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GLYCOENGINEERING IMMUNOGLOBULIN E

CLAIM OF PRIORITY

This application claims the benefit of U.S. Provisional Patent Application Serial Nos. 62/808,449, filed on February 21, 2019, and 62/808,456, filed on February 21, 2019.

5 The entire contents of the foregoing are hereby incorporated by reference.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under Grant Nos. AR068272 and AI139669 awarded by the National Institutes of Health (NIH). The Government has certain rights in the invention.

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TECHNICAL FIELD

This disclosure relates to glycoengineering immunoglobulin E (IgE), and methods of utilizing glycoengineering for treating various diseases or disorders. Also provided herein are methods for diagnosing allergies.

BACKGROUND

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Allergic disease is a global health burden affecting almost one in three individuals worldwide. Mechanistically, IgE antibodies bind to the surface of mast cells or basophils that express the IgE high affinity receptor, FcεRI³. Subsequent exposure to allergen crosslinks cell-bound IgE, leading to cellular activation and release of allergic mediators including histamine, prostaglandins, and leukotrienes³. This cascade culminates in the canonical symptoms of allergic disease, the most severe of which is anaphylaxis. While IgE that recognizes otherwise innocuous allergens is well established as the causative agent of most allergic diseases^{1,3}, testing for allergic disease remains relatively inaccurate⁴⁻⁶, and curative therapies, including oral immunotherapy, are cumbersome, and only partially effective⁸⁻¹⁰. Further, allergen-specific IgE is detected in many people who do not express allergic symptoms¹¹. Thus, while IgE is absolutely necessary for triggering the allergic cascade, it is not clear how IgE causes allergic disease in some circumstances and not others.

SUMMARY

Approximately one-third of the world's population suffers from allergies^{1,2}. Allergen exposure crosslinks mast cell- and basophil-bound immunoglobulin E (IgE), triggering the release of inflammatory mediators, including histamine³. Although IgE is absolutely required for allergies, it is not understood why total and allergen-specific IgE concentrations do not reproducibly correlate with allergic disease⁴⁻⁶. It is well-established that glycosylation of IgG dictates its effector function and has disease-specific patterns. However, whether IgE glycans differ in disease states or impact biological activity is completely unknown⁷. We therefore unbiasedly examined glycosylation patterns of total IgE from peanut-allergic and non-atopic individuals. This revealed an increase in sialic acid content on total IgE from peanut allergic individuals compared to non-atopic subjects. Sialic acid removal from IgE attenuated effector cell degranulation and anaphylaxis in multiple functional models of allergic disease. Therapeutic interventions, including sialic acid removal from cell-bound IgE with a FcεRI targeted-neuraminidase, or administration of asialylated IgE, markedly reduced anaphylaxis. Together, these results reveal a role for IgE glycosylation, and specifically sialylation, in regulating allergy and anaphylaxis, and establish IgE sialylation as a biomarker and therapeutic target for allergies.

Thus, provided herein are fusion polypeptides comprising: an Immunoglobulin E (IgE) or IgG antibody Fc domain region; and a sialidase or a functional portion thereof, preferably wherein the sialidase or a functional portion thereof can hydrolyze alpha-(2->3)-, alpha-(2->6)-, alpha-(2->8)-glycosidic linkages of terminal sialic residues on IgE. In some embodiments, the sialidase is NEU1, NEU2, NEU3, NEU4, or *Vibrio cholerae* serotype O1 sialidase. In some embodiments, the sialidase is a human sialidase. In some embodiments, the fusion polypeptide comprises an IgE CH2 region, an IgE CH3 region, and/or an IgE CH4 region; or an IgG CH2 and CH3 region.

Also provided herein are polynucleotides encoding the fusion polypeptides described herein, vectors comprising polynucleotides encoding the fusion polypeptides, and cells comprising the vectors, and optionally expressing the fusion polypeptides described herein.

Further, provided herein are methods for treating a subject having an IgE-mediated disorder. The methods include administering to the subject an effective amount of a composition comprising a fusion protein described herein. In some embodiments, the IgE-mediated disorder is an allergic disorder. In some embodiments, the allergic disorder is an anaphylactic allergy. In some embodiments, the allergic disorder is asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, or urticaria.

Additionally, provided herein are methods for preparing glycoengineered IgE, e.g., a composition comprising glycoengineered IgE, the method comprising: providing a composition comprising IgE, preferably human IgE, obtained from a plurality of subjects, contacting the IgE with a sialidase under conditions and for a time sufficient to remove sialylation, e.g., a desired amount of sialylation, from the IgE; thereby preparing glycoengineered IgE. In some embodiments, the method further comprises formulating the glycoengineered IgE for intravenous administration. In addition, provided herein are compositions comprising the glycoengineered IgE prepared by a method described herein, and a pharmaceutically acceptable carrier. In some embodiments, the compositions are formulated for intravenous administration.

Also provided herein are methods for treating a subject having an IgE-mediated disorder. The methods include administering to the subject an effective amount of a composition comprising glycoengineered IgE as described herein.

Further, provided herein are fusion proteins, glycoengineered IgE, and compositions comprising a fusion polypeptide and/or glycoengineered IgE as described herein, optionally with a pharmaceutically acceptable carrier, and the use of these compositions, fusion proteins, glycoengineered IgE and in treating a subject having an IgE-mediated disorder.

In some embodiments, the IgE-mediated disorder is an allergic disorder, e.g., an anaphylactic allergy. In some embodiments, the allergic disorder is asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, or urticaria.

This disclosure relates to glycoengineering, and methods of utilizing glycoengineering for treating various diseases or disorders (e.g., IgE-mediated disorders).

In another aspect, the disclosure relates to a fusion polypeptide having an antibody heavy chain CH2 region; an antibody heavy chain CH3 region; and a catalytic

domain of sialidase, wherein the catalytic domain of sialidase removes sialic acid from a glycoprotein.

In some embodiments, the sialidase is NEU1, NEU2, NEU3, NEU4, or *Vibrio cholerae* serotype O1 sialidase. In some embodiments, the sialidase is a human sialidase.

5 In some embodiments, the fusion polypeptide has an IgG CH2 region, and an IgG CH3 region.

In some embodiments, the fusion polypeptide has an IgE CH2 region, an IgE CH3 region, and an IgE CH4 region.

10 In another aspect, the disclosure provides a polynucleotide encoding the fusion polypeptide as described herein.

In another aspect, the disclosure also relates to a vector having a polynucleotide sequence encoding the fusion polypeptide as described herein.

In one aspect, the disclosure relates to a cell having the vector as described herein, and the vector optionally expresses the fusion polypeptide as described herein.

15 In one aspect, the disclosure relates to a heteromultimer that has a first fusion polypeptide having an antibody heavy chain CH2 region, an antibody heavy chain CH3 region, and a catalytic domain of mannosidase, wherein the catalytic domain of mannosidase removes mannose from a glycoprotein; and a second fusion polypeptide having an antibody heavy chain CH2 region, an antibody heavy chain CH3 region, and a
20 catalytic domain of sialidase, wherein the catalytic domain of sialidase removes sialic acid from a glycoprotein.

In some embodiments, the heteromultimer is a heterodimer, and the first fusion polypeptide associates with the second fusion polypeptide, thereby forming the heterodimer.

25 In some embodiments, the mannosidase is MAN1B1 or MAN2A1.

In some embodiments, the sialidase is NEU1, NEU2, NEU3, NEU4, or *Vibrio cholerae* serotype O1 sialidase.

In some embodiments, the first fusion polypeptide and the second polypeptide each has a human IgE CH2 region, a human IgE CH3 region, and a human IgE CH4
30 region.

In some embodiments, the first fusion polypeptide and the second polypeptide each has a human IgG CH2 region, and a human IgG CH3 region.

In another aspect, the disclosure also relates to methods of treating a subject having an IgE-mediated disorder. The methods involve administering to the subject an
5 effective amount of a composition having the heteromultimer as described herein.

In some embodiments, the IgE-mediated disorder is an allergic disorder.

In some embodiments, the IgE-mediated disorder is an autoimmune disease.

In some embodiments, the IgE-mediated disorder is anaphylaxis.

In some embodiments, the allergic disorder is asthma. In some embodiments, the
10 allergic disorder is atopic dermatitis. In some embodiments, the allergic disorder is allergic rhinitis, allergic conjunctivitis, eczema, or urticaria.

In one aspect, the disclosure relates to methods of treating a subject having an IgE-mediated disorder. The methods involve administering to the subject an effective amount of one or both of the following:

(a) a first polypeptide having a catalytic domain of mannosidase; and

(b) a second polypeptide having a catalytic domain of sialidase,

wherein the catalytic domain of the mannosidase removes mannose from a glycoprotein, and the catalytic domain of sialidase removes sialic acid from a glycoprotein.

In some embodiments, the first polypeptide further has a human IgE CH2 region, a human IgE CH3 region, and a human IgE CH4 region.

In some embodiments, the first polypeptide further has a human IgG CH2 region, and a human IgG CH3 region.

In some embodiments, the second polypeptide further has a human IgE CH2 region, a human IgE CH3 region, and a human IgE CH4 region.

In some embodiments, the second polypeptide further has a human IgG CH2 region, and a human IgG CH3 region.

In some embodiments, the IgE-mediated disorder is an allergic disorder. In some embodiments, the IgE-mediated disorder is an autoimmune disease. In some
embodiments, the IgE-mediated disorder is anaphylaxis.

In some embodiments, the allergic disorder is asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, or urticaria.

In one aspect, the disclosure provides a heteromultimer that has a first fusion polypeptide having a collagen trimerizing domain and a catalytic domain of mannosidase; a second fusion polypeptide having a collagen trimerizing domain and a catalytic domain of sialidase; and a third fusion polypeptide having a collagen trimerizing domain, wherein the first fusion polypeptide, the second fusion polypeptide, and the third fusion polypeptide bind to each other, forming the heteromultimer.

In some embodiments, the third fusion polypeptide further has a catalytic domain of sialidase. In some embodiments, the third fusion polypeptide further has a catalytic domain of mannosidase.

In another aspect, the disclosure relates to a heteromultimer that has a tetramer having four streptavidin polypeptides; and four polypeptides, wherein each of the four polypeptides is linked with biotin, and one or more of the four polypeptides has a catalytic domain of mannosidase or a catalytic domain of sialidase, wherein each of the four polypeptides binds to the tetramer having the four streptavidin polypeptides.

In some embodiments, each of the four polypeptides has a catalytic domain of mannosidase or a catalytic domain of sialidase. In some embodiments, each of the four polypeptides has a catalytic domain of mannosidase. In some embodiments, each of the four polypeptides has a catalytic domain of sialidase.

In some embodiments, two of the four polypeptides each has a catalytic domain of mannosidase, and two of the four polypeptides each has a catalytic domain of sialidase.

In one aspect, the disclosure also relates to a heteromultimer that has an antibody or antibody fragment thereof; a catalytic domain of mannosidase; and/or a catalytic domain of sialidase, wherein the catalytic domain of mannosidase and the catalytic domain of sialidase each is linked to the antibody or antibody fragment thereof.

In some embodiments, the heteromultimer has an antibody, and the antibody has two antibody heavy chains, and two antibody light chains.

In some embodiments, the catalytic domain of mannosidase is linked to C-terminus of the antibody heavy chain. In some embodiments, the catalytic domain of mannosidase is linked to C-terminus of the antibody light chain.

In some embodiments, the catalytic domain of sialidase is linked to C-terminus of the antibody heavy chain. In some embodiments, the catalytic domain of sialidase is linked to C-terminus of the antibody light chain.

