious agents through supplementation with recombinant LGALS3BP.

## BACKGROUND OF THE INVENTION

Failure of the immune system can manifest either through the inability to defend the host against infectious agents or, conversely, through a mistaken recognition of self as a breach of tolerance thus giving rise to autoimmune pathologies. Autoimmune pathologies are generally caused by a combination of genetic and environmental factors and can be grossly classified into pathologies mediated by T cells or B cells. Autoreactive pathogenic T cells recognize a target cell by binding the T-cell receptor to the appropriate combination of MHC I molecule and autoantigen-derived peptides resulting in a direct killing of target cells via a number different mechanisms. Development of type-1 diabetes and primary biliary cirrhosis are representative examples of pathologies mediated by autoreactive T cells.

The common feature of B cell associated autoimmunity is the presence of autoantibodies that are directed against functional structures of the cell (nucleic acids, nuclear proteins, receptors, ion channels). By binding to their targets, autoantibodies can mediate cytotoxic destruction of cells by complement activation and/or antibody-dependent cell-mediated cytotoxicity (ADCC) or by blocking the target's function. Pathogenic autoantibodies mediate development of a number of diseases including Graves' disease (anti-thyroid-stimulating hormone Abs), myasthenia gravis (anti-acetylcholine receptor Abs), vasculitis and Wegener's granulomatosis (anti-ANCA Abs) neuromyelitis optica (anti-aquaporin-4 Abs), primary sclerosing cholangitis (anti-neutrophil cytoplasmic Ab, anti-SM Ab). Other autoimmune diseases are caused by a pathogenic action of immune complexes of autoantibodies with their target molecules, e.g. SLE, Sjoegren's syndrome and lupus nephritis (anti-DNA, anti-RNA, anti-histone, anti-Ro, anti-La, anti-phospholipid Abs), subset of rheumatoid arthritis (anti-citrullinated protein, anti-RF, anti-CarP Abs).

Therapeutic approaches for treatment of autoimmune diseases have a rather limited efficacy. The traditional treatment regimens rely on action of steroids and various cytotoxic and cytostatic immunosuppressants that should eliminate rapidly proliferating autoreactive immune cells and thus slow down development of autoimmune processes. The most commonly used drugs for treatment of autoimmune diseases, i.e., cortisone/prednisone, methotrexate, mycophenolate mofetil, chloroquine and azathioprine exhibit limited therapeutic efficacy and are accompanied by numerous adverse effects.

More targeted approaches focus on elimination of autoantibody production and hold better therapeutic promise. Belimumab (trade name Benlysta, previously known as LymphoStat-B), a human monoclonal antibody that inhibits B-cell activating factor (BAFF), also known as B-lymphocyte stimulator (BlyS), a cytokine important for B-cell differentiation and survival, is an approved therapy for adult patients with active, autoantibody positive SLE, and which demonstrates only modest efficacy. Several other biologic therapies attempting to eliminate B cells and, by consequence, the associated pathogenic autoantibodies have focused on cell surface receptors and molecules that are present on human B cells. The anti-CD20 targeting antibody rituximab (and similarly additional biologics,-for example, ocrelizumab, obinutuzumab and ofatumumab) was designed to recognize antibody-producing B cells and eliminate them via ADCC. Although no anti-CD20 antibodies have been approved for treatment of SLE, they are often prescribed off-label for treatment of SLE and other autoimmune diseases. In addition,

biologics targeting additional surface molecules on human B cells, CD19 and CD22 (epratuzumab) , are or were undergoing clinical development, albeit thus far with limited or no clinical effect. The common drawback of the B cell targeting strategies is thought to be the absence of their targets on the surface long-lived plasma cells. The CD19-/CD38hi/CD138+ plasma cells reside in bone marrow and are the source of the majority of the long-lived Ab responses. Therapeutics that could block their activity or lead to their elimination to suppress pathogenic autoantibody production are not currently identified.

Systemic lupus erythematosus (SLE) is a representative autoimmune disorder characterized by formation of autoantibody-containing immune complexes (ICs) that trigger inflammation, tissue damage and premature mortality. SLE ICs often contain nucleic acids that are recognized by numerous innate immune receptors that can initiate pathological mechanisms leading to production of cytokines, interferons and ultimately to immune responses leading to organ damage. Due to the great clinical diversity and idiopathic nature of SLE, management of idiopathic SLE depends on its specific manifestations and severity. Therefore, medications suggested to treat SLE generally are not necessarily effective for the treatment of all manifestations of and complications resulting from SLE, e.g., LN. LN usually arises early in the disease course, within 5 years of diagnosis. The pathogenesis of LN is believed to derive from deposition of immune complexes in the kidney glomeruli that initiates an inflammatory response. An estimated 30-50% of patients with SLE develop nephritis that requires medical evaluation and treatment. LN is a progressive disease, running a course of clinical exacerbations and remissions.

While many patients fail to respond or respond only partially to the standard of care medications listed above, the long-term use of high doses of corticosteroids and cytotoxic therapies may have profound side effects such as bone marrow depression, increased infections with opportunistic organisms, irreversible ovarian failure, alopecia and increased risk of malignancy. Infectious complications coincident with active SLE and its treatment with immunosuppressive medications are the most common cause of death in patients with SLE. Therefore, there is a need for alternative therapeutic agents to treat SLE, and in preferred embodiments LN, wherein said therapeutic agents are associated with fewer side effects than current standards of care.

SUMMARY OF THE INVENTION

The subject of this application, LGALS3BP, is identified as a B-cell associated target whose functional blockade leads to elimination of activated B cells as well as long-lived plasma cells. While it is not intended the claimed methods of the present invention be limited to any specific mechanism, B cell activation and production of antibodies is regulated at many levels. In one instance B cells get activated by various T cell-dependent stimuli (e.g., CD40 ligation) as well as T cell-independent stimuli (various TLR ligands, polysaccharides, etc.). As shown in the Experimental section of the instant application, TLR7 agonists provide examples of a B cell stimulant as a representative case of B cell activating agents that can induce production of antibodies.

Autoantibody production is widely observed clinically, yet only a small percentage of the population who produce autoantibodies will develop SLE. Moreover, the autoantibody repertoire in SLE is restricted and seems to be enriched for antibodies that recognize autoantigens on proteins that are associated with nucleic acids. The majority of SLE patients have documented production of antibodies against DNA, RNP or both. Autoantigens associated with nucleic acids activate autoreactive B cells and allow them to escape peripheral tolerance checkpoints and differentiate into autoantibody-secreting cells.

Following antigen recognition and uptake of nucleic acid-cell debris complexes the nucleic acids are recognized, in part, by endosomal toll-like receptors (e.g., TLR3, TLR7, TLR8 and TLR9). Stimulation of TLRs in B cells leads to their activation and maturation and increased production of antibodies as well as numerous cytokines. The relative contribution of individual TLRs in the development of SLE has been observed in many mouse SLE models. Moreover, the activity of TLR7, an RNA receptor, plays a major role and gene knock out as well as use of TLR7 inhibitors significantly attenuates disease progression. Also, increased TLR7 activity either by overexpression of TLR7 gene or by systemic administration of small molecule TLR7 agonists leads to induction of SLE-like symptoms.

Nucleic acids present in SLE immune complexes can also be recognized by TLRs in dendritic cells. Stimulation of TLR7 in plasmacytoid dendritic cells leads to production of large amounts of type I interferon. Type I IFN is a cytokine that is involved in antiviral defense by activating a set of genes (interferon target genes) that contribute to control of the virus spread and

preservation of host integrity. These genes are often seen activated in SLE patients. Type I IFN plays a role in activating B cells and their expansion and differentiation into Ig-producing cells.

In view of the key role TLR7 stimulation plays in the activity of B cells, embodiments of the present invention describe screens which identify proteins that can modulate production of antibodies. These screens identified proteins and pathways useful in the pharmacological modulation of autoantibody production in the treatment of SLE. A library of plasmids coding for secreted proteins for transient production of cell culture supernatants enriched for these proteins was used and, subsequently, the activity of these proteins in a cellular system with primary B cells stimulated with a small molecule TLR7 ligand using IgG production as a readout to score efficacy. This screen identified a number of proteins that either increase or decrease production of IgGs. Embodiment of the present invention describe proteins not previously associated with B cell biology which include, in a preferred embodiment, LGALS3BP.

LGALS3BP (Mac2-BP, p90) is a ubiquitously expressed gene that belongs to the scavenger receptor family, originally identified as a protein secreted by certain types of tumor cells LGALS3BP expression levels are closely correlated with tumor progression. Apart from its direct effect on tumor cell proliferation/survival, LGALS3BP can also upregulate expression of vascular endothelial growth factor and promote angiogenesis. Its levels are augmented during HIV-1 infection and its activity is believed to reduce infectivity of HIV-1 through interference with the maturation and incorporation of envelope proteins into virions. Analysis of liver biopsies of hepatitis C patients suggested a direct role of LGALS3BP in hepatitis C-related fibrosis. In addition, increased levels of plasma LGALS3BP were also observed in SLE patients. LGALS3BP may contribute to increased cardiovascular complications in SLE, as it can facilitate thrombus formation and attachment of thrombi to endothelial cells. Serum levels of LGALS3BP were also found to be increased in patients with Behcet's disease and correlated with disease activity.

A variety of proteins that interact with and mediate the function of LGALS3BP have been described, including galectins, lectins, integrins and others. LGALS3BP contains several protein-protein interaction domains (SRCR, BTB, POZ) that are likely involved in numerous interactions with cellular proteins in a cell-specific manner.

In one embodiment of the present invention methods are described, wherein, LGALS3BP promotes IgG production in primary B cells stimulated with TLR7 ligand under conditions such that LGALS3BP-neutralizing antibodies significantly reduce IgG production from B cells stimulated with TLR7 ligand or via BCR-ligation. Transcriptome analysis of various immune cells in SLE revealed that LGALS3BP mRNA levels are increased relative to healthy donors and correlate with expression levels of interferon regulated genes.

While it is not intended that the claimed embodiments of the present invention be limited to any specific mechanism (in particular any suggestion that TLR7 must exert, exclusively, a stimulatory effect) the effects that LGALS3BP exert in IgG production in B cells and provides validation for the use of LGALS3BP neutralizing antibodies in the treatment of SLE, LN and potentially other autoimmune diseases such as rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, diabetes mellitus, myasthenia gravis, vasculitis, primary sclerosing cholangitis, autoimmune thyroiditis, Sjogren's Syndrome, Wegener's granulomatosis, Graves' disease, Hashimoto's thyroiditis, autoimmune thrombocytopenic purpura, anti-phospholipid syndrome, neuromyelitis optica and primary sclerosing cholangitis.

Outside of autoimmunity however, augmentation of a naturally occurring or vaccine-induced pathogen-directed humoral immune responses may be beneficial and indeed may be necessary to provide protective immunity against bacteria, parasites or viruses in an infectious disease setting. In this regard, for example, strategies to enhance the efficacy of recombinant protein subunit vaccines without sacrificing safety are of great interest, because immune responses, elicited by these (i.e. against malaria) are typically of weaker magnitude and durability relative to more potent live attenuated or recombinant vectors. In such cases, recombinant LGALS3BP supplementation to enhance humoral immunity and anti-pathogen responses will be beneficial in supporting host defense.

In one embodiment the present invention describes a method for modulating LGALS3BP in a subject presenting symptoms of an immune disorder, inflammatory response or autoimmune disease comprising administering an anti-LGALS3BP antibody to said subject under conditions such that at least one symptom of said immune disorder, inflammatory response or disease said is improved.

In one embodiment the present invention describes a method for modulating LGALS3BP in a

subject presenting symptoms of the disease states consisting essentially of Graves' disease, myasthenia gravis, vasculitis and Wegener's granulomatosis, neuromyelitis optica, primary sclerosing cholangitis, Sjoegren's syndrome, lupus nephritis and rheumatoid arthritis comprising administering an anti-LGALS3BP antibody to said subject under conditions such that at least one symptom of one of said disease states said is improved.

In a preferred embodiment the present invention describes treating a patient with SLE, comprising administering to the patient a therapeutically effective amount of an anti-LGALS3BP antibody. In one embodiment the anti-LGALS3BP antibody is effective to: (a) inhibit progression of nephritis; (b) stabilize nephritis; or, (c) reverse nephritis, in the patient. In another embodiment, the amount of anti- LGALS3BP antibody is effective to (a) inhibit progression of proteinuria; (b) stabilize proteinuria; or, (c) reverse proteinuria, in the patient.

In one embodiment the present invention describes treating a patient with SLE, comprising administering to the patient a therapeutically effective amount of an anti-LGALS3BP antibody at a dose effective to stabilize or decrease, in the patient, a clinical parameter selected from; (a) the patient's blood concentration of urea, creatinine or protein; (b) the patient's urine concentration of protein or blood cells; (c) the patient's urine specific gravity; (d) the amount of the patient's urine; (e) the patient's clearance rate of inulin, creatinine, urea or p-aminohippuric acid; (f) hypertension in the patient; (g) edema in the patient; and, (h) circulating autoantibody levels in the patient.

In one embodiment the present invention describes administration of recombinant LGALS3BP as an adjuvant to enhance the activity of a virally-directed vaccine by augmenting a protective antibody responses.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Fig. 1A** shows the data from primary human B cells that were isolated and stimulated with a small molecule TLR7 agonist and cultured for 5 days. A library of conditioned cell culture supernatants with secreted proteins was added and IgG secretion and cell viability (CTG, CellTiter-Glo) measured at the end of culture.

**Fig. 1B** shows data from different cellular subsets which were isolated by FACS from healthy controls (first data point in each cellular subset) and lupus nephritis patients with increasing levels of type I IFN (data points 2-4). RNA expression was analyzed by RNA-seq. Normalized FPKM expression values are presented on the graph.

**Fig. 1C** shows purified recombinant LGALS3BP that was added to purified human B cells stimulated with small molecule TLR7 agonist, CpG (ODN2006) or anti-IgM/CD40L/CpG (ODN2006). IgG was measure by AlphaLISA 5 days after stimulation.

**Fig. 1D** shows human PBMCs that were stimulated with small molecule TLR7 agonist and RNA isolated 5h later. Gene expression analysis was performed by RNA-seq and expression levels analyzed as normalized FPKM values.

**Fig. 2A-1 and Fig. 2A-2** show data from B cells stimulated with small molecule TLR7 agonist in the presence of increasing concentrations of purified recombinant LGALS3BP. B cell activation was measured 16h later by flow cytometry quantifying CD69 expression.

**Fig. 2B** presents data from experiments, wherein, an anti-LGALS3BP antibody was tested for specificity in a western blot with recombinant LGALS3BP (recLGALS3BP) and human plasma.

**Fig. 2C** shows localization of LGALS3BP as detected using anti-LGALS3BP antibody compared to CD19 B cell and DAPI nuclear stain.

**Fig. 3A-1 and Fig. 3A-2** show data from isolated primary human B cells that were stimulated with small molecule TLR7 agonist in the presence of potential LGALS3BP inhibitors and controls (left). Anti-LGALS3BP antibody was added to primary human B cells activated with CpG or anti-IgM/CD40L/CpG (right). IgG secretion was measured 5 days later by AlphaLISA.

**Fig. 3B-1** shows data from primary human B cells that were activated with small molecule TLR7 agonist in the presence of potential LGALS3BP inhibitors and controls. IgM secretion was measured 5 days later by AlphaLISA.

**Fig. 3B-2** shows data from primary human B cells that were activated with small molecule TLR7 agonist in the presence of potential LGALS3BP inhibitors and controls. B cell viability was measured 5 days later by CellTiter-Glo.

**Fig. 3B-3** shows data from primary human B cells that were activated with small molecule TLR7 agonist in the presence of potential LGALS3BP inhibitors and controls. IL-6 secretion was measured 2 days after stimulation by AlphaLISA.

**Fig. 3C-1** shows data from B cell activation in the presence of potential LGALS3BP inhibitors and controls as measured 16 hours after activation by quantification of CD69 expression by flow cytometry.

**Fig. 3C-2** shows data from B cell activation in the presence of potential LGALS3BP inhibitors and controls as measured 16 hours after activation by quantification of CD69 expression shown are percentages of cells that have upregulated CD69.

**Fig. 3C-3** shows data from B cell activation in the presence of potential LGALS3BP inhibitors and controls as measured 16 hours after activation by quantification of CD69 expression shown are mean fluorescence intensity (MFI) of CD69 detection on all B cells.

**Fig. 3D-1 and Fig. 3D-2** show data from experiments, wherein, an anti-LGALS3BP antibody was added to unstimulated primary human B cells and the subsequent viability of these B cells was measured 2 days later using CellTtiter-Glo.

**Fig. 4A** shows data from experiments, wherein, kidneys and spleens were collected from female MRL/lpr mice at 14 weeks of age (early disease). Tissue homogenates were analyzed by NanoString for expression of LGALS3BP and compared to C57BL/6 healthy control mice. Alternatively, RNA was isolated from blood or spleen samples of mice treated with pristane or PBS or from blood, spleen, or kidney of BXSB-Yaa old diseased mice or young control mice. Presented LGALS3BP gene expression levels were measured by QPCR and normalized to Hprt.

**Fig. 4B** shows data from experiments, wherein, SJL mice were immunized with proteolipid protein (PLP) to induce experimental autoimmune encephalomyelitis ("EAE"). On day 7 and 14 SJL-PLP EAE diseased mice were euthanized and lumbar spinal cords were collected. RNA was purified and analyzed by NanoString for expression of LGALS3BP and compared to naïve non-immunized healthy control mice. In the experiments described in Fig. 4A and 4B each experimental group contained 5 mice or more and diseased mice were compared to healthy controls with a non-paired Student's t test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

**Fig. 4C** presents "IFN gene signature scores". These scores were calculated based on the expression of 5 genes known to be interferon regulated (USP18, IRF7, IFIT1, OAS3, BST2). Mice were then grouped in 4 quartiles based on these scores and plotted against average LGALS3BP expression relative to healthy control mice.

**Fig. 5A** shows LGALS3BP expression by QPCR using RNA extracted from *in vitro* differentiated primary human macrophages activated with indicated stimuli for 6h. Expression between samples was normalized using HPRT1 as a housekeeping gene.

**Fig. 5B** shows LGALS3BP measured by ELISA in supernatants of *in vitro* differentiated primary human macrophages activated with indicated stimuli for 20h.

**Fig. 6A** shows primary B cells isolated from healthy controls (HC) and SLE patient blood were stimulated with TLR7 agonist in the presence (stim + Ab) or absence (stim only) of anti-

LGALS3BP antibody. IgM was measured in cultures after 5 days of stimulation. * P<0.05; **P<0.01 two-tailed paired student's t test.

**Fig. 6**B shows primary B cells isolated from healthy controls (HC) and SLE patient blood were stimulated with TLR7 agonist in the presence (stim + Ab) or absence (stim only) of anti-LGALS3BP antibody. IgG was measured in cultures after 5 days of stimulation. * P<0.05; **P<0.01 two-tailed paired student's t test.

**Fig. 7A-1 and Fig. 7A-2** shows data which validates the ability of anti-LGALS3BP antibody treatment to reduce antibody titers irrespective of specificity. B cells from healthy controls (HC) and SLE patients were stimulated with TLR7 agonist for 5 days and cell culture supernatants analyzed for 128 autoantibody specificities (IgM and IgG). Number of autoantigens recognized was calculated as specificities with a signal to noise ratio >3. Specificities with positive signal in unstimulated B cells + anti-LGALS3BP antibody were filtered out.

**Fig. 7B** shows a heatmap of antibody titers represented as z scores (sample – $avg_{all}$)/$std_{all}$. Each column represents one donor stimulated with TLR7 agonist with (+ Ab) or without (-) anti-LGALS3BP antibody. * P<0.05 two-tailed paired student's t test.

**Fig. 8A-1**, **Fig. 8A-2 and Fig. 8A-3** present data showing that anti-LGALS3BP antibody treatment reduces the viability of plasma cells. Freshly isolated B cells from healthy volunteers were differentiated into plasma cells in a two-step, 7 day protocol in the presence of cytokines driving B cell activation (step 1) and B cell differentiation (step 2). Flow cytometry of *in vitro* differentiated human antibody secreting cells (ASC), plasmablasts (PB) plasma cells (PC). Cells were pre-gated on $CD19^+$ B cells.

**Fig. 8B** shows day 7 differentiated plasma cells which were cultured in the presence or absence of anti-LGALS3BP antibody. Viability was measured by CellTiter-Glo (ATP production) after 4 days. * P<0.05 two-tailed paired student's t test.

**Fig. 9A-1 and Fig. 9A-2** show how anti-LGALS3BP antibody treatment induces apoptosis preferentially in B cells. Freshly isolated PBMCs from healthy donors were incubated in the presence or absence of anti-LGALS3BP antibody (aLGALS3BP), isotype control (Rabbit IgG), glycerol control or hydroxychloroquine analog (HCQ analog) for 3 days. In Fig. 9A-1, Annexin V and 7-AAD were measured by flow cytometry together with markers for B (CD19) and T (CD3) cells.

**Fig. 9B-1 and Fig. 9B-2** show average frequencies of Annexin V-positive apoptotic cells from 4 donors. Relative frequencies of B and T cells in total PBMCs. Frequencies were normalized to no treatment control.

**Fig. 10A-1, Fig. 10A-2 and Fig 10A-3** confirm that anti-LGALS3BP antibody SP-2 does not reduce B cell viability or antibody production. Freshly isolated B cells from healthy volunteers were stimulated with TLR7 agonist in the presence or absence of anti-LGALS3BP antibody SP-2 or PBS control for 5 days

**Fig. 10B** show how IgM and IgG were measured in cell culture supernatants by AlphaLISA, viability of cells by CellTiter-Glo (CTG).

DETAILED DESCRIPTION

Embodiments of the present invention are based on the role that LGALS3BP plays in IgG production and the implications of the same for the treatment of SLE and, more particularly, LN. These therapeutic embodiments of the present invention are validated by data showing the following. LGALS3BP is one of the most differentially regulated genes between lupus nephritis patients and healthy controls across multiple cell types. LGALS3BP closely correlates with IFN-inducible genes and is upregulated in human PBMCs after TLR7 stimulation. LGALS3BP enhances IgG secretion in ex-vivo stimulated primary human B cells. LGALS3BP is present on the surface of B cells and all other PBMCs. Blockade of LGALS3BP with antibody or lactose abrogates IgG production. LGALS3BP antibody blockade does not require the inhibitory FcγRIIb on B cells. LGALS3BP blockade specifically reduces viability of cultured primary human B cells with only a small effect on primary monocytes or total PBMCs and that LGALS3BP is upregulated in mouse models of SLE and EAE.

An LGALS3BP polypeptide refers to full length polypeptide sequence, as well as subsequences, fragments or portions, and modified forms and variants of LGALS3BP polypeptide, unless the context indicates otherwise. Such LGALS3BP subsequences, fragments, modified forms and variants have at least a part of, a function or activity of an unmodified or reference LGALS3BP protein. In particular embodiments a modified form or variant retains, at least a part of, a function or activity of an unmodified or reference protein. A "functional polypeptide" or "active polypeptide" refers to a modified polypeptide or a subsequence thereof. For example, a functional or active LGALS3BP polypeptide or a subsequence thereof possesses at least one partial function or activity (e.g., biological activity) characteristic of a native wild type or full length counterpart polypeptide, for example LGALS3BP, as disclosed herein, which function or activity can be identified through an assay. Embodiments of the present invention, therefore,

contemplate modified forms and variants of LGALS3BP polypeptide sequences, and subsequences, which modified forms or variants typically retain, at least a part of, one or more functions or activities of an unmodified or reference LGALS3BP polypeptide sequence.

As disclosed herein, particular non-limiting examples of a function or activity of LGALS3BP polypeptide is to modulate aberrant immune response, immune disorder, inflammatory response, or inflammation, or an autoimmune response, disorder or disease. In one embodiment said autoimmune disease is SLE. In a preferred embodiment said autoimmune disease is LN. While it is not intended that the present invention be limited to any specific mechanism additional, non-limiting, examples of a function or activity of LGALS3BP polypeptide is to modulate the expression of IgG.

An exemplary full length human LGALS3BP polypeptide sequence (SEQ ID NO: 1) is as follows:

MTPPRLFWVWLLVAGTQGVNDGDMRLADGGATNQGRVEIFYRGQWGTVCDNLWDLTDASVVC
RALGFENATQALGRAAFGQGSGPIMLDEVQCTGTEASLADCKSLGWLKSNCRHERDAGVVCT
NETRSTHTLDLSRELSEALGQIFDSQRGCDLSISVNVQGEDALGFCGHTVILTANLEAQALW
KEPGSNVTMSVDAECVPMVRDLLRYFYSRRIDITLSSVKCFHKLASAYGARQLQGYCASLFA
ILLPQDPSFQMPLDLYAYAVATGDALLEKLCLQFLAWNFEALTQAEAWPSVPTDLLQLLLPR
SDLAVPSELALLKAVDTWSWGERASHEEVEGLVEKIRFPMMLPEELFELQFNLSLYWSHEAL
FQKKTLQALEFHTVPFQLLARYKGLNLTEDTYKPRIYTSPTWSAFVTDSSWSARKSQLVYQS
RRGPLVKYSSDYFQAPSDYRYYPYQSFQTPQHPSFLFQDKRVSWSLVYLPTIQSCWNYGFSC
SSDELPVLGLTKSGGSDRTIAYENKALMLCEGLFVADVTDFEGWKAAIPSALDTNSSKSTSS
FPCPAGHFNGFRTVIRPFYLTNSSGVD

Definitions

A "polypeptide" refers to two, or more, amino acids linked by an amide or equivalent bond. A polypeptide can also be referred to herein, inter alia, as a protein, peptide, or an amino acid sequence. Polypeptides include at least two, or more, amino acids bound by an amide bond, or equivalent. Polypeptides can form intra or intermolecular disulfide bonds. Polypeptides can also form higher order structures, such as multimers or oligomers, with the same or different polypeptide, or other molecules.

The terms “patient” and “subject” are used in this disclosure to refer to a mammal being treated or in need of treatment for a condition such as SLE or LN. The terms include human patients and volunteers, non-human mammals such as a non-human primates, large animal models and rodents.

"Administering" or "administration of" a drug to a patient refers to direct administration, which may be administration to a patient by a medical professional or may be self-administration, and/or indirect administration, which may be the act of prescribing a drug. For example, a physician or clinic that instructs a patient to self-administer a drug or provides a patient with a prescription for a drug is administering the drug to the patient.

The terms "dose" and "dosage" refer to a specific amount of active or therapeutic agent(s) for administration at one time. A "dosage form" is a physically discrete unit that has been packaged or provided as unitary dosages for subjects being treated. It contains a predetermined quantity of active agent calculated to produce the desired onset, tolerability, and therapeutic effect.

A "therapeutically effective amount" of a drug refers to an amount of a drug that, when administered to a patient to treat a conditions such as SLE and LN, will have a beneficial effect, such as alleviation, amelioration, palliation or elimination of one or more symptoms, signs, or laboratory markers associated with the active or pathological form of the condition.

## EXAMPLES

The following examples are intended for illustration only and should not be construed to limit the scope of the claimed invention.

### EXAMPLE 1: LGALS3BP Enhances IgG Secretion in B Cells Activated With a TLR7 Agonist

To identify secreted proteins that affect IgG production by B cells a selection of proteins from the human secretome in an IgG secretion assay were screened using primary human B cells. B cells from healthy volunteers were exposed to 1400 recombinantly expressed secreted proteins before activation with a TLR7 small molecule agonist. After 5 days IgG was measured to identify proteins that enhance or inhibit IgG secretion. Besides B cell stimulatory cytokines such as IL-2 and IL10, this experiment demonstrated that LGALS3BP enhanced IgG secretion by 4.1-fold, while cell viability and metabolic activity (ATP measured by CellTiter-Glo assay) doubled (**Fig. 1a**). LGALS3BP was independently identified as the most differentially regulated gene in blood from lupus nephritis patients compared to healthy volunteers. LGALS3BP was upregulated in all cell types analyzed and correlated with the patient's interferon signature (**Fig. 1b**).

The enhanced IgG production (1.6-fold) was confirmed using purified recombinant LGALS3BP on B cells from 6 more healthy volunteer human subjects (**Fig. 1c**). Similar increases in IgG were observed when B cells were stimulated with the TLR9 agonist CpG (1.9-fold) or an activation cocktail with anti-IgM, CD40L and CpG (1.2-fold). PBMCs were simulated from healthy volunteers with a small molecule agonist to test if the activation protocol could enhance LGALS3BP expression *in vitro* **(Fig. 1b)**. Baseline expression values were comparable to those found in cells directly *ex vivo*. TLR7 stimulation did increase the expression levels by more than 3-fold. This finding provides an explanation for the variable effect the addition of exogenous LGALS3BP had on B cells from different donors. LGALS3BP was identified as one of the most differentially expressed gene in different immune cell types from LN patients compared to healthy volunteers and found an enhancing role for the secreted protein in antibody production.

LGALS3BP has an IRF binding site consistent with regulation by type I interferons. To determine which pathways can induce LGALS3BP expression, primary human monocytes were differentiated into macrophages *in vitro* and subsequently were stimulated with IFN-α, IFN-γ, TLR4 agonist (LPS), TLR7/8 agonist (resiquimod) and TLR9 agonist (CpG). IFN-α, IFN-γ and LPS induced LGALS3BP mRNA expression (**Fig. 5A**) and increased secretion of the protein (**Fig. 5B**). All stimuli induced secretion of IL-6. This indicates that not only type I interferons can drive LGALS3BP expression but also IFN-γ and other innate triggers.

Based on location of histone acetylation sites, LGALS3BP expression is regulated by factors binding to 4 different regions in the LGALS3BP gene: at the promoter start site, in an upstream enhancer (region 5 K upstream), in an intronic site, or in the 3' UTR. Motif scanning by 3 different methods identified likely immune-relevant transcriptional regulators. IRFs, AP-1, and STATs as well as other important factors such as NF-KB were found in and around the LGALS3BP gene locus. Prediction of transcription factor binding suggests that LGALS3BP expression is regulated by interferons through interferon regulatory factors (IRFs) as well as other immune stimuli that activate STATs, NF-kB, and AP-1.

**EXAMPLE 2: LGALS3BP is Present on the B Cell Surface but Does not Increase B Cell Activation**

To investigate if addition of LGALS3BP affects activation of naïve B cells CD69 expression was measured 16h after stimulation with TLR7 agonist. All B cells had increased CD69 expression compared to non-stimulated cells but no change was seen upon addition of various concentrations of recombinant LGALS3BP (**Fig. 2A-1 and Fig. 2A-2**). The localization of endogenous LGALS3BP in primary human B cells with an antibody specific for LGALS3BP was then evaluated (**Fig. 2b**). These studies confirmed that LGALS3BP is present on the B cell surface as well as on all other cell types found in PBMCs (**Fig. 2c**).

**EXAMPLE 3: Anti-LGALS3BP Inhibits IgG Secretion Through Induction of B Cell and Plasma Cell Apoptosis.**

The effect of anti-LGALS3BP antibodies on IgG secretion by primary human B cells was evaluated. IgG secretion by TLR7 activated B cells was inhibited by almost 90% in presence of anti-LGALS3BP antibody or anti-LGALS3BP F(ab')$_2$ (74%) to exclude inhibition through FcγRIIb present on B cells (**Fig. 3A-1 and 3A-2**). Lactose, a known ligand for LGALS3BP had the same but weaker effect (59% inhibition), while sucrose did not inhibit IgG secretion. The same inhibitory effect of the LGALS3BP antibody was observed when B cells were activated with CpG (94%) or anti-IgM/CD40L/CpG (77%). IgM secretion was inhibited by antibody blockade as well excluding a role of LGALS3BP in isotype switching (**Fig. 3B-1, Fig. 3B-2 and Fig. 3B-3**). Measuring ATP as a readout for cell number and viability showed a close correlation with IgG secretion, thereby, implicating LGALS3BP in B cell survival and/or proliferation. IL-6 secretion was measured to investigate if LGALS3BP blockade interferes with TLR7 activation and signaling thereby reducing B cell proliferation. A 37% decrease in IL-6 production was observed 48h after B cell stimulation in the presence of anti-LGALS3BP antibody. This reduction was LGALS3BP specific and not mediated through FcγRIIb given the same effect was measured in the presence of Fc block or with anti-LGALS3BP F(ab')$_2$. Lactose also had the same effect, thereby, excluding a direct effect of the antibody through cross-linking the surface-bound protein. Non-stimulated primary human B cells do not proliferate and have limited survival *in vitro*. To test if anti-LGALS3BP antibodies reduce B cell survival by blocking B cell activation CD69 upregulation was measured 16h after activation with TLR7 agonist (**Fig. 3C-1, Fig. 3C-2 and Fig. 3C-3**). No difference in percentage of CD69$^+$ activated cells or expression levels of CD69 was observed when an anti-LGALS3BP antibody was added. LGALS3BP blockade inhibits IgG secretion independent of the stimulation protocol used. To determine if

LGALS3BP blockade has an effect on B cell survival in the absence of stimulation additional experiments were conducted. Adding the antibody to non-stimulated B cells reduced viability by 66% (**Fig. 3D-1 and Fig. 3D-2**). This effect was most pronounced in B cells. Anti-LGALS3BP treatment of total PBMCs or monocytes showed a 37.5% and 39% reduction in viability. Together these results confirm an anti-apoptotic role of LGALS3BP during B cell homeostasis, activation, proliferation and differentiation.

Dysregulated B cell tolerance is a key driver of SLE pathogenesis. To address if anti-LGALS3BP treatment has the same effect on SLE B cells as observed in B cells from healthy donors, the B cell stimulation experiments were repeated in B cells from SLE donors. A significant reduction in IgM production was observed when the cells were stimulated with TLR7 agonist in the presence of anti-LGALS3BP antibody (**Fig. 6A** and **Fig. 6B**). There was reduction in IgG secretion, although not significant, accounted for due to the fact that B cells from SLE donors did not raise much IgG in response to TLR7 stimulation. These experiments confirm that the inhibitory effect of anti-LGALS3BP treatment is conserved in SLE B cells. Supernatants from TLR7-stimulated B cells on a 128 autoantigen protein microarray were analyzed (**Table 1**). Anti-LGALS3BP treatment reduced the number of autoantigens recognized by IgM antibodies (**Fig. 7B**) and uniformly reduced the IgM titers of all autoantigens, confirming that no specificity escapes anti-LGALS3BP treatment (**Fig. 7B**). These data confirm that anti-LGALS3BP treatment uniformly reduces antibody production by healthy as well as SLE patient B cells irrespective of specificity.

SLE patients usually have pre-existing long-lived plasma cells at the time when diagnosed with the disease. Treatments that deplete B cells are able to reduce antibody titers depending on the specificity. dsDNA-specific antibodies for example are reduced with B cell depletion, while others, such as RNP-specific ones remain elevated. Long-lived plasma cells, on the other hand, are not depleted and continue to secrete antibodies. An in vitro system to differentiate plasma cells from primary human B cells from healthy donors was designed to test if anti-LGALS3BP treatment has an effect on plasma cell viability (**Fig. 8A-1, Fig. 8A-2 and Fig. 8A-3**). The differentiated plasma cells were then exposed to anti-LGALS3BP antibodies for 4 days and viability was assessed indirectly by measuring ATP production. A significant reduction in plasma cell viability was observed, thereby, validating the therapeutic effect of anti-LGALS3BP treatment on long-lived plasma cells (**Fig. 8B**).

In order to determine if this reduced viability was due to necrosis or apoptosis of the targeted cells, PBMCs from healthy donors were incubated with anti-LGALS3BP antibodies for 4 days and subsequently annexin V surface expression and cell permeability (7-AAD) were measured by flow cytometry. Anti-LGALS3BP treatment induced expression of annexin V, which is consistent with cell death by apoptosis (**Fig. 9A-1, Fig. 9A-2, Fig. 9B-1 and Fig. 9B-2** ). Glycerol or control rabbit IgG did not produce the same effect, while high doses of a hydroxychloroquine analog also induced apoptosis. Comparing the frequency of B and T cells, the treatment affected B cells more than T cells in accordance with the prior observation that PBMCs or monocytes are not as susceptible to treatment as B cells.

These results confirm an anti-apoptotic role of LGALS3BP during B cell homeostasis, activation, proliferation and differentiation.