As used herein, the term “multimer” refers to a protein having two or more polypeptides or a polypeptide complex formed by two or more polypeptides. The polypeptides can associate with each other, forming a quaternary structure.

As used herein, the term “heteromultimer” refers to a multimer having more than
5 one type of polypeptides.

As used herein, the term “homodimer” refers to a multimer having two identical polypeptides.

As used herein, the term “heterodimer” refers to a multimer having two polypeptides, and the two polypeptides are different.

10 As used herein, the term “luminal domain” or “enzymatic luminal domain” refers to the portion of a glycosylation enzyme that is located within the lumen of the Golgi apparatus in its native state. The enzymatic luminal domain of a glycosyltransferase is usually the soluble portion of the glycosylation enzyme.

As used herein, the term “soluble portion” or “soluble domain” refers to the
15 portion of glycosylation enzyme that is soluble. For trans-Golgi glycosylation enzymes, the soluble portions are often the enzymatic luminal domains of the glycosylation enzymes. For non-trans-Golgi glycosylation enzymes, the entire glycosylation enzymes can be soluble. Thus, in some embodiments, the soluble portion can be the entire glycosylation enzyme or part of the glycosylation enzyme.

20 As used herein, the term “catalytic domain” refers to a portion of a protein that has a catalytic activity.

As used herein, the term “antibody-mediated disorder” refers to a disorder caused by or characterized by an increased level or an increased activity of an antibody.

As used herein, the term “IgE-mediated disorder” refers to a disorder caused by or
25 characterized by an increased level or an increased activity of IgE.

As used herein, the term “linked” refers to being covalently or non-covalently associated, e.g., by a chemical bond (e.g., a peptide bond, or a carbon–carbon bond), by hydrophobic interaction, by Van der Waals interaction, and/or by electrostatic interaction.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIGs. 1A-I. Glycan composition of IgE. A, A schematic of human IgE with N-linked glycosylation sites identified. Sites occupied by complex, biantennary glycans are noted by closed circles, oligomannose glycans by hatched circles, and unoccupied by X. Complex biantennary and oligomannose glycan schematics are shown at black and hatched circles, respectively; squares, GlcNAc; dark grey circles, mannose; triangle, fucose; light grey circles, galactose; grey diamonds, sialic acid. B, Total IgE titers from non-atopic (n = 17) and peanut allergic (n = 13) individuals; $**P = 0.0085$. C, Allergen-specific IgE levels for Ara h 2 (peanut), Der p 1 (dust mite), Fel d 1 (cat), and Bet v 1 (birch pollen) from non-atopic (n = 17) as compared to allergic (n = 13) subjects; $*P = 0.0192$. D, Allergen-specific IgE as a fraction (%) of total IgE across non-atopic (n = 17) and allergic (n = 13) individuals; $**P = 0.0086$. E-I, Quantified glycan residues per IgE molecule on total IgE from non-atopic and peanut allergic individuals by glycopeptide mass spectrometry, showing total mannose (e, n = 15 for non-atopic and 14 for allergic individuals, $P = 0.8467$), fucose (f, n = 10 for non-atopic and 11 for allergic individuals, $P = 0.0720$), biGlcNAc (g, n = 10 for non-atopic and 11 for allergic individuals, $*P = 0.0491$), terminal galactose (h, n = 14 for non-atopic and 19 for allergic individuals, $****P < 0.0001$), and terminal sialic acid (i, n = 9 for non-atopic and 11 for allergic individuals, $***P = 0.0009$). Data plotted are mean $\pm$ s.e.m., n.s., not significant and P values were determined by unpaired two tailed t test.

FIGs. 2A-B. Sialic acid and galactose distinguish allergic from non-atopic IgE. A, Receiver operating characteristic curve (ROC) for total number of variable IgE glycan moieties. ROC was performed for total IgE glycans isolated from allergic subjects as compared to non-atopic controls. Sialic acid (non-atopic $n = 9$, allergic = 11); Galactose (non-atopic $n = 14$, allergic = 19); Fucose (non-atopic $n = 14$, allergic = 15); biGlcNac (non-atopic $n = 14$, allergic = 19); Oligomannose (non-atopic $n = 15$, allergic = 14). B, Glycopeptide mass spectrometry analysis of site-specific N-glycan structures on total IgE from non-atopic (N) and allergic (A) individuals; Site 140 (non-atopic $n=11$, allergic $n=11$), Site 168 (non-atopic $n=13$, allergic $n=15$), Site 218 (non-atopic $n=11$, allergic $n=17$), Site 265 (non-atopic $n=12$, allergic $n=19$), Site 371 (non-atopic $n=12$, allergic $n=15$), Site 394 (non-atopic $n=12$, allergic $n=11$). The specific glycan structures per group are detailed in FIG. 7G. **** $P < 0.0001$, *** $P = 0.0006$. P values are determined by two-way ANOVA followed by Tukey's multiple comparison test.

FIGs. 3A-N. Sialic acid removal attenuates IgE. A, SNA lectin blot specific for $\alpha 2,6$ -sialic acid and coomassie protein loading control of OVA-specific buffer-treated S^{ia} mIgE and NEU-treated A^s mIgE. Images are representative of at least four independent digests. B, Quantification of ear blue coloration and representative ear images following OVA-induced PCA by PBS ($n = 2$), OVA-specific S^{ia} mIgE ($n = 8$), OVA-specific A^s mIgE ($n = 12$), OVA-specific $Re-S^{ia}$ mIgE ($n = 4$). Data are representative of three experiments. **** $P < 0.0001$; ns, $P = 0.9933$ (one-way ANOVA with Tukey's multiple comparison test). C, Left, mean fluorescent intensity (MFI) and right, representative histograms of anti-mIgE on dermal mast cells following sensitization by PBS ($n = 5$), OVA-specific S^{ia} mIgE ($n = 6$), or OVA-specific A^s mIgE ($n = 6$) in mouse ears. ns, $P = 0.9017$ (one-way ANOVA with Tukey's multiple comparison test). D, Binding of OVA-specific S^{ia} mIgE and A^s mIgE to OVA as determined by ELISA. $n = 2$ replicates and are representative of three experiments. ns, $P > 0.8040$ for all concentrations tested (two-way ANOVA with Sidak's multiple comparisons test). E, F, Temperature change (E) and serum histamine quantified at defined intervals (F) following DNP-induced PSA in mice intravenously sensitized with PBS ($n = 3$), DNP-specific S^{ia} mIgE ($n = 5$), or DNP-specific A^s mIgE ($n = 5$). Data are representative of three experiments. *** $P = 0.0005$, **** $P < 0.0001$, *** $P = 0.0007$ (two-way ANOVA with Tukey's multiple comparison test). G, Serum levels of

DNP-specific ^{Sia}mIgE ($n = 4$), or DNP-specific ^{As}mIgE ($n = 5$) at defined intervals after intraperitoneal systemic administration as determined by ELISA. Data are representative of three experiments. ns, $P > 0.7948$ for all time points (two-way ANOVA with Sidak's multiple comparisons test). H, Temperature change following PFA elicited by oral administration of TNP-OVA in mice sensitized with PBS ($n = 2$), TNP-specific ^{Sia}mIgE ($n = 4$), or TNP-specific ^{As}mIgE ($n = 4$). Data are representative of three experiments. $**P = 0.0057$ and 0.0035 , $****P < 0.0001$, $***P = 0.0005$ (two-way ANOVA with Tukey's multiple comparison test). I, SNA lectin blot and coomassie loading control of OVA-specific buffer-treated ^{Sia}hIgE and NEU-treated ^{As}hIgE. Images are representative of at least four independent batches. J, OVA-induced β -hexosaminidase release by LAD2 mast cells sensitized with PBS, OVA-specific ^{Sia}hIgE or OVA-specific ^{As}hIgE. $n = 3$ replicates and are representative of six experiments. $****P < 0.0001$; ns, $P > 0.9999$ (two-way ANOVA with Tukey's multiple comparison test). K, β -hex degranulation after OVA stimulation of peripheral blood mononuclear cell-derived human mast cells sensitized with OVA-specific ^{Sia}hIgE and ^{As}hIgE. Mean and s.e.m. are plotted. $****P < 0.0001$; ns, $P = 0.9995$ (two-way ANOVA with Sidak's multiple comparison test). L, Basophils expressing surface CD63 (left) and representative FACS plots following OVA stimulation on CD123⁺HLADR⁻ peripheral blood mononuclear cells sensitized with PBS ($n = 1$), OVA-specific ^{Sia}hIgE ($n = 4$) or OVA-specific ^{As}hIgE ($n = 4$). Data are representative of four experiments. $****P < 0.0001$, $**P = 0.0017$; ns, $P = 0.3829$ (two-way ANOVA with Tukey's multiple comparison test). M, N, Binding kinetics of analytes OVA-specific ^{Sia}hIgE or OVA-specific ^{As}hIgE to ligands hFc ϵ RI α (m) or OVA (n) loaded on biosensors. Analytes kinetics were performed with 3-fold serial dilution of analytes from 90 nM to 1 nM. Data are representative of three experiments. All data plotted are mean $\pm$ s.e.m.

FIGs. 4A-I. Asialylated IgE modulation of anaphylaxis. A, Immunoblots of phosphorylated and total Syk and β -actin in LAD2 mast cells sensitized with PBS, OVA-specific ^{Sia}hIgE or OVA-specific ^{As}hIgE after OVA stimulation for the indicated times. Images are representative of three independent experiments. B, OVA-induced Ca²⁺ flux traces showing fluo-4 fluorescence over fluorescence at time = 0 (F/F₀, left) and quantified maximal Ca²⁺ changes after OVA stimulation as measured by the difference

between maximum (Fmax) over F0 (right) in Fluo-4 loaded LAD2 mast cells sensitized with PBS, OVA-specific ^{Sia}hIgE or OVA-specific ^{As}hIgE. Data are representative of three independent experiments. *P = 0.0346 (two-tailed paired t-test). C, OVA-elicited degranulation in LAD2 mast cells sensitized with OVA-specific ^{Sia}hIgE and treated with ^{Sia}Fetuin or ^{As}Fetuin. *n* = 3 replicates and are representative of three experiments. *P = 0.0248, ****P < 0.0001 (two-way ANOVA with Sidak's multiple comparison test). D, Quantification of ear blue coloration (left) and representative ear images (right) following OVA-induced PCA of mice sensitized with PBS (*n* = 2), OVA-specific ^{Sia}mIgE (20 ng, *n* = 6), both OVA-specific ^{Sia}mIgE (20 ng) + ^{As}mIgE (200 ng) (*n* = 3), or both OVA-specific ^{Sia}mIgE (20 ng) + mIgE isotype control (200 ng) (*n* = 3). Data are representative of three experiments. *P = 0.0478 and 0.0321; ns, *P* = 0.9733 (one-way ANOVA with Tukey's multiple comparison test). E, Temperature change following DNP-induced PSA in mice receiving DNP-specific ^{Sia}mIgE on day 0 and PBS (*n* = 6 for e), OVA-specific ^{Sia}mIgE (*n* = 7 for e), or OVA-specific ^{As}mIgE (*n* = 7 for e) on day 1. E, ***P = 0.0001, *P = 0.0211 and 0.0278. (two-way ANOVA with Tukey's multiple comparison test). F, Schematics of NEU^{Fcε}. Neuraminidase was linked to IgE Fc Cε2-4 by a peptide linker. G, OVA-induced β-hexosaminidase release by LAD2 mast cells sensitized with OVA-specific ^{Sia}hIgE and treated with PBS, NEU^{Fcε}, heat-inactivated NEU^{Fcε} (H-I NEU^{Fcε}) or IgE isotype control. *n* = 3 replicates and are representative of three experiments. ****P < 0.0001 (two-way ANOVA with Tukey's multiple comparison test). H, Peanut-induced β-hexosaminidase release by LAD2 mast cells sensitized with peanut allergic ^{Sia}hIgE treated with PBS, NEU^{Fcε}, or IgE isotype control. *n* = 3 replicates and are representative of three experiments. ****P < 0.0001 (two-way ANOVA with Tukey's multiple comparison test). I, Temperature change following OVA-induced PSA in mice receiving OVA-specific ^{Sia}mIgE on day 0 and PBS, NEU^{Fcε}, or IgE isotype control on day 1. *n* = 4 per group and data are representative of three experiments. ***P = 0.0008 and 0.0003, *P = 0.0184 (two-way ANOVA with Tukey's multiple comparison test). All data plotted are mean ± s.e.m.

FIGs. 5A-F. Functional aspects of allergen specific human IgE. A, Strategy for enriching IgE from human sera. B, Degranulation of human LAD2 mast cells sensitized with PBS, non-atopic or peanut allergic IgE stimulated by anti-human IgE and

determined by β -hexosaminidase release. STATS C, Quantified MFI (left) and representative histograms (right) of anti-hIgE on human LAD2 mast cells sensitized with PBS, non-atopic, or allergic hIgE. STATS D, Binding of anti-hIgE from b to ^{Sia}hIgE and ^{As}hIgE as determined by ELISA shows no sialic acid dependent binding effects. n = 2
 5 replicates and are representative of three experiments. E, Specific glycans on IgE do not differ significantly between male and female subjects (n = 9 males, n = 12 females). F, Number of biGlcNAc residues differs significantly between 0-9 years old (n = 2) and subjects of ages 10-19 (n = 2, *P = 0.0228), 20-29 (n = 6, *P = 0.0295) and 30-39 (n = 7, *P = 0.0019) respectively. Sialic acid, galactose and fucose do not differ across age
 10 groups. Data are presented as the mean $\pm$ SEM; ns, not significant, *P<0.05, **P<0.01, ***P<0.0001 as determined by unpaired t test.

FIGs. 6A-E. Complex glycans observed on native human IgE. A, Representative MS/MS spectrum for N265 A2F glycopeptide showing B and Y ions from glycosidic bond cleavage as well as B ions from peptide bond cleavage. The Y1 ion used for
 15 quantification of glycopeptides is circled. B, Extracted ion chromatograms for IgE N265 sialylation variants from an allergic patient and non-allergic donor. C, Extracted ion chromatograms for IgE N168 sialylation variants from an allergic patient and non-allergic donor. D, Extracted ion chromatograms site specific N-glycosylation from chymotryptic digest of the IgE myeloma sample used as a standard. E, Extracted ion
 20 chromatograms site specific N-glycosylation from tryptic digest of the IgE myeloma sample used as a standard.

FIGs. 7A-G. Site-specific characterization of total IgE from peanut allergic and non-atopic individuals. A, Occupancy of N-linked glycosylation sites by glycans on non-atopic and allergic IgE; Site 140 (non-atopic n = 15, allergic n = 13), Site 168 (non-atopic
 25 n = 16, allergic n = 14), Site 218 (non-atopic n = 15, allergic n = 15), Site 265 (non-atopic n = 12, allergic n = 15), Site 371 (non-atopic n = 15, allergic n = 15), Site 383 (non-atopic n = 16, allergic n = 15), Site 394 (non-atopic n = 13, allergic n = 16). B, Configuration of oligomannose residues at site 394 does not differ between non-atopic (n = 23) and allergic (n = 18) groups. C, Total number of fucose residues per site of IgE, non-atopic,
 30 allergic; Site 140 (non-atopic n = 15, allergic n = 13), Site 168 (non-atopic n = 15, allergic n = 17), Site 218 (non-atopic n = 15, allergic n = 19), Site 265 (non-atopic n = 12,

allergic n = 18), Site 371 (non-atopic n = 15, allergic n = 17). D, biGlcNAc residues by site of IgE, non-atopic compared to allergic; Site 140 (non-atopic n = 15, allergic n = 13), Site 168 (non-atopic n = 16, allergic n = 17), Site 218 (non-atopic n = 15, allergic n = 19), Site 265 (non-atopic n = 16, allergic n = 20), Site 371 (non-atopic n = 16, allergic n = 17).

5 E, Total galactose residues from non-atopic and allergic subjects; Site 140 (non-atopic n = 15, allergic n = 14 STATS), Site 168 (non-atopic n = 15, allergic n = 17), Site 218 (non-atopic n = 15, allergic n = 19), Site 265 (non-atopic n = 12, allergic n = 19, $**P = 0.0014$), Site 371 (non-atopic n = 15, allergic n = 17). F, Quantified sialic acid residues by IgE glycosylation site, non-atopic compared to allergic subjects; Site 140 (non-atopic
10 n = 14, allergic n = 13), Site 168 (non-atopic n = 15, allergic n = 13, $*P = 0.0375$), Site 218 (non-atopic n = 15, allergic n = 17), Site 265 (non-atopic n = 12, allergic n = 19, $*P = 0.0132$), Site 371 (non-atopic n = 15, allergic n = 17). G, Representative structures for complex N-glycans. Data plotted are mean $\pm$ s.e.m. P values are determined by two-way ANOVA followed by Sidak's multiple comparison test.

15 FIGs. 8A-C. IgE has α 2,6-linked sialic acid. A, Protein gel stain and lectin blots of IVIG, native human IgE purified from allergic patients, and fetuin. Lectin SNA was used for α 2,6- and MALI for α 2,3-linked sialic acids detection. B, HPLC glycan traces of undigested or allergic human IgE or fetuin digested with sialidase from *Arthrobacter ureafaciens* for releasing α 2,3-, α 2,6-, α 2,8- and α 2,9-linked sialic acids or sialidase from
20 *Streptococcus pneumoniae* for releasing α 2,3-linked sialic acids. C, HPLC glycan traces of undigested or recombinant OVA-specific mIgE digested with sialidase from *Arthrobacter ureafaciens* for releasing α 2,3-, α 2,6-, α 2,8- and α 2,9-linked sialic acids.

FIGs. 9A-B PCA and dermal mast cell loading of Sia mIgE and As mIgE. A, Quantitation of vascular leakage by Evan's blue dye (left) and representative ear images
25 from 3 mice (right) after PCA with PBS, or Sia mIgE and As mIgE specific for DNP. $n = 6$ and are representative of three experiments. Mean and s.e.m. are plotted. $**P = 0.0047$ (two-tailed unpaired t -test). B, Gating strategy for IgE loading on mouse skin ear mast cells. Representative FACS plots used to identify mast cells in mouse ears and determine IgE levels on mouse ear mast cells. SSC, side scatter.

30 FIGs. 10A-C. PSA reaction and serum levels of Sia mIgE and As mIgE after systemic sensitization. OVA-elicited anaphylaxis as measured by temperature drop in

mice sensitized with PBS, OVA-specific ^{Sia}mIgE ($n = 4$ for a and 6 for b) or ^{As}mIgE ($n = 5$ for a and 6 for b) by intravenous (a) or intraperitoneal (b) injection. Data are representative of 3 independent experiments. Mean and s.e.m. are plotted. For A, **** $P < 0.0001$ (two-way ANOVA with Tukey's multiple comparison test). For B, * $P = 0.0270$ and 0.0122, ** $P = 0.0012$ and 0.0018, **** $P < 0.0001$ (two-way ANOVA with Tukey's multiple comparison test). C, Serum levels of DNP-specific ^{Sia}mIgE and ^{As}mIgE in mice at defined time after systemically administration as determined by ELISA. $n = 4$ for all group.

FIGs. 11A-C. FACS analysis of LAD2 mast cell loading of ^{Sia}hIgE and ^{As}hIgE, PBMC-derived mast cells, and primary basophils. A, MFI (left) and representative histogram (right) of surface-bound hIgE on LAD2 mast cells following sensitization with PBS, OVA-specific ^{Sia}hIgE or OVA-specific ^{As}hIgE. $n = 3$ replicates and are representative of three experiments. **** $P < 0.0001$, * $P = 0.0134$ (one-way ANOVA with Tukey's multiple comparison test). B, Phenotypic staining by FACS of peripheral blood mononuclear cell-derived human mast cells. C, Gating strategy for basophil activation assay. Representative FACS plots used to determine basophil activation from PBMC.

FIG. 12. | OVA-specific PSA of OVA-specific SiamIgE, or OVA-specific AsmIgE isotype controls from Fig. 4E. Temperature change following OVA-induced PSA in mice receiving DNP-specific SiamIgE on day 0 and PBS, OVA-specific SiamIgE, or OVA-specific AsmIgE on day 1. $n = 4$ for all groups. **** $P < 0.0001$ (two-way ANOVA with Tukey's multiple comparison test). All data plotted are mean $\pm$ s.e.m and are representative of three experiments.

FIGs. 13A-G. Characterization of NEU^{Fce}. A, Protein gel stain (left) and immunoblot for mIgE (right) of native and denatured NEU^{Fce}. B, Binding kinetics of analyte NEU^{Fce} to ligand hFcεRIα on biosensor. Analytes kinetics were performed with 3-fold serial dilution of analyte from 24 to 0.3 nM. Data are representative of three experiments. C, MFI of surface-bound NEU^{Fce} on LAD2 mast cells following overnight sensitization by FACS analysis. $n = 3$ replicates and are representative of three experiments. D-G, Sialidase activity of NEU^{Fce} determined by digestion of mIgE or fetuin overnight (D-F) and detection of protein loading by coomassie (D), terminal α2,6-sialic

acid by SNA (E), and terminal galactose by ECL (F) or by the amount of substrate 2-O-(p-Nitrophenyl)- α -D-N-acetylneuraminic acid digested by NEU^{Fce} in a colorimetric assay (G).

FIG. 14 lists the amino acid sequences of several exemplary glycosylation enzymes.

FIGs. 15A-B lists the amino acid sequences of exemplary fragment crystallizable region (Fc) of several human and mouse immunoglobulin E (IgE, FIG. 15A) and IgG (FIG. 15B).

FIG. 16 lists the amino acid sequences of several exemplary glycosylation enzyme-Fc fusion proteins.

FIG. 17 lists the amino acid sequences of exemplary dog glycosylation enzymes: Canine NEU1 (SEQ ID NO: 45); Canine NEU2 (SEQ ID NO: 46); Canine NEU3 (SEQ ID NO: 47).

FIG. 18 lists the amino acid sequences of exemplary cat glycosylation enzymes: Feline NEU1 (SEQ ID NO: 48); Feline NEU2 (SEQ ID NO: 49); Feline NEU3 (SEQ ID NO: 50); Feline NEU4 (SEQ ID NO: 51).

FIG. 19 lists the amino acid sequences of exemplary cow IgE and glycosylation enzymes: Bovine IgE heavy chain constant region (SEQ ID NO: 52); Bovine Sialidase-1 (NEU1) -lysosomal (SEQ ID NO: 52); Bovine Sialidase-3 (NEU3) – Plasma membrane (SEQ ID NO: 53);.

FIG. 20 lists the amino acid sequences of exemplary horse IgE and glycosylation enzymes: Equine IgE heavy chain constant region (SEQ ID NO: 55); Equine Neuraminidase (NEU1) - lysosomal (SEQ ID NO: 56); Equine Neuraminidase (NEU2) – cytosolic (SEQ ID NO: 57); Equine Neuraminidase (NEU3) - membrane (SEQ ID NO: 58).