**Table 1: List of Antigens on the Autoantigen Array**

| | | | |
|---|---|---|---|
| Aggrecan | dsRNA | La/SSB | Ro/SSA(60KDa) |
| Alpha Fodrin(Sptan1) | dsDNA | Laminin | S100 |
| Alpha-actinin | EBNA1 | LC1 | Scl-70 |
| Amyloid | Elastin | LKM1 | Sm |
| AQP4 recombinant | Entaktin EDTA | M2 antigen | Sm/RNP |
| BP1 | Factor I | Matrigel | SmD |
| C1q | Factor P | MDA5 | SmD1 |
| Cardiolipin | Factor B | Mi-2 | SmD2 |
| CENP-A | Factor D | Mitochondrial antigen | SmD3 |
| CENP-B | Factor H | MPO | SP100 |
| Chondroitin Sulfate C | Fibrinogen IV | Muscarinic receptor | Sphingomyelin |
| Chromatin | Fibrinogen S | Myelin basic protein (MBP) | SPR54 |
| Collagen I | Fibronectin | Myelin-associated glycoprotein-FC | ssDNA |
| Collagen II | GBM (disso) | Myosin | T1F1 GAMMACollagen |
| Collagen III | Genomic DNA | Nucleolin | Thyroglobulin |
| Collagen IV | Gliadin (IgG) | Nucleosome antigen | TNFa |
| Collagen V | Glycated Albumin | Nup62 | Topoisomerase I |
| Collagen VI | GP2 | PCNA | TPO |
| Complement C1q | gP210 | Peroxiredoxin 1 | TTG |
| Complement C3 | Histone H1 | Phophatidylinositol | U1-snRNP-68 |
| Complement C3a | Histone H2A | PL-12 | U1-snRNP-A |
| Complement C3b | Histone H2B | PL-7 | U1-snRNP-BB' |
| Complement C4 | Histone H3 | PM/Scl-100 | U1-snRNP-C |
| Complement C5 | Histone H4 | PM/Scl-75 | Vimentin |
| Complement C6 | Hemocyanin | POLB | Vitronectin |
| Complement C7 | Heparan HSPG | PR3 | β2-glycoprotein I |
| Complement C8 | Heparin | Proteoglycan | β2-microglobulin |
| Complement C9 | Heparan Sulfate | Prothrombin protein | IgA - human and mouse |
| CPR antigen(human) | Histone (total) | Ribo phosphoprotein P1 | IgE- human |
| Cytochrome C | Intrinsic Factor | Ribo phosphoprotein P2 | IgG - human and mouse |
| Decorin-bovine | Jo-1 | Ribo phosphoprotein P0 | IgM - human and mouse |
| DGPS | KU (P70/P80) | Ro/SSA (52KDa) | Anti-IgG, IgA and anti-IgM |

**EXAMPLE 4: LGALS3BP Expression is Increased in Mouse Models of SLE and EAE Model**

The following experiments tested if the increase of LGALS3BP expression in lupus nephritis patients is conserved in mouse models of SLE. MRL/lpr mice have a mutation in Fas resulting in a defect in lymphocyte apoptosis which ultimately manifests in an SLE-like autoimmune disease. Comparison of MRL/lpr and wildtype C57/BL6 animals showed a significant increase in LGALS3BP expression in kidneys and spleens of diseased animals (**Fig. 4A**). The same was observed in an induced mouse model of SLE where intraperitoneal injection of pristane leads to autoantibodies, proteinuria and nephritis. These mice also develop an IFN signature detectable in blood and spleen similar to the IFN-induced genes observed in SLE human patients. BXSB/Yaa mice have a duplication of a genetic region that spans the innate RNA sensor TLR7 and develop SLE-like symptoms. TLR7 is known to play an important role in SLE and TLR7 activation leads to the secretion of type I IFNs. Knowing that LGALS3BP expression is inducible by TLR7 stimulation and that its expression correlates with the IFN signature in lupus nephritis human patients LGALS3BP expression was measured across multiple organs in BXSB/Yaa mice. A significant increase in LGALS3BP mRNA was found only in kidney samples of mice that had developed nephritis. Two mice had low nephritis scores and did not show an increase in LGALS3BP expression. In order to evaluate if LGALS3BP expression tracked with IFN-regulated genes, "IFN gene signature scores" were calculated based on the expression of 5 genes (*usp18, irf7, ifit1, oas3, bst2*). These scores confirmed the same correlation of LGALS3BP expression with IFN scores found in LN patients. Upregulation of IFN-induced genes was also limited to the kidney, further validating the link of LGALS3BP to the IFN response. LGALS3BP was also found to be differentially expressed in multiple sclerosis (MS) human patients and in EAE mice (Raddatz *et al.*, PLUS ONE 2014). This finding was confirmed by immunizing SJL mice with proteolipid protein (PLP) to induce EAE. LGALS3BP expression was significantly increased 14 days after induction of disease (**Fig. 4C**).

**EXAMPLE 5: Galectin-3 Inhibition Does Not Reduce B Cell Viability and Antibody Production**

Primary B cells from healthy human donors were stimulated in the presence of galectin-3 inhibitors in order to determine if galectin-3 plays a role in the function of LGALS3BP in B cell biology. Specifically, freshly isolated B cells from healthy volunteers were pre-incubated with

galectin-3 (Gal-3) inhibitors for 30 minutes before stimulation with TLR7 agonist for 5 days. Supernatants were harvested and IgG measured by AlphaLISA. Cell viability was measured by CellTiter-Glo (ATP production). None of the inhibitors had an effect on B cell viability or antibody production, indicating that galectin-3 is not directly involved in antibody production by B cells (**Table 2**).

**Table 2: Galectin-1 and Galectin-3 Inhibitors do not Induce B cell Apoptosis and Reduction in Antibody Secretion.**

| Compound | Inhibits | IgG production | Viability |
|---|---|---|---|
| LacNAc, N-Acetyl-D-lactosamine | Gal-3 | > 10 μM | > 10 μM |
| Pectin *(Pienta KJ et al. J Natl Cancer Inst. 1995)* | Gal-3 | > 10 μM | > 10 μM |
| Beta n-propal lactoside | Gal-3 | > 10 μM | > 10 μM |

**EXAMPLE 6: SP-2, an Anti-LGALS3BP Tumor-Inhibitory Antibody Does Not Affect B Cell Viability or Antibody Production**

LGALS3BP has been reported to play a role in cancer and SP-2, an anti-LGALS3BP antibody inhibits tumor growth and angiogenesis. SP-2 was tested in a B cell stimulation system and no effect on B cell viability or antibody production was observed (**Fig. 10A-1, Fig. 10A-2, Fig. 10A-3 and 10B-1**). Moreover, SP-2 targets the C-terminal domain of LGALS3BP, while the antibody that inhibits B cell viability and antibody production was raised against domain 2, indicating separate functions for different domains of the protein.

For all purposes in the United States of America, each and every publication and patent document cited herein is incorporated by reference for all purposes as if each such publication or document was specifically and individually indicated to be incorporated, herein, by reference.

While the invention has been described with reference to the specific embodiments, changes can be made and equivalents can be substituted to adapt to a particular context or intended use, thereby achieving benefits of the invention without departing from the scope of the claims that follow.

# CLAIMS

1. A method for modulating LGALS3BP in a subject presenting symptoms of an immune disorder, inflammatory response or autoimmune disease comprising administering an anti-LGALS3BP antibody to said subject under conditions such that at least one symptom of said immune disorder, inflammatory response or disease said is improved.

2. The method of claim 1, wherein, said immune disorder, inflammatory response or autoimmune disease is selected from the group consisting essentially of Graves' disease, myasthenia gravis, vasculitis and Wegener's granulomatosis, neuromyelitis optica, primary sclerosing cholangitis, Sjoegren's syndrome, lupus nephritis and rheumatoid arthritis.

3. A method of treating a patient with SLE, comprising administering to the patient a therapeutically effective amount of an anti-LGALS3BP antibody.

4. The method of claim 3 wherein the amount of anti-LGALS3BP antibody is effective to: (a) inhibit progression of nephritis; (b) stabilize nephritis; or, (c) reverse nephritis, in the patient.

5. The method of claim 3 wherein the amount of anti- LGALS3BP antibody is effective to (a) inhibit progression of proteinuria; (b) stabilize proteinuria; or, (c) reverse proteinuria, in the patient.

6. The method of claim 3 wherein the amount of anti- LGALS3BP antibody is effective to stabilize or decrease, in the patient, a clinical parameter selected from; (a) the patient's blood concentration of urea, creatinine or protein; (b) the patient's urine concentration of protein or blood cells; (c) the patient's urine specific gravity; (d) the amount of the patient's urine; (e) the patient's clearance rate of inulin, creatinine, urea or p-aminohippuric acid; (f) hypertension in the patient; (g) edema in the patient; and, (h) circulating autoantibody levels in the patient.

7. A method of using recombinant LGALS3BP as an adjuvant to enhance the activity of a virally-directed vaccine.

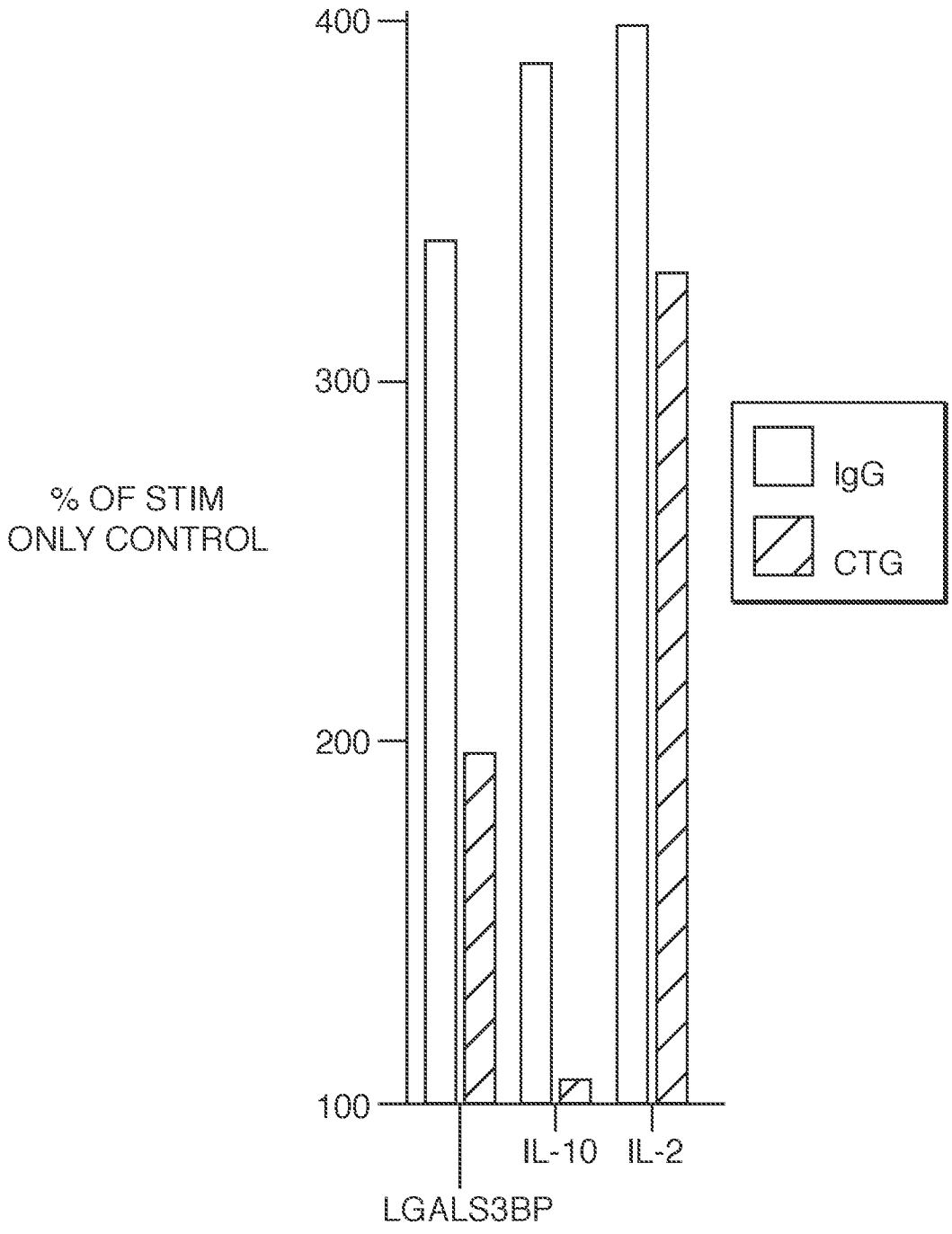
400
300
200
100
% OF STIM
ONLY CONTROL
IgG
CTG
LGALS3BP
IL-10
IL-2

FIG. 1A

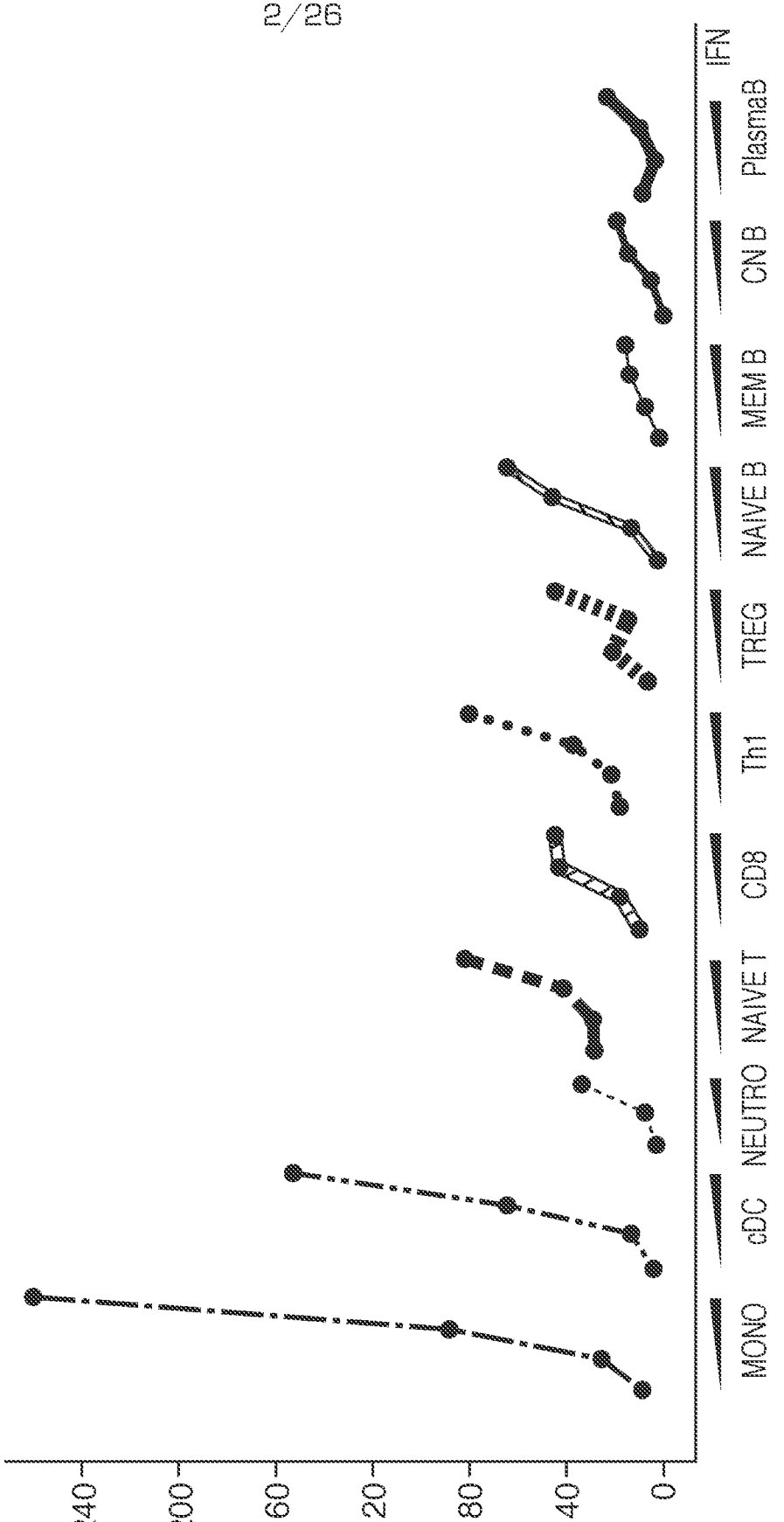
AVG LGALS3BP FPKM
240
200
160
120
80
40
0
MONO
cDC
NEUTRO
NAIVE T
CD8
Th1
TREG
NAIVE B
MEM B
CN B
PlasmaB
IFN

FIG. 1B

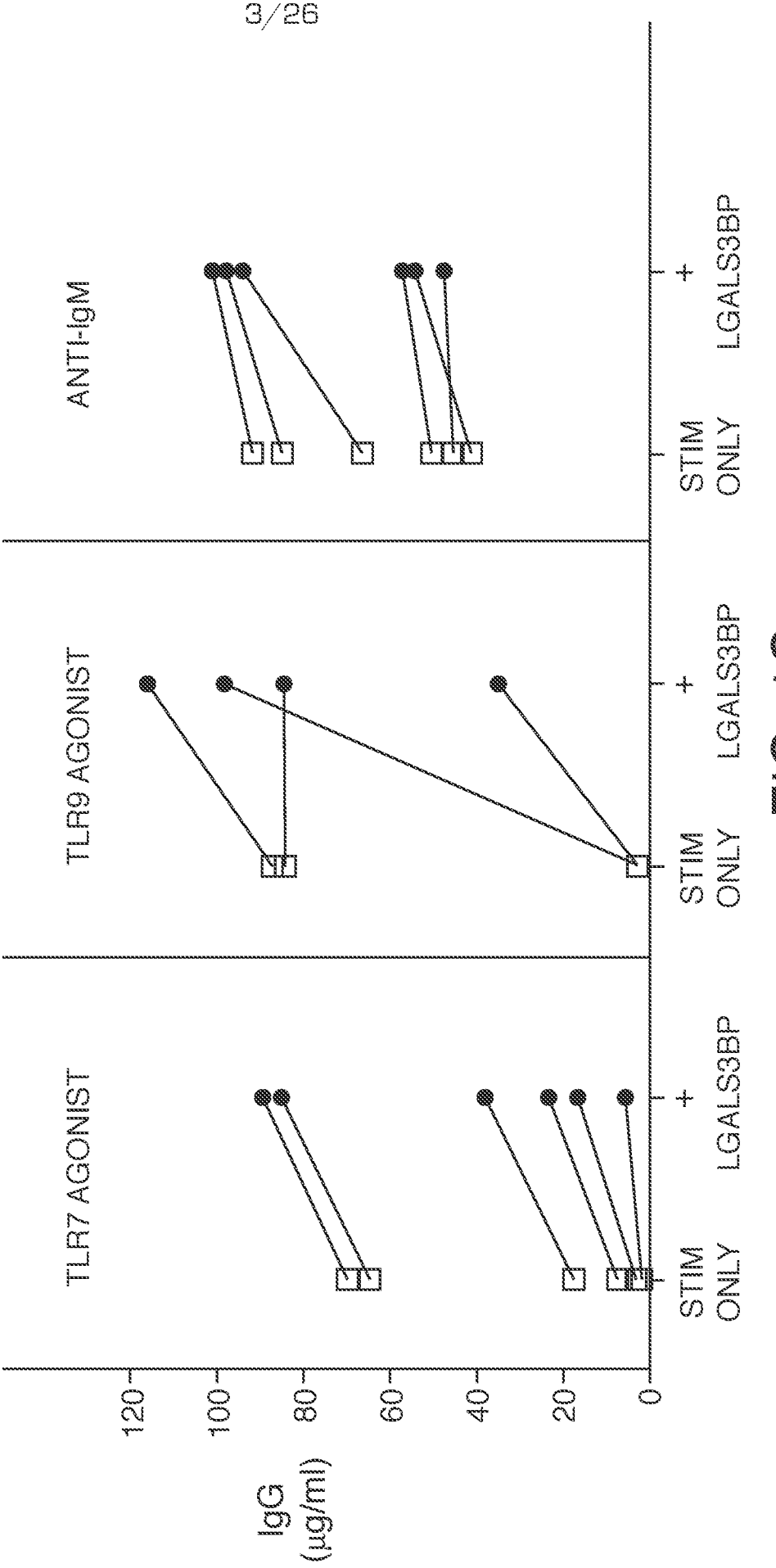
IgG (µg/ml)
0
20
40
60
80
100
120
TLR7 AGONIST
TLR9 AGONIST
ANTI-IgM
STIM ONLY
+
LGALS3BP

FIG. 1C

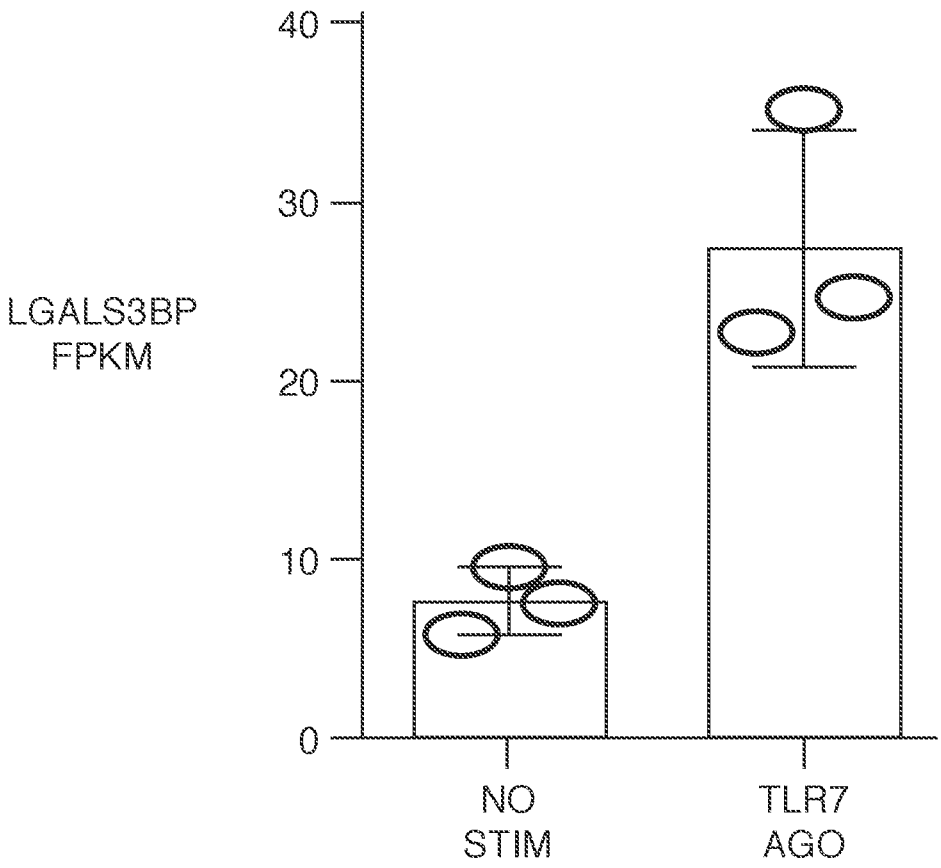


FIG. 1D

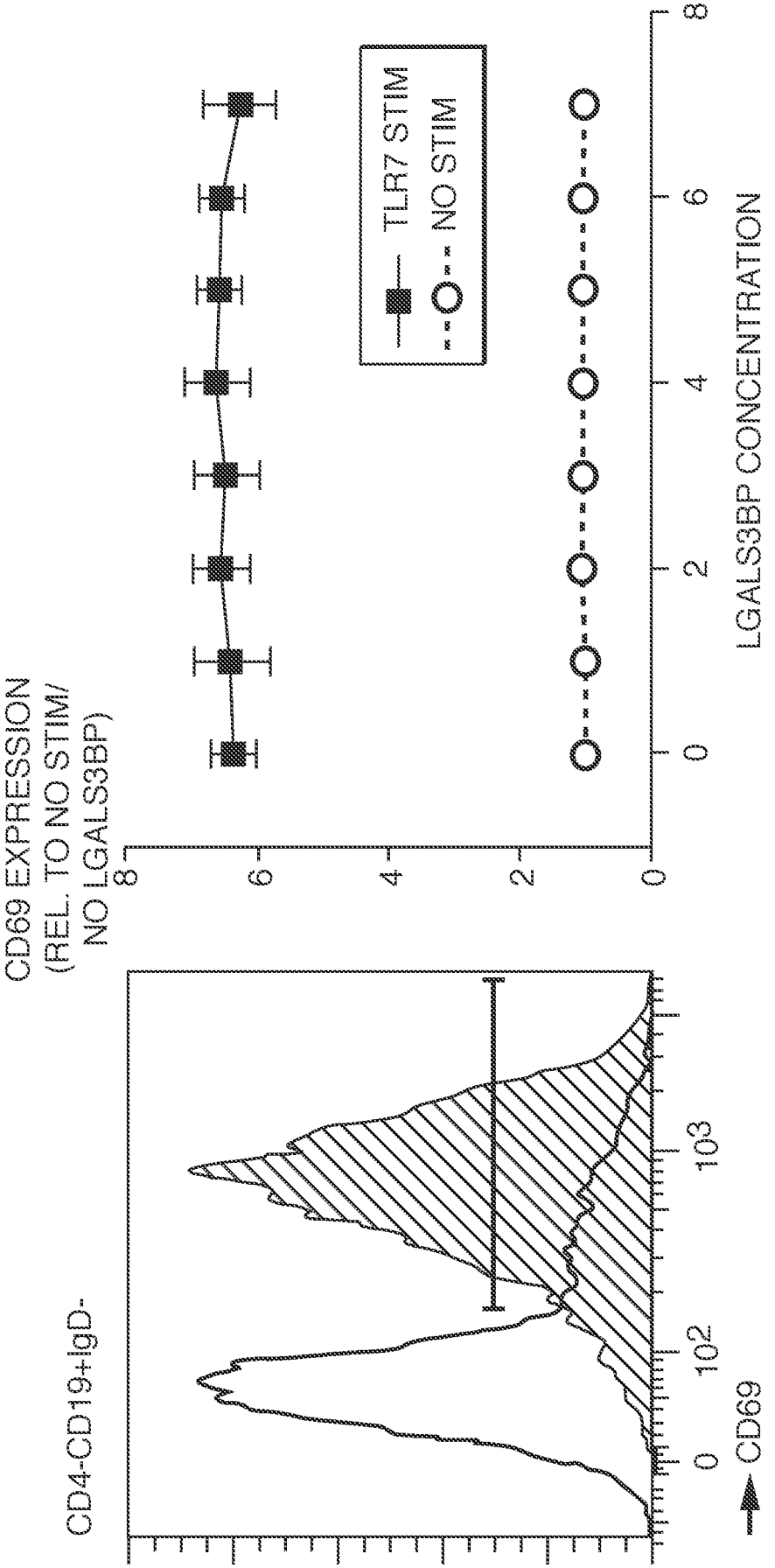


FIG. 2A-2




FIG. 2A-1

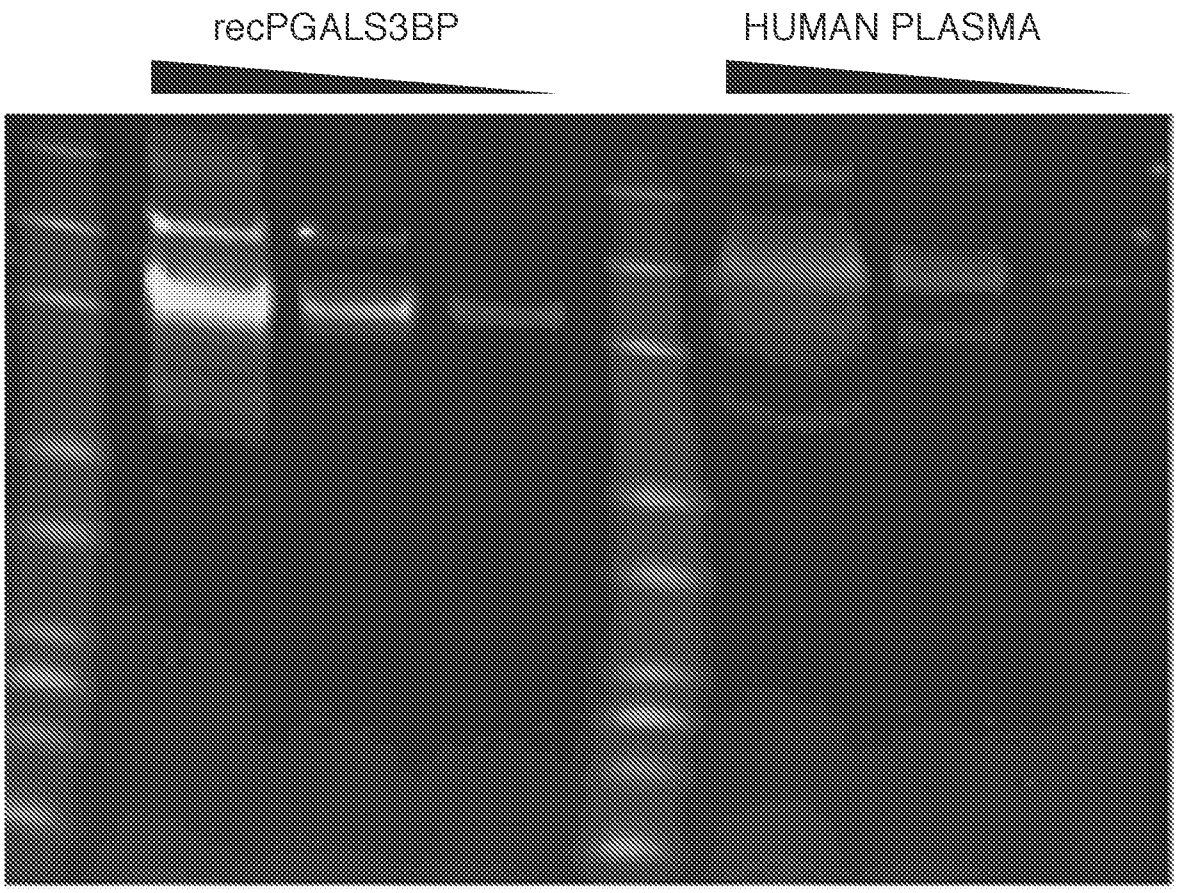


FIG. 2B

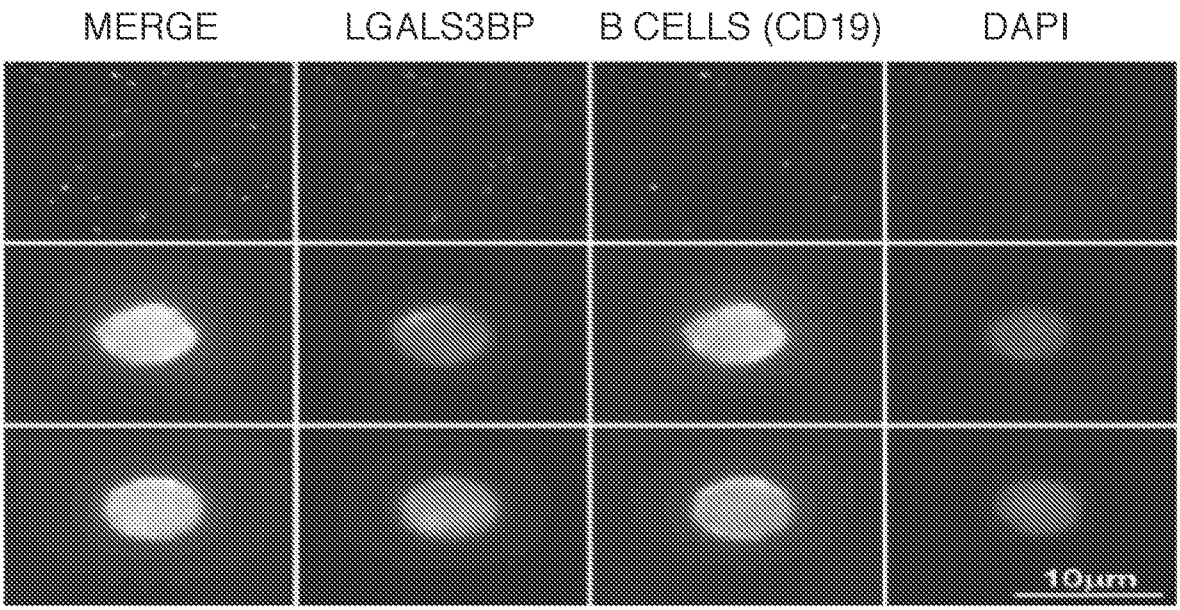


FIG. 2C

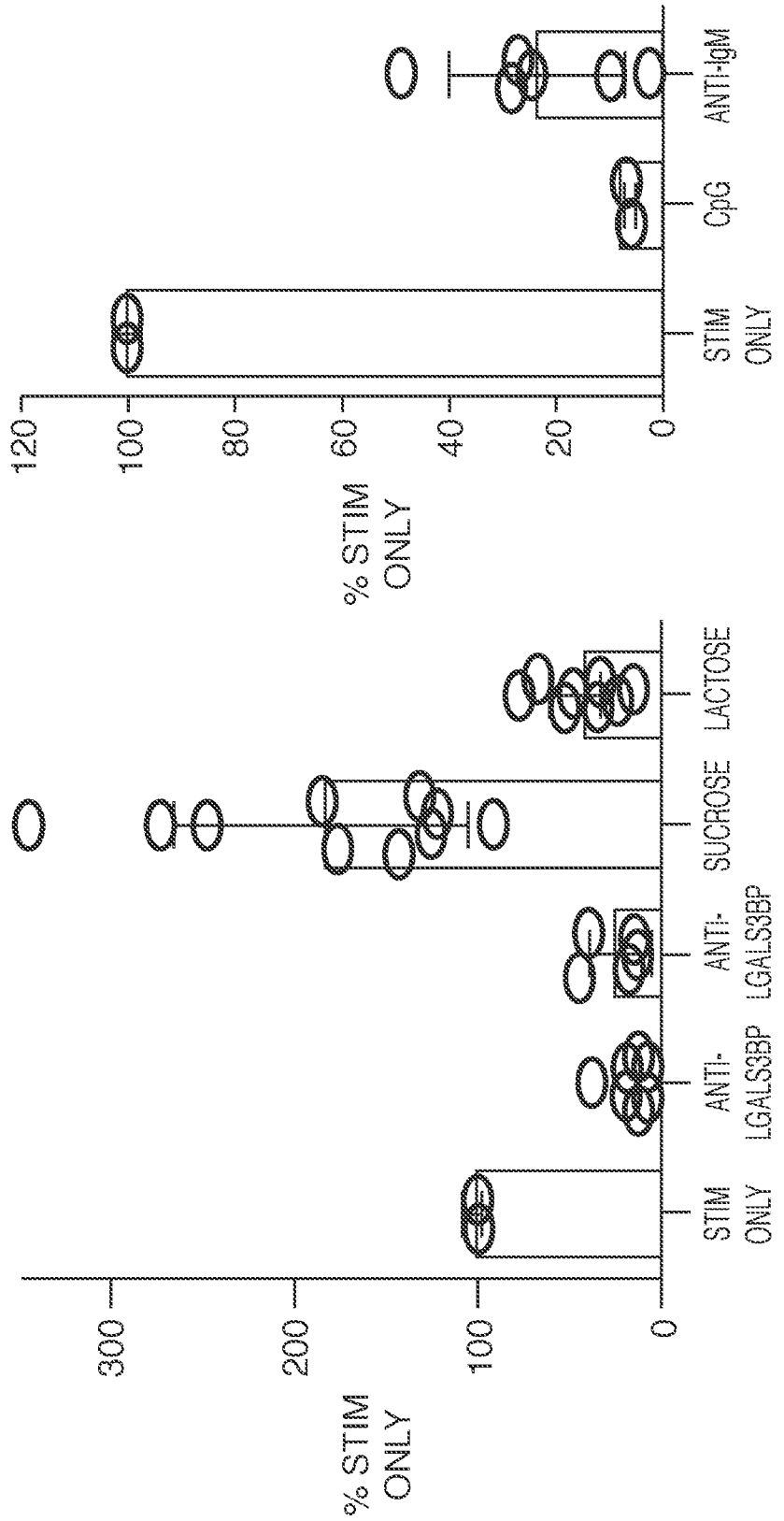


FIG. 3A-1

FIG. 3A-2

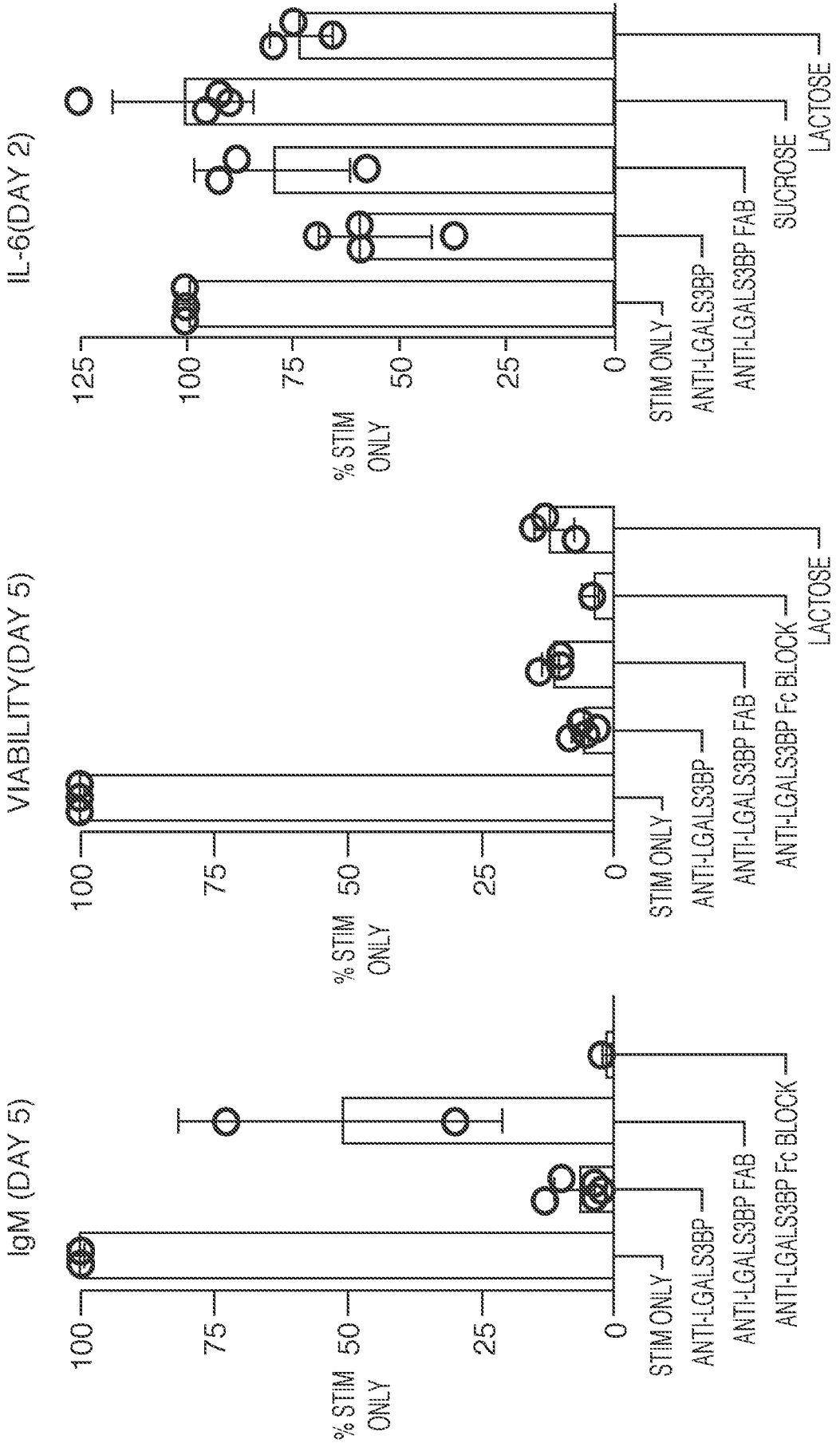


FIG. 3B-1

FIG. 3B-2

FIG. 3B-3

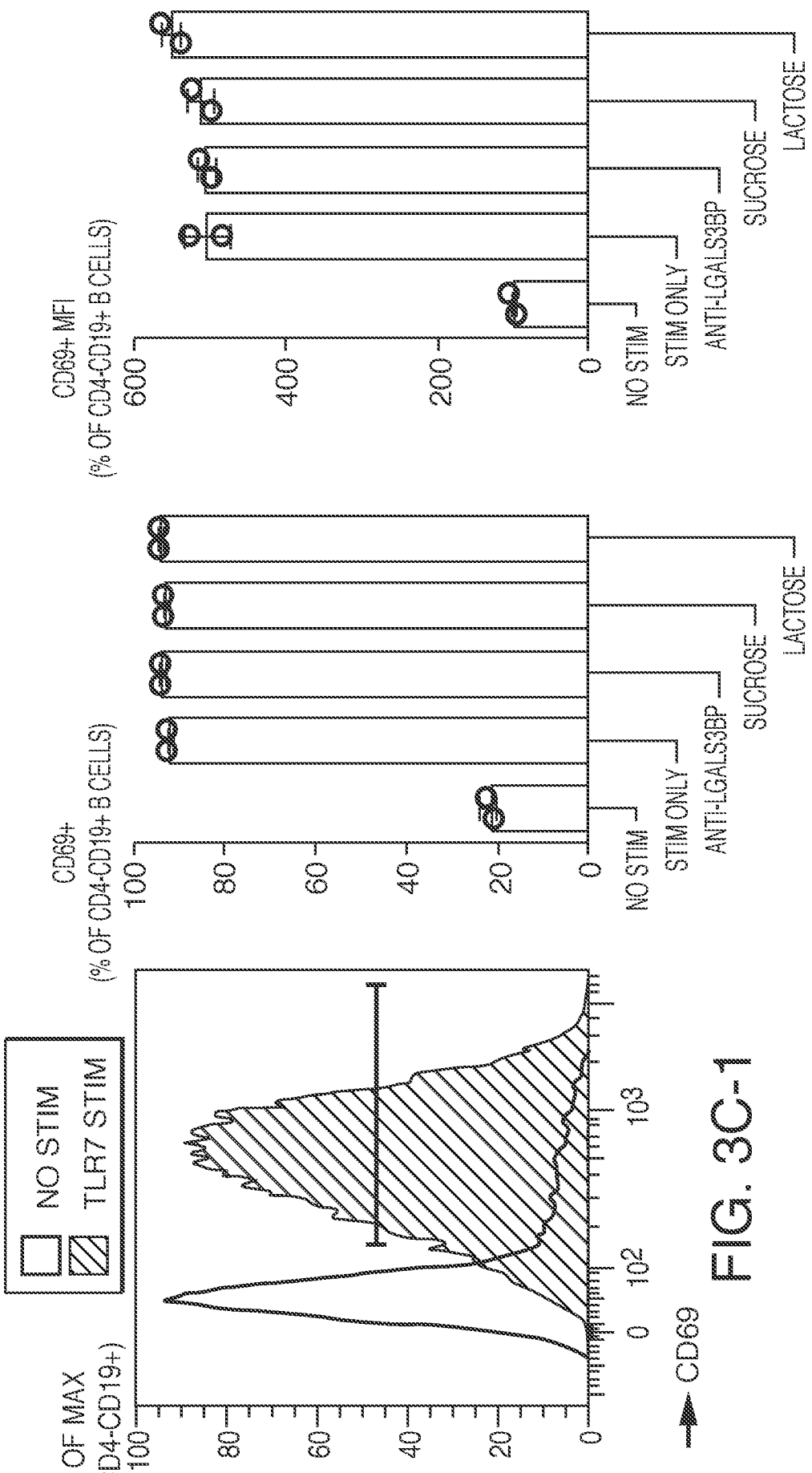


FIG. 3C-1

FIG. 3C-2

FIG. 3C-3

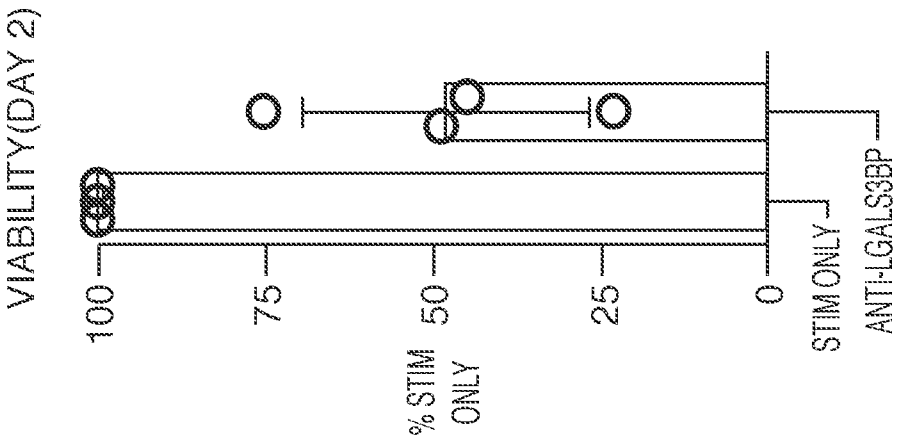


FIG. 3D-1

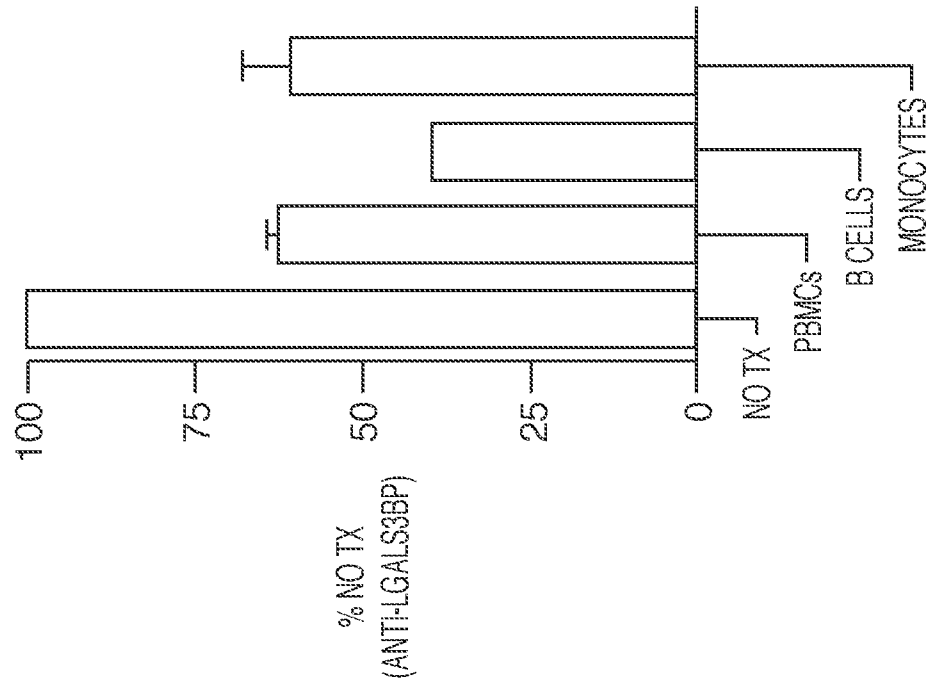


FIG. 3D-2

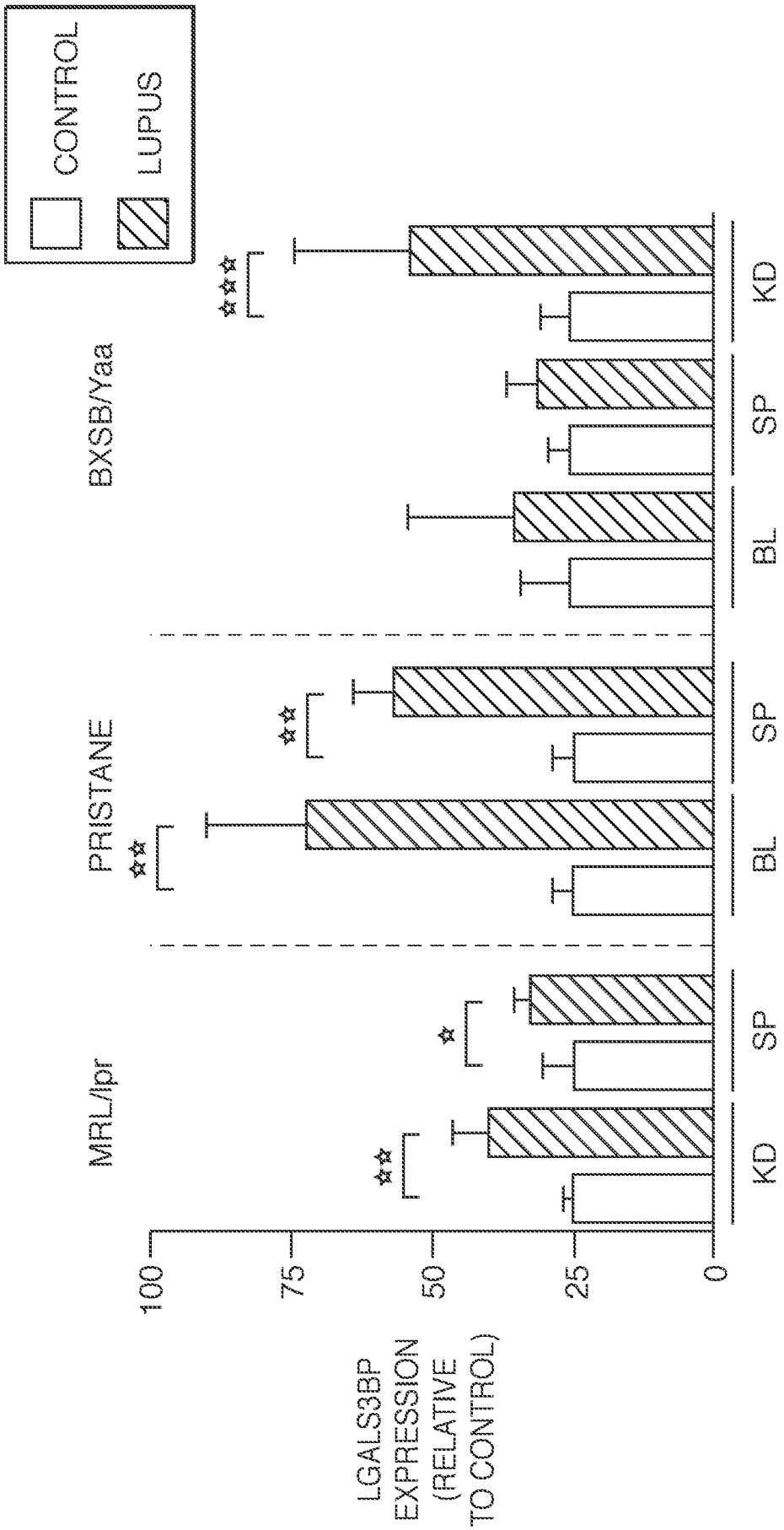
CONTROL
LUPUS
LGALS3BP EXPRESSION (RELATIVE TO CONTROL)
100
75
50
25
0
MRL/lpr
PRISTANE
BXSB/Yaa
KD
SP
BL
SP
KD
SP
BL
SP
KD

FIG. 4A

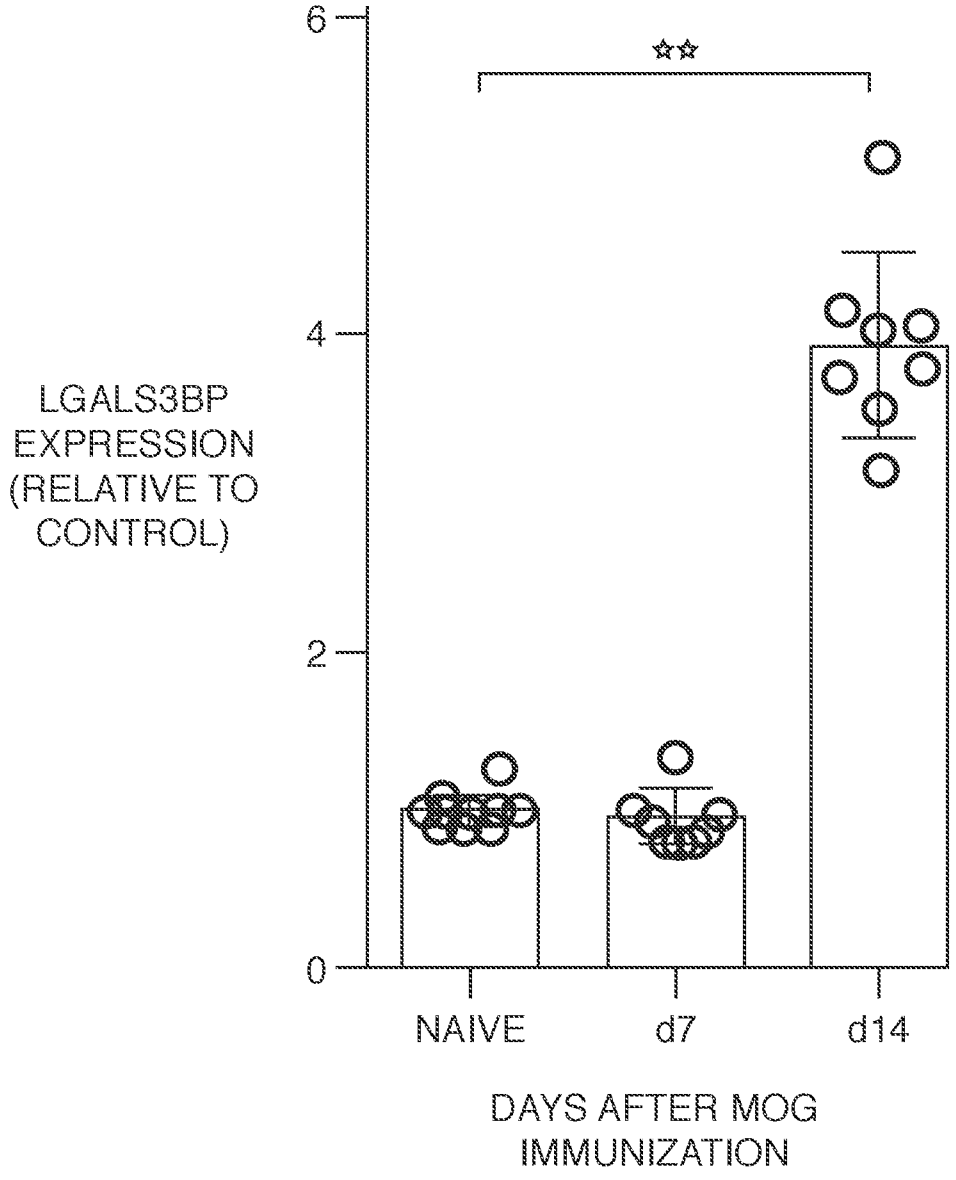
6
4
2
0
**
LGALS3BP EXPRESSION (RELATIVE TO CONTROL)
NAIVE
d7
d14
DAYS AFTER MOG IMMUNIZATION

FIG. 4B

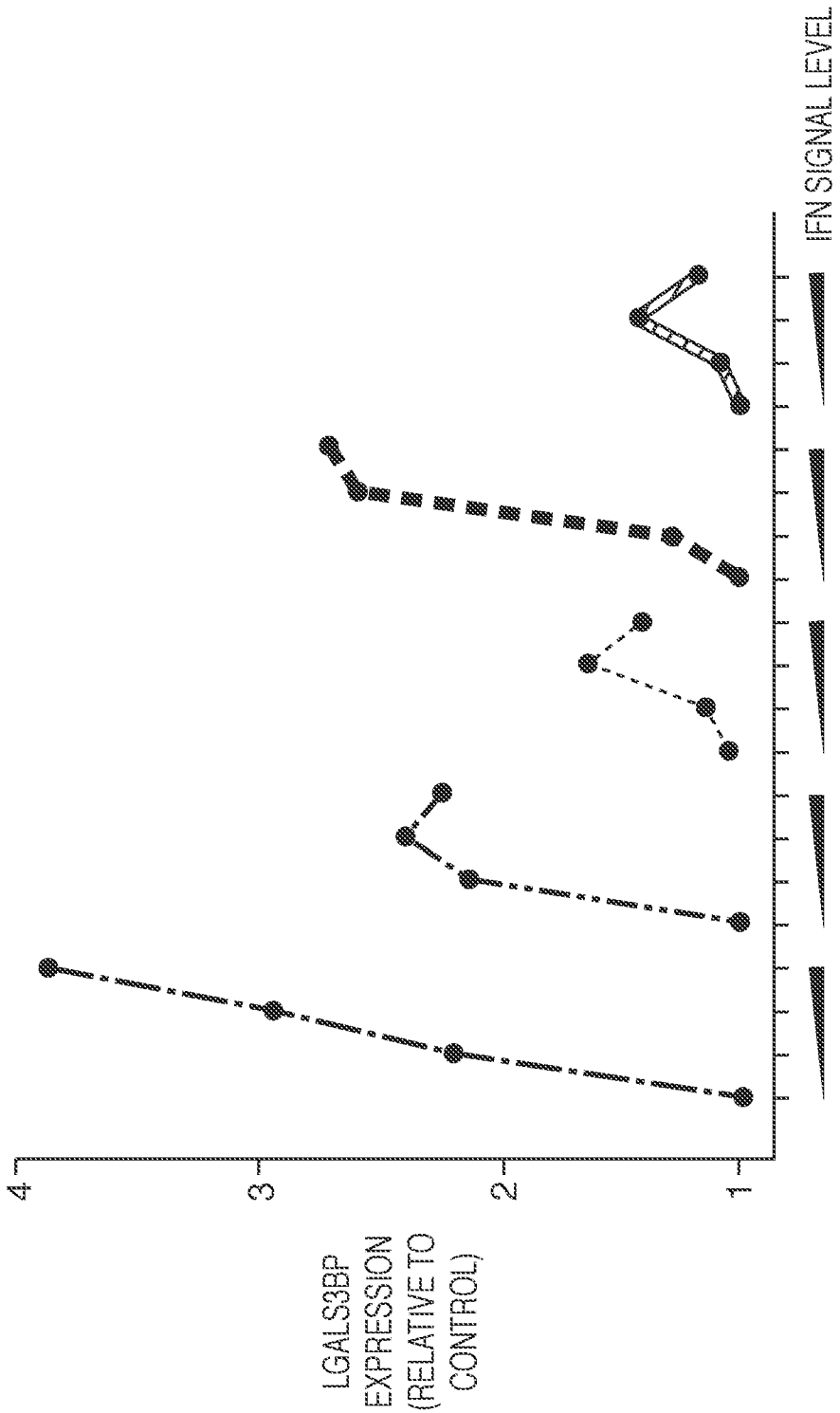
LGALS3BP EXPRESSION (RELATIVE TO CONTROL)
4
3
2
1
IFN SIGNAL LEVEL

FIG. 4C

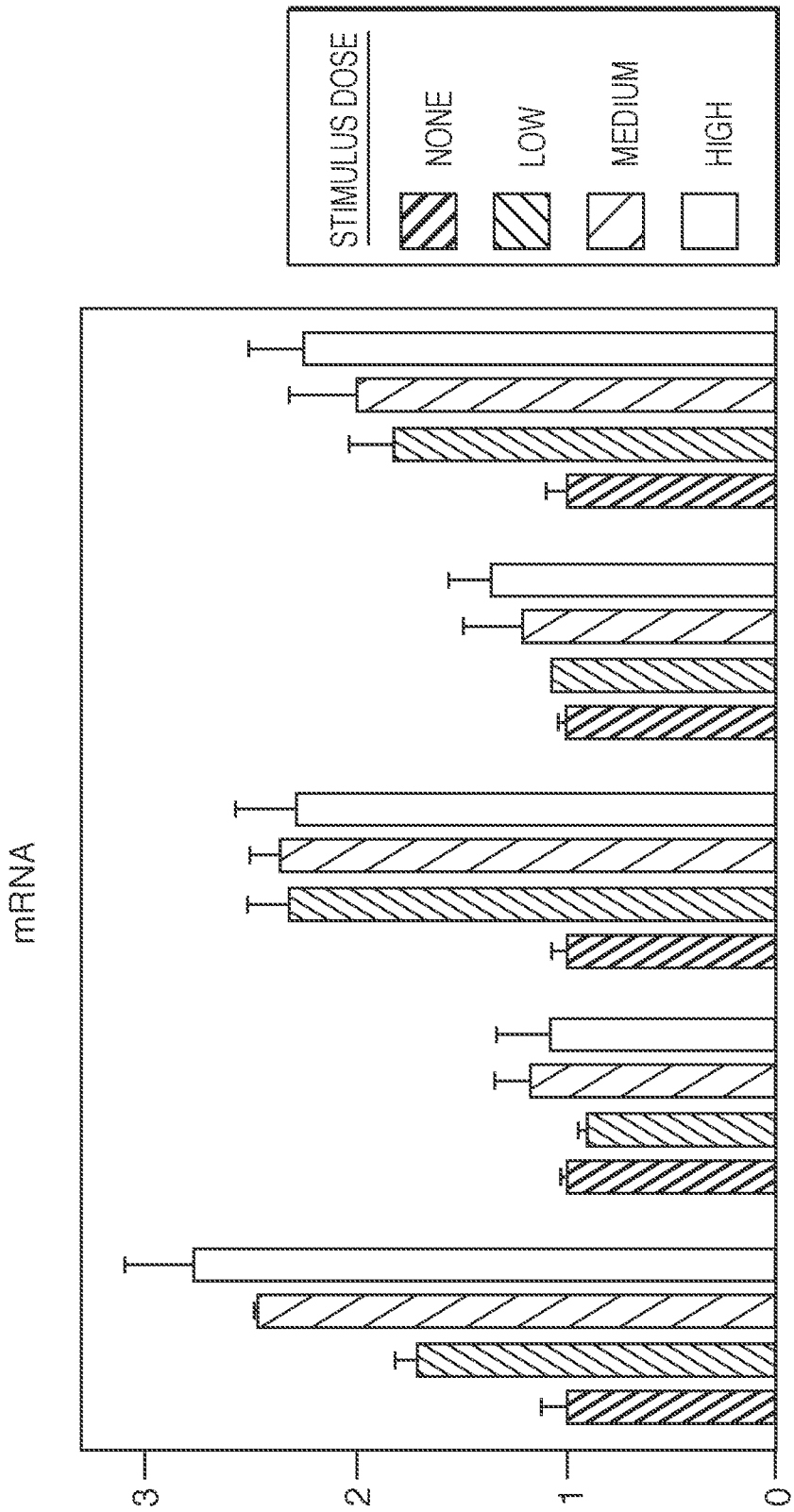
STIMULUS DOSE
NONE
LOW
MEDIUM
HIGH
mRNA
LGALS3BP EXPRESSION (RELATIVE TO CONTROL)
0
1
2
3
IFNα
TLR9
TLR4
TLR7/8
IFNγ

FIG. 5A

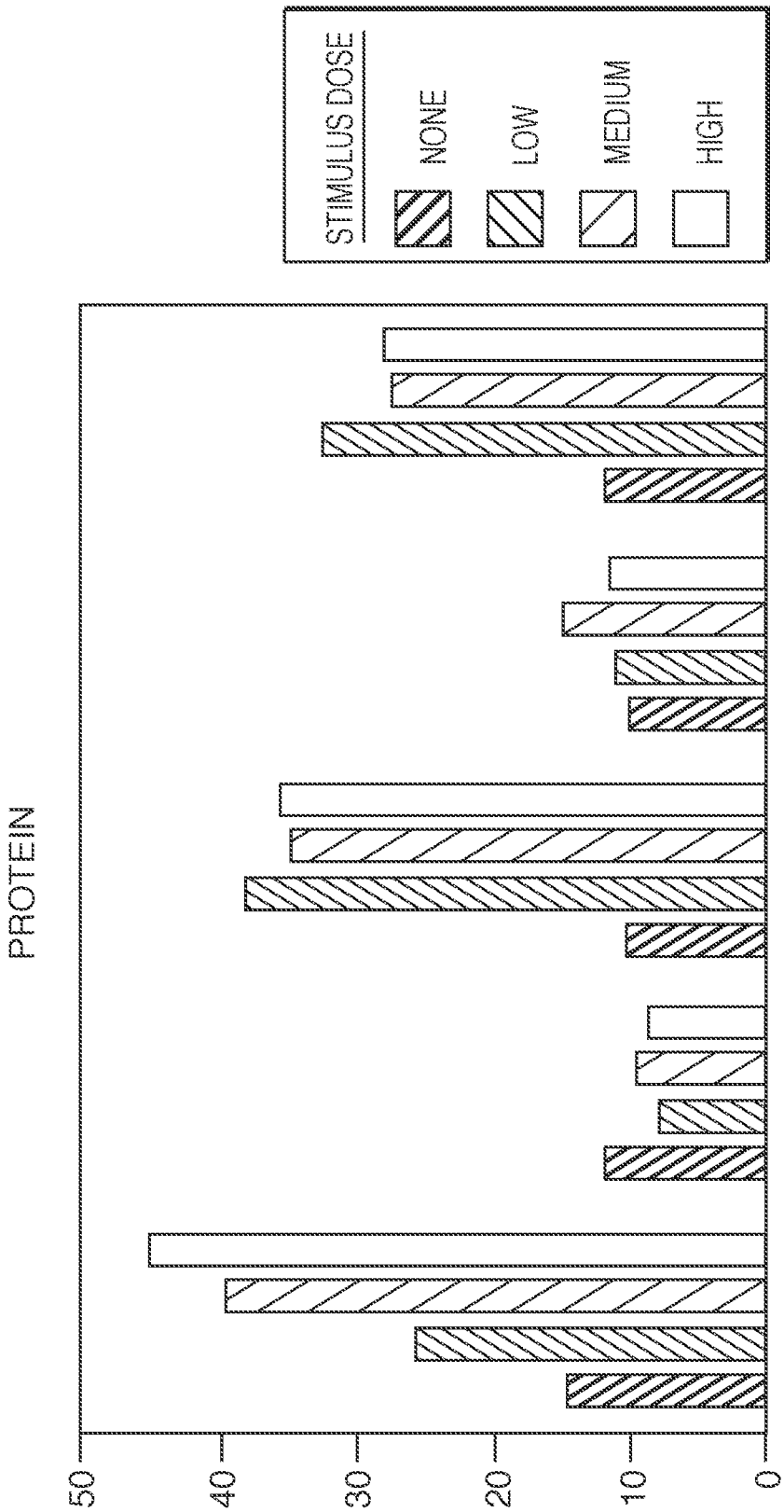
STIMULUS DOSE
NONE
LOW
MEDIUM
HIGH
PROTEIN
LGALS3BP (ng/ml)
0
10
20
30
40
50
IFNα
TLR9
TLR4
TLR7/8
IFNγ

FIG. 5B

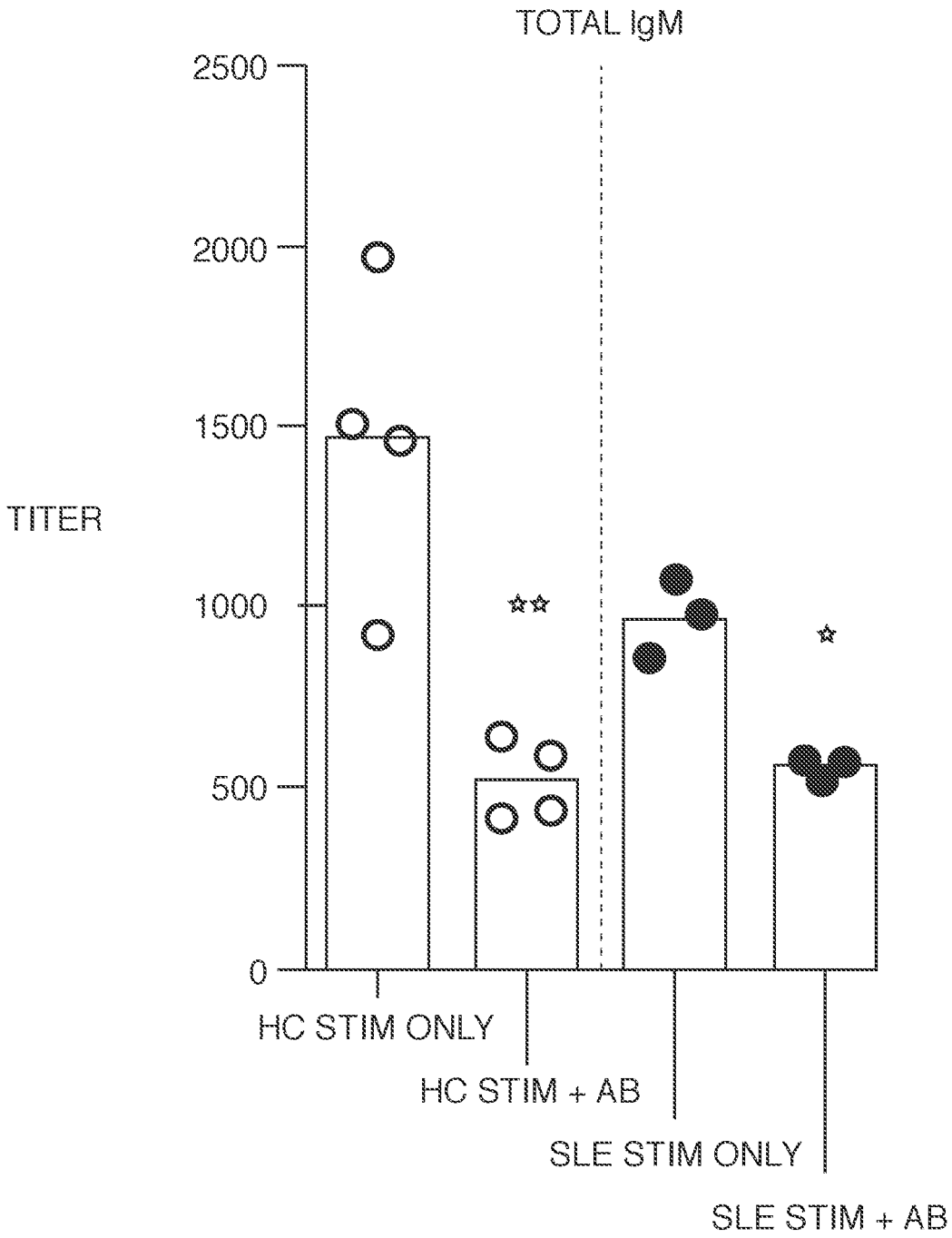
TOTAL IgM
2500
2000
1500
1000
500
0
TITER
☆☆
☆
HC STIM ONLY
HC STIM + AB
SLE STIM ONLY
SLE STIM + AB

FIG. 6A

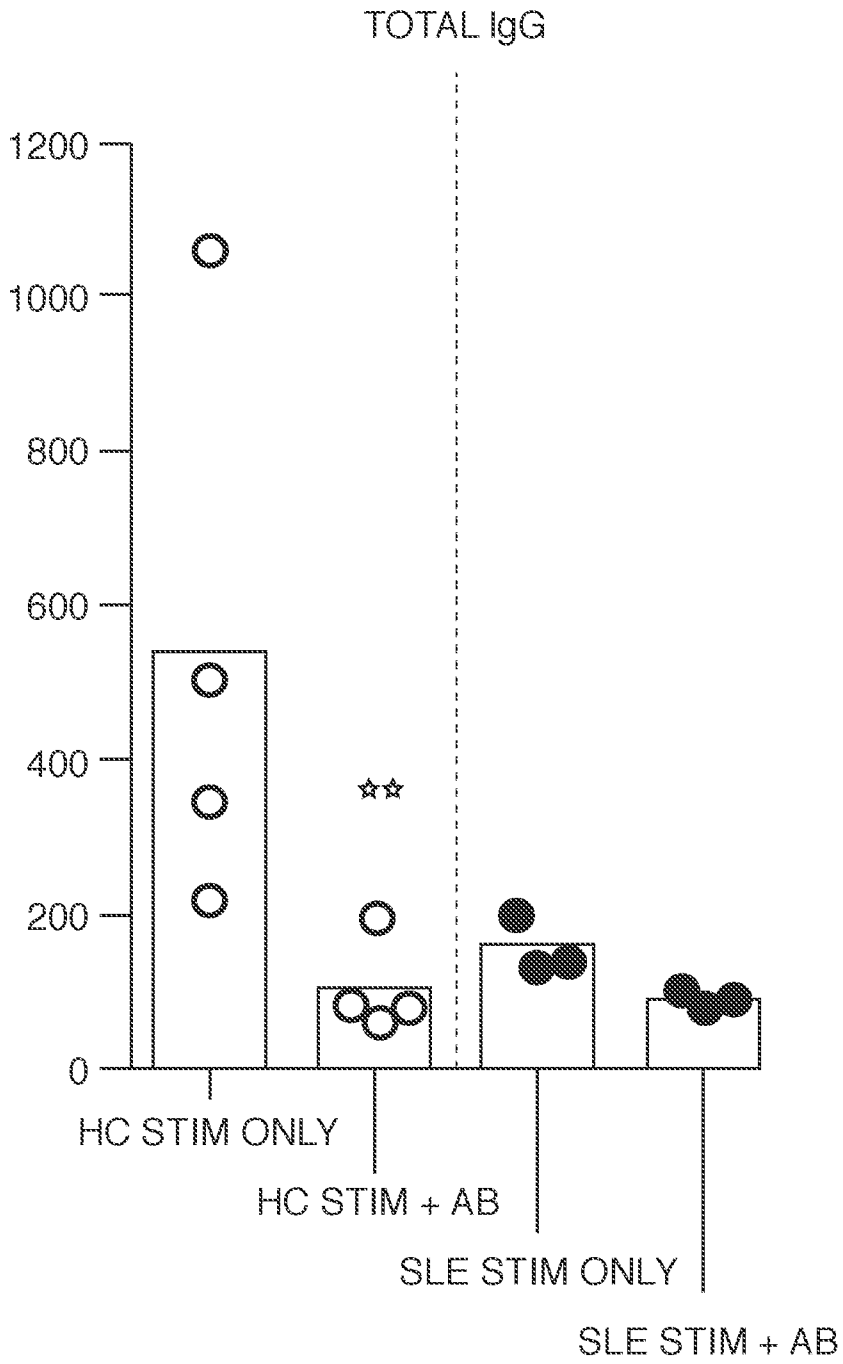
TOTAL IgG
1200
1000
800
600
400
200
0
HC STIM ONLY
HC STIM + AB
SLE STIM ONLY
SLE STIM + AB