FIG. 21 lists an exemplary sequence encoding hNEU1_hIgEFc (SEQ ID NO: 59).

FIG. 22 lists an exemplary sequence encoding hNEU2_hIgEFc (codon optimized for mammalian expression) (SEQ ID NO: 60).

FIG. 23 lists an exemplary sequence encoding hNEU3_hIgEFc (SEQ ID NO: 61).

FIG. 24 lists an exemplary sequence encoding hNEU4_hIgEFc (SEQ ID NO: 62).

FIG. 25 lists an exemplary sequence encoding hNEU1_mIgEFc (SEQ ID NO: 63).

FIG. 26 lists an exemplary sequence encoding hNEU2_mIgEFc (Codon optimized for mammalian expression) (SEQ ID NO:64).

FIG. 27 lists an exemplary sequence encoding hNEU3_mIgEFc (SEQ ID NO:65).

FIG. 28 lists an exemplary sequence encoding hNEU4_mIgEFc (SEQ ID NO:66).

5

DETAILED DESCRIPTION

IgE-mediated allergic diseases are multifactorial, with a broad range of clinical presentations. While the presence of peanut-specific IgE associates with peanut allergy, there is a high rate of false positive allergy test results^{4,6,9,35}. Many non-mutually exclusive mechanisms for this discrepancy exist, including differences in IgE affinity or epitope diversity for allergens, mast cell numbers, FcεRI expression levels, Syk signaling, allergen-specific IgG antibodies, anti-IgE antibodies, and regulatory T cells numbers^{36,37}. While IgE from primary allergic samples is severely limited because of its low serum concentrations, recent studies have identified and sequenced B cells that produce peanut-specific antibodies IgE^{9,38}. However, the role of post-translation modifications of the IgE constant chains, including glycosylation, in regulating allergic disease has not been considered. As demonstrated herein, sialic acid content on total IgE distinguishes peanut-allergic and non-atopic IgE. Further, IgE-mediated allergic reactions are attenuated through removal of sialic acid from IgE or administration of asialylated glycoproteins. The sialic acid content and its role in regulating IgE in other atopies and non-atopic conditions is not known³⁹⁻⁴¹. Glycoengineering has been applied to tailor therapeutic IgGs with desirable pro- and anti-inflammatory functions^{18,20}. The present studies revealed that modulating IgE sialic acid content can attenuate anaphylaxis and affirms the application of glycoengineering to allergic disease. Thus, the sialic acid content on IgE can be used as a biomarker for allergic disease, and modulating the IgE sialylation axis presents a powerful means to attenuate allergies and anaphylaxis.

The present disclosure shows engineered glycosylation enzymes can modulate antibody effector function by engineering antibody glycans *in vivo* for various therapeutic effects. As shown herein, sialic acid in IgE glycans are important for IgE functions; the present disclosure further provides engineered glycosylation enzymes for modulating IgE, e.g., by removing sialic acid (neuraminidase or sialidase) from IgE Fcs, and thus inhibiting IgE pro-allergic function or activity.

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Thus, the present disclosure relates to methods and compositions comprising a fusion peptide comprising a catalytic domain of a deglycosylation enzyme (e.g., neuraminidase or sialidase) fused to Fc (e.g., IgG Fc or IgE Fc). The methods and compositions described herein can be used to modulate IgE effector function for various therapeutic effects.

Glycans on IgE

The proteins and cells that make up the human body are decorated by sugars often referred to as glycans (Varki, A. *Glycobiology* 3, 97-130 (1993)). Glycans can be linked to many types of biological molecule to form glycoconjugates. The enzymatic process that links sugars/saccharides to themselves and to other molecules is known as glycosylation. Glycoproteins, proteoglycans, and glycolipids are the most abundant glycoconjugates found in mammalian cells.

Glycans have an important role in the function of many proteins. Glycans are saccharides (i.e., a plurality of monosaccharides linked glycosidically) that form the carbohydrate portion of glycoconjugates (e.g., glycoproteins, glycopeptides, peptidoglycans, glycolipids, glycosides and lipopolysaccharides). They can be added to proteins in the endoplasmic reticulum, and further modified as proteins travel through the Golgi apparatus. Precursor glycan structures can be attached to asparagine (N-linked), serine or threonine (O-linked), phospholipids (GPI), tryptophan (C-linked), or by phosphodiester bonds (phosphoglycosylation).

Immunoglobulin E (IgE) has two heavy chains (ϵ chain) and two light chains, with the ϵ chain containing 4 Ig-like constant domains (C ϵ 1, C ϵ 2, C ϵ 3, C ϵ 4; also referred to as CH1, CH2, CH3, CH4). IgE antibodies are primary mediators of allergic disease, and are heavily glycosylated with 7 N-linked glycosylation sites distributed across its four constant regions (C ϵ 1-C ϵ 4). The distinct glycans on IgE play important and divergent roles in allergic inflammation. Removal of the conserved oligomannose in the constant domains (e.g., C ϵ 1, C ϵ 2, C ϵ 3, C ϵ 4) prevents binding to the high affinity receptor Fc ϵ RI on Fc ϵ RI-expressing cells (e.g., mast cells and basophils), therefore can inhibit the function or activity of IgE.

Analysis of the glycosylation of human serum IgE indicated that oligomannose structures are present on IgE. In fact, IgE is the most heavily glycosylated monomeric immunoglobulin in mammals. There are six complex-type biantennary (N140, N168, N218, N265, N371, N383) and one oligomannose-type (N394) conserved N-linked glycosylation sites on the constant region of each heavy chain of IgE. The total glycan weight on ϵ heavy chains contributes to ~12% of the molecular weight of IgE.

The composition of the single N-linked glycan on IgG antibodies profoundly influences its biological activity, and impacts the outcome of many diseases, including Dengue hemorrhagic fever¹², *Mycobacterium tuberculosis* latency¹³, Influenza vaccination¹⁴, Rheumatoid Arthritis^{7,15}, and Granulomatosis with polyangiitis^{16,17}. For example, IgG with afucosylated glycans gain affinity to the activating Fc receptor, Fc γ RIIIA, 50-fold, making IgG markedly more cytotoxic *in vivo*¹⁸. Conversely, terminal sialylation of the IgG glycan converts IgG into anti-inflammatory mediators, and is thought to be responsible for the immunomodulatory activity of high dose intravenous immunoglobulin^{19,20}. IgE is the most heavily glycosylated monomeric immunoglobulin with seven asparagine (N)-linked glycosylation sites distributed across the heavy chains of human IgE (hIgE)^{7,21}. However, whether particular IgE glycans are associated with allergic disease, or impact IgE function, is completely unknown. IgE is the least abundant antibody class in circulation, and, as such, analysis of hIgE glycosylation has been restricted to samples from subjects with myelomas, hyper IgE syndromes, hyperimmune syndromes pooled from multiple donors, or recombinant IgE²¹⁻²⁴. These studies revealed a single N-linked oligomannose glycan at N394 on IgE, N383 is unoccupied, and the remaining five sites are occupied by complex antennary glycans (Fig. 1a)²⁵. Previously, we and others demonstrated the oligomannose at N394 was required for appropriate IgE folding and Fc ϵ RI binding^{23,26} to initiate effector functions.

IgE Fc glycans can be removed by enzymatic treatment with mannosidase, neuraminidase, Endo F, and/or PNGase F. The enzymatic treatment can inhibit binding of IgE molecules or IgE-Fc fragments to Fc ϵ RI. Mutagenesis of the conserved N394 site, which corresponds to N297 on IgG Fc, also reduces the binding to Fc ϵ RI.

A detailed description regarding glycans on IgE and the functions thereof can be found, e.g., in Arnold, et al., "The glycosylation of human serum IgD and IgE and the

accessibility of identified oligomannose structures for interaction with mannan-binding lectin." *The Journal of Immunology* 173.11 (2004): 6831-6840; Shade, et al., "A single glycan on IgE is indispensable for initiation of anaphylaxis." *Journal of Experimental Medicine* 212.4 (2015): 457-467; Shade, et al., "Antibody glycosylation and inflammation." *Antibodies* 2.3 (2013): 392-414; and Plomp, et al., "Site-specific N-glycosylation analysis of human immunoglobulin E." *Journal of proteome research* 13.2 (2013): 536-546; each of which is incorporated herein by reference in its entirety.

Glycosylation enzymes

Glycosylation enzymes are responsible for the reaction in which a carbohydrate, *i.e.* a glycosyl donor, is attached to a hydroxyl or other functional group of another molecule (a glycosyl acceptor, e.g., proteins, lipids, and glycans). There are many different kinds of glycosylation enzymes, e.g., α -2,6 sialyltransferase (ST6GAL1), β -1,4-galactosyltransferase 1 (B4GALT1), mannosyl-oligosaccharide 1,2- α -mannosidase (MAN1B1), α -mannosidase 2 (MAN2A1), human sialidase-1 (NEU1), human sialidase-2 (NEU2), human sialidase-3 (NEU3), human sialidase-4 (NEU4), *Vibrio cholerae* serotype O1 sialidase, *Elizabethkingia meningoseptica* Endo F1, endo- β -N-acetylglucosaminidase (Endo S), etc. As shown herein, sialic acid removal attenuated IgE effector functions. Thus, provided herein are fusion proteins in which soluble portions (or the enzymatic luminal domains) or the catalytic domains of sialidases can be fused with Fc (e.g., IgG Fc or IgE Fc), or other appropriate peptides to form multimers, and can be used in any methods described herein.

Sialidase

Sialidases (also known as neuraminidases) hydrolyze α -(2 $\rightarrow$ 3)-, α -(2 $\rightarrow$ 6)-, α -(2 $\rightarrow$ 8)-glycosidic linkages of terminal sialic residues in oligosaccharides, glycoproteins, glycolipids, colominic acid and synthetic substrates. There are four types of human sialidases. They are classified according to their major intracellular location as intralysosomal (NEU1), cytosolic (NEU2), plasma membrane (NEU3) and lysosomal or mitochondrial membrane (NEU4) associated sialidases. These human isoforms are distinct from each other in their enzymatic properties as well as their substrate specificity. The sequences for NEU1 (SEQ ID NO: 1), NEU2 (SEQ ID NO: 2), NEU3 (SEQ ID NO:

3) and NEU4 (SEQ ID NO: 4) are shown in **FIG. 14**. A detailed description of human sialidases and their functions can be found, e.g., in Magesh, et al. "Homology modeling of human sialidase enzymes NEU1, NEU3 and NEU4 based on the crystal structure of NEU2: hints for the design of selective NEU3 inhibitors." Journal of Molecular Graphics and Modelling 25.2 (2006): 196-207, which is incorporated by reference in its entirety.

Sialidases can also be found in bacteria, e.g., *Vibrio cholerae*. *Vibrio cholerae* is a Gram-negative, comma-shaped bacterium. Some strains of *V. cholerae* can cause cholera. *Vibrio cholerae* serotype O1 sialidase has been suggested to be a pathogenic factor in microbial infections. It facilitates cholera toxin binding to host intestinal epithelial cells by converting cell surface polysialogangliosides to GM1 monogangliosides. The sequence for *Vibrio cholerae* serotype O1 sialidase is shown in **FIG. 14** (SEQ ID NO: 5). The function and the properties of *Vibrio cholerae* serotype O1 sialidase are known in the art, and are described, e.g., in Jermyn, William S., and E. Fidelma Boyd.

"Characterization of a novel *Vibrio* pathogenicity island (VPI-2) encoding neuraminidase (nanH) among toxigenic *Vibrio cholerae* isolates." Microbiology 148.11 (2002): 3681-3693; and Xiao, Han, et al. "Precision glycocalyx editing as a strategy for cancer immunotherapy." Proceedings of the National Academy of Sciences (2016): 201608069; each of which is incorporated herein by reference in its entirety.