FIG. 6B

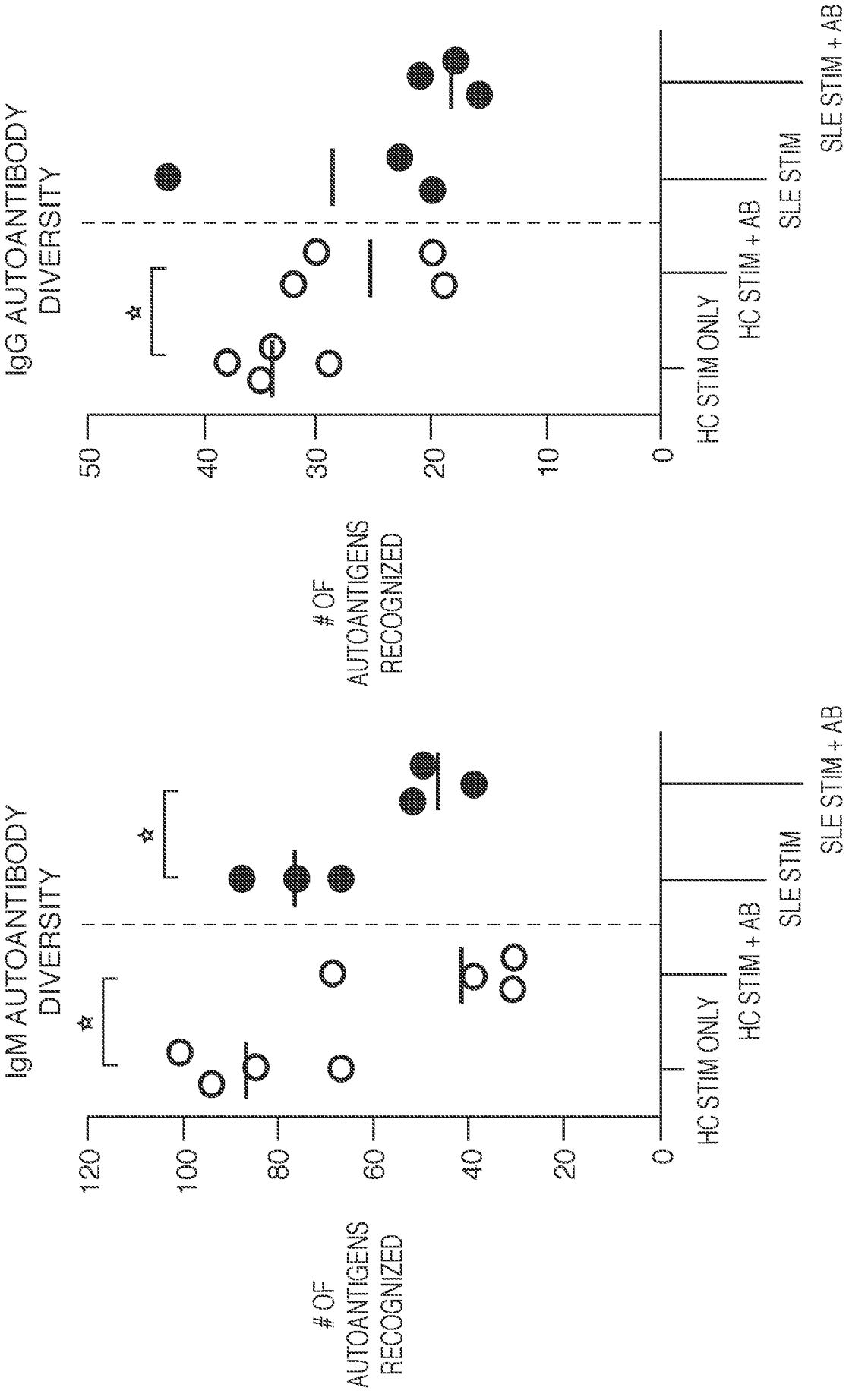


FIG. 7A-1

FIG. 7A-2

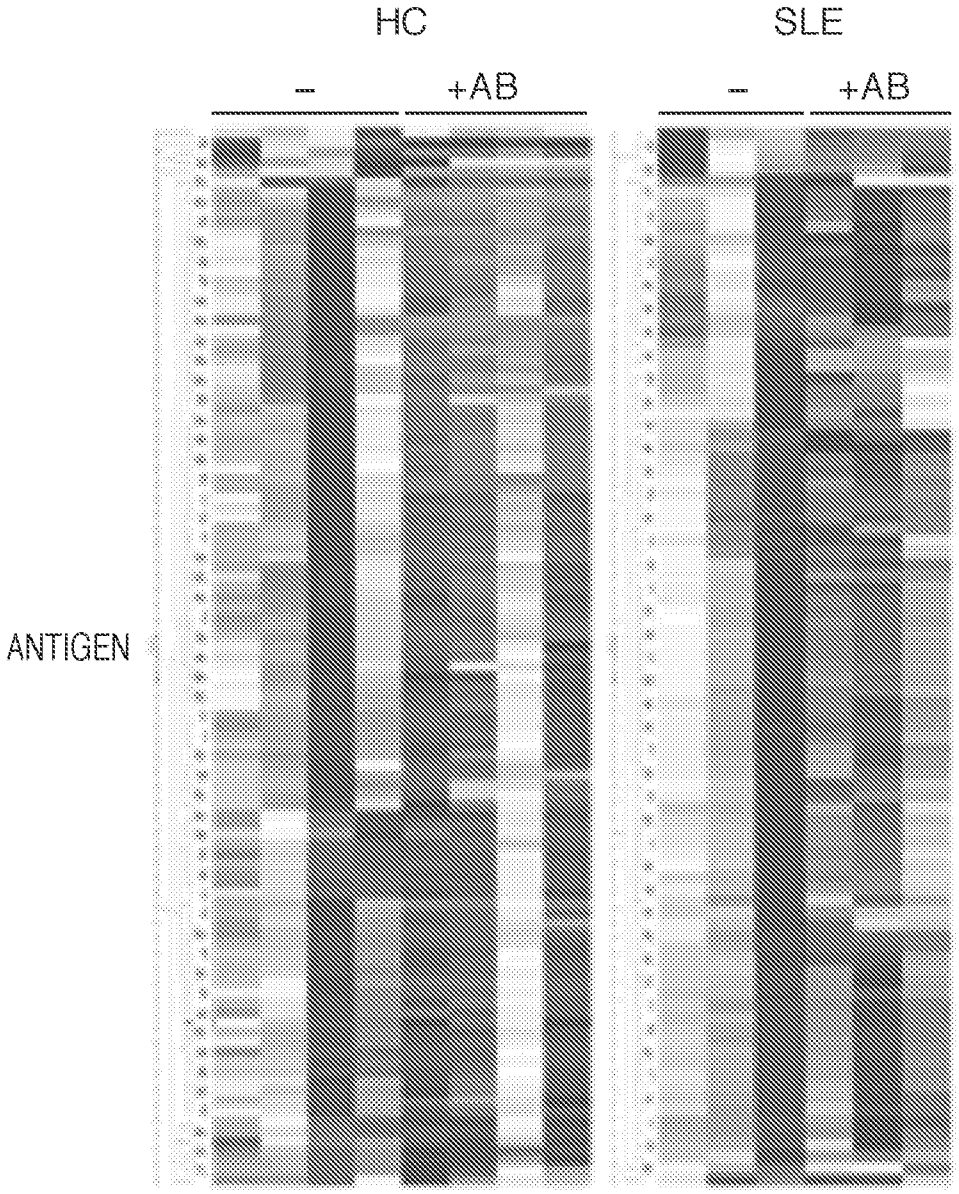


FIG. 7B

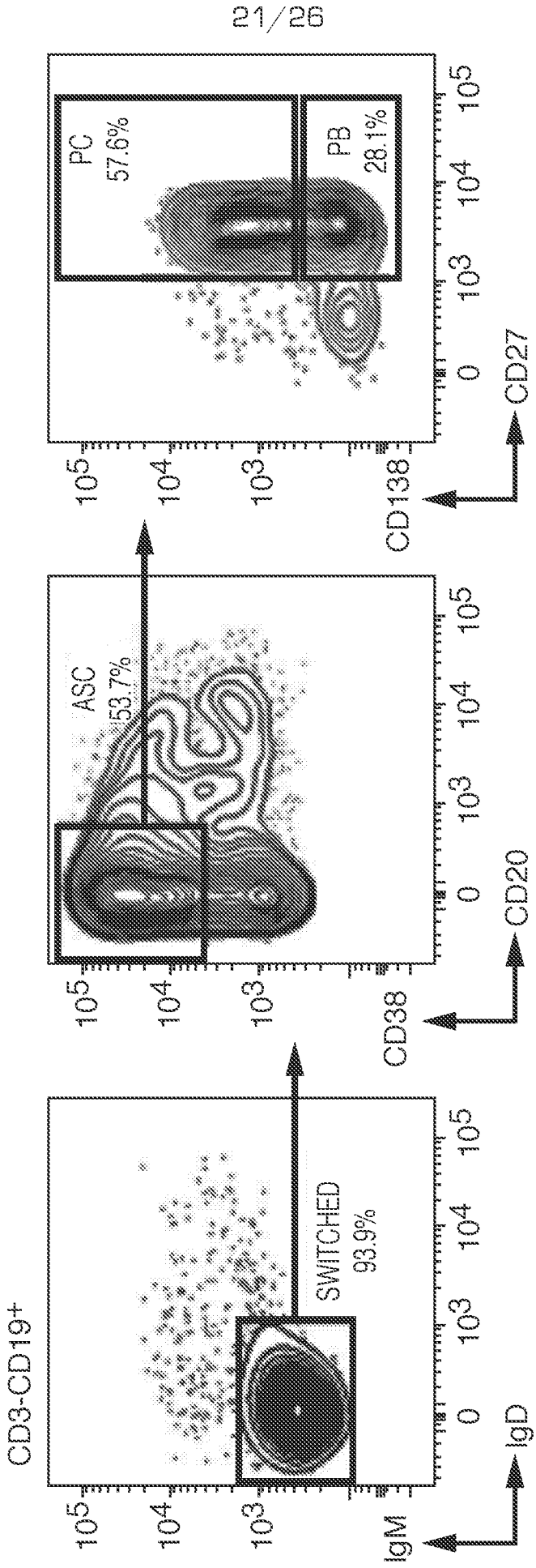


FIG. 8A-1

FIG. 8A-2

FIG. 8A-3

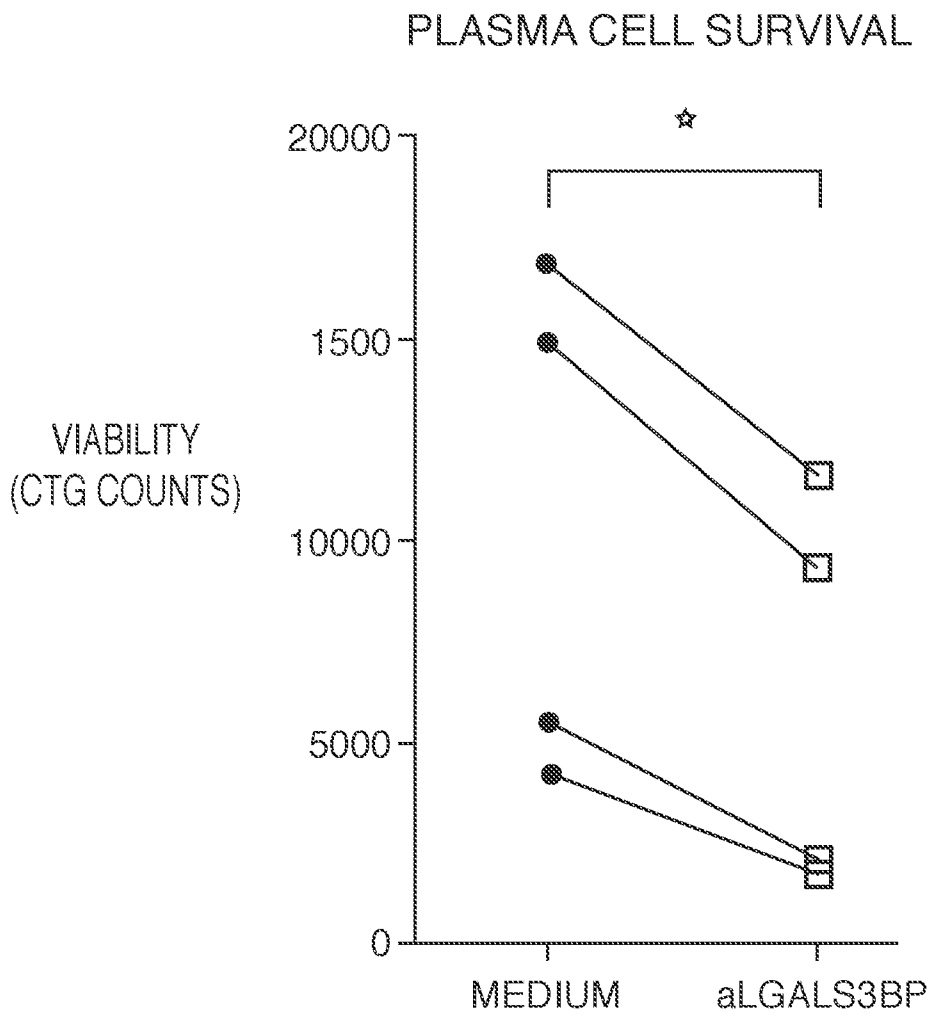
PLASMA CELL SURVIVAL
VIABILITY
(CTG COUNTS)
20000
1500
10000
5000
0
MEDIUM
aLGALS3BP

FIG. 8B

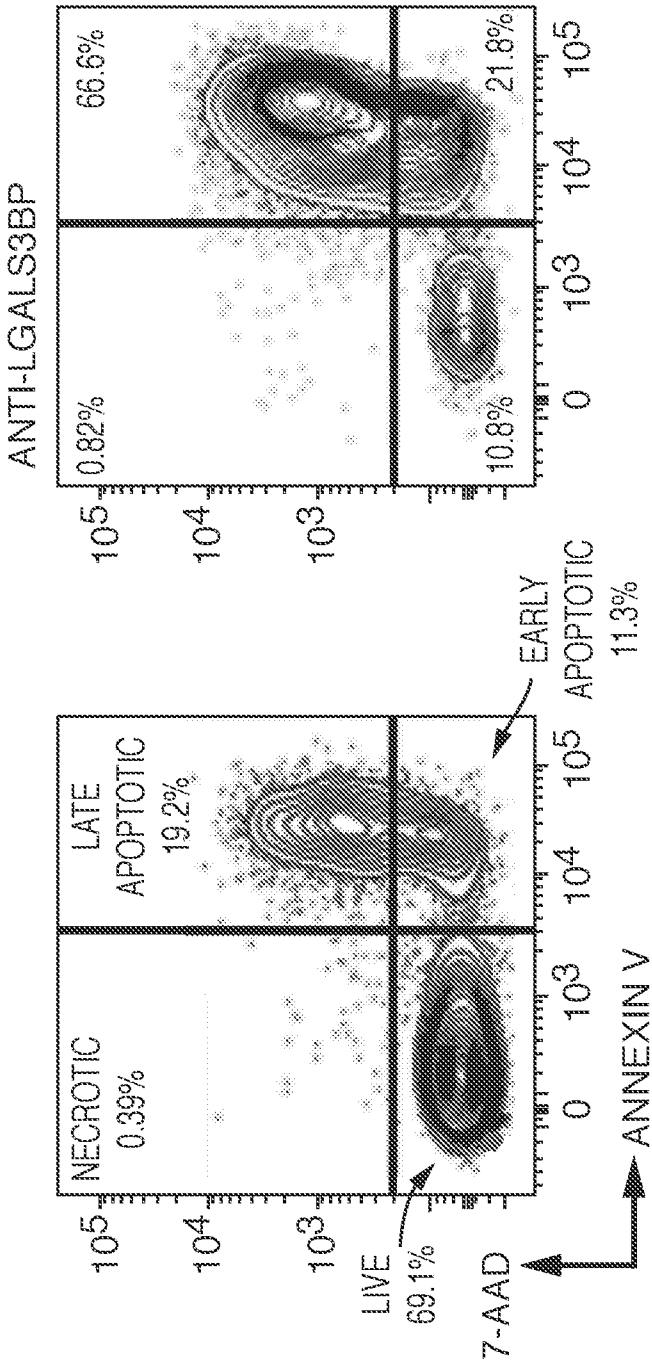


FIG. 9A-2

FIG. 9A-1

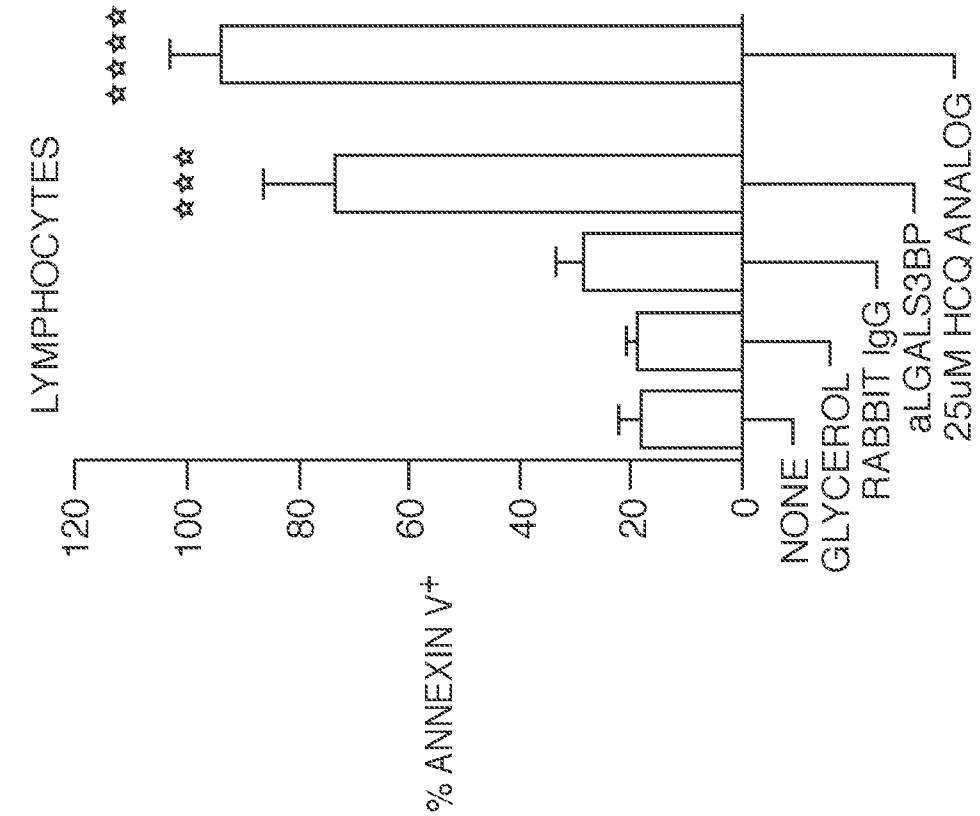


FIG. 9B-1

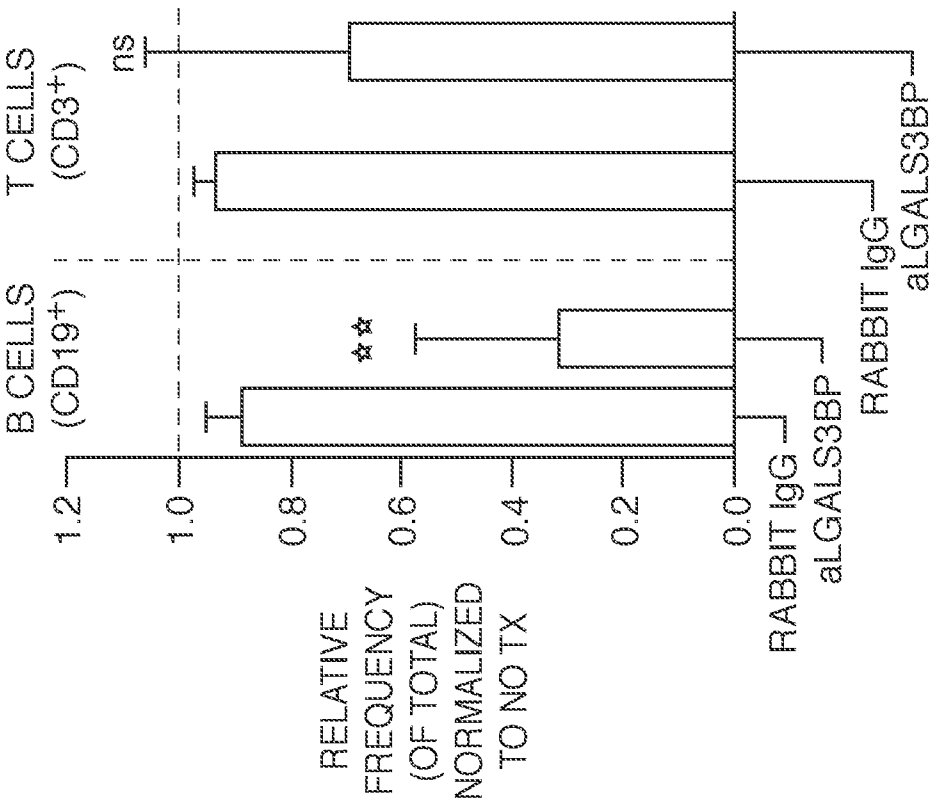


FIG. 9B-2

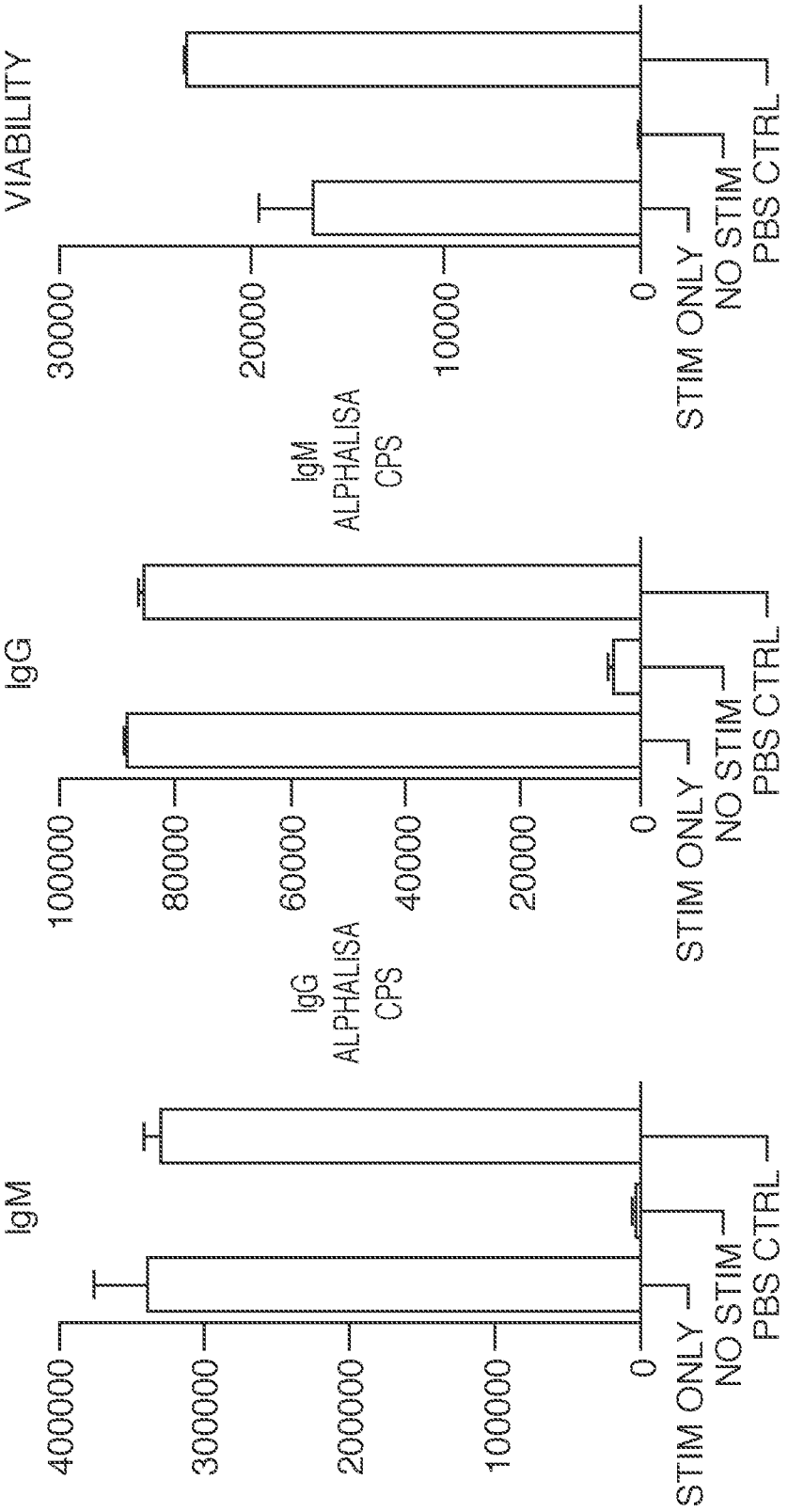


FIG. 10A-1




FIG. 10A-2




FIG. 10A-3

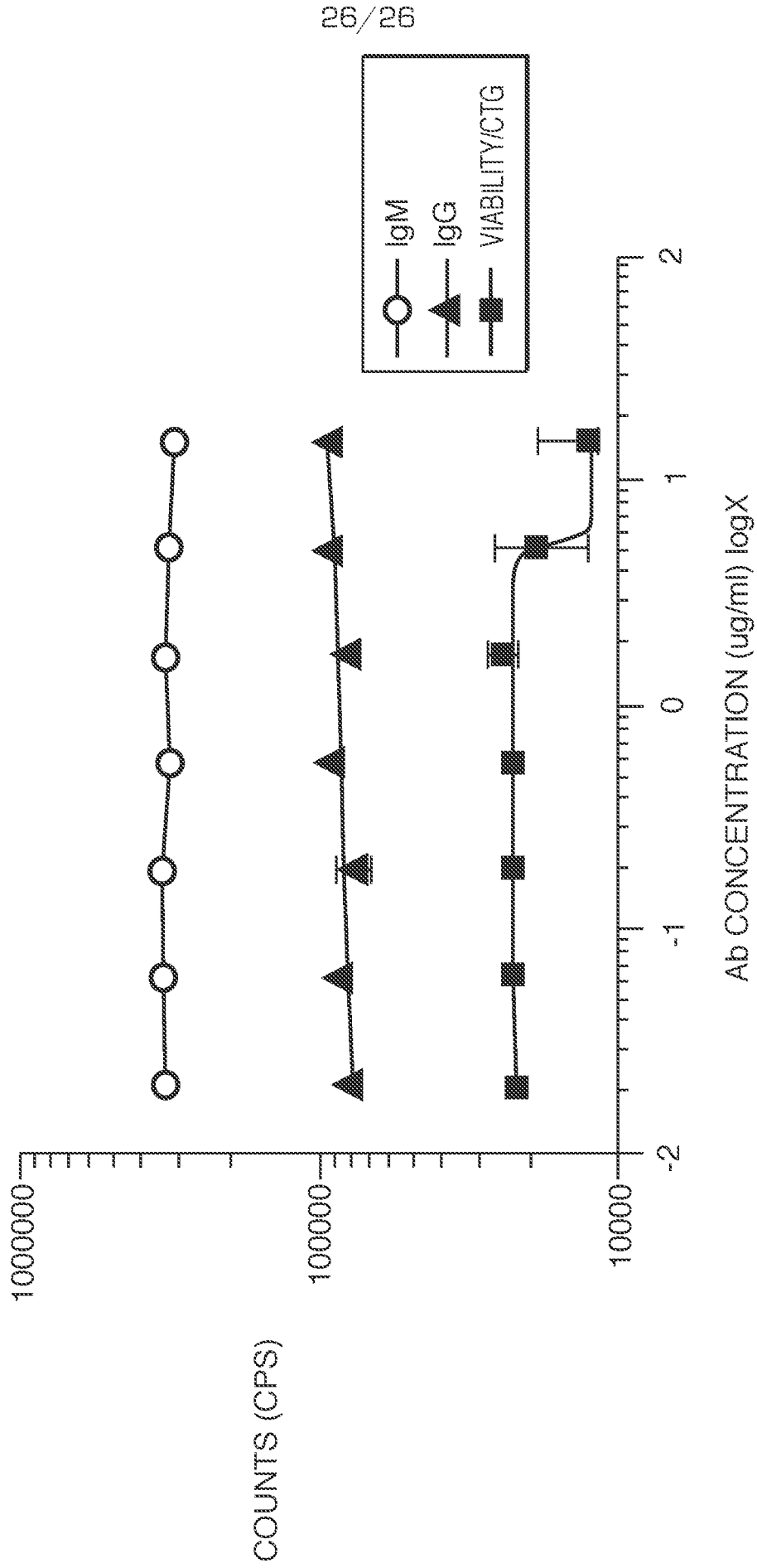
IgM
IgG
VIABILITY/CTG
COUNTS (CPS)
1000000
100000
10000
-2
-1
0
1
2
Ab CONCENTRATION (ug/ml) logX

FIG. 10B

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/049378

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 2011/126833 A2 (MASSACHUSETTS INST TECHNOLOGY [US]; GEN HOSPITAL CORP [US]; MILWID JOH) 13 October 2011 (2011-10-13) paragraph [0085]; claims 34,36,50-57 table 3 | 1-6 |
| Y | WO 2011/119185 A1 (JOLLA INST ALLERGY IMMUNOLOG [US]; SHAKED IFTACH [US]; LEY KLAUS [US]) 29 September 2011 (2011-09-29) claims 1-42 | 1-6 |
| A | ES 2 244 270 A1 (UNIV MADRID AUTONOMA [ES]; CONSEJO SUPERIOR INVESTIGACION; LAUZURICA G) 1 December 2005 (2005-12-01) claims 1-25 | 1-6 |
| Y | WO 2004/076682 A2 (SURROMED INC [US]) 10 September 2004 (2004-09-10) page 27, line 29 - line 31; claim 1 | 1-6 |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 11 November 2016 | 20/01/2017 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Klee, Barbara |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/049378

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-6

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/** 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6

A method for modulating LGALS3BP in a subject presenting symptoms of an immune disorder, inflammatory response or autoimmune disease comprising administering an anti-LGALS3BP antibody to said subject under conditions such that at least one symptom of said immune disorder, inflammatory response or disease said is improved.

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2. claim: 7

A method of using recombinant LGALS3BP as an adjuvant to enhance the activity of a virally-directed vaccine.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/049378

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2011126833 A2 | 13-10-2011 | EP 2552472 A2 | 06-02-2013 |
| | | JP 2013527834 A | 04-07-2013 |
| | | US 2013052198 A1 | 28-02-2013 |
| | | WO 2011126833 A2 | 13-10-2011 |
| WO 2011119185 A1 | 29-09-2011 | EP 2566878 A1 | 13-03-2013 |
| | | US 2013095127 A1 | 18-04-2013 |
| | | WO 2011119185 A1 | 29-09-2011 |
| ES 2244270 A1 | 01-12-2005 | ES 2244270 A1 | 01-12-2005 |
| | | ES 2244297 A1 | 01-12-2005 |
| | | JP 2011148829 A | 04-08-2011 |
| WO 2004076682 A2 | 10-09-2004 | NONE | |

(19)中华人民共和国国家知识产权局

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62/212,163 2015.08.31 US

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PCT/US2016/049378 2016.08.30

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代理人 余颖

(51)Int.Cl.
*A61K 39/00*(2006.01)
*C07K 16/28*(2006.01)

权利要求书1页 说明书11页
序列表3页 附图30页

(54)发明名称

调节LGALS3BP以治疗系统性红斑狼疮的方法

(57)摘要

本发明的实施方式描述了调节LGALS3BP的方法及其抗体治疗包括系统性红斑狼疮和狼疮性肾炎的自身免疫疾病的用途。

CN 107921112 A

1.在表现出免疫失调、炎性反应或自身免疫疾病症状的对象中调节LGALS3BP的方法，包括在各种条件下给予所述对象抗LGALS3BP抗体以致至少一种所述免疫失调、炎性反应或疾病症状得到改善。

2.如权利要求所述1的方法，所述免疫失调、炎性反应或自身免疫疾病选自主要由以下各项组成的集合：格氏病，重症肌无力，脉管炎和韦格纳肉芽肿，视神经脊髓炎，原发性硬化性胆管炎，干燥综合征，狼疮性肾炎和类风湿性关节炎。

3.治疗SLE患者的方法，包括给予患者治疗有效量的抗LGALS3BP抗体。

4.如权利要求3所述的方法，其中抗LGALS3BP抗体的量在患者中有效(a)抑制肾炎的进展，(b)稳定肾炎，或(c)逆转肾炎。

5.如权利要求3所述的方法，其中抗LGALS3BP抗体的量在患者中有效(a)抑制蛋白尿的进展，(b)稳定蛋白尿，或(c)逆转蛋白尿。

6.如权利要求3所述的方法，其中抗LGALS3BP抗体的量在患者中有效稳定或降低选自以下所述的临床参数：(a)患者的血液中尿素、肌酸酐或蛋白质浓度；(b)患者的尿液中蛋白质或血细胞浓度；(c)患者的尿比重；(d)患者的尿量；(e)患者的菊粉、肌酸酐、尿素或对氨马尿酸清除率；(f)患者的高血压；(g)患者的水肿；和(h)患者的循环自身抗体水平。

7.使用重组LGALS3BP作为佐剂来增强病毒导向疫苗的活性的方法。

# 调节LGALS3BP以治疗系统性红斑狼疮的方法

[0001] 优先权声明

[0002] 本PCT专利申请要求2015年8月31日提交的第62/212,163号美国临时专利申请的优先权,该临时申请经援引全部纳入。

[0003] 序列表

[0004] 本申请包含以ASCII格式电子提交的序列表,经援引全部纳入。所述ASCII文件制作于2016年8月23日,原名"P15167WO_SEQ_LISTING.txt",共5,320字节。

## 发明领域

[0005] 本发明主要涉及在各种条件下调节(包括但不限于降低、减少、抑制、阻遏、限制或控制)LGALS3BP活性致多种自身免疫病相关自身抗体产生减少的方法,或者,通过补充重组LGALS3BP放大和增强针对致病感染原的天然抗体分泌或疫苗应答的方法。

## 背景技术

[0006] 免疫系统失调即可表现为无法保护宿主抵抗感染原,也可相反地表现为误将自身识别为排斥对象从而引起自身免疫病。自身免疫病一般由多种遗传和环境因素共同造成,可大致分为T细胞介导型或B细胞介导型。自身反应性致病性T细胞通过T细胞受体结合相应的MHC-I分子与自体抗原衍生肽组合来识别靶细胞,通过多种不同的机制直接杀死靶细胞。I型糖尿病和原发性胆汁性肝硬化的形成是自身反应性T细胞介导疾病的代表例。

[0007] B细胞相关自身免疫的共同特征是存在针对细胞功能性结构(核酸、核蛋白、受体、离子通道)的自身抗体。通过结合各自靶标,自身抗体能够通过补体激活作用和/或抗体依赖性细胞介导细胞毒效应(ADCC)或通过阻抑靶标的功能来介导细胞毒性细胞破坏。致病性自身抗体介导多种疾病的发生发展,包括:格氏(Graves')病(抗促甲状腺激素抗体),重症肌无力(抗乙酰胆碱受体抗体),脉管炎和韦格纳(Wegener's)肉芽肿(抗ANCA抗体),视神经脊髓炎(抗水通道蛋白-4抗体),原发性硬化性胆管炎(抗中性粒细胞胞浆抗体,抗SM抗体)。其他自身免疫疾病是由自身抗体与其靶分子的免疫复合物的致病作用引起的,例如SLE、干燥综合征(Sjoegren's syndrome)和狼疮性肾炎(抗DNA、抗RNA、抗组蛋白、抗Ro、抗La、抗磷脂抗体)、类风湿性关节炎亚类(抗瓜氨酸化蛋白、抗RF、抗CarP抗体)。

[0008] 自身免疫疾病治疗方法的疗效相当有限。传统治疗方法依赖于类固醇和各种细胞毒性和细胞抑制性免疫抑制剂的作用,它们应迅速清除扩增性自身反应性免疫细胞从而延缓自身免疫进程的发展。最常用的自身免疫疾病治疗药物,如可的松/泼尼松、氨甲喋呤、霉酚酸酯、氯喹和硫唑嘌呤,疗效有限,并伴有多种副作用。