Thus, exemplary neuraminidases useful in the methods and compositions described herein include human NEU1, NEU2, NEU3, and NEU4; and *Vibrio cholerae* serotype O1 sialidase. See, e.g., FIG. 14.

NEU1 can include, e.g., human NEU1, e.g., the full length soluble NEU1 (SEQ ID NO: 1) or an active portion thereof comprising the luminal domain of human NEU1 (amino acids: 48 – 415 of SEQ ID NO: 1) and/or the catalytic domain residues of human NEU1 (including catalytic amino acid residues: R78, R97, D103, D135, S156, E264, R280, Q282, R342, Y370, and E394 of SEQ ID NO: 1).

NEU2 can include, e.g., human NEU2, e.g., the full length, soluble NEU2 (SEQ ID NO: 2) or an active portion thereof comprising the active site residues of human NEU2 (amino acids: R21, D46, M85, E111, Y179, Y181, L217, R237, R283, S288, and Y377 of SEQ ID NO: 2).

NEU3 can include, e.g., human NEU3, e.g., the full length human NEU3 (SEQ ID NO: 3) or an active portion thereof comprising the putative catalytic active sites of human NEU3 (amino acids: R25, R45, D50, M87, N88, R108, Q115, A160, E225, R235, R340, Y370, and E387 of SEQ ID NO: 3).

5 NEU4 can include, e.g., human NEU4, e.g., the full length NEU4 (SEQ ID NO: 4) or an active portion thereof comprising the catalytic active sites of human NEU4 (amino acids: R35, R55, D59, N88, V117, E234, R254, P256, R381, Y431, and E452 of SEQ ID NO: 4).

10 *Vibrio cholerae* serotype O1 sialidase can include, e.g., the full length sialidase (SEQ ID NO: 5) or an active portion thereof comprising the catalytic active sites of sialidase (AA25-781, as the first 24AA correspond to the signal peptide).

The active portions retain the ability of the full-length proteins to hydrolyze alpha-(2->3)-, alpha-(2->6)-, alpha-(2->8)-glycosidic linkages of terminal sialic residues on IgE.

15 The enzymes, the soluble portions thereof (or the luminal domains), the catalytic domains thereof, active sites, and catalytic amino acid residues of these glycosylation enzymes are described, e.g., in Seyrantepe, Volkan, et al. "Neu4, a novel human lysosomal lumen sialidase, confers normal phenotype to sialidosis and galactosialidosis cells." *Journal of Biological Chemistry* 279.35 (2004): 37021-37029; Chavas, Leonard
20 MG, et al. "Crystal Structure of the Human Cytosolic Sialidase Neu2 - Evidence For The Dynamic Nature Of Substrate Recognition." *Journal of Biological Chemistry* 280.1 (2005): 469-475; MONTI, Eugenio, et al. "Identification and expression of NEU3, a novel human sialidase associated to the plasma membrane." *Biochemical Journal* 349.1 (2000): 343-351; and Seyrantepe, Volkan, et al. "Molecular pathology of NEU1 gene in
25 sialidosis." *Human mutation* 22.5 (2003): 343-352; each of which is incorporated by reference herein in its entirety.

In some embodiments, the sialidase used in the present methods is not receptor destroying enzyme (RDE) (II). Yamazaki et al., *J Biol Chem.* 2019 Apr 26;294(17):6659-6669. Epub 2019 Mar 4.

Nucleic Acid Sequences and Amino Acid Sequences

This disclosure provides various nucleic acid sequences and amino acid sequences.

In some embodiments, the nucleic acid sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any of the nucleic acid sequences disclosed herein. In some embodiments, the nucleic acid sequence is identical to any of the sequences described in this disclosure.

In some embodiments, the amino acid sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any of the amino acid sequences disclosed herein. In some embodiments, the amino acid sequence is identical to any of the sequences described in this disclosure.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes is at least 80% of the length of the reference sequence, and in some embodiments is at least 90%, 95%, or 100%. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid “identity” is equivalent to amino acid or nucleic acid “homology”). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. For purposes of the present disclosure, the comparison of sequences and determination of percent identity between two sequences can be accomplished using a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

Neu-IgE Fc Fusion Proteins

Described herein are fusion proteins comprising the Fc region of IgE, preferably human IgE, fused at the N or C terminus to a neuraminidase, referred to herein as Neu-IgE Fc Fusion Proteins. Exemplary sequences of Neu-IgE Fc Fusion Proteins are shown in Fig. 16. A schematic is shown in Fig. 4F.

The neuraminidases, e.g., full proteins or active portions thereof can be fused to IgE, or a part thereof. The neuraminidases can be fused to IgG Fc. Fc fusions have a number of advantages: the soluble protein will have an extended serum half-life (e.g., more than 5 days, 10 days, 14 days, or 20 days), and also will form a dimer. In some embodiments, these fusion polypeptides can form homodimers or heterodimers, depending on the glycosylation target.

The IgE Fc can be the Fc region of any IgE known in the art. For example, the IgE Fc can be a human IgE-Fc (e.g., comprising SEQ ID NO: 6), a mouse IgE-Fc (e.g., comprising SEQ ID NO: 7), a canine IgE Fc (e.g., comprising SEQ ID NO: 8), or a feline IgE Fc (e.g., comprising SEQ ID NO: 9). See Fig. 15A. Preferably, the species of the immunoglobulins is chosen to correspond with the species of the subject to whom the fusion protein will be administered.

In some embodiments, the peptides comprise an IgE antibody epsilon chain Cε2, Cε3, and/or Cε4 region, and an enzymatic luminal domains or a catalytic domain of neuraminidase (e.g., NEU1, NEU2, NEU3, NEU4, *Vibrio cholerae* serotype O1 sialidase). In some embodiments, the peptide has the amino acid sequence that is set forth in SEQ ID NOS: 1, 2, 3, 4, or 5, e.g., amino acids 48 – 415 of SEQ ID NO: 1, amino acids 1 – 380 (full length) of SEQ ID NO:2, , amino acids 1 – 428 (full length) of SEQ ID NO:3, amino acids 1 – 484 (full length) of SEQ ID NO:4, amino acids 25 – 781 of SEQ ID NO: 5, or amino acids 557 – 747 of SEQ ID NO: 5.

Although fusion proteins comprising IgE Fc are exemplified herein, fusion proteins comprising IgG Fc are also described herein. Thus, in some embodiments the neuraminidase can be fused to IgG (e.g., IgG1, IgG2, IgG3, IgG4) or a part thereof. In some embodiments, the neuraminidase can be fused to the Fc portion of an IgG (e.g., IgG1, IgG2, IgG3, IgG4). Fc fusions have a number of advantages: the soluble protein will have an extended serum half-life (e.g., more than 5 days, 10 days, 14 days, or 20

days), and also will form a dimer. In some embodiments, these fusion polypeptides can form homodimers or heterodimers, depending on the glycosylation target.

The IgG Fc can be the Fc region of any IgG known in the art. For example, the IgG Fc can be a human IgG1-Fc (e.g., comprising SEQ ID NO: 10), a human IgG2-Fc (e.g., comprising SEQ ID NO: 11), a human IgG3-Fc (e.g., comprising SEQ ID NO: 12), a human IgG4-Fc (e.g., comprising SEQ ID NO: 13), a mouse IgG1-Fc (e.g., comprising SEQ ID NO: 14), a mouse IgG2a-Fc (e.g., comprising SEQ ID NO: 15), a mouse IgG2b-Fc (e.g., comprising SEQ ID NO: 16), a mouse IgG3-Fc (e.g., comprising SEQ ID NO: 17), a canine IgG-A Fc (e.g., comprising SEQ ID NO: 18), or a feline IgG1 Fc (e.g., comprising SEQ ID NO: 19). See, e.g., FIG. 15B. Preferably, the species of the immunoglobulins is chosen to correspond with the species of the subject to whom the fusion protein will be administered.

In some embodiments, these polypeptides can form a homodimer. The homodimer can have two enzymatic luminal domains (or catalytic domains) of mannosidase. In some other cases, the homodimer can have two enzymatic luminal domains (or catalytic domains) of sialidase or neuraminidase. In some embodiments, these polypeptides can form a heterodimer. In some embodiments, the heterodimer can have one enzymatic luminal domain (or catalytic domain) of mannosidase and one enzymatic luminal domain (or catalytic domain) of sialidase or neuraminidase.

In some embodiments, the peptides comprise an enzymatic luminal domain or a catalytic domain of sialidase or neuraminidase (e.g., NEU1, NEU2, NEU3, NEU4, *Vibrio cholerae* serotype O1 sialidase), and an IgE antibody heavy chain CH2 region, an IgE antibody heavy chain CH3 region, and/or an IgE antibody heavy chain CH3 region..

FIG. 16 shows several examples of glycosylation enzyme - Fc fusion proteins, including human NEU1 – human IgE Fc (hNEU1-hIgE Fc, SEQ ID NO: 20); IgE Fc - NEU2 fusion protein (SEQ ID NO: 21); hNEU2-hIgE Fc (SEQ ID NO: 22); hNEU3-hIgE Fc (SEQ ID NO: 23); hNEU4-hIgE Fc (SEQ ID NO: 24) human IgE Fc- Sialidase (*Vibrio cholerae* serotype O1 sialidase) fusion protein (SEQ ID NO: 25); human NEU1 – mouse IgE Fc (hNEU1-mIgEFc, SEQ ID NO: 26); human NEU2 – mouse IgE Fc (hNEU2-mIgEFc, SEQ ID NO: 27); hNEU3 – mIgE Fc (SEQ ID NO: 28); hNEU4 – mIgE Fc (SEQ

ID NO:29), canine IgE - NEU2 fusion protein (SEQ ID NO: 30), and feline IgE Fc-NEU2 fusion protein (SEQ ID NO: 31).

In some embodiments, the peptide can comprise IgE antibody heavy chain constant regions (e.g., CH1, CH2, CH3 and/or CH4) and/or glycosylation enzymes
5 derived from non-human animals (e.g., dog, cat, cow, or horse; see FIGs. 17-20).

Exemplary sequences In some embodiments, canine IgE-NEU2 has a sequence that is set forth in SEQ ID NO: 30., feline IgE1-NEU2 can have a sequence that is set forth in SEQ ID NO: 32.

In some embodiments, these peptides can additionally include signal sequences,
10 e.g., IL2-signal sequence (e.g., MYRMQLLSIALSLALVTNS, SEQ ID NO: 32), a secretion signal (e.g., MPLLLLLPLLWAGALA, SEQ ID NO:33), or κ -signal sequence (e.g., METDTLLLWVLLLWVPGSTGDAAQPARRAVRSLVPSSDP, SEQ ID NO: 34). These signal sequences usually present at the N-terminus of the peptides.

In some embodiments, the fusion proteins also include one or more flexible
15 linkers. The linkers can be used to attach the separate parts of the fusion protein together. In some embodiments, the linker is a peptide linker. Peptide linkers can be from about 2-100, 10-50, or 15-30 amino acids long. In some embodiments, peptide linkers may be at least 2, 4, 5, 6, 10, 15, or at least 20 amino acids long and/or up to 20, 25, 35, 40, 60, 80, 90, or no more than 100 amino acids long. In some embodiments, the linker is a peptide
20 linker comprising one or more glycines and/or serines, e.g., a single or repeating GGGGS (SEQ ID NO: 35), GGGS (SEQ ID NO: 36), GS, GGGGGG (SEQ ID NO: 37), GSGGS (SEQ ID NO: 38), GGSG (SEQ ID NO: 39), GGSGG (SEQ ID NO: 40), GSGSG (SEQ ID NO: 41), GSGGG (SEQ ID NO: 42), GGGSG (SEQ ID NO: 43), and/or GSSSG (SEQ ID NO: 44) sequence(s). Other linkers are known in the art. Intact antibodies with
25 desired specificity can also be fused to glycosylation enzymes, enabling specific targeting of the enzymes. Further, similar protein fusions can be generated using dog/cat/horse/cow equivalent/homologous antibodies or glycosylation enzymes, enabling treatment of non-human animals (e.g., pets and livestock).

Glycoengineered Intravenous IgE (gIVIE)

Also provided herein are glycoengineered intravenous IgE (gIVIE) compositions. Analogous to the intravenous immunoglobulin (IVIg) compositions presently in clinical use, the compositions can comprise normal polyspecific obtained from large numbers of healthy donors. The compositions can be polyclonal natural antibodies synthesized, in response to immune stimuli (antigens and T cells), by plasma B cells. Methods for the production of therapeutic IVIG compositions are known in the art (see, e.g., Afonso and João, *Biomolecules*. 2016 Mar; 6(1): 15 and references cited therein) and can be adapted for production of IVIE, e.g., as shown in Fig. 5A and described herein, or by other methods, e.g., as described in Kleine-Tebbe et al., *J Immunol Methods*. 1995 Feb 27;179(2):153-64. After obtaining the IgE, they are treated with sufficient neuraminidase (e.g., NEU1, NEU2, NEU3, NEU4, *Vibrio cholerae* serotype O1 sialidase) to remove sialic acid from the IgE, to produce a gIVIE composition, e.g., that attenuates IgE effector functions *in vivo*.

IgE-mediated disorders

IgE is known to mediate allergic responses and is produced by B cells in both membrane-bound and secretory form. IgE binds to B-cells through its Fc region to a low affinity IgE receptor, known as FcεRII. Upon exposure to an allergen, B-cells bearing a surface-bound IgE molecule specific for the allergen are activated and further develop into IgE-secreting plasma cells. The secreted IgE molecules, which are specific for the allergen, circulate through the bloodstream and become bound to the surface of mast cells in tissue and basophils in bloodstream through the high affinity receptor, known as FcεRI. This binding by allergen-specific IgE, sensitizes the mast cells and basophils for the allergen. Subsequent exposure to the allergen causes cross-linking of FcεRI on basophils and mast cells, leading to up-regulation of the granular molecule CD63 and the release of a number of factors, such as histamine, platelet activating factors, eosinophil and neutrophil chemotactic factors, and cytokines such as IL-3, IL-4, IL-5 and GM-CSF.

As used herein, the term “IgE-mediated response” refers to responses of IgE receptor expressing cells (e.g., basophils and mast cells) induced directly or indirectly by IgE. In some embodiments, the response can be observed (e.g., degranulation) and/or

measured by up-regulation of the granular molecule CD63, or the release of one or more of histamine, platelet activating factors, eosinophil and neutrophil chemotactic factors, and cytokines such as IL-3, IL-4, IL-5 and GM-CSF. In some embodiments, IgE-mediated responses include e.g., degranulation, up-regulation of the granular molecule CD63, and/or the release of histamine from basophils. In some embodiments, IgE-mediated responses can cause allergic reactions.

As used herein, the term “attenuating an IgE-mediated response” refers to the extent, occurrence and/or frequency of an IgE-mediated response that is reduced by the methods as described herein, e.g., by administering an agent as described herein as compared to without administering the agent. The extent of reduction can be statistically significant and in certain embodiments, by at least 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90% or greater.

The IgE-mediated disorder is characterized by abnormal responses mediated by IgE. In some embodiments, the abnormal responses mediated by IgE are due to overproduction of IgE and/or hypersensitivity of basophils or mast cells to IgE. Thus, IgE-mediated disorders include, e.g., (1) allergic disorders (e.g., asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, urticaria, food allergy and seasonal allergy, as well as anaphylactic shock); (2) autoimmune disorders (e.g., lupus, rheumatoid arthritis, psoriasis); and (3) anaphylaxis, etc. A detailed description regarding IgE-mediated disorder and IgE-mediated response can be found, e.g., in US 8828394 B2, which is incorporated herein by reference in its entirety.

IgE that can specifically recognize an allergen has a unique long-lived interaction with its high-affinity receptor FcεRI so that basophils and mast cells, capable of mediating inflammatory reactions, become "primed", ready to release chemicals like histamine, leukotrienes, and certain interleukins. These chemicals cause many of the symptoms associated with allergy, such as airway constriction in asthma, local inflammation in eczema, increased mucus secretion in allergic rhinitis, and increased vascular permeability, which allow other immune cells to gain access to tissues, but which can lead to a potentially fatal drop in blood pressure as in anaphylaxis.

Anaphylaxis is a serious allergic reaction that is rapid in onset and may cause death. It typically causes e.g., an itchy rash, throat or tongue swelling, shortness of

breath, vomiting, lightheadedness, and low blood pressure. These symptoms typically come on over minutes to hours. When anaphylaxis occurs, IgE binds to the antigen. The antigen-bound IgE then activates FcεRI receptors on mast cells and basophils. This leads to the release of inflammatory mediators such as histamine. These mediators
5 subsequently increase the contraction of bronchial smooth muscles, trigger vasodilation, increase the leakage of fluid from blood vessels, and cause heart muscle depression.

As histamine is central to the pathogenesis of allergic disorders, e.g., asthma and atopic dermatitis, by attenuating IgE-mediated responses such as histamine release, the present method is also effective in treating allergic disorders.

10 Thus in some embodiments, the fusion proteins described herein can be used to target FcεRI-expressing cells.

Methods of Treatment

The methods described herein include methods for treating IgE-mediated
15 disorders, e.g., allergies, e.g., anaphylactic allergies, and methods for attenuating IgE-mediated responses. Generally, the methods include administering a therapeutically effective amount of compositions comprising Neu-IgE Fc fusion proteins or gIVIE as described herein, to a subject who is in need of, or who has been determined to be in need of, such treatment. In some embodiments, the subject can be allergic to a food antigen,
20 e.g., eggs, milk, peanuts, soy, fish, shellfish, tree nuts, and/or wheat, or to an environmental allergen, e.g., dust mite excretions, pollen, pet dander, or royal jelly, *inter alia*. See, e.g., Valenta et al., *Gastroenterology*. 2015 May; 148(6): 1120–1131.e4.

As used in this context, to “treat” means to ameliorate at least one symptom of the disorders or the diseases. Often, the treatment results in an improvement in the
25 symptoms. In some embodiments, the treatment can result in a reduction of histamine release. In some embodiments, one or more of the clinical symptoms are ameliorated or reduced, the duration being shortened, the frequency of the occurrence of the symptoms is reduced, or the clinical symptoms are prevented from manifesting.

As used herein, the terms “subject” and “patient” are used interchangeably
30 throughout the specification and describe an animal, human or non-human, e.g., a mammal, to whom treatment according to the methods of the present invention is

provided. Veterinary and non-veterinary applications are contemplated by the present invention. Human patients can be adult humans or juvenile humans (e.g., humans below the age of 18 years old). In addition to humans, patients include but are not limited to mice, rats, hamsters, guinea-pigs, rabbits, ferrets, cats, dogs, and primates. Included are, for example, non-human primates (e.g., monkey, chimpanzee, gorilla, and the like), rodents (e.g., rats, mice, gerbils, hamsters, ferrets, rabbits), lagomorphs, swine (e.g., pig, miniature pig), equine, canine, feline, bovine, and other domestic, farm, and zoo animals. Thus, in some embodiments, the glycosylation enzymes, the antibodies, or the parts thereof (e.g., Fc regions of the antibodies or the catalytic domain of the glycosylation enzymes) as described herein can also derive from these non-human animals. The present disclosure further provides the amino acid sequences of the glycosylation enzymes, and the antibodies or the parts thereof that derive from some of these non-human animals. For example, **FIGs. 15A-B and 17** list exemplary amino acid sequences of dog IgE and IgG heavy chain constant regions, dog NEU1, dog NEU2, and dog NEU3. **FIGs. 15A-B and 18** list exemplary amino acid sequences of cat IgE and IgG heavy chain constant regions, cat NEU1, cat NEU2, cat NEU3 and cat NEU4. **FIG. 19** lists exemplary amino acid sequences of cow IgE heavy chain constant region, cow NEU1, and cow NEU3. **FIG. 20** lists exemplary amino acid sequences of horse IgE heavy chain constant region, horse NEU1, horse NEU2, and horse NEU3.

In some embodiments, the subject is a human (e.g., male human or female human) with an age over 6 months old, 12 months old, 2 years old, 5 years old, 6 years old, 10 years old, 12 years old, 16 years old, 18 years old, 25 years old, 30 years old, 40 years old, 50 years old, 60 years old, 70 years old, or 80 years old.

As used herein, the terms “therapeutically effective” and “effective amount”, used interchangeably, applied to a dose or amount refers to a quantity of a composition, compound or pharmaceutical formulation that is sufficient to result in a desired activity upon administration to a subject in need thereof. Within the context of the present disclosure, the term “therapeutically effective” refers to that the composition, compound or pharmaceutical formulation, in a sufficient amount, can reduce or eliminate at least one symptom or one condition of the disorders as described herein, delay or reduce risk or frequency of symptoms, or delay or reduce risk of progression.

Expression Systems

To use the fusion proteins or peptides as described herein, it may be desirable to express them from a nucleic acid that encodes them. This can be performed in a variety of ways. For example, the nucleic acid encoding the fusion proteins or peptides can be cloned into an intermediate vector for transformation into prokaryotic or eukaryotic cells for replication and/or expression. Intermediate vectors are typically prokaryote vectors, e.g., plasmids, or shuttle vectors, or insect vectors, for storage or manipulation of the nucleic acid encoding the fusion proteins or peptides for production. The nucleic acid encoding the fusion proteins or peptides can also be cloned into an expression vector, for administration to a plant cell, animal cell, preferably a mammalian cell or a human cell, fungal cell, bacterial cell, or protozoan cell.

To obtain expression, a sequence encoding a fusion protein or peptide is typically subcloned into an expression vector that contains a promoter to direct transcription.

Suitable bacterial and eukaryotic promoters are well known in the art and described, e.g., in Sambrook et al., *Molecular Cloning, A Laboratory Manual* (3d ed. 2001); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel et al., eds., 2010). Bacterial expression systems for expressing the engineered protein are available in, e.g., *E. coli*, *Bacillus* sp., and *Salmonella* (Palva et al., 1983, *Gene* 22:229-235). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available. In some embodiments, the fusion proteins and peptides are expressed by transfection of HEK-293T cells, Expi293 cells, or CHO cells with vectors comprising the polynucleotides encoding fusion proteins and peptides as described in this disclosure.

The promoter used to direct expression of a nucleic acid depends on the particular application. For example, a strong constitutive promoter is typically used for expression and purification of fusion proteins. In contrast, when a vector encoding the fusion protein or peptide is to be administered *in vivo*, either a constitutive or an inducible promoter can be used, depending on the particular need. In some embodiments, the promoter for administration of the vector encoding the fusion protein or peptide can be a weak

promoter, such as HSV TK or a promoter having similar activity. The promoter can also include elements that are responsive to transactivation, e.g., hypoxia response elements, Gal4 response elements, lac repressor response element, and small molecule control systems such as tetracycline-regulated systems and the RU-486 system (see, e.g., Gossen & Bujard, 1992, Proc. Natl. Acad. Sci. USA, 89:5547; Oligino et al., 1998, Gene Ther., 5:491-496; Wang et al., 1997, Gene Ther., 4:432-441; Neering et al., 1996, Blood, 88:1147-55; and Rendahl et al., 1998, Nat. Biotechnol., 16:757-761).