[0009] 更高靶向性的方法侧重消除自身抗体的产生,因而具有更好的治疗前景。贝利单抗(Belimumab)(商品名"Benlysta",曾被称为"LymphoStat-B"),一种人单克隆抗体,其抑制B细胞激活因子(BAFF),又称B-淋巴细胞刺激因子(BlyS),是B细胞分化和存活的重要细胞因子之一,是已获批的活动期自身抗体阳性SLE成人患者的疗法,疗效中等。其他几种生物疗法试图消灭B细胞,继而消灭聚焦于人B细胞上的细胞表面受体和分子的相关致病性自

身抗体。抗CD20靶向性抗体利妥昔单抗(Rituximab)(和类似的其他生物制剂如奥克雷珠单抗(ocrelizumab)、奥妥珠单抗(obinutuzumab)和奥伐单抗(ofatumumab))被设计为识别产抗体B细胞并通过ADCC消灭它们。虽然还没有抗CD20抗体获批用于治疗SLE,但它们经常超说明书地被处方用于治疗SLE及其他自身免疫疾病。此外,靶向人B细胞上其他表面分子(例如CD19和CD22)的生物制剂(如依帕珠单抗epratuzumab)正在或已在临床开发之中,尽管迄今临床效果有限或尚未见临床效果。B细胞靶向策略共同的弱点被认为是长寿浆细胞表面上没有他们的靶标。CD19-/CD38hi/CD138+浆细胞处于骨髓中,是大多数长寿抗体反应的源头。迄今还没有能够抑制它们的活性或导致其消亡从而抑制致病性自身抗体产生的治疗手段。

[0010] 系统性红斑狼疮(SLE)是一种代表性的自身免疫失调,特征为形成含自身抗体-的免疫复合物(IC),这些复合物触发炎症、组织破坏或过早死亡。SLE IC常含有多种先天免疫受体识别的核酸,这些受体会引发病理机制,引起细胞因子、干扰素的产生以及最终导致器官伤害的免疫反应。由于SLE的显著临床差异及其特发性,特发性SLE的处理主要根据其具体临床表现和严重程度。所以,SLE的建议用药通常不一定对SLE的各种临床表现或SLE引起的并发症都有效,例如LN。LN通常发生于病程早期起,确诊后5年内。LN的发病被认为是源于免疫复合物在肾小球内沉积引起炎性反应。估计30-50%的SLE患者患上肾炎,需要医学鉴定和治疗。LN是进行性疾病,会经历反复加重和缓解。

[0011] 虽然以上所述标准治疗对许多患者无效或仅部分有效,长期使用高剂量皮质类固醇和细胞毒性治疗药会有严重的副作用,例如骨髓抑制、条件致病性生物体感染增加、不可逆性卵巢衰竭、脱发和恶性疾病风险增加。与活动期SLE重合的感染性并发症和用免疫抑制药物治疗是SLE患者最常见的死因。所以需要更多治疗药物选择来治疗SLE尤其是LN,这些治疗药物比当前标准治疗更少副作用。

## 发明概要

[0012] 本发明的主题LGALS3BP是一个B-细胞相关靶标,它的功能性阻断导致活化B细胞和长寿浆细胞消亡。虽然本发明的方法不受任何具体机制的局限,B细胞活化和抗体产生受到了多个水平的调控。例子之一,B细胞被各种T细胞依赖性刺激(如CD40配基化)和T细胞-非依赖性刺激(各种TLR配体、多糖等)活化。如本申请实验部分所示,TLR7激动剂提供了B细胞刺激因子的例子,作为能够诱导抗体生产的B细胞活化剂的代表例。

[0013] 临床上可广泛观察到自身抗体的产生,但产生自身抗体的群体中仅很小百分比会发展为SLE。而且,SLE的自身抗体库是限制性的,似乎仅富集识别与核酸相结合蛋白质上自体抗原的抗体。大多数SLE患者被记录曾产生抗DNA、RNP或两者的抗体。与核酸结合的自体抗原激活自身反应性B细胞,并使他们得以逃脱外周耐受检查点,分化为自身抗体分泌细胞。

[0014] 抗原识别和核酸-细胞碎片复合物吞噬之后,核酸部分地被内体类铎受体(如TLR3、TLR7、TLR8和TLR9)识别。B细胞内的TLR刺激引起B细胞活化和成熟以及抗体和多种细胞因子生产增加。在许多小鼠模型研究中观察到单个TLR在SLE发展的相对贡献。而且,TLR7(RNA受体)的活性起主要作用,基因敲除和使用TLR7抑制剂显著缓解疾病进程。并且,TLR7基因过表达或系统性服用小分子TLR7激动剂所致TLR7活性升高引起SLE样症状诱导。

[0015] SLE免疫复合物内的核酸还被树突细胞的TLR所识别。浆细胞样树突细胞内的TLR7刺激引起I型干扰素大量产生。I型IFN是一种细胞因子，通过激活参与控制病毒扩散和维持宿主完整性的一组基因(干扰素靶基因)来参与抗病毒防御。SLE患者中常可见这些基因被激活。I型IFN参与B细胞活化、扩增和分化为Ig-生产细胞。

[0016] 鉴于TLR7刺激在B细胞活化中的关键作用，本发明的实施方式描述了鉴定能够调节抗体产生的蛋白质的筛选方法。这些筛选方法鉴定能够在SLE治疗中用于药理调节自身抗体产生的蛋白质和路径。采用编码分泌蛋白质的质粒文库瞬态生产细胞培养上清液并从中富集所述蛋白质，然后，在细胞系统中检测这些蛋白质的活性，其中，用小分子TLR7配体刺激原代B细胞，以IgG生产作为读数来评定效果得分。该筛选方法鉴定了一批或者提高或者降低IgG生产的蛋白质。本发明实施方式描述了此前未与B细胞生物学关联的蛋白质，这包括优选实施方式中的LGALS3BP。

[0017] LGALS3BP(Mac2-BP，p90)是泛表达基因，属于清道夫受体家族，原先被认为是某类肿瘤细胞分泌的蛋白质。LGALS3BP表达水平与肿瘤的进展密切相关。除了直接作用于肿瘤细胞繁殖/存活，LGALS3BP还会上调血管内皮生长因子的表达和促进血管生成。HIV-1感染期间，其水平升高，据信其活性通过干扰包膜蛋白质成熟和纳入病毒粒子来降低HIV-1的感染性。丙型肝炎患者的肝脏活组织切片分析提示LGALS3BP直接参与丙型肝炎相关性纤维化。此外，SLE患者中也观察到血浆LGALS3BP水平升高。LGALS3BP可能与SLE中的心血管并发症增加有关，因为它促进血栓形成和血栓向内皮细胞的附着。白塞病患者中也可见LGALS3BP血清水平升高，且与疾病活跃性相关。

[0018] 已知多种与LGALS3BP相互作用并介导其功能的蛋白质，包括半乳凝素、凝集素、整联蛋白等。LGALS3BP含有多个蛋白质-蛋白质相互作用结构域(SRCR、BTB、POZ)，它们可能以细胞特异性方式参与多种与细胞蛋白质之间的相互作用。

[0019] 本发明实施方式之一中描述的方法中，LGALS3BP在各种条件下促进经TLR7配体刺激的原代B细胞中IgG的生产，LGALS3BP-中和抗体因此显著减少经TLR7配体刺激或BCR-配基化作用下B细胞的IgG生产。对SLE中多种免疫细胞的转录组分析揭示，LGALS3BP mRNA相比健康志愿者水平升高，并与受干扰素调节基因的表达水平相关联。

[0020] 虽然无意于将本发明的实施方式局限于任何具体机制(尤其任何关于TLR7一定发挥了唯一性刺激作用的提示)，LGALS3BP在B细胞IgG生产中的作用为LGALS3BP中和抗体能够用于治疗SLE、LN、可能还有其他自身免疫疾病提供了验证，所述疾病例如类风湿性关节炎、青少年类风湿性关节炎、屑病性关节炎、糖尿病、重症肌无力、脉管炎、原发性硬化性胆管炎、自身免疫性甲状腺炎、干燥综合症、韦格纳氏肉芽肿病、格氏病、桥本氏甲状腺炎、自身免疫性血小板减少性紫癜、抗磷脂综合征、视神经脊髓炎和原发性硬化性胆管炎。

[0021] 然而，自身免疫之外，为了在感染性疾病中提供抗细菌、寄生虫或病毒的保护性免疫，放大天然存在或疫苗诱导的病原导向的体液免疫反应可能是有利的而且实际上可能是必需。就此而言，例如，增强重组蛋白亚基疫苗的效力而不牺牲安全性的策略备受关注，因为相比更强的活减毒或重组载体，它们引发的免疫反应(即抗疟疾)通常程度和持久性都较弱。此类情形中，补充重组LGALS3BP来增强体液免疫和抗病原反应将有利于支持宿主防御。

[0022] 本发明实施方式之一中描述了在表现出免疫失调、炎性反应或自身免疫疾病症状的对象中调节LGALS3BP的方法，包括在各种条件下给予所述对象抗LGALS3BP抗体以致所述

免疫失调、炎性反应或疾病的至少一种症状得到改善。

[0023] 实施方式之一中本发明描述了在表现出以下集合中疾病的症状的对象中调节LGALS3BP的方法，包括在各种条件下给予所述对象抗LGALS3BP抗体以致所述疾病的至少一种症状得到改善，所述集合主要由以下疾病构成：格氏病、重症肌无力、脉管炎和韦格纳(Wegener's)肉芽肿、视神经脊髓炎、原发性硬化性胆管炎、干燥综合征、狼疮性肾炎和类风湿性关节炎。

[0024] 优选实施方式之一中，本发明描述了对SLE患者的治疗，包括给予患者治疗有效量的抗LGALS3BP抗体。实施方式之一中，抗LGALS3BP抗体能够在患者中有效地：(a)抑制肾炎的进展，(b)稳定肾炎，或(c)逆转肾炎。

[0025] 另一实施方式中，抗LGALS3BP抗体的量能够在患者中有效地：(a)抑制蛋白尿的进展，(b)稳定蛋白尿，或(c)逆转蛋白尿。

[0026] 实施方式之一中，本发明描述了对SLE患者的治疗，包括给予患者治疗有效量的抗LGALS3BP抗体，给药剂量能够在患者中有效地稳定或降低选自以下所述的临床参数：(a)患者的血液中尿素、肌酸酐或蛋白质浓度，(b)患者的尿液中蛋白质或血细胞浓度，(c)患者的尿比重，(d)患者的尿量，(e)患者的菊粉、肌酸酐、尿素或对氨马尿酸清除率，(f)患者的高血压，(g)患者的水肿，和(h)患者的循环自身抗体水平。

[0027] 实施方式之一中，本发明描述给予重组LGALS3BP作为佐剂通过放大保护性抗体反应来增强病毒导向疫苗的活性。

## 附图说明

[0028] 图1A显示原代人B细胞的数据，所述细胞分离后用小分子TLR7激动剂刺激并培养5天。加入含分泌蛋白质的条件细胞培养物上清液库，在培养终点测定IgG分泌和细胞活力(CTG，CellTiter-Glo)。

[0029] 图1B显示不同细胞亚组的数据，所述细胞亚组用FACS分离自健康对照(每个细胞亚组的第一个数据点)和I型IFN水平升高的狼疮性肾炎患者(数据点2-4)。RNA表达用RNA-seq来分析。图上所示为标准化后的FPKM表达值。

[0030] 图1C显示纯化重组LGALS3BP加入受小分子TLR7激动剂CpG(ODN2006)或抗IgM/CD40L/CpG(ODN2006)刺激的纯化人B细胞。刺激后5天用Alpha-LISA测定IgG。

[0031] 图1D显示人PBMC用小分子TLR7激动剂刺激，并于5小时后分离RNA。基因表达分析用RNA-seq进行，分析表达水平并标准化为FPKM值。

[0032] 图2A-1和图2A-2显示递增浓度纯化重组LGALS3BP存在下用小分子TLR7激动剂刺激的B细胞数据。16小时后通过流式细胞术定量测定CD69表达来测定B细胞活化。

[0033] 图2B为以下实验的数据：用重组LGALS3BP(recLGALS3BP)和人血浆进行蛋白质印迹来测定抗LGALS3BP抗体的特异性。

[0034] 图2C显示用抗LGALS3BP抗体测定的LGALS3BP定位，与CD19B细胞和DAPI核染色比较。

[0035] 图3A-1和图3A-2显示分离的原代人B细胞的数据，所述细胞在可能的LGALS3BP抑制剂和对照(左)存在下用小分子TLR7激动剂刺激。将抗LGALS3BP抗体加入CpG或抗IgM/CD40L/CpG(右)活化的原代人B细胞。5天后用Alpha-LISA测定IgG分泌。

[0036] 图3B-1显示原代人B细胞数据，所述细胞在可能的LGALS3BP抑制剂和对照存在下用小分子TLR7激动剂活化。5天后用Alpha-LISA测定IgG分泌。

[0037] 图3B-2显示原代人B细胞数据，所述细胞在可能的LGALS3BP抑制剂和对照存在下用小分子TLR7激动剂活化。5天后用CellTiter-Glo测定B细胞活力。

[0038] 图3B-3显示原代人B细胞数据，所述细胞在可能的LGALS3BP抑制剂和对照存在下用小分子TLR7激动剂活化。刺激后2天，用Alpha-LISA测定IL-6分泌。

[0039] 图3C-1显示在可能的LGALS3BP抑制剂和对照存在下，活化后16小时，通过流式细胞术定量检测CD69表达测定的B细胞活化数据。

[0040] 图3C-2显示在可能的LGALS3BP抑制剂和对照存在下，活化后16小时，通过定量检测CD69表达测定的B细胞活化数据，显示的是CD69上调细胞百分比。

[0041] 图3C-3显示在可能的LGALS3BP抑制剂和对照存在下，活化后16小时，通过定量检测CD69表达来测定的B细胞活化数据，显示的是全体B细胞上CD69检测平均荧光强度(MFI)。

[0042] 图3D-1和图3D-2显示以下实验所得数据：将抗LGALS3BP抗体加入无刺激原代人B细胞，2天后用CellTtiter-Glo检测这些B细胞的活力。

[0043] 图4A显示以下实验所得数据：采集14周龄雌性MRL/lp小鼠(早期疾病)的肾脏和脾脏。对组织匀浆进行NanoString分析，测定LGALS3BP表达，并与C57BL/6健康对照小鼠进行比较。或者，从经降植烷或PBS处理小鼠的血液或脾脏样品、或从BXSB-Yaa老年患病小鼠或年轻对照小鼠的血液、脾脏或肾脏分离RNA。显示的LGALS3BP基因表达水平用QPCR测定并相对于Hprt标准化。

[0044] 图4B显示以下实验所得数据：用蛋白脂蛋白(PLP)免疫SJL小鼠以诱导实验性自身免疫性脑脊髓炎(“EAE”)。第7天和第14天，SJL-PLP EAE患病小鼠被人道处死，采集腰椎脊髓。纯化RNA，用NanoString分析测定LGALS3BP表达，并与空白非免疫健康对照小鼠进行比较。图4A和4B所述实验中，各实验组包含至少5只小鼠，用非配对斯氏t检验进行患病小鼠与健康对照的比较。*p<0.05，**p<0.01，***p<0.001。

[0045] 图4C显示“IFN基因签名得分”。这些得分是根据5个已知受干扰素调节的基因(USP18、IRF7、IFIT1、OAS3、BST2)的表达计算的。然后根据以上得分对小鼠进行四分位分组，并对LGALS3BP表达制图，所述LGALS3BP表达为相对于健康对照小鼠的表达。

[0046] 图5A显示QPCR测定的LGALS3BP表达，采用提取自用所示刺激因子活化了6小时的体外分化后原代人巨噬细胞的RNA。各样本的表达用HPRT1作为管家基因进行标准化。

[0047] 图5B显示ELISA测得的用所示刺激因子活化了20小时的体外分化后原代人巨噬细胞上清液中的LGALS3BP。

[0048] 图6A显示分离自健康对照(HC)和SLE患者血液的原代B细胞在有(刺激+抗体)或无抗LGALS3BP抗体(仅刺激)条件下用TLR7激动剂刺激。刺激5天后，测定培养物中的IgM。*P<0.05；**P<0.01双尾配对斯氏t检验。

[0049] 图6B显示分离自健康对照(HC)和SLE患者血液的原代B细胞在有(刺激+抗体)或无抗LGALS3BP抗体(仅刺激)条件下用TLR7激动剂刺激。刺激5天后，测定培养物中的IgG。*P<0.05；**P<0.01双尾配对斯氏t检验。

[0050] 图7A-1和图7A-2显示验证抗LGALS3BP抗体处理能够降低抗体(不论特异性)滴度的数据。健康对照(HC)和SLE患者的B细胞用TLR7激动剂刺激5天，用培养物上清液分析128

种自身抗体特异性(IgM和IgG)。计算特异性信噪比>3的被识别自体抗原数量。过滤掉非刺激B细胞+抗LGALS3BP抗体中信号阳性的特异性。

[0051] 图7B显示z值(样本-平均值$_{全体}$)/标准差$_{全体}$表示的抗体滴度的热图。每个柱代表一个用TLR7激动剂刺激且有(+抗体)或没有(-)抗LGALS3BP抗体的样本。*P<0.05双尾配对斯氏t检验。

[0052] 图8A-1、图8A-2和图8A-3的数据显示:抗LGALS3BP抗体处理降低浆细胞活力。新鲜分离自健康志愿者的B细胞按7天方案分两步,在促进B细胞活化(第1步)和B细胞分化(第2步)的细胞因子存在下分化为浆细胞。体外分化的人抗体分泌细胞(ASC)、浆母细胞(PB)浆细胞(PC)的流式细胞计数。细胞预先经$CD19^{+}$B细胞门控。

[0053] 图8B显示第7天的分化后浆细胞,细胞培养在有或无抗LGALS3BP抗体条件下。4天后,CellTiter-Glo测定细胞活力(ATP生产)。*P<0.05双尾配对斯氏t检验。

[0054] 图9A-1和图9A-2显示抗LGALS3BP抗体处理如何优先诱导B细胞凋亡。新鲜分离自健康供体的PBMC在有或无抗LGALS3BP抗体(aLGALS3BP)、同种型对照(兔IgG)、甘油对照或羟氯喹类似物(HCQ类似物)条件下培养3天。图9A-1中,用流式细胞术测定膜联蛋白V和7-AAD,同时测定B细胞标志(CD19)和T细胞标志(CD3)。

[0055] 图9B-1和图9B-2显示4个供体的膜联蛋白V-阳性凋亡细胞的平均频率。总PBMC中B细胞和T细胞的相对频率。这些频率相对于无处理对照进行标准化。

[0056] 图10A-1、图10A-2和图10A-3确认:抗LGALS3BP抗体SP-2不降低B细胞活力和抗体生产。新鲜分离自健康志愿者的B细胞在有或无抗LGALS3BP抗体SP-2或PBS对照条件下用TLR7激动剂刺激5天。

[0057] 图10B显示Alpha-LISA测定的细胞培养上清液中的IgM和IgG,以及CellTiter-Glo(CTG)测定的细胞活力。

[0058] 详细描述

[0059] 本发明的实施方式是基于LGALS3BP在IgG产生中的作用及其在治疗SLE尤其LN中的意义。本发明的这些治疗性实施方式得到后文数据的支持。LGALS3BP是狼疮性肾炎患者与健康对照之间在多种细胞类型中差异调控最显著的基因之一。LGALS3BP与IFN-诱导性基因密切相关,并且在受TLR7刺激后的人PBMC中上调。LGALS3BP在离体受激原代人B细胞中增强IgG分泌。LGALS3BP存在于B细胞及其他所有PBMC表面。抗体或乳糖造成的LGALS3BP阻断会消除IgG产生。LGALS3BP抗体阻断不需要B细胞上的抑制性FcγRIIb。LGALS3BP阻断特异性降低培养的原代人B细胞的活力,但对原代单核细胞或总PBMC只有弱效,并且,SLE和EAE小鼠模型中的LGALS3BP上调。

[0060] LGALS3BP多肽指全长多肽序列以及亚序列、片段或部分,还有LGALS3BP多肽的修饰形式和变体,除非文中另作说明。所述LGALS3BP亚序列、片段、修饰形式和变体至少具有参照LGALS3BP蛋白的一部分、功能或活性。具体实施方式中,修饰形式或变体保留非修饰或参照蛋白的至少一部分、功能或活性。"功能性多肽"或"活性多肽"指它们的修饰多肽或亚序列。例如,功能性或活性LGALS3BP多肽或其亚序列具有天然野生型或全长对应多肽(如LGALS3BP)的至少一项部分特征性功能或活性(如生物活性),如后文所述,该功能或活性可通过实验鉴定。所以,本发明实施方式包括LGALS3BP多肽序列和亚序列的修饰形式和变体,所述修饰形式或变体一般保留非修饰或参照LGALS3BP多肽序列的至少一部分、一项或多项

功能或活性。

[0061] 如本文所述，LGALS3BP多肽功能或活性的非限定性例子是调节异常免疫反应，免疫失调，炎性反应，或炎症，或自身免疫反应、失调或疾病。实施方式之一中，所述自身免疫疾病是SLE。优选实施方式之一中，所述自身免疫疾病是LN。尽管无意于将本发明局限于任何具体机制，LGALS3BP多肽功能或活性的其他非限定性例子是调节IgG的表达。

[0062] 一例全长人LGALS3BP多肽序列(SEQ ID NO:1)如下所述：

[0063] MTPPRLFWVWLLVAGTQGVNDGDMRLADGGATNQGRVEIFYRGQWGTVCDNLWDLTDASVVCRALGFEN
ATQALGRAAFGQGSGPIMLDEVQCTGTEASLADCKSLGWLKSNCRHERDAGVVCTNETRSTHTLDLSRELSEALGQI
FDSQRGCDLSISVNVQGEDALGFCGHTVILTANLEAQALWKEPGSNVTMSVDAECVPMVRDLLRYFYSRRIDITLSS
VKCFHKLASAYGARQLQGYCASLFAILLPQDPSFQMPLDLYAYAVATGDALLEKLCLQFLAWNFEALTQAEAWPSVP
TDLLQLLLPRSDLAVPSELALLKAVDTWSWGERASHEEVEGLVEKIRFPMMLPEELFELQFNLSLYWSHEALFQKKT
LQALEFHTVPFQLLARYKGLNLTEDTYKPRIYTSPTWSAFVTDSSWSARKSQLVYQSRRGPLVKYSSDYFQAPSDYR
YYPYQSFQTPQHPSFLFQDKRVSWSLVYLPTIQSCWNYGFSCSSDELPVLGLTKSGGSDRTIAYENKALMLCEGLFV
ADVTDFEGWKAAIPSALDTNSSKSTSSFPCPAGHFNGFRTVIRPFYLTNSSGVD

[0064] 定义

[0065] "多肽"指通过酰胺键或与之相当的键相连的两个或更多个氨基酸。本文中，多肽也指蛋白质、肽或氨基酸序列等。多肽包括通过酰胺键或与之相当的键相连的至少两个或更多个氨基酸。多肽可形成分子内或分子间二硫键。多肽还可形成高级结构，例如与相同或不同多肽或其他分子形成多聚体或寡聚体。

[0066] "患者"和"对象"在本文中指接受针对某状况(如SLE或LN)处理或治疗的哺乳动物。这包括人患者和志愿者，非人哺乳动物，如非人灵长类动物、大型动物模型和啮齿类动物。

[0067] "给予"患者药物或"给药"指直接给药，即由医疗工作者或患者自己施用药物，和/或间接给药，例如开具药物处方。例如，医师或临床工作者指导患者自行施用药物或向患者提供药物处方，这些都是向患者给药。

[0068] "剂"和"剂量"指活性或治疗性物质一次给药的具体量。"剂型"是一个物理上独立的单位，它呈包装形式或以整体剂量形式给予接受治疗的对象。它包含预定量的活性物质，所述预定量经计算能够提供所需的起效、耐受和治疗效果。

[0069] "治疗有效量"的药物指一定量的药物，给予患者来治疗某状况如SLE和LN会产生有益效果，例如缓解，改善，缓和或消除与该状况的活跃度或病理状态相关的一种或多种症状、表现或实验室指标。

## 实施例

[0070] 以下实施例仅用于说明，不应理解为对权利要求所述本发明的限定。

[0071] 实施例1:LGALS3BP增强TLR7激动剂活化的B细胞中的IgG分泌

[0072] 为了鉴定影响B细胞IgG生产的分泌蛋白，对选自IgG分泌试验中人分泌蛋白组的蛋白质用原代人B细胞进行筛选。将健康志愿者的B细胞暴露于1400重组表达的分泌蛋白，然后用TLR7小分子激动剂活化。5天后，通过测定IgG来鉴定增强或抑制IgG分泌的蛋白质。B细胞刺激性细胞因子如IL-2和IL10之外，该实验表明，LGALS3BP令IgG分泌增强4.1倍，细胞

活力和代谢活性(据CellTiter-Glo实验检测的ATP)提高一倍(图1a)。LGALS3BP被独立鉴定为狼疮性肾炎患者血液中相比健康志愿者差异调节最显著的基因。LGALS3BP在全部接受分析的细胞类型中都上调,并且与患者的干扰素签名相关(图1b)。

[0073] 对另外6名人健康志愿者对象的B细胞使用纯化重组LGALS3BP证实了IgG生产增强(1.6倍)(图1c)。用TLR9激动剂CpG(1.9倍)或含抗IgM、CD40L和CpG的活化混合物(1.2倍)激活B细胞时,观察到相似的IgG升高。用小分子激动剂刺激健康志愿者的PBMC,用以检验该活化方案能否体外增强LGALS3BP表达(图1b)。基线表达值与直接离体细胞中的相当。TLR7刺激的确使表达水平提高超过3倍。这一发现为添加外源LGALS3BP对不同供体来源B细胞的效果差异提供了解释。LGALS3BP被认为是LN患者不同免疫细胞类型中与健康志愿者相比差异表达最显著的基因之一,并发现该分泌蛋白在抗体生产中有增强作用。

[0074] LGALS3BP有一个IRF结合位点,这与受I型干扰素调节是一致的。为了确定哪条路径能够诱导LGALS3BP表达,令原代人单核细胞体外分化为巨噬细胞,然后用IFN-α、IFN-γ、TLR4激动剂(LPS)、TLR7/8激动剂(瑞喹莫德)和TLR9激动剂(CpG)刺激。IFN-α、IFN-γ和LPS诱导LGALS3BP mRNA表达(图5A)并提高该蛋白分泌(图5B)。全部刺激因子都诱导IL-6分泌。这提示,不仅I型干扰素能够促进LGALS3BP表达,IFN-γ和其他先天因子也有激发作用。

[0075] 根据组蛋白乙酰化位点,LGALS3BP表达受结合LGALS3BP基因4个不同区域的因子调节:启动子启动位点,上游增强子内(5K上游区),内含子位点内,或3'UTR内。3种不同方法的基序(Motif)筛选鉴定到了可能免疫相关的转录调节因子。LGALS3BP基因座内和基因座周围发现了IRF、AP-1和STAT以及其他重要因子如NF-KB。关于转录因子的预测提示LGALS3BP表达通过干扰素调节因子(IRF)和活化STAT、NF-kB和AP-1的其他免疫刺激因子受干扰素调节。

[0076] 实施例2:LGALS3BP位于B细胞表面但不增强B细胞活化

[0077] 为了研究添加LGALS3BP是否影响空白B细胞的活化,TLR7激动剂刺激后16小时测定CD69表达。全部B细胞的CD69表达相比无刺激细胞都升高,但添加不同浓度的重组LGALS3BP未见变化(图2A-1和图2A-2)。然后,用LGALS3BP特异性抗体评测内源LGALS3BP在原代人B细胞中的位置(图2b)。结果证实LGALS3BP位于B细胞表面,且存在于PBMC中所有其他细胞类型上(图2c)。

[0078] 实施例3:抗LGALS3BP通过诱导B细胞和浆细胞凋亡抑制IgG分泌

[0079] 就抗LGALS3BP抗体对原代人B细胞IgG分泌的作用进行了评测。TLR7活化B细胞的IgG分泌在抗-LGALS3BP抗体存在下抑制几乎达90%,抗LGALS3BP $F(ab')_2$(74%)存在下也抑制,说明不是通过B细胞上FcγRIIb的抑制(图3A-1和3A-2)。LGALS3BP的已知配体之一乳糖可见同样的抑制但较弱(59%抑制),但蔗糖对IgG分泌没有抑制。当B细胞用CpG(94%)或抗IgM/CD40L/CpG(77%)活化时可以看到与LGALS3BP抗体相同的抑制效果。IgM分泌同样因抗体阻断而被抑制,说明LGALS3BP不参与同种型转换(图3B-1、图3B-2和图3B-3)。测定ATP作为细胞数量和活力的数据,可见与IgG分泌密切相关,这提示LGALS3BP影响B细胞存活和/或繁殖。通过测定IL-6分泌来检查LGALS3BP阻断是否干扰TLR7活化及信号传导并因此降低B细胞繁殖。抗LGALS3BP抗体存在下刺激B细胞,48小时后测得IL-6生产降低37%。这一IL-6生产降低是LGALS3BP特异性的而不是FcγRIIb介导的,因为在Fc阻抑条件下或抗LGALS3BP $F(ab')_2$存在下测得相同结果。乳糖也有同样效果,由此说明不是抗体通过交联表面结合蛋

白质的直接结果。在体外，无刺激原代人B细胞不繁殖，且存活能力有限。为了检测抗LGALS3BP抗体是否通过阻抑B细胞活化降低B细胞存活，TLR7激动剂活化后16小时，测定CD69上调(图3C-1，图3C-2和图3C-3)。加入抗LGALS3BP抗体时，$CD69^{+}$活化细胞百分比和CD69表达水平未见改变。LGALS3BP阻断抑制IgG分泌不依赖于具体使用的刺激方案。进一步实验确定无刺激时LGALS3BP阻断是否对B细胞存活有作用。将抗体加入无刺激B细胞令活力降低66％(图3D-1和图3D-2)。这一结果在B细胞中最显著。对总PBMC或单核细胞进行抗LGALS3BP处理显示37.5％和39％的活力下降。综合以上结果确认LGALS3BP在B细胞的自身稳定、活化、繁殖和分化过程中有抗凋亡作用。

[0080] B细胞耐受失调是SLE发病中的关键驱动因素之一。为了确定抗LGALS3BP处理对SLE B细胞是否有如同健康供体B细胞中所见相同的效果，在SLE供体的B细胞中重复B细胞刺激实验。抗LGALS3BP抗体存在下用TLR7激动剂刺激细胞时可见IgM生产显著降低(图6A和图6B)。IgG分泌也有下降，但不显著，因为SLE供体的B细胞应TLR7刺激产生的IgG不多。这些实验证明，抗LGALS3BP处理抑制性效果在SLE B细胞中得以保留。

[0081] 对TLR7-刺激B细胞的上清液在128个自体抗原蛋白的微阵(表1)上进行了分析。抗LGALS3BP处理减少被IgM抗体识别的自体抗原数量(图7B)，一致性地降低全部自体抗原的IgM滴度，这证明抗LGALS3BP处理下没有特异性逃逸(图7B)。以上数据证明抗LGALS3BP处理一致性地降低健康者和SLE患者B细胞的抗体生产，与特异性无关。

[0082] SLE患者在确诊时通常具有预存长寿浆细胞。去B细胞治疗能够根据特异性降低抗体滴度。例如，去B细胞降低dsDNA-特异性抗体，但其他抗体例如RNP-特异性抗体仍然升高。另一方面，长寿浆细胞未被去除而持续分泌抗体。设计了一个健康志愿者原代人B细胞中浆细胞的体外分化系统，用来测试抗LGALS3BP处理是否对浆细胞活力有作用(图8A-1、图8A-2和图8A-3)。然后，将分化后的浆细胞暴露于抗LGALS3BP抗体共4天，通过测定ATP生产间接评价活力。观察到浆细胞活力明显下降，由此证实抗LGALS3BP处理对长寿浆细胞有治疗效果(图8B)。

[0083] 为了确定这一活力下降是否是因为靶细胞坏死或凋亡，健康志愿者PBMC与抗LGALS3BP抗体一起培养4天，然后通过流式细胞术测定膜联蛋白V表面表达和细胞透性(7-AAD)。抗LGALS3BP处理诱导膜联蛋白V表达，这与细胞因凋亡所致死亡一致(图9A-1、图9A-2、图9B-1和图9B-2)。甘油和对照兔IgG无此效果，高剂量羟氯喹类似物也诱导凋亡。比较B细胞和T细胞的频率，处理对B细胞的影响大于对T细胞的影响，这与之前的观察结果即PBMC和单核细胞不像B细胞那样对处理易感一致。

[0084] 以上结果确认LGALS3BP在B细胞的自身稳定、活化、繁殖和分化过程中有抗凋亡作用。

[0085] 表1：自体抗原阵列上的抗原表

[0086]

| | | | |
|---|---|---|---|
| 聚集蛋白聚糖 | dsRNA | La/SSB | Ro/SSA(60KDa) |
| α胞衬蛋白(Sptan1) | dsDNA | 层粘蛋白 | S100 |
| α-辅肌动蛋白 | EBNA1 | LC1 | Scl-70 |
| 淀粉样蛋白 | 弹力蛋白 | LKM1 | Sm |
| AQP4 重组 | 巢蛋白 EDTA | M2 抗原 | Sm/RNP |
| BP1 | 因子 I | 基质胶 | SmD |
| C1q | 因子 P | MDA5 | SmD1 |
| 心磷脂 | 因子 B | Mi-2 | SmD2 |
| CENP-A | 因子 D | 线粒体抗原 | SmD3 |
| CENP-B | 因子 H | MPO | SP100 |

[0087]

| | | | |
|---|---|---|---|
| 硫酸软骨素 C | 纤维蛋白原 IV | 毒蕈碱受体 | 鞘磷脂 |
| 染色质 | 纤维蛋白原 S | 髓鞘碱性蛋白(MBP) | SPR54 |
| 胶原 I | 纤连蛋白 | 髓鞘碱相关糖蛋白-FC | ssDNA |
| 胶原 II | GBM (disso) | 肌球蛋白 | T1F1γ胶原 |
| 胶原 III | 基因组 DNA | 核仁素 | 甲状腺球蛋白 |
| 胶原 IV | 醇溶蛋白(IgG) | 核小体抗原 | TNFa |
| 胶原 V | 糖化白蛋白 | Nup62 | 拓扑异构酶 I |
| 胶原 VI | GP2 | PCNA | TPO |
| 补体 C1q | gP210 | 过氧化物氧化还原酶 1 | TTG |
| 补体 C3 | 组蛋白 H1 | 磷脂酰肌醇 | U1-snRNP-68 |
| 补体 C3a | 组蛋白 H2A | PL-12 | U1-snRNP-A |
| 补体 C3b | 组蛋白 H2B | PL-7 | U1-snRNP-BB' |
| 补体 C4 | 组蛋白 H3 | PM/Scl-100 | U1-snRNP-C |
| 补体 C5 | 组蛋白 H4 | PM/Scl-75 | 波形蛋白 |
| 补体 C6 | 血蓝蛋白 | POLB | 玻连蛋白 |
| 补体 C7 | 乙酰肝素 HSPG | PR3 | β2- 糖蛋白 I |
| 补体 C8 | 肝素 | 蛋白多糖 | β2-微球蛋白 |
| 补体 C9 | 硫酸乙酰肝素 | 凝血素蛋白 | IgA - 人和鼠 |
| CPR 抗原(人) | 组蛋白 (总) | 核糖(Ribo)磷蛋白 P1 | IgE- 人 |
| 细胞色素 C | 内因子 | 核糖磷蛋白 P2 | IgG - 人和鼠 |
| 核心蛋白聚糖-牛 | Jo-1 | 核糖磷蛋白 P0 | IgM - 人和鼠 |
| DGPS | KU (P70/P80) | Ro/SSA (52KDa) | 抗 IgG、IgA 和抗 IgM |

[0088] 实施例4:小鼠SLE模型和EAE模型中LGALS3BP表达升高

[0089] 以下实验检验狼疮性肾炎患者中的LGALS3BP表达升高是否在小鼠SLE模型中保守。MRL/lpr小鼠具有Fas内突变,造成淋巴细胞凋亡缺陷,最终表现为SLE-样自身免疫疾病。MRL/lpr与野生型C57/BL6动物比较显示患病动物的肾脏和脾脏中LGALS3BP表达显著升高(图4A)。腹腔内注射降植烷诱导自身抗体、蛋白尿和肾炎的小鼠诱导SLE模型中可见相同结果。这些小鼠还形成了于血液和脾脏测到的IFN签名,与SLE人患者中所见IFN-诱导基因相似。BXSB/Yaa小鼠具有双倍重复的跨RNA传感器TLR7基因区,形成了SLE-样症状。已知TLR7在SLE中起重要作用,TLR7活化引起I型IFN分泌。知道LGALS3BP表达是TLR7刺激诱导的而且该表达与狼疮性肾炎人患者的IFN签名相关,于是测定了BXSB/Yaa小鼠多种器官内的LGALS3BP表达。LGALS3BP mRNA显著升高只见于肾炎小鼠的肾脏样本。两个小鼠肾炎得分低,未显示LGALS3BP表达升高。为了评价LGALS3BP表达是否与IFN-调节基因同踪,根据五基因(usp18、irf7、ifit1、oas3、bst2)表达计算"IFN基因签名得分"。所得分值证实LN患者中LGALS3BP表达与IFN得分之间存在同样的关联。IFN-诱导型基因同样限于肾脏,进一步确认LGALS3BP与IFN反应相关。还发现LGALS3BP在多发性硬化症(MS)人患者与EAE小鼠之间差异表达(Raddatz等,PLUS ONE 2014)。蛋白脂蛋白(PLP)免疫SJL小鼠诱导EAE证实了这一发

现。疾病诱导后14天，LGALS3BP表达显著升高(图4C)。

[0090] 实施例5:半乳凝素-3抑制不降低B细胞活力和抗体生产

[0091] 健康人供体的原代B细胞在半乳凝素-3抑制剂存在下接受刺激，目的在于确定半乳凝素-3是否参与LGALS3BP在B细胞生物学中的功能。具体地说，自健康志愿者新鲜分离的B细胞与半乳凝素-3(Gal-3)抑制剂预培养30分钟，然后用TLR7激动剂刺激5天。收集上清液，用AlphaLISA测定IgG。用CellTiter-Glo测定细胞活力(ATP生产)。抑制剂都对B细胞活力和抗体生产没有作用，提示半乳凝素-3不直接参与B细胞的抗体生产(表2)。

[0092] 表2:半乳凝素-1和半乳凝素-3抑制剂不诱导B细胞凋亡和抗体分泌减少

[0093]

| 化合物 | 抑制 | IgG 生产 | 活力 |
|---|---|---|---|
| LacNAc, N-乙酰-D-氨基乳糖苷 | Gal-3 | > 10 μM | > 10 μM |
| 果胶 *(Pienta KJ 等. J Natl Cancer Inst. 1995)* | Gal-3 | > 10 μM | > 10 μM |
| β 正-丙二醇乳糖苷(β n-propal lactoside) | Gal-3 | > 10 μM | > 10 μM |

[0094] 实施例6:抗LGALS3BP肿瘤抑制抗体SP-2不影响B细胞活力和抗体生产

[0095] 有报道称LGALS3BP参与癌症而抗LGALS3BP抗体SP-2抑制肿瘤生长和血管生成。在B细胞刺激系统中进行了SP-2试验，未见对B细胞活力和抗体生产的影响(图10A-1、图10A-2、图10A-3和10B-1)。而且，SP-2靶向的是LGALS3BP的C末端结构域，而抑制B细胞活力和抗体生产的抗体则是针对结构域2产生的，这提示该蛋白质的不同结构域各有不同的功能。

[0096] 出于在美国的任何目的，本文中援引的所有和每一篇公开文献和专利文献均被纳入用于所有目的，如同被针对性地、各自通过援引纳入。

[0097] 以上结合具体实施方式对本发明进行了描述，然而，根据实际情况或用途可以进行改变和等同替换从而实现本发明的有益效果，这些都在权利要求范围之内。

序列表

<110> 默克专利有限公司(MERCK PATENT GMBH)

<120> 调节LGALS3BP以治疗系统性红斑狼疮的方法

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Gln Gly Val Asn Asp Gly Asp Met Arg Leu Ala Asp Gly Gly Ala Thr
20 25 30
Asn Gln Gly Arg Val Glu Ile Phe Tyr Arg Gly Gln Trp Gly Thr Val
35 40 45
Cys Asp Asn Leu Trp Asp Leu Thr Asp Ala Ser Val Val Cys Arg Ala
50 55 60
Leu Gly Phe Glu Asn Ala Thr Gln Ala Leu Gly Arg Ala Ala Phe Gly
65 70 75 80
Gln Gly Ser Gly Pro Ile Met Leu Asp Glu Val Gln Cys Thr Gly Thr
85 90 95
Glu Ala Ser Leu Ala Asp Cys Lys Ser Leu Gly Trp Leu Lys Ser Asn
100 105 110
Cys Arg His Glu Arg Asp Ala Gly Val Val Cys Thr Asn Glu Thr Arg
115 120 125
Ser Thr His Thr Leu Asp Leu Ser Arg Glu Leu Ser Glu Ala Leu Gly
130 135 140
Gln Ile Phe Asp Ser Gln Arg Gly Cys Asp Leu Ser Ile Ser Val Asn
145 150 155 160
Val Gln Gly Glu Asp Ala Leu Gly Phe Cys Gly His Thr Val Ile Leu
165 170 175
Thr Ala Asn Leu Glu Ala Gln Ala Leu Trp Lys Glu Pro Gly Ser Asn

180 185 190

Val Thr Met Ser Val Asp Ala Glu Cys Val Pro Met Val Arg Asp Leu

195 200 205

Leu Arg Tyr Phe Tyr Ser Arg Arg Ile Asp Ile Thr Leu Ser Ser Val

210 215 220

Lys Cys Phe His Lys Leu Ala Ser Ala Tyr Gly Ala Arg Gln Leu Gln

225 230 235 240

Gly Tyr Cys Ala Ser Leu Phe Ala Ile Leu Leu Pro Gln Asp Pro Ser

245 250 255

Phe Gln Met Pro Leu Asp Leu Tyr Ala Tyr Ala Val Ala Thr Gly Asp

260 265 270

Ala Leu Leu Glu Lys Leu Cys Leu Gln Phe Leu Ala Trp Asn Phe Glu

275 280 285

Ala Leu Thr Gln Ala Glu Ala Trp Pro Ser Val Pro Thr Asp Leu Leu

290 295 300

Gln Leu Leu Leu Pro Arg Ser Asp Leu Ala Val Pro Ser Glu Leu Ala

305 310 315 320

Leu Leu Lys Ala Val Asp Thr Trp Ser Trp Gly Glu Arg Ala Ser His

325 330 335

Glu Glu Val Glu Gly Leu Val Glu Lys Ile Arg Phe Pro Met Met Leu

340 345 350

Pro Glu Glu Leu Phe Glu Leu Gln Phe Asn Leu Ser Leu Tyr Trp Ser

355 360 365

His Glu Ala Leu Phe Gln Lys Lys Thr Leu Gln Ala Leu Glu Phe His

370 375 380

Thr Val Pro Phe Gln Leu Leu Ala Arg Tyr Lys Gly Leu Asn Leu Thr

385 390 395 400

Glu Asp Thr Tyr Lys Pro Arg Ile Tyr Thr Ser Pro Thr Trp Ser Ala

405 410 415

Phe Val Thr Asp Ser Ser Trp Ser Ala Arg Lys Ser Gln Leu Val Tyr

420 425 430

Gln Ser Arg Arg Gly Pro Leu Val Lys Tyr Ser Ser Asp Tyr Phe Gln

435 440 445

Ala Pro Ser Asp Tyr Arg Tyr Tyr Pro Tyr Gln Ser Phe Gln Thr Pro

450 455 460

Gln His Pro Ser Phe Leu Phe Gln Asp Lys Arg Val Ser Trp Ser Leu

465 470 475 480

Val Tyr Leu Pro Thr Ile Gln Ser Cys Trp Asn Tyr Gly Phe Ser Cys

485 490 495

Ser Ser Asp Glu Leu Pro Val Leu Gly Leu Thr Lys Ser Gly Gly Ser
500 505 510
Asp Arg Thr Ile Ala Tyr Glu Asn Lys Ala Leu Met Leu Cys Glu Gly
515 520 525
Leu Phe Val Ala Asp Val Thr Asp Phe Glu Gly Trp Lys Ala Ala Ile
530 535 540
Pro Ser Ala Leu Asp Thr Asn Ser Ser Lys Ser Thr Ser Ser Phe Pro
545 550 555 560
Cys Pro Ala Gly His Phe Asn Gly Phe Arg Thr Val Ile Arg Pro Phe
565 570 575
Tyr Leu Thr Asn Ser Ser Gly Val Asp
580 585

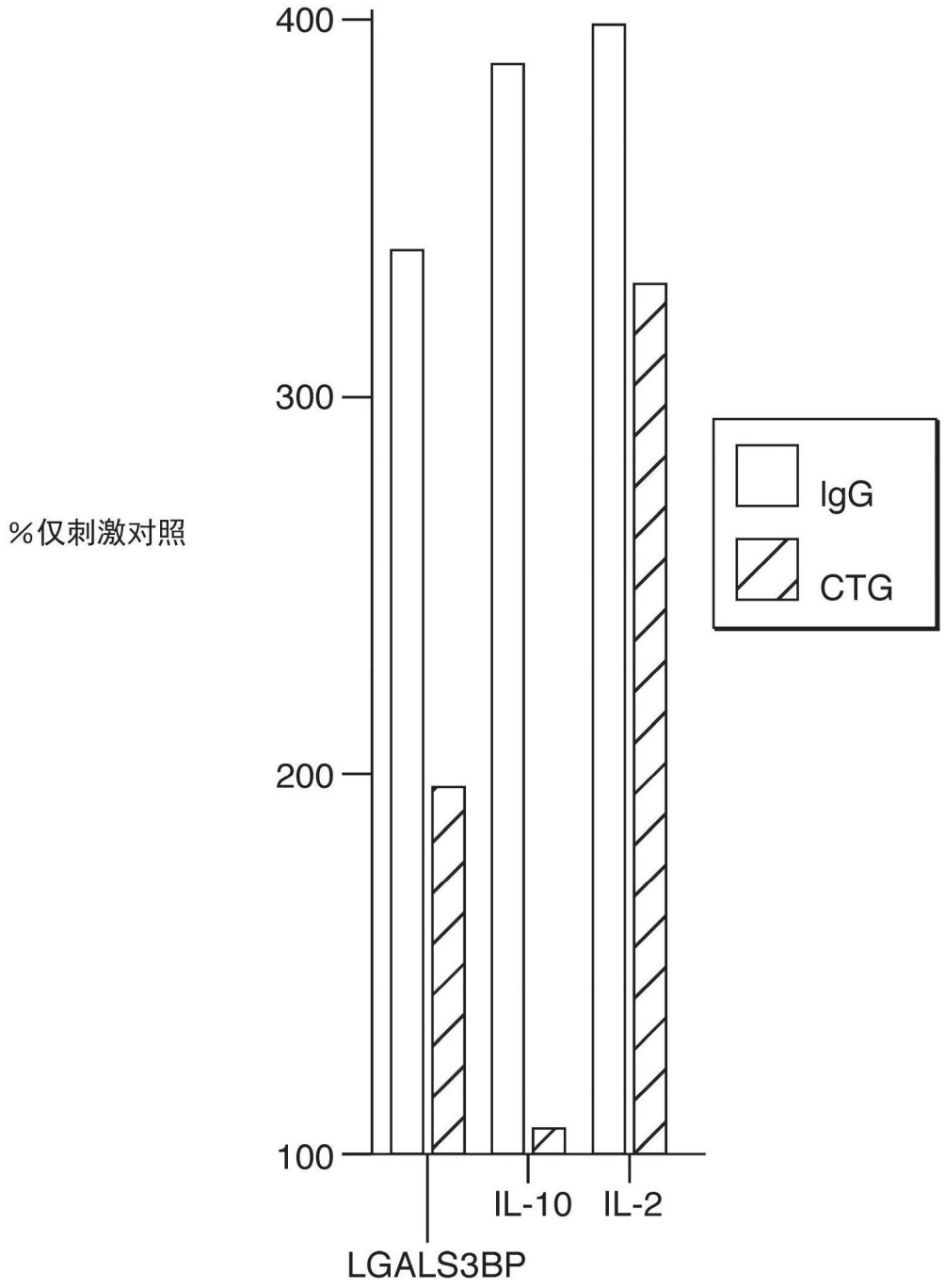


图1A

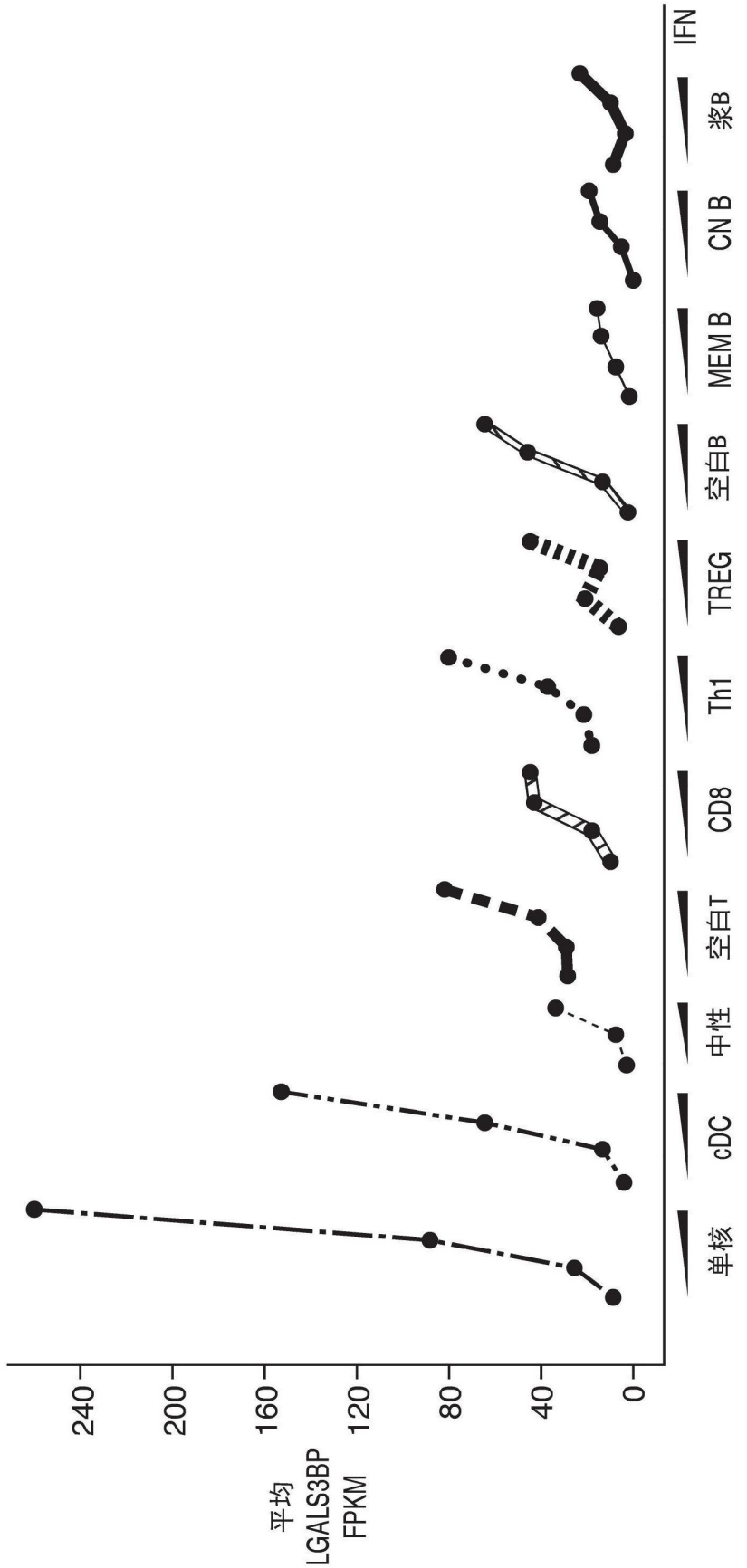
平均
LGALS3BP
FPKM
240
200
160
120
80
40
0
单核
cDC
中性
空白T
CD8
Th1
TREG
空白B
MEM B
CN B
浆B
IFN

图1B

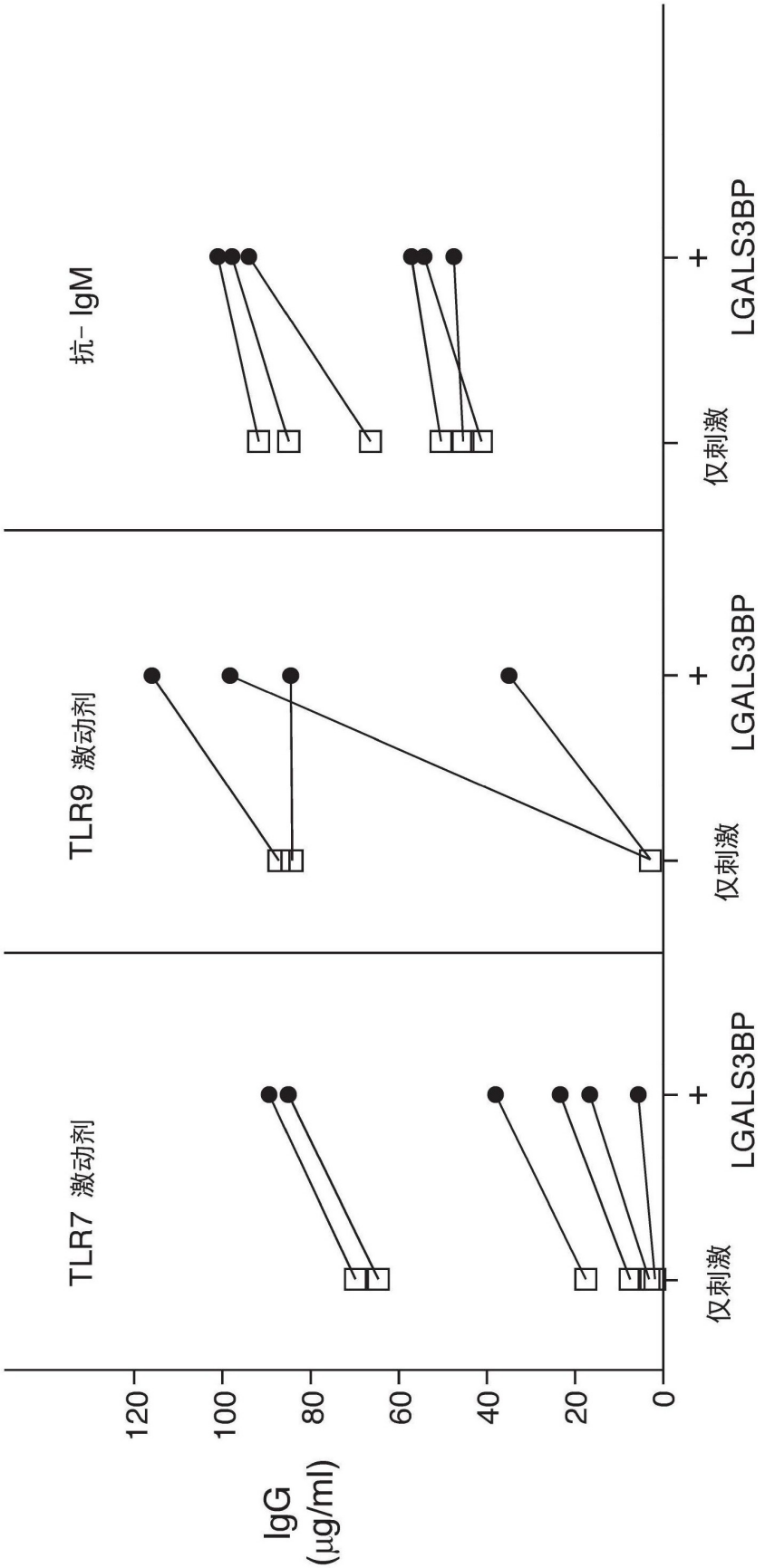
IgG (μg/ml)
0
20
40
60
80
100
120
TLR7 激动剂
TLR9 激动剂
抗- IgM
仅刺激
+
LGALS3BP

图1C

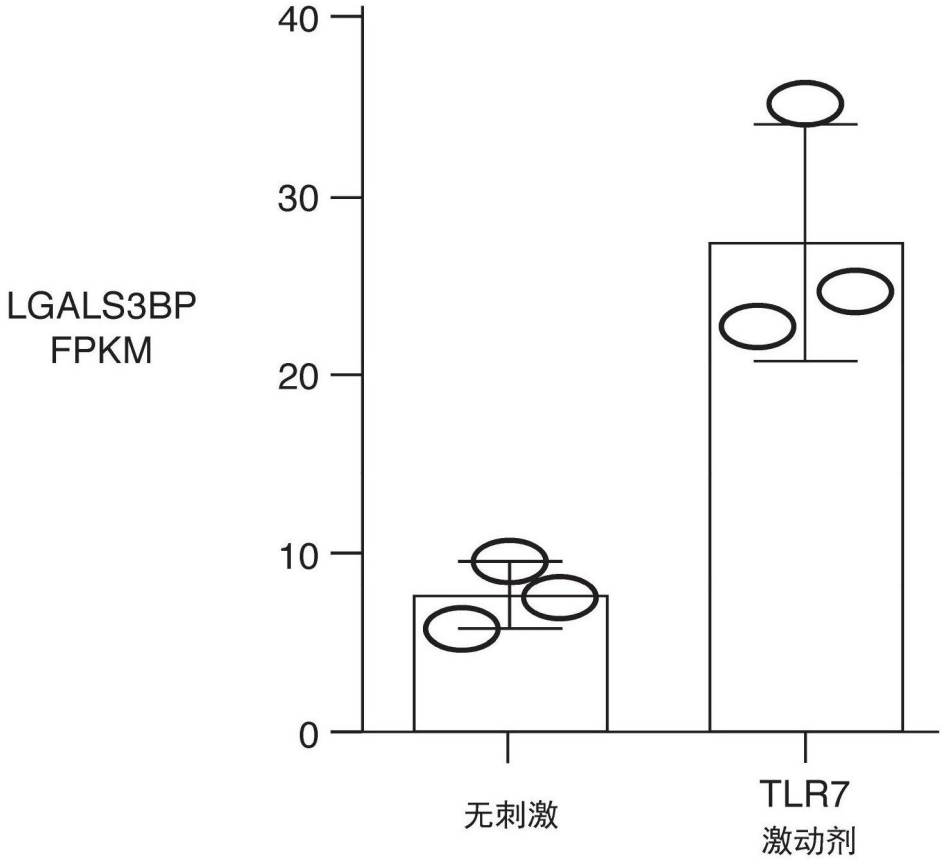


图1D

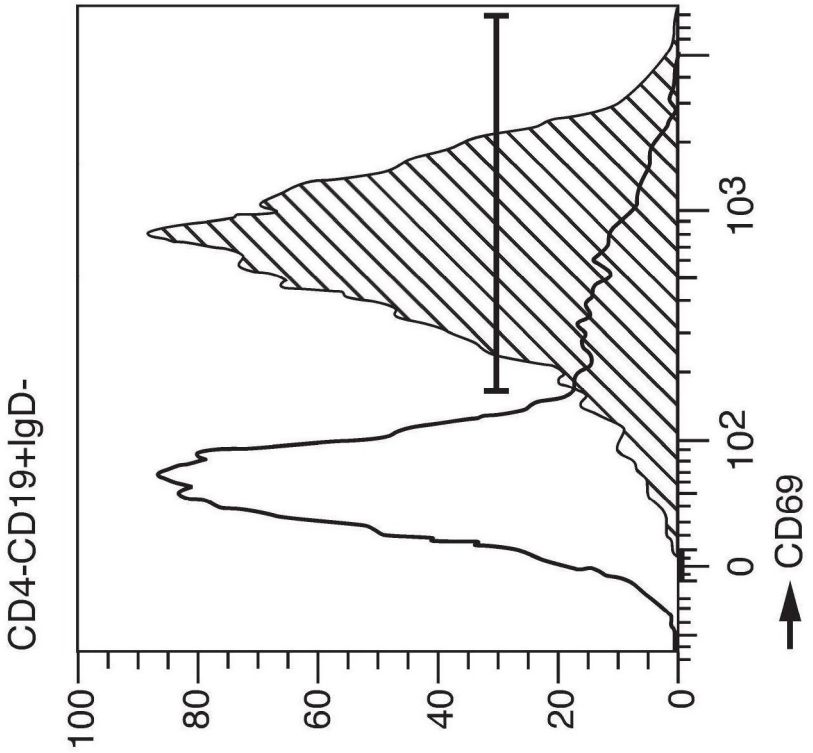


图2A-1

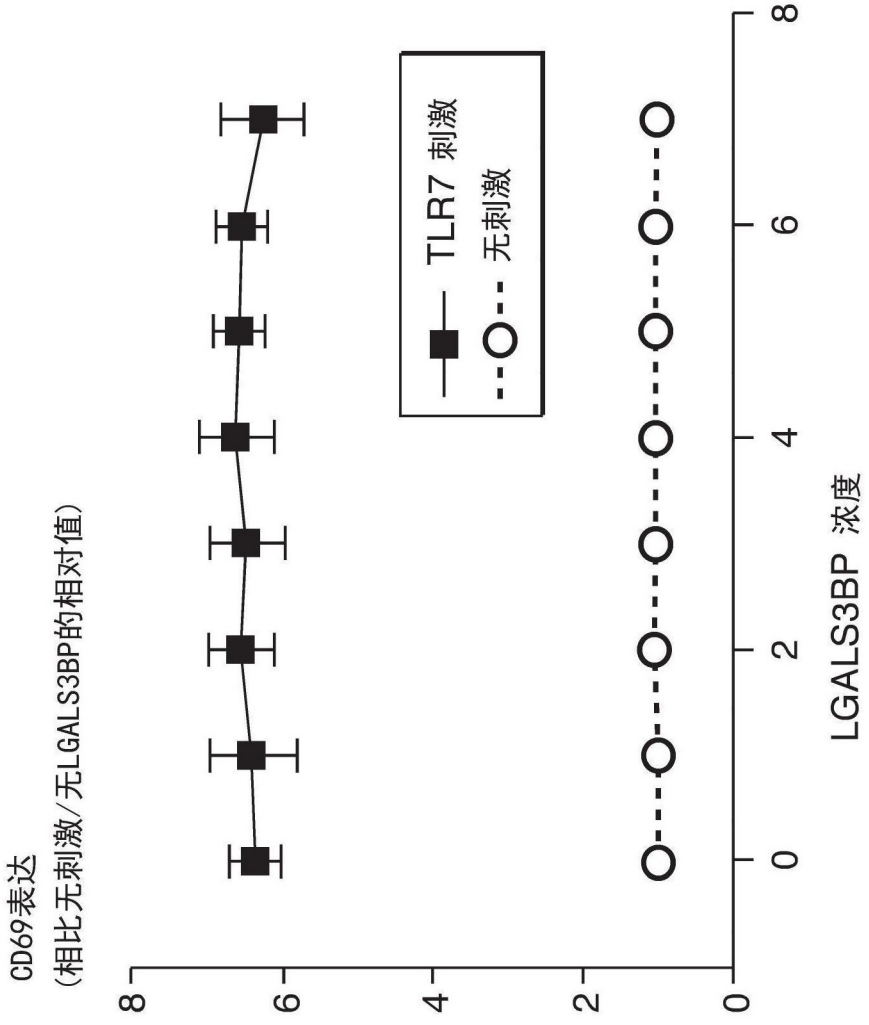
CD69表达
(相比无刺激/无LGALS3BP的相对值)
8
6
4
2
0
TLR7 刺激
无刺激
0
2
4
6
8
LGALS3BP 浓度