In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the nucleic acid in host cells, either prokaryotic or eukaryotic. A typical expression cassette thus contains a promoter operably linked, e.g., to the nucleic acid sequence encoding the fusion protein or peptide, and any signals required, e.g., for efficient polyadenylation of the transcript, transcriptional termination, ribosome binding sites, or translation termination. Additional elements of the cassette may include, e.g., enhancers, and heterologous spliced intronic signals.

The particular expression vector used to transport the genetic information into the cell is selected with regard to the intended use, e.g., expression in plants, animals, bacteria, fungus, protozoa, etc. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and commercially available tag-fusion expression systems such as GST and LacZ.

Expression vectors containing regulatory elements from eukaryotic viruses are often used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A+, pMTO10/A+, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV40 early promoter, SV40 late promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

The vectors for expressing the fusion protein or peptide can include RNA Pol III promoters to drive expression of the guide RNAs, e.g., the H1, U6 or 7SK promoters.

These human promoters allow for expression of fusion protein or peptide in mammalian cells following plasmid transfection.

Some expression systems have markers for selection of stably transfected cell lines such as thymidine kinase, hygromycin B phosphotransferase, and dihydrofolate reductase. High yield expression systems are also suitable, such as using a baculovirus vector in insect cells, with the encoding sequence under the direction of the polyhedrin promoter or other strong baculovirus promoters.

The elements that are typically included in expression vectors also include a replicon that functions in *E. coli*, a gene encoding antibiotic resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of recombinant sequences.

Standard transfection methods are used to produce bacterial, mammalian, yeast or insect cell lines that express large quantities of protein, which are then purified using standard techniques (see, e.g., Colley et al., 1989, J. Biol. Chem., 264:17619-22; Guide to Protein Purification, in Methods in Enzymology, vol. 182 (Deutscher, ed., 1990)). Transformation of eukaryotic and prokaryotic cells are performed according to standard techniques (see, e.g., Morrison, 1977, J. Bacteriol. 132:349-351; Clark-Curtiss & Curtiss, Methods in Enzymology 101:347-362 (Wu et al., eds, 1983)).

Any of the known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, nucleofection, liposomes, microinjection, naked DNA, plasmid vectors, viral vectors, both episomal and integrative, and any of the other well-known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (see, e.g., Sambrook et al., supra). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing the fusion protein or peptide.

The present disclosure also includes the vectors and cells comprising the vectors, as well as kits comprising the proteins and nucleic acids described herein, e.g., for use in various methods as described herein.

Dosage

An “effective amount” is an amount sufficient to effect beneficial or desired results. For example, a therapeutic amount is one that achieves the desired therapeutic effect. This amount can be the same or different from a prophylactically effective amount, which is an amount necessary to prevent onset of disease or disease symptoms. An effective amount can be administered in one or more administrations, applications or dosages. A therapeutically effective amount of polypeptides, multimers, or compositions (i.e., an effective dosage) depends on the polypeptides, multimers, or compositions that are selected. The compositions can be administered one from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the polypeptides, multimers, or compositions described herein can include a single treatment or a series of treatments.

Dosage, toxicity and therapeutic efficacy of the polypeptides, multimers, or compositions can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Polypeptides, multimers, or compositions which exhibit high therapeutic indices are preferred. While polypeptides, multimers, or compositions that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets polypeptides, multimers, or compositions to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of polypeptides, multimers, or compositions lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending

upon the dosage form employed and the route of administration utilized. For any polypeptides, multimers, or compositions used in the methods as described in this disclosure, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test polypeptide, 5 multimer, or composition which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Pharmaceutical Compositions and Methods of Administration

The methods described herein include the use of pharmaceutical compositions comprising fusion proteins as described in this disclosure as an active ingredient as well as the compositions themselves.

15 Pharmaceutical compositions typically include a pharmaceutically acceptable carrier. As used herein, the language “pharmaceutically acceptable carrier” includes saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.

20 Pharmaceutical compositions are typically formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration.

25 Methods of formulating suitable pharmaceutical compositions are known in the art, see, e.g., Remington: The Science and Practice of Pharmacy, 21st ed., 2005; and the books in the series Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs (Dekker, NY). For example, solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, 30 glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite;

chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes
5 or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water,
10 Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for
15 example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and
20 antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum
25 monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating polypeptides, multimers, or compositions as described in this disclosure in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by
30 incorporating the polypeptides, multimers, or compositions into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those

enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

5 Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active agents can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included
10 as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or agents of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a
15 sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the composition can be delivered in the form of an aerosol spray from a pressured container or dispenser that contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer. Such methods include those
20 described in U.S. Patent No. 6,468,798.

In some embodiments, the polypeptides or multimers are prepared with carriers that will protect the polypeptides or multimers against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene
25 vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques, or obtained commercially, e.g., from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to selected cells with monoclonal antibodies to cellular antigens) can also be used as pharmaceutically acceptable carriers. These can be
30 prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Methods of Diagnosis

Included herein are methods for diagnosing allergy. The methods rely on
5 detection of a sialylation levels on IgE. The methods include obtaining a sample from a subject, and evaluating the presence and/or level of sialylation on IgE, e.g., on total IgE, or on allergen-specific IgE (i.e., IgE that binds specifically to a selected allergen) in the sample, and comparing the presence and/or level with one or more references, e.g., a control reference that represents a normal level of sialylation on IgE e.g., a level in an
10 unaffected (non-allergic) subject, and/or a disease reference that represents a level of the proteins associated with allergy e.g., a level in a subject having an allergy, e.g., an anaphylactic allergy. Suitable reference values can include those shown in Figures 1I, and 2B, showing total IgE titers and Ara h 2 titers.

As used herein the term “sample”, when referring to the material to be tested for
15 the presence of a biological marker using the method of the invention, includes whole blood, plasma, or serum. The type of sample used may vary depending upon the clinical situation in which the method is used. Various methods are well known within the art for the identification and/or isolation and/or purification of IgE from a sample. In some embodiments, the methods include isolating antigen-specific IgE, e.g., by purifying total
20 IgE, and then enriching antigen/allergen-specific IgE using antigen/allergen -coupled beads.

The presence and/or level of sialylation on IgE can be evaluated using methods known in the art, e.g., using standard electrophoretic and quantitative immunoassay methods, including but not limited to, Western blot; enzyme linked immunosorbent assay
25 (ELISA); radio-immunoassay; immunohistochemistry (IHC); or mass spectrometry (Kim (2010) Am J Clin Pathol 134:157-162; Yasun (2012) Anal Chem 84(14):6008-6015; Brody (2010) Expert Rev Mol Diagn 10(8):1013-1022; Philips (2014) PLOS One 9(3):e90226; Pfaffe (2011) Clin Chem 57(5): 675-687).

In some embodiments, an ELISA method may be used, wherein the wells of a
30 microtiter plate are coated with an antibody against which the protein is to be tested. The sample containing or suspected of containing the biological marker is then applied to the

wells. After a sufficient amount of time, during which antibody-antigen complexes would have formed, the plate is washed to remove any unbound moieties, and a detectably labelled molecule is added. Again, after a sufficient period of incubation, the plate is washed to remove any excess, unbound molecules, and the presence of the labeled molecule is determined using methods known in the art. Variations of the ELISA method, such as the competitive ELISA or competition assay, and sandwich ELISA, may also be used, as these are well-known to those skilled in the art.

Mass spectrometry, and particularly matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and surface-enhanced laser desorption/ionization mass spectrometry (SELDI-MS), is useful for the detection of biomarkers of this invention. (See U.S. Patent No. 5,118,937; 5,045,694; 5,719,060; 6,225,047)

In some embodiments, the presence and/or level of sialylation on IgE is comparable to the presence and/or level of the protein(s) in the disease reference, and the subject has or has had one or more symptoms associated with an allergic reaction, then the subject can be diagnosed with an allergy, e.g., an anaphylactic allergy. In some embodiments, the subject has no overt signs or symptoms of allergy or allergic reaction, but the presence and/or level of sialylation on IgE is comparable to the presence and/or level of the protein(s) in the disease reference, then the subject has an increased risk of developing an allergy, e.g., an anaphylactic allergy. In some embodiments, once it has been determined that a person has an allergy, e.g., an anaphylactic allergy, or has an increased risk of developing an allergy, e.g., an anaphylactic allergy, then a treatment, e.g., as known in the art or as described herein, can be administered.

Suitable reference values can be determined using methods known in the art, e.g., using standard clinical trial methodology and statistical analysis. The reference values can have any relevant form. In some cases, the reference comprises a predetermined value for a meaningful level of sialylation on IgE, e.g., a control reference level that represents a normal level of sialylation on IgE, e.g., a level in an unaffected subject or a subject who is not at risk of developing an allergy as described herein, and/or a disease reference that represents a level of the proteins associated with conditions associated with allergy or anaphylactic allergy, e.g., a level in a subject having an allergy (e.g., an anaphylactic allergy).

The predetermined level can be a single cut-off (threshold) value, such as a median or mean, or a level that defines the boundaries of an upper or lower quartile, tertile, or other segment of a clinical trial population that is determined to be statistically different from the other segments. It can be a range of cut-off (or threshold) values, such as a confidence interval. It can be established based upon comparative groups, such as where association with risk of developing disease or presence of disease in one defined group is a fold higher, or lower, (e.g., approximately 2-fold, 4-fold, 8-fold, 16-fold or more) than the risk or presence of disease in another defined group. It can be a range, for example, where a population of subjects (e.g., control subjects) is divided equally (or unequally) into groups, such as a low-risk group, a medium-risk group and a high-risk group, or into quartiles, the lowest quartile being subjects with the lowest risk and the highest quartile being subjects with the highest risk, or into n-quantiles (i.e., n regularly spaced intervals) the lowest of the n-quantiles being subjects with the lowest risk and the highest of the n-quantiles being subjects with the highest risk.

In some embodiments, the predetermined level is a level or occurrence in the same subject, e.g., at a different time point, e.g., an earlier time point.

Subjects associated with predetermined values are typically referred to as reference subjects. For example, in some embodiments, a control reference subject does not have a disorder described herein (e.g. an allergy, e.g., an anaphylactic allergy). In some cases it may be desirable that the control subject is non-allergic, and in other cases it may be desirable that a control subject has an allergy, e.g., to a different allergen, or a non-anaphylactic allergy.

A disease reference subject is one who has (or has an increased risk of developing) an allergy, e.g., an anaphylactic allergy. An increased risk is defined as a risk above the risk of subjects in the general population.

Thus, in some cases the level of sialylation on IgE in a subject being less than or equal to a reference level of sialylation on IgE is indicative of a clinical status (e.g., indicative of a disorder as described herein, e.g., an allergy, e.g., an anaphylactic allergy. In other cases the level of sialylation on IgE in a subject being greater than or equal to the reference level of sialylation on IgE is indicative of the absence of disease or normal risk of the disease. In some embodiments, the amount by which the level in the subject is the

less than the reference level is sufficient to distinguish a subject from a control subject, and optionally is a statistically significantly less than the level in a control subject. In cases where the level of sialylation on IgE in a subject being equal to the reference level of sialylation on IgE the “being equal” refers to being approximately equal (e.g., not statistically different).