图2A-2

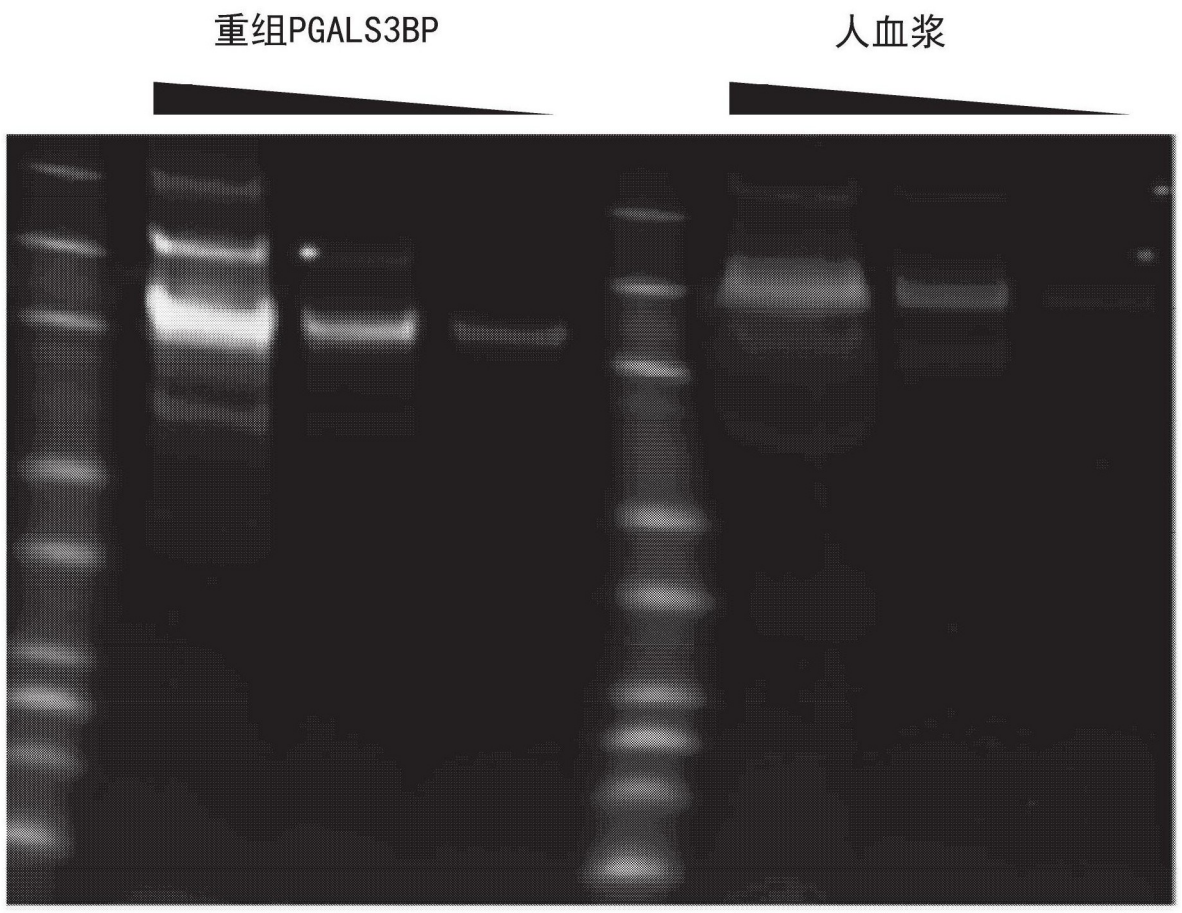


图2B

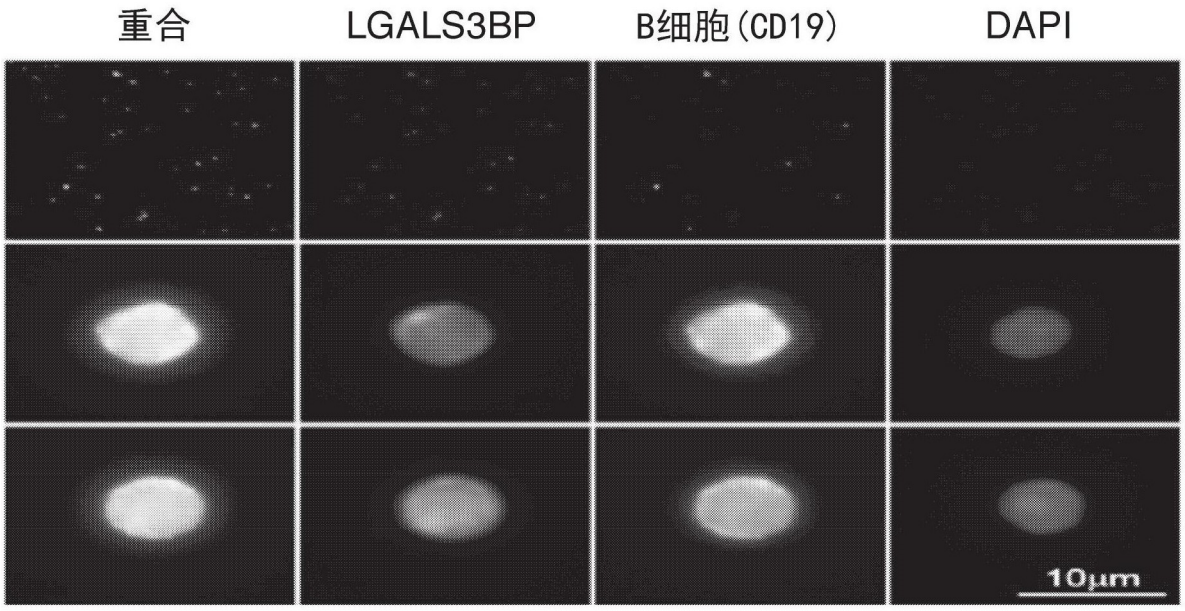


图2C

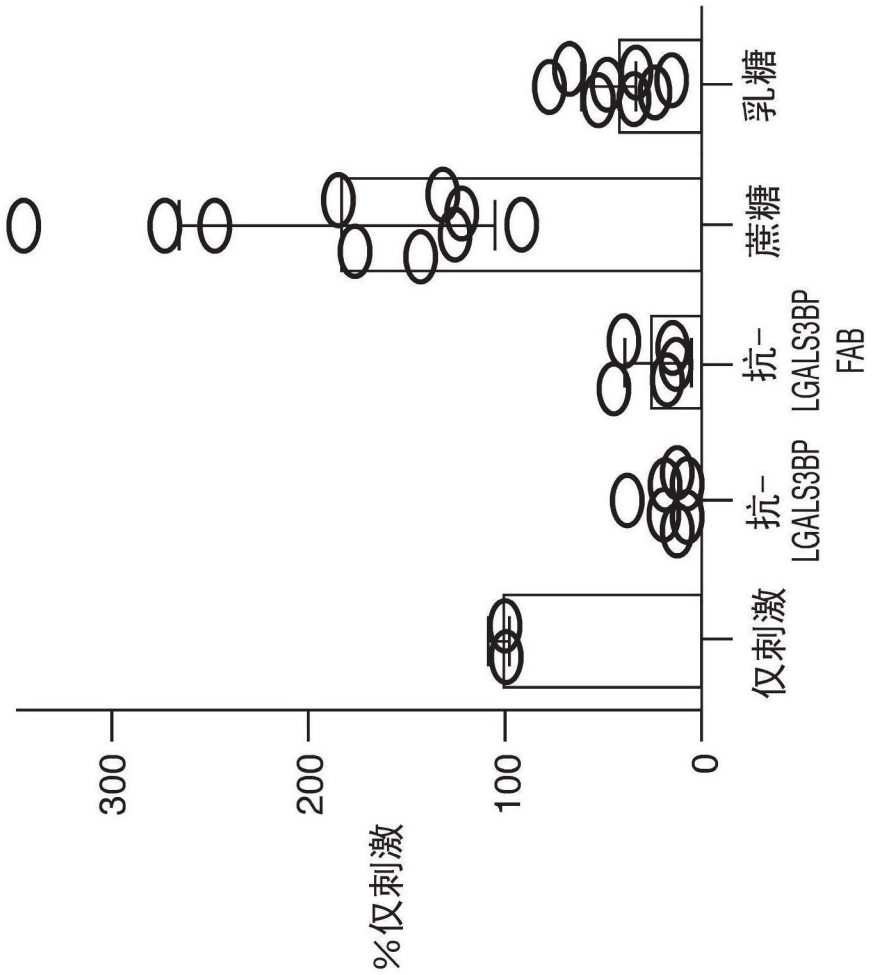


图3A-1

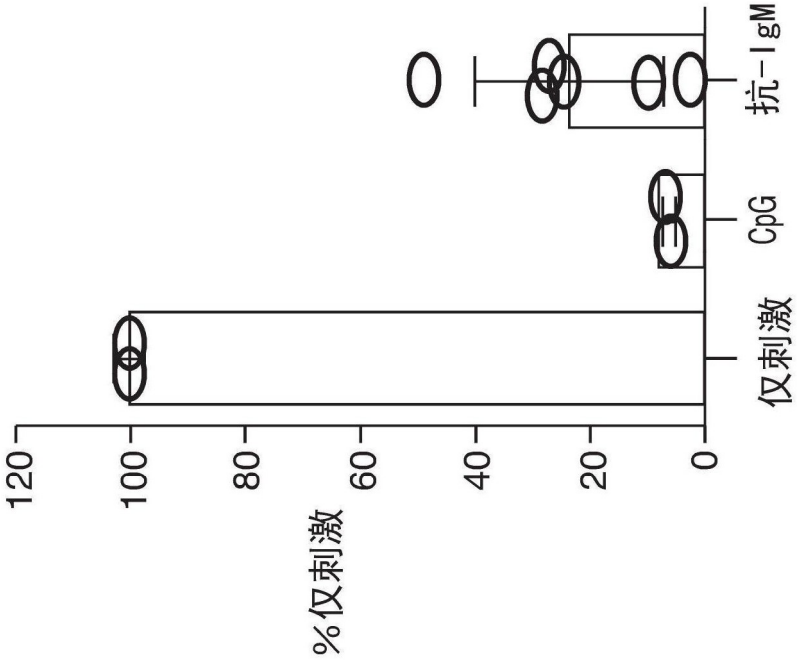


图3A-2

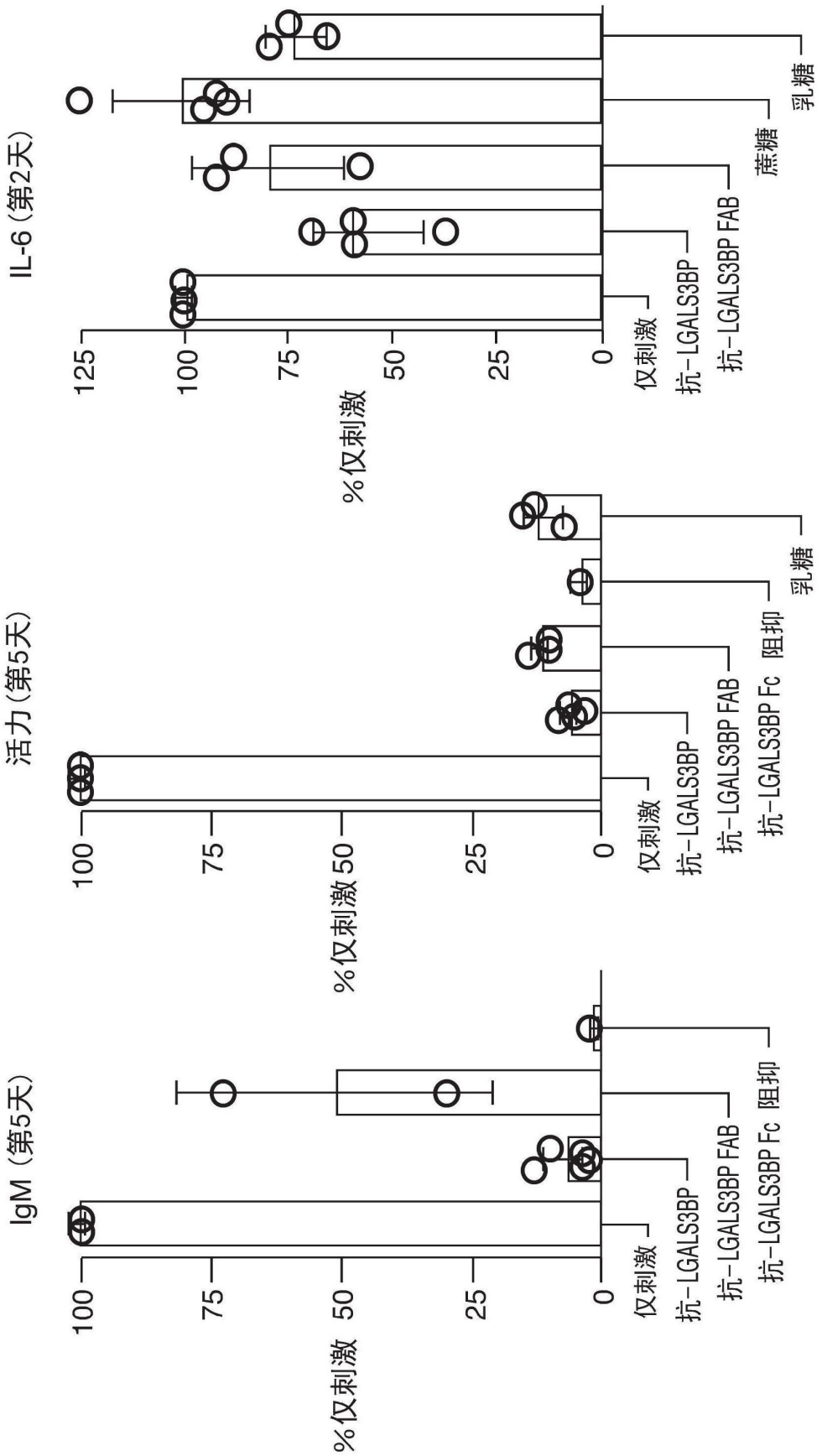


图 3B-1

图 3B-2

图 3B-3

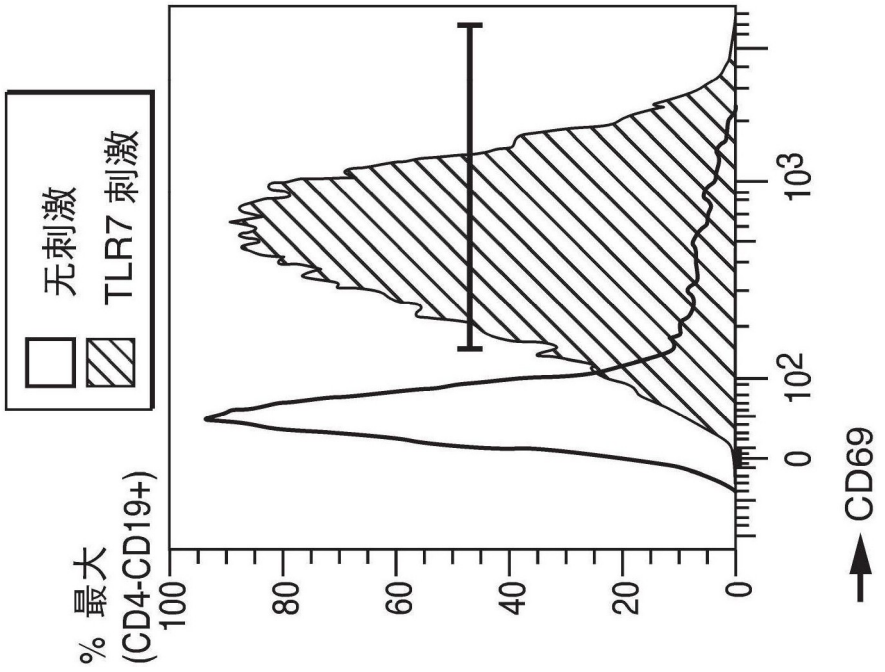
无刺激
TLR7 刺激
% 最大
(CD4-CD19+)
100
80
60
40
20
0
0
$10^2$
$10^3$
CD69

图3C-1

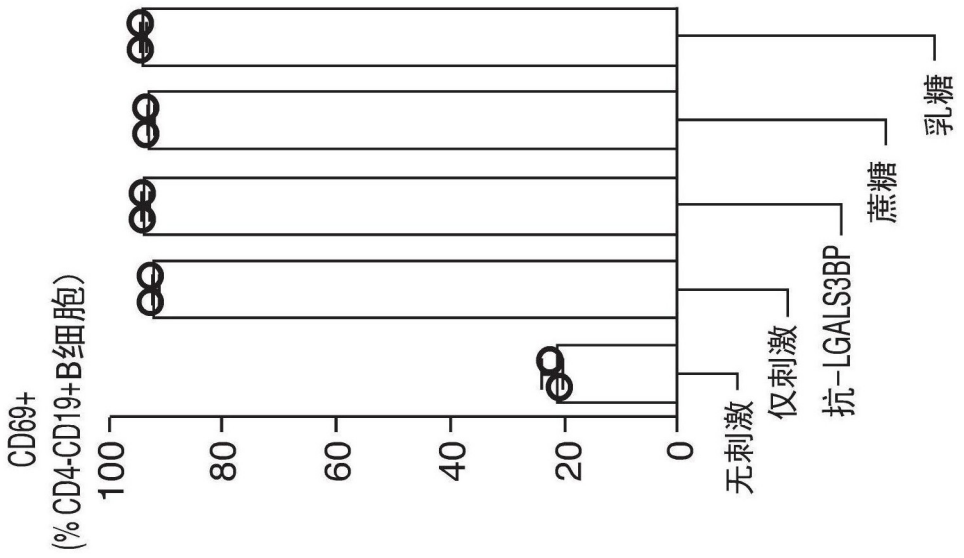
CD69+
(% CD4-CD19+B细胞)
100
80
60
40
20
0
无刺激
仅刺激
抗-LGALS3BP
蔗糖
乳糖

图3C-2

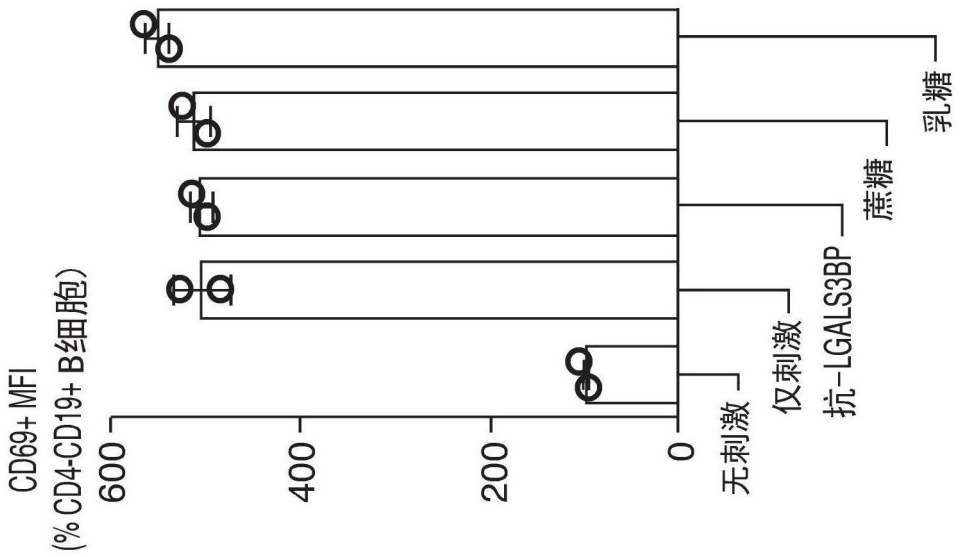
CD69+ MFI
(% CD4-CD19+ B细胞)
600
400
200
0
无刺激
仅刺激
抗-LGALS3BP
蔗糖
乳糖

图3C-3

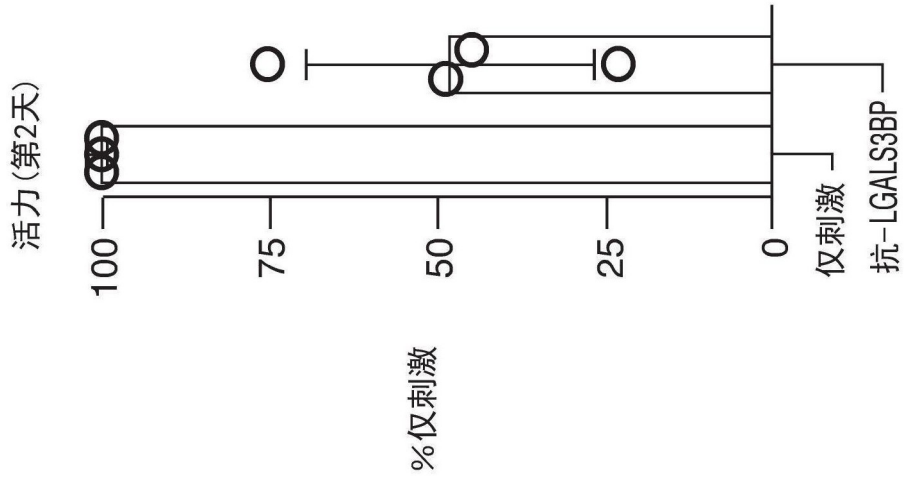
活力(第2天)
100
75
50
25
0
%仅刺激
仅刺激
抗-LGALS3BP

图3D-1

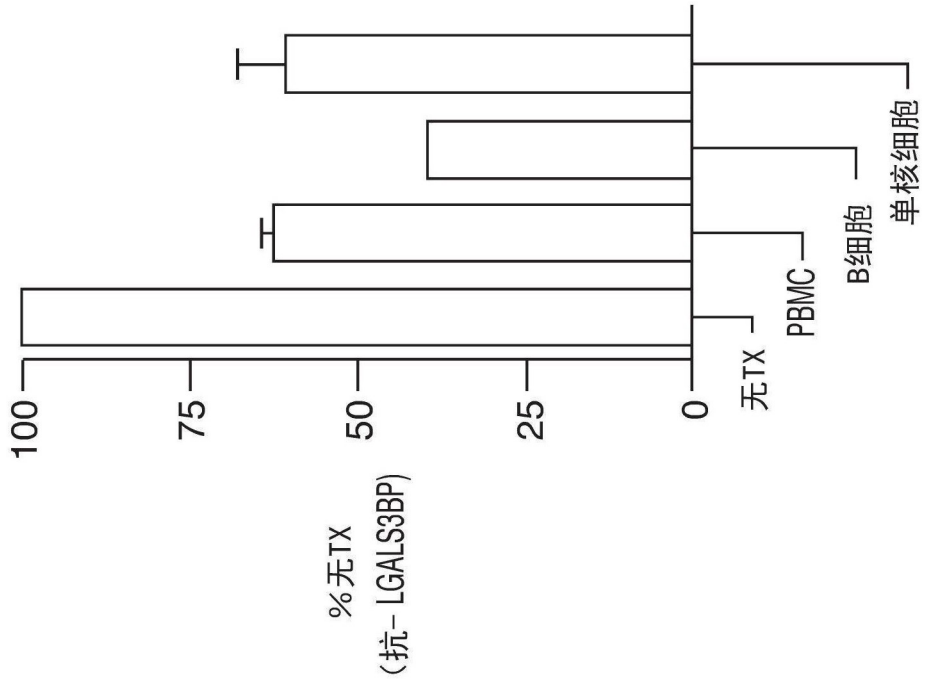


图3D-2

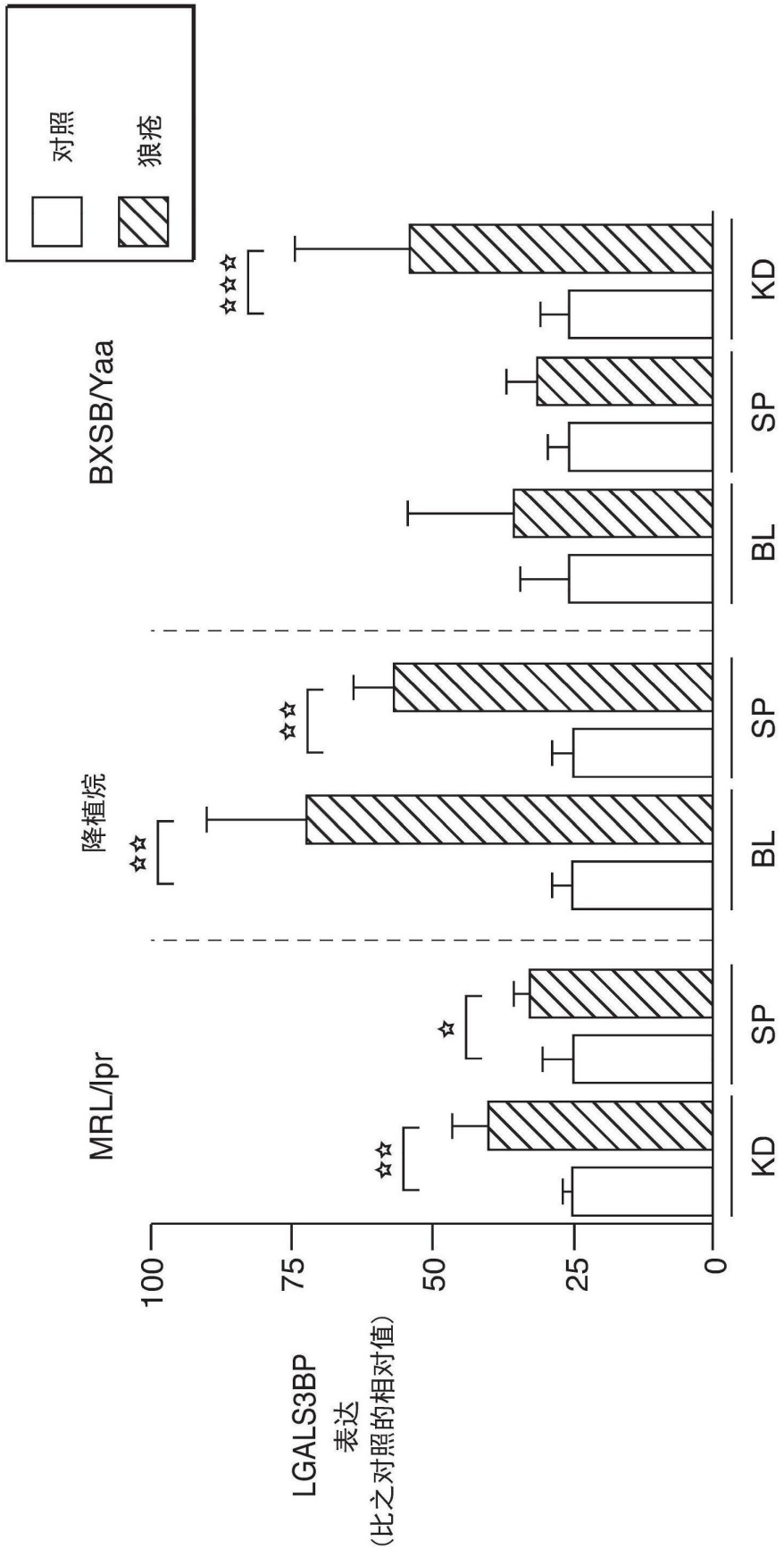
对照
狼疮
MRL/lpr
降植烷
BXSB/Yaa
KD
SP
BL
SP
KD
SP
BL
SP
KD
☆☆
☆
☆☆
☆☆
☆☆☆
100
75
50
25
0
LGALS3BP
表达
(比之对照的相对值)

图4A

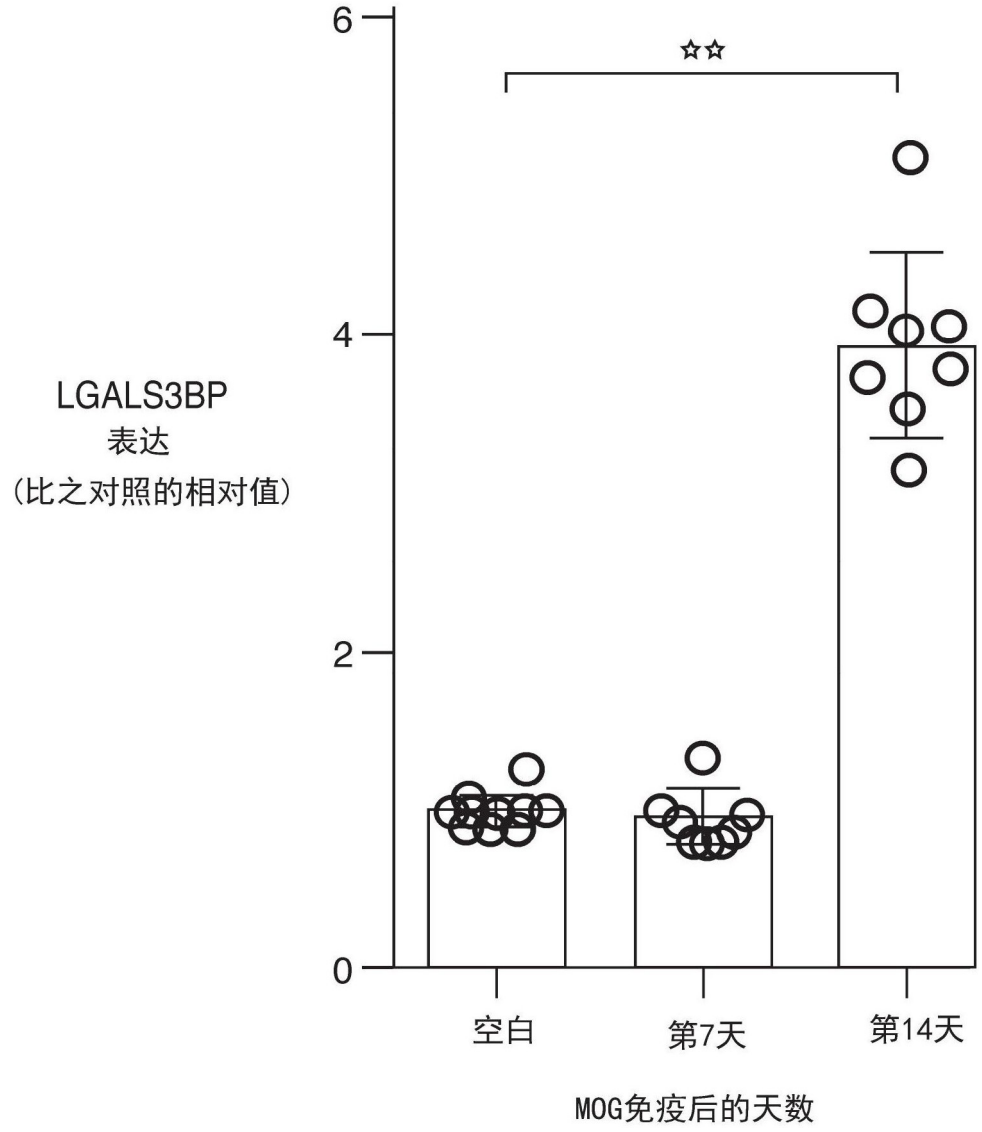


图4B

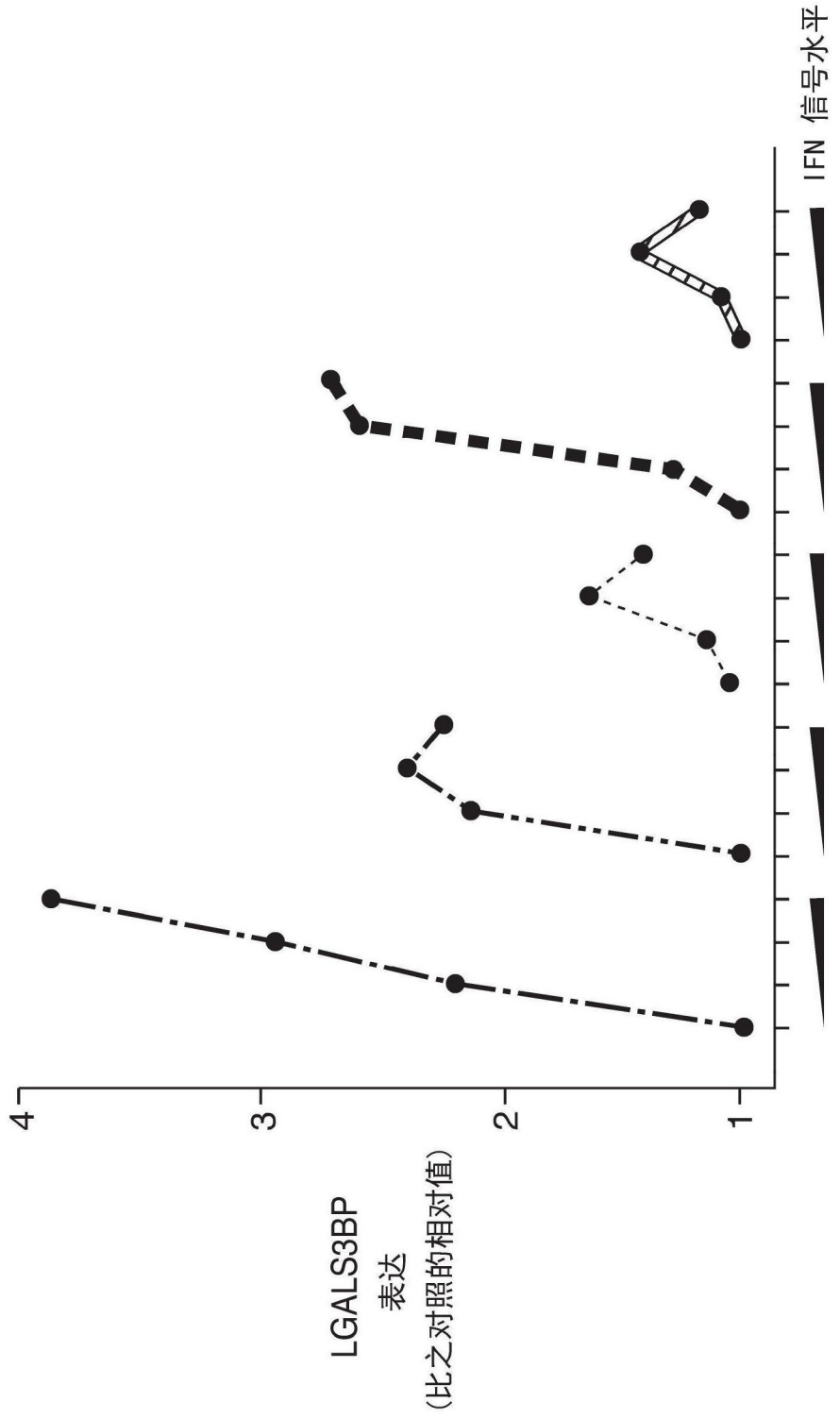


图4C

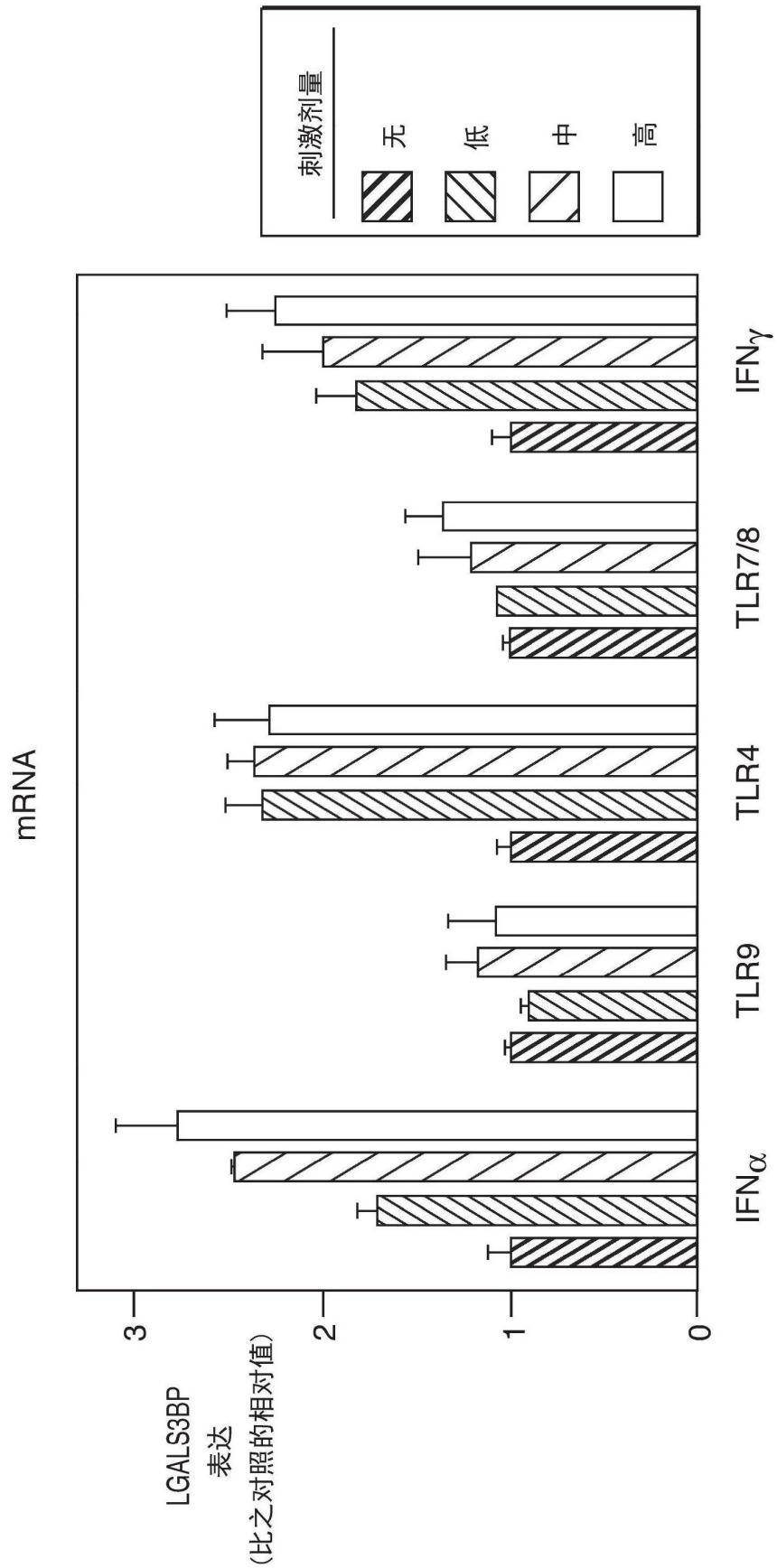
刺激剂量
无
低
中
高
mRNA
LGALS3BP
表达
(比之对照的相对值)
0
1
2
3
IFNα
TLR9
TLR4
TLR7/8
IFNγ

图5A

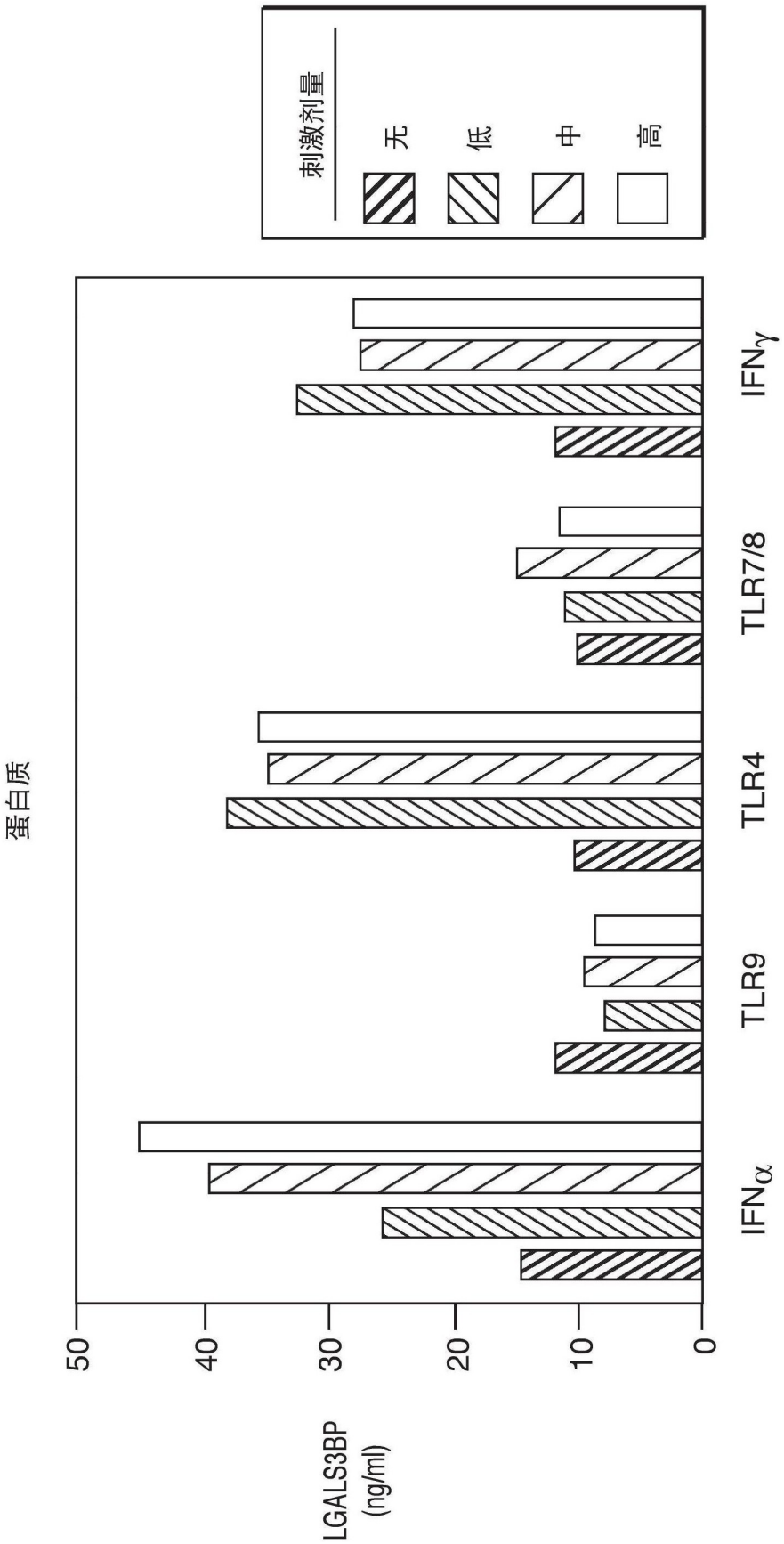
刺激剂量
无
低
中
高
蛋白质
IFNγ
TLR7/8
TLR4
TLR9
IFNα
50
40
30
20
10
0
LGALS3BP
(ng/ml)

图5B

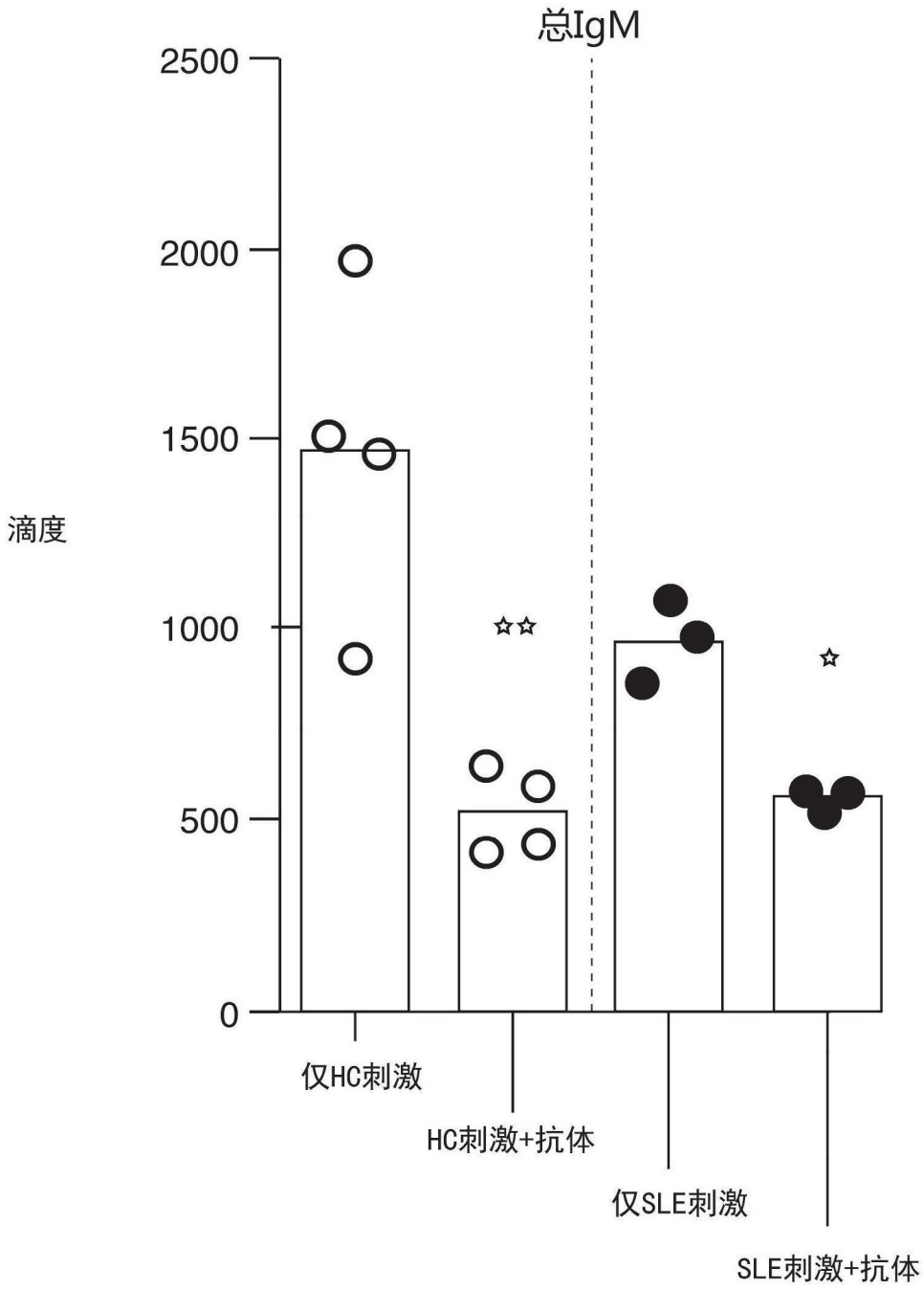
总IgM
2500
2000
1500
1000
500
0
滴度
仅HC刺激
HC刺激+抗体
仅SLE刺激
SLE刺激+抗体

图6A

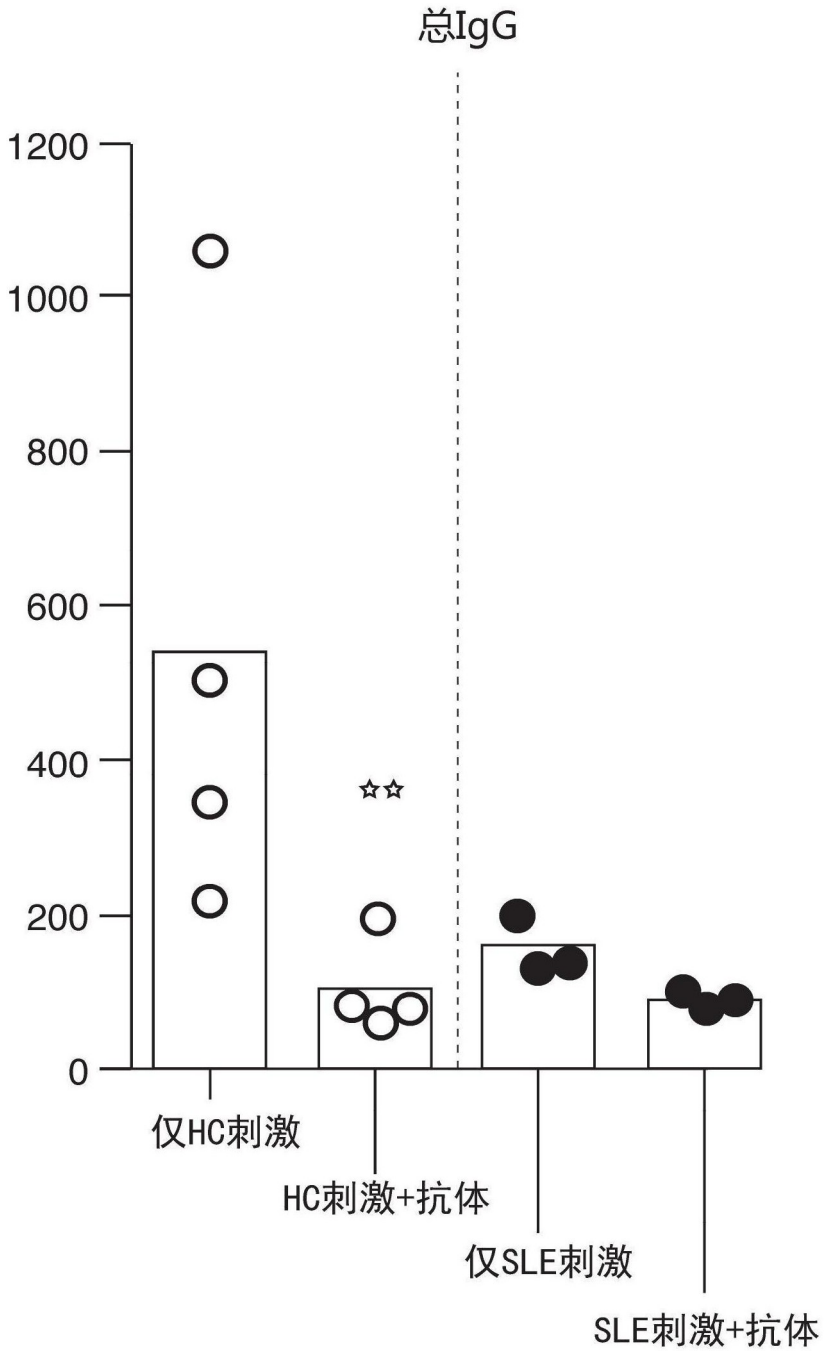


图6B

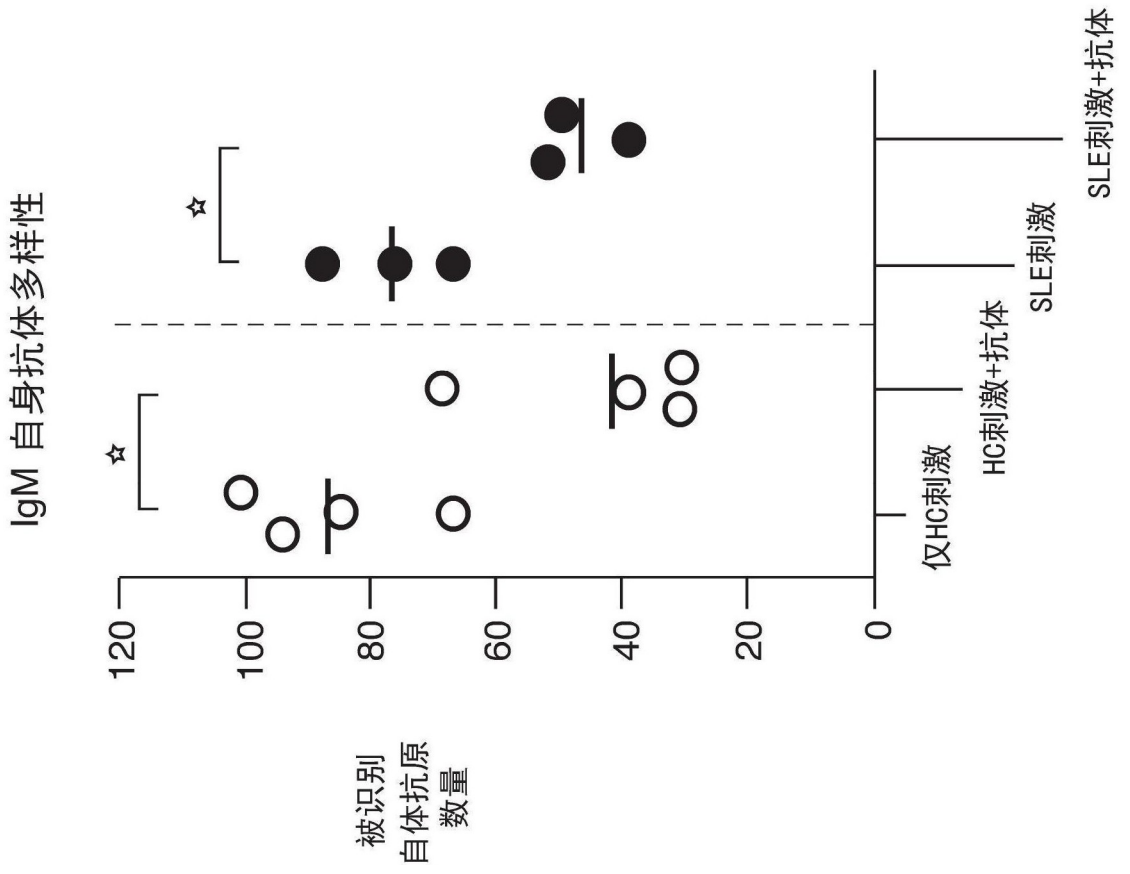
IgM 自身抗体多样性
被识别
自体抗原
数量
120
100
80
60
40
20
0
仅HC刺激
HC刺激+抗体
SLE刺激
SLE刺激+抗体

图7A-1

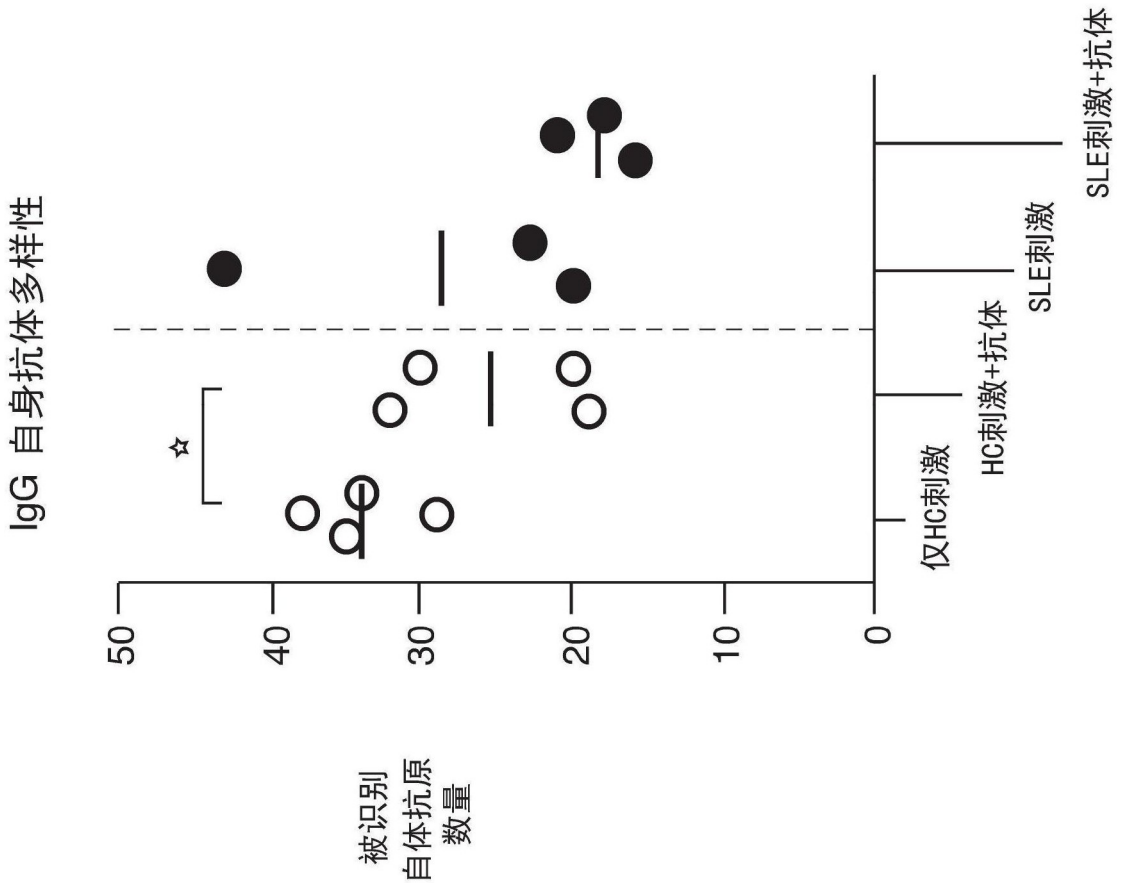
IgG 自身抗体多样性
被识别
自体抗原
数量
50
40
30
20
10
0
仅HC刺激
HC刺激+抗体
SLE刺激
SLE刺激+抗体

图7A-2

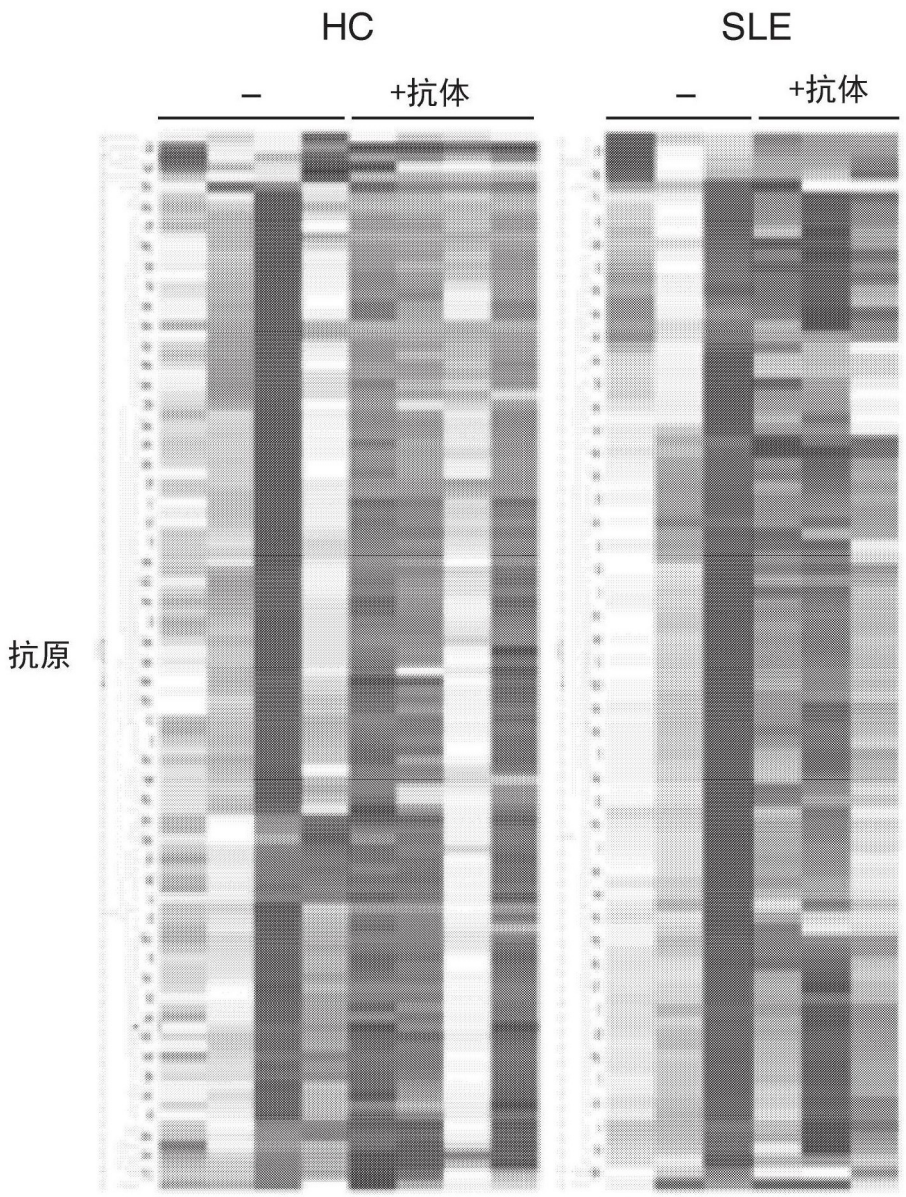


图7B

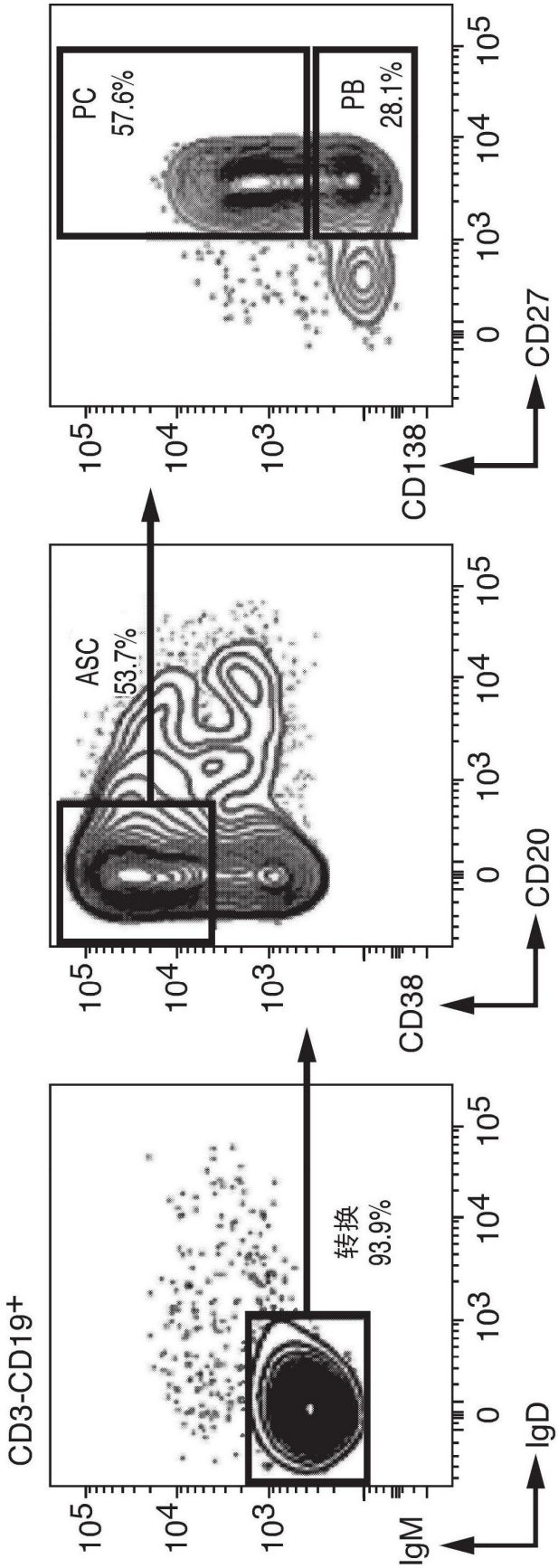


图 8A-1

图 8A-2

图 8A-3

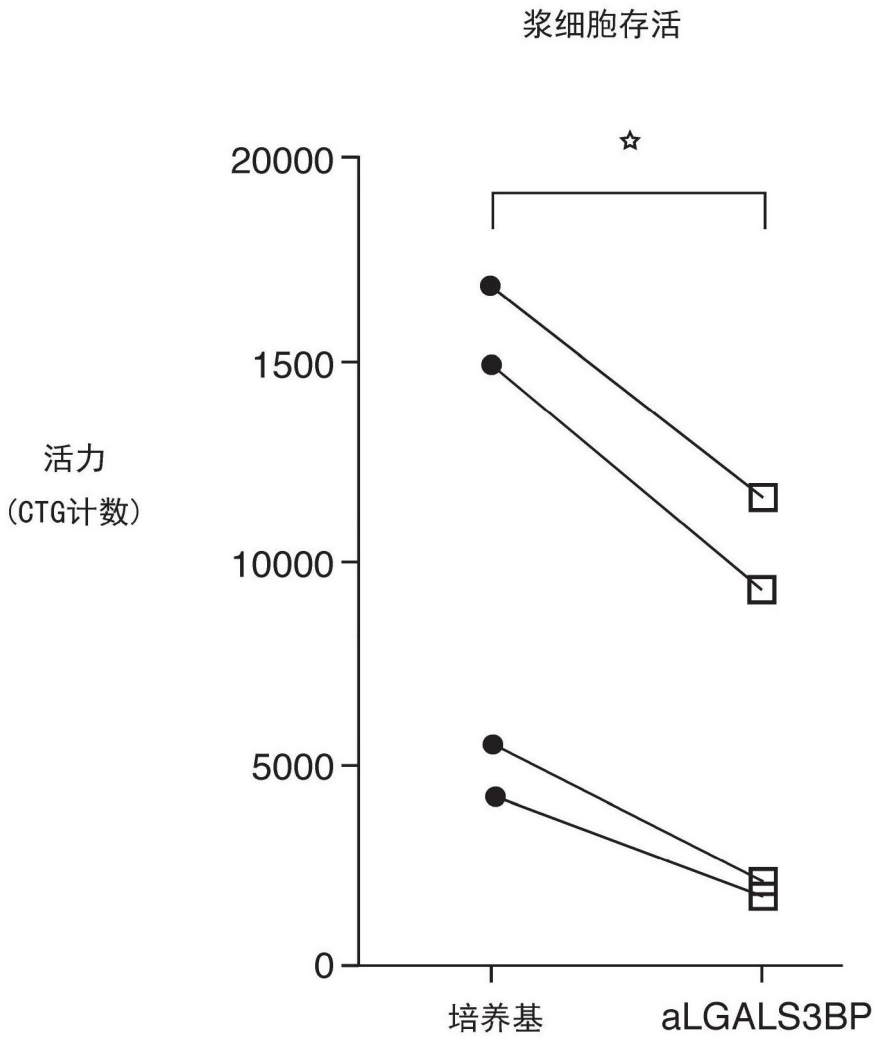


图8B

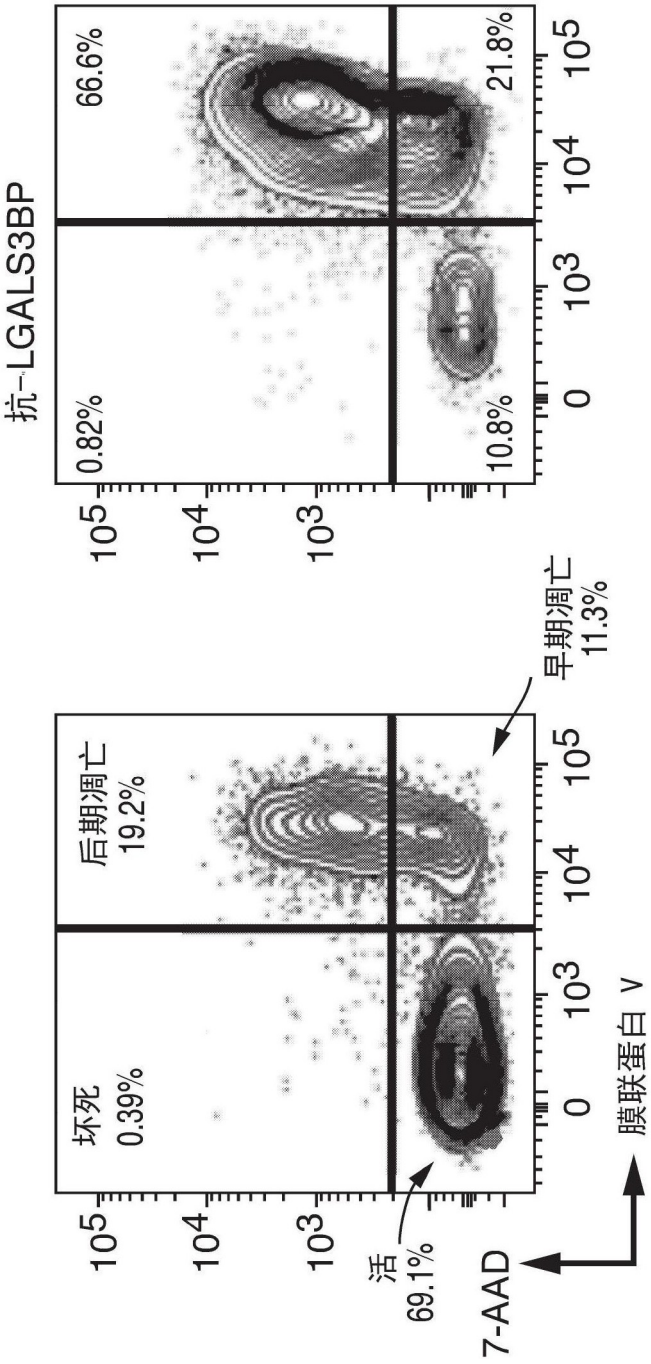
抗-LGALS3BP
66.6%
21.8%
0.82%
10.8%
坏死
0.39%
后期凋亡
19.2%
早期凋亡
11.3%
活
69.1%
7-AAD
膜联蛋白 V
0
$10^3$
$10^4$
$10^5$

图 9A-2

图 9A-1

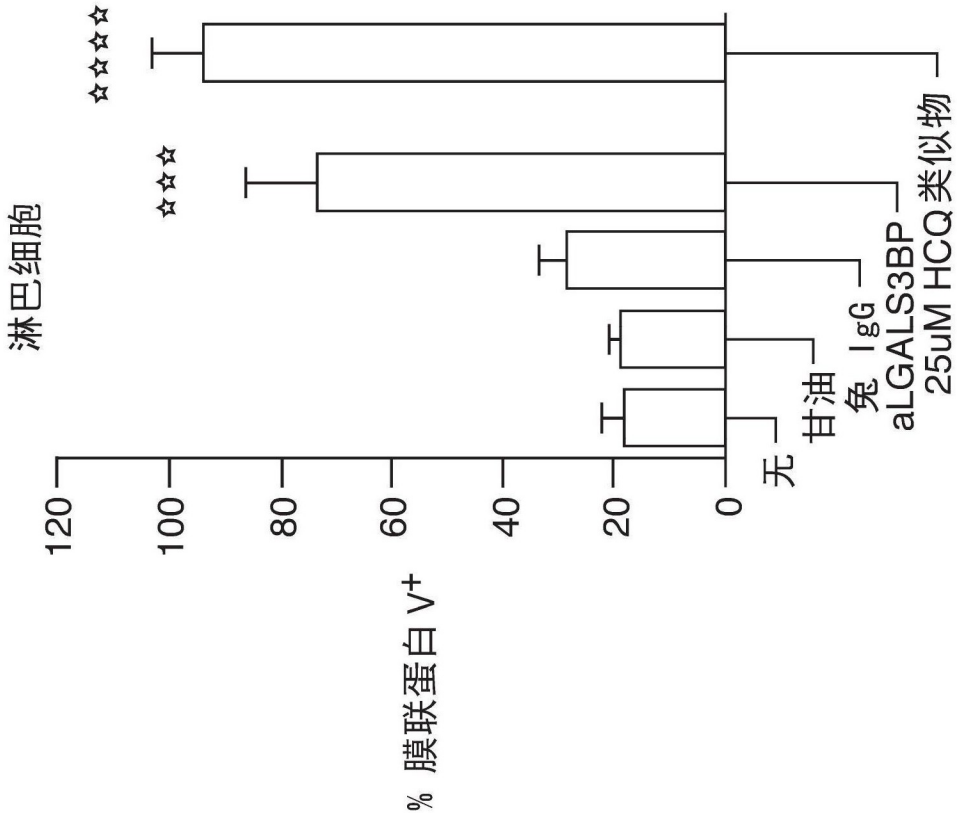
淋巴细胞
☆☆☆☆
☆☆☆
% 膜联蛋白 V+
120
100
80
60
40
20
0
无
甘油
兔 IgG
aLGALS3BP
25uM HCQ类似物

图9B-1

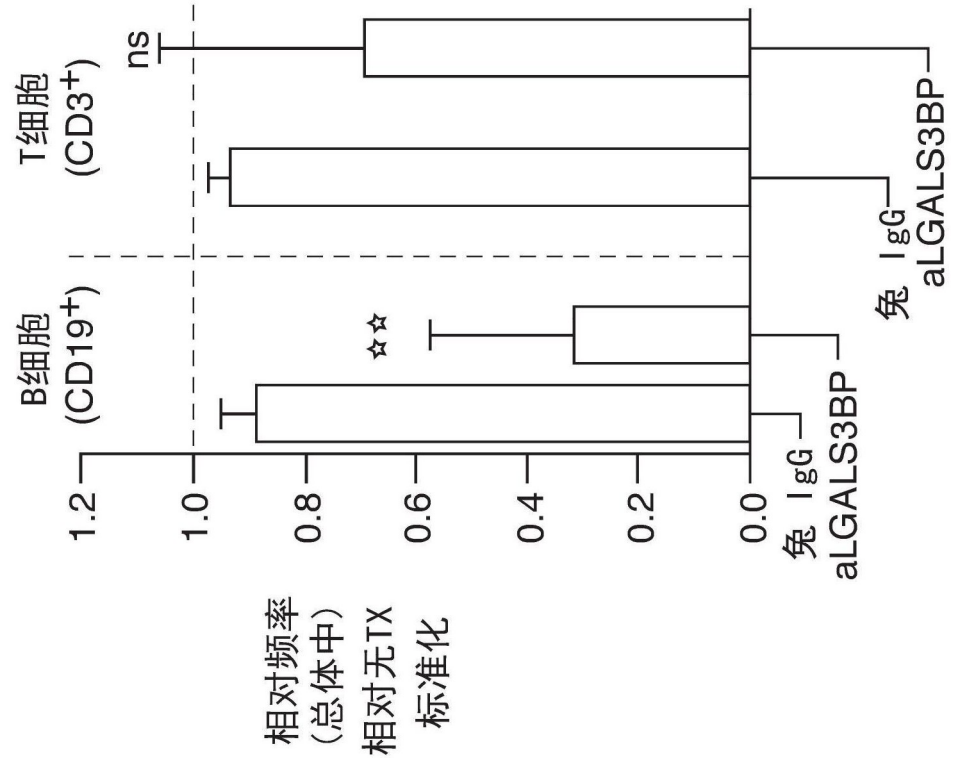
T细胞
(CD3+)
B细胞
(CD19+)
ns
☆☆
相对频率
(总体中)
相对无TX
标准化
1.2
1.0
0.8
0.6
0.4
0.2
0.0
兔 IgG
aLGALS3BP
兔 IgG
aLGALS3BP

图9B-2

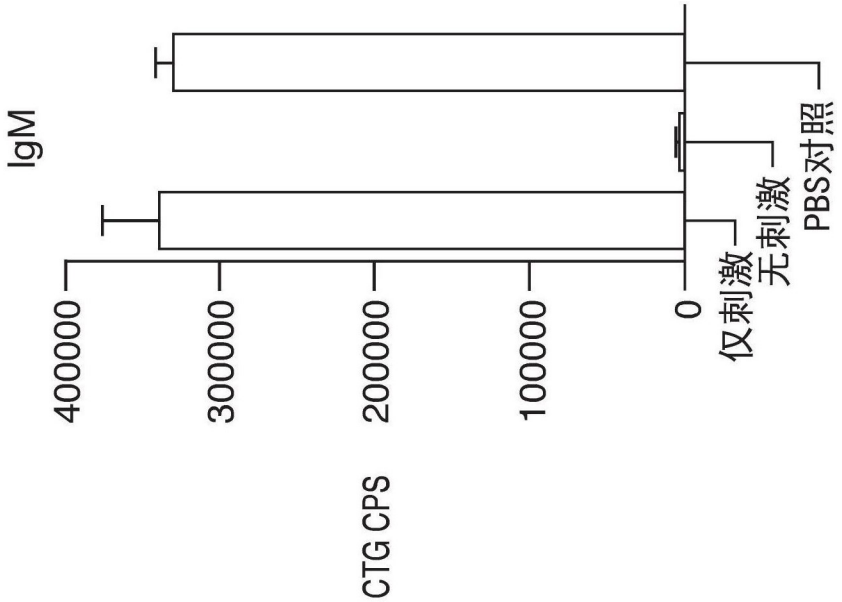
IgM
400000
300000
200000
100000
0
CTG CPS
仅刺激
无刺激
PBS对照

图10A-1

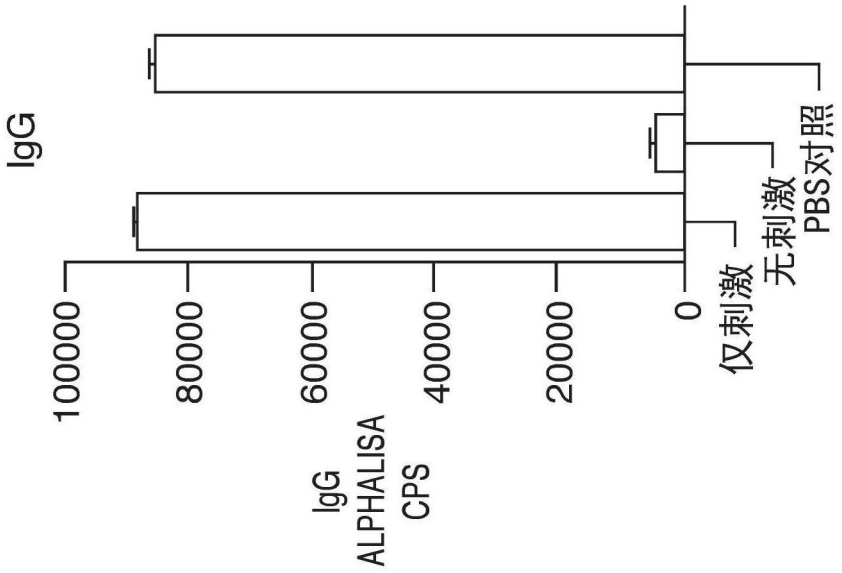
IgG
100000
80000
60000
40000
20000
0
IgG
ALPHALISA
CPS
仅刺激
无刺激
PBS对照

图10A-2

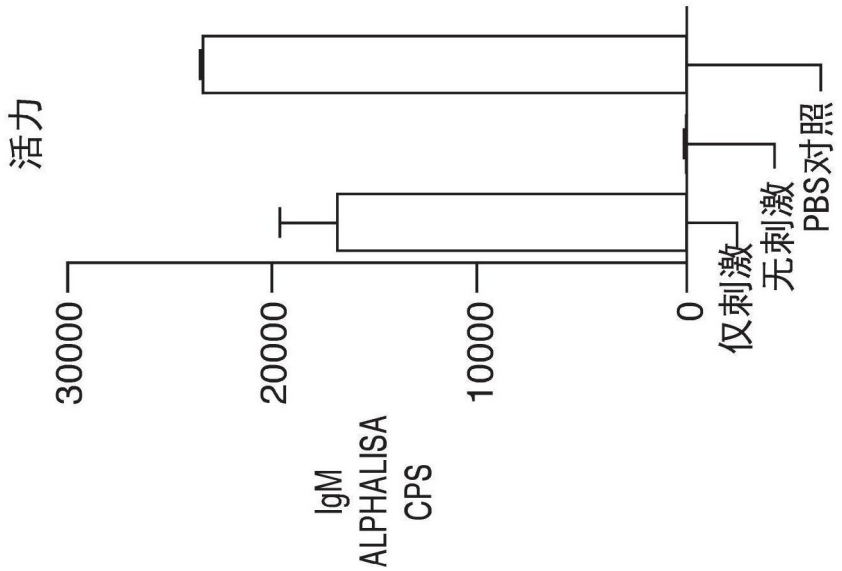


图10A-3

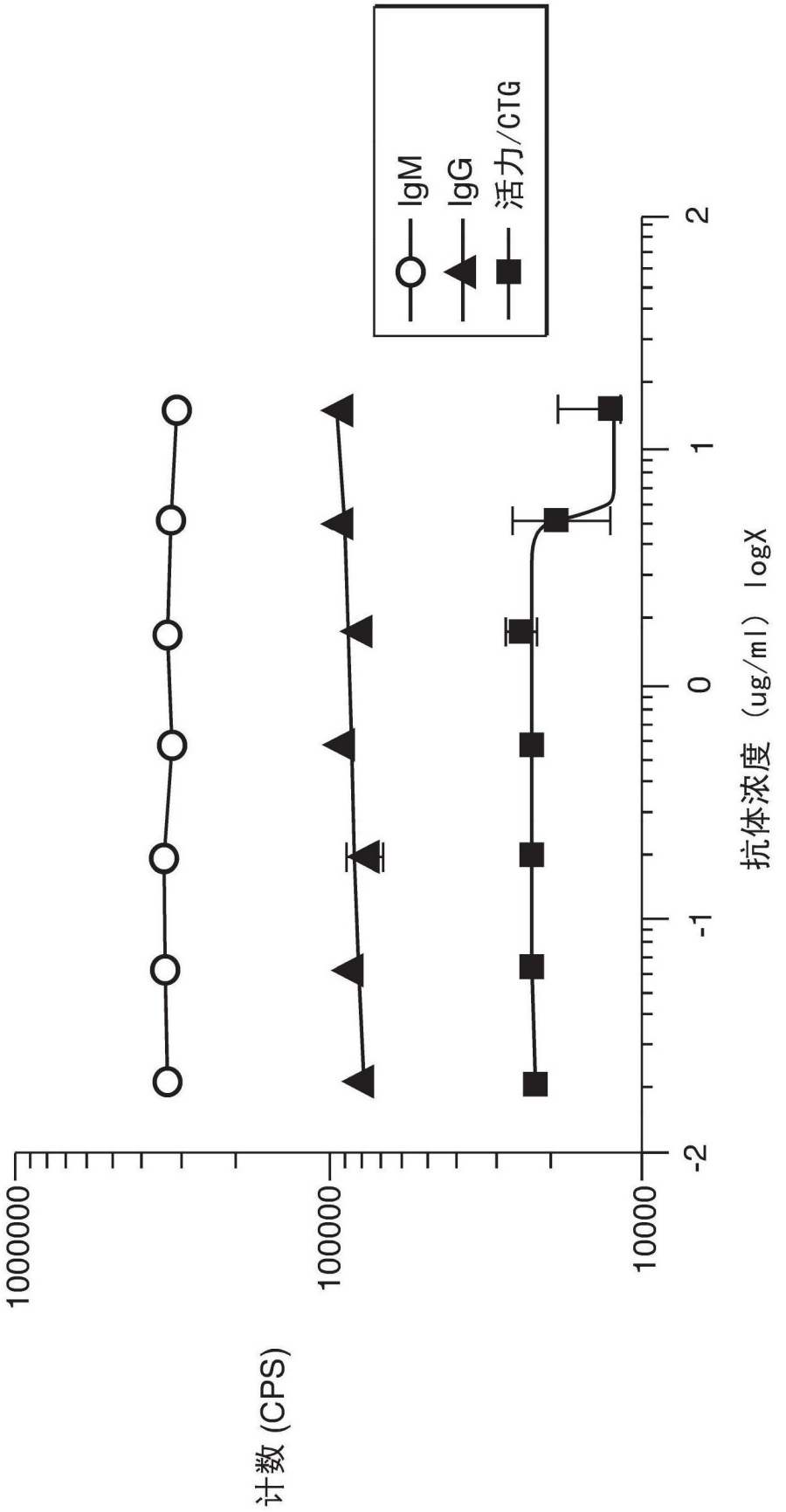
IgM
IgG
活力/CTG
1000000
100000
10000
计数 (CPS)
-2
-1
0
1
2
抗体浓度 (ug/ml) logX

图10B