The predetermined value can depend upon the particular population of subjects (e.g., human subjects) selected. For example, an apparently healthy non-allergic population may have a different ‘normal’ range of levels of sialylation on IgE than will a population of subjects which have, are likely to have, or are at greater risk to have, an allergy, e.g., an anaphylactic allergy. Accordingly, the predetermined values selected may take into account the category (e.g., sex, age, health, risk, presence of other diseases) in which a subject (e.g., human subject) falls. Appropriate ranges and categories can be selected with no more than routine experimentation by those of ordinary skill in the art.

In characterizing likelihood, or risk, numerous predetermined values can be established.

Upon diagnosis with an allergy, e.g., an anaphylactic allergy, the subject can be administered or prescribed a treatment, e.g., avoidance of the allergen, immunotherapy (e.g., oral, sublingual, or subcutaneous immunotherapy, e.g., Sublingual immunotherapy (SLIT)), and/or a pharmacological treatment, e.g., a chronically administered treatment (e.g., corticosteroids, antihistamines, Leukotriene receptor antagonists (LTRAs), Anti-IgE antibody) or an acutely-administered treatment (e.g., epinephrine or a rapid-acting bronchodilator). See, e.g., Min, Allergy Asthma Immunol Res. 2010 Apr; 2(2): 65–76.

In some embodiments, the methods rely on the observation that Human IgE has seven N-linked glycosylation sites, 5 of which are occupied by complex biantennary glycans. One site is occupied by an oligomannose glycan, and one site is unoccupied. On complex biantennary glycans, sialic acid is attached to galactose. Thus provided herein is an *in vitro* assay method to determine the pathogenicity of circulating IgEs in allergic humans. The method can include measuring the levels of terminal sialic acid sugar residues or terminal galactose residues on sera IgEs, isolated from said humans, wherein higher levels of sialylation (higher sialylation correlates with less terminal galactose, and vice versa) predict susceptibility to a pathogenic reaction (e.g., anaphylaxis) in said

allergic humans. In some embodiments, the *in vitro* assay is an ELISA in which total IgE is captured, and sialylation levels are quantified by the ratio of the amount of IgE-bound labelled lectin, specific for terminal sialic acid or terminal galactose, normalized to the amount of total anti-IgE detection antibody is bound. In some embodiments, the measurement for the amount of IgE-bound labelled lectin is by, but not limited to, fluorescence or a colorimetric enzymatic reaction. Also provided is an *in vitro* assay method to determine the pathogenicity to a specific allergen of circulating IgEs in humans; the method can include measuring the levels of sialic acid sugar residues or galactose residues on said IgEs, isolated from the sera of human patients and which bind to a specific allergen, wherein higher levels of sialylation on allergen-bound IgEs predict susceptibility to a pathogenic reaction (e.g. anaphylaxis) to said allergen. In some embodiments, the *in vitro* assay is an ELISA in which allergen-specific IgE is captured, and sialylation levels are quantified by the ratio of the amount of IgE-bound labelled lectin, specific for terminal sialic acid or terminal galactose, normalized to the amount of total anti-IgE detection antibody is bound. In some embodiments, the measurement for the amount of IgE-bound labelled lectin is by, but not limited to, fluorescence or a colorimetric enzymatic reaction.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1. IgE Sialylation is a Determinant of Allergic Pathogenicity

Methods

The following materials and methods were used in this Example.

IgE Antibodies

All human samples were collected under IRB approved protocols. Serum samples from MGH IRB approved and consented peanut allergic were collected prior to treatment. Peanut allergy was confirmed by clinical history, allergen-specific IgE screening, and double-blind placebo-controlled oral challenge (PNOIT2, NCT01750879, Table 1). IRB-approved non-atopic adults were recruited (Research Blood Components, Boston, MA) on the basis of self-identification as non-allergic donors. Non-atopy was

confirmed by clinical history, and allergen-specific IgE screening (Table 1). Total IgE, Ara h 2-specific IgE, Fel d 1-specific IgE, Der p 1-specific IgE, and Bet v 1-specific IgE were determined by ImmunoCap Assay (Phallion, Thermo Scientific) according to manufacturer's protocols. Primary IgE was enriched from serum samples by serially
 5 depleting IgG by protein G agarose (GE Healthcare) followed by anti-IgE conjugated NHS-beads (GE Healthcare). IgE purity was confirmed by protein electrophoresis and coomassie gel staining. Recombinant OVA-specific IgE was generated as described²³. Briefly, cDNA sequences for generating OVA-specific heavy ϵ and light κ chain of mouse and human IgE²³ were cloned into pcDNA3.4 using restriction enzyme sites XbaI
 10 and AgeI. To generate recombinant OVA-specific mouse or human IgE, plasmids containing OVA-specific heavy and light chain were transiently co-transfected at 1:1 ratio using Expi293 Expression System Kit (Life Technologies) according to the manufacturer's protocol. The cells expressing IgE were selected by addition of 400 μ g/mL G418 in the culture media for two weeks and maintained before expanding to a
 15 larger scale production. OVA-specific IgE was purified from cell culture supernatant by OVA-coupled agarose beads²³.

Table 1. Patient Demographic Data

Sample #	Diagnosis	Gender	Age	Other Atopies*
73	Allergic	M	22	Yes
10	Allergic	F	25	Yes
22	Allergic	F	19	Yes
34	Allergic	F	30	Yes
51	Allergic	F	15	Yes
60	Allergic	F	40	Yes
61	Allergic	F	27	Yes
67	Allergic	F	52	Yes
84	Allergic	M	22	Yes
97	Allergic	F	36	Yes
24	Allergic	M	36	Yes
33	Allergic	F	30	Yes
34	Allergic	F	16	Yes
69	Allergic	M	15	Yes
80	Allergic	F	8	Yes
95	Allergic	M	8	Yes
97	Allergic	F	36	Yes
100	Allergic	F	22	Yes
105	Allergic	F	22	Yes
111	Allergic	F	22	Yes

Sample #	Diagnosis	Gender	Age	Other Atopies*
106	Allergic	F	32	Yes
149	Non-atopic	M	27	No
349	Non-atopic	F	35	No
241	Non-atopic	M	29	No
528	Non-atopic	M	38	No
53208	Non-atopic	M	36	No
53209	Non-atopic	M	37	No
53210	Non-atopic	M	39	No
53211	Non-atopic	F	31	No
53195	Non-atopic	M	60	No
57543	Non-atopic	M	69	No
57544	Non-atopic	F	27	No
57546	Non-atopic	M	22	No
57699	Non-atopic	M	58	No
57713	Non-atopic	M	32	No
57714	Non-atopic	M	28	
56986	Non-atopic	M	29	
56988	Non-atopic	F	50	
57527	Non-atopic	F	29	

*Includes reports of allergic asthma, allergic rhinitis, other food dermatitis.

ELISAs

Sandwich ELISA for quantifying mIgE and OVA-specific binding were
 5 conducted as previously described²³. Briefly, 96-well Nunc plates were coated with goat
 polyclonal anti-mouse IgE (Bethyl Laboratories) or OVA and blocked with BSA in PBS
 (1% BSA for mIgE and 2% for OVA) prior to sample incubation. Samples were probed
 with goat polyclonal anti-mouse IgE-HRP (2 ng/ml; Bethyl Laboratories) and the
 reactions were detected by 3,3',5,5'-tetramethylbenzidine (TMB; Thermo Fisher
 10 Scientific) and stopped by 2 M sulfuric acid, and the absorbance was measured at 450
 nm.

Glycopeptide Mass Spectrometry and Glycan Analysis

Site specific glycosylation was quantified for IgE isolated from non-allergic
 donors and from peanut allergic donors using nano LC-MS/MS following enzymatic
 15 digestion of the proteins as described previously, with minor modifications²²⁻²⁴ (Tables 2,
 3).

The isolated polyclonal primary hIgE and myeloma hIgE (Sigma Aldrich AG30P)
 was prepared for proteolysis by denaturing the protein in 6M guanidine HCl followed by

reduction with dithiothreitol and alkylation with iodoacetamide followed by dialysis into 25mM ammonium bicarbonate pH 7.8. Proteolysis was done with either trypsin to quantify N218, N371 and N394 or chymotrypsin to quantify N140, N168 and N265. For the tryptic digest IgE was incubated with trypsin (Trypsin Gold Promega) at a 1:50 enzyme to substrate ratio overnight at 37C. For the chymotryptic digest IgE was incubated with chymotrypsin (Sequencing Grade Promega) at a 1:100 enzyme to substrate ratio for 4 hours at 25C. Both enzymes were quenched with formic acid added to 2% w/w. The separation was performed on a Thermo EasySpray C18 nLC column 0.75umx50cm using water and acetonitrile with 0.1% formic acid for mobile phase A and mobile phase B respectively. A linear gradient from 1% to 35% mobile phase B was run over 75 minutes. Mass spectra were recorded on a Thermo Q Exactive mass spectrometer operated in positive mode using data independent acquisition (DIA) targeting the masses shown. Glycopeptides were quantified based on the extracted ion area of the Y1 ion (Figs. 6A-E). The relative abundance was calculated for all identified glycan species for each site. Myeloma IgE (Sigma Aldrich AG30P) was run prior to paired sample sets to monitor retention time shifts and ensure consistency in the analytical results across the sample set. The percentage of glycan moieties at each site was calculated using the relative abundance of each glycan. For example, if a particular site was determined to have 60% monosialylated, fucosylated glycans (A1F), and 40% of disialylated, fucosylated glycans (A2F), the number of sialic acids at one site would be 1.4 (0.6x1+0.4x2), and total 2.8 sialic acids per molecule accounting for two sites.

Mice

Five- to six-week-old female BALB/c mice were purchased from the Jackson Laboratory and used in these studies. All mice were housed in specific pathogen-free conditions according to the National Institutes of Health (NIH), and all animal experiments were conducted under protocols approved by the MGH IACUC. For all experiments, age- and sex-matched mice were randomized allocating to experimental group, with 4-5 mice per group, and repeated at least three independent times.

Passive Cutaneous Anaphylaxis (PCA) was conducted as previously described²³. In brief, monoclonal ^{Sia}mIgE or ^{As}mIgE specifically for OVA or dinitrophenyl (DNP, clone SPE-7; Sigma-Aldrich) was injected intradermally in the mice ears. For

experiments where OVA-specific ^{As}mIgE was added to OVA-specific ^{Sia}mIgE, a mIgE isotype control (clone MEA-36, Biolegend) was included. The next day mice were intravenously challenged with PBS containing 125 µg OVA (Sigma-Aldrich) or DNP-Human Serum Albumin (DNP-HSA; Sigma-Aldrich) and 2% Evans blue dye in PBS. 45 min after challenge, the ears were excised and minced before incubation in N,N-dimethyl-formamide (EMD Millipore) at 55°C for 3 h. The degree of blue dye in the ears was quantitated by the absorbance at 595 nm.

Passive Systemic Anaphylaxis (PSA) was elicited as previously described with minor modifications^{42,43}. Briefly, mice were injected intravenously with monoclonal mIgE specific for OVA or DNP (clone SPE-7; Sigma-Aldrich) in PBS and challenged the next day intravenously with PBS containing 1 mg OVA (Sigma-Aldrich) or DNP-HSA (Sigma-Aldrich). For examining the therapeutic potential of ^{As}mIgE, mice that had been injected intravenously with 10 µg DNP-specific mIgE (clone SPE-7; Sigma-Aldrich) the first day were injected intravenously with PBS, 20 µg OVA-specific ^{Sia}mIgE or 20 µg OVA-specific ^{As}mIgE the next day and challenged with 1 mg DNP-HSA or OVA (Sigma-Aldrich) the third day. For testing the therapeutic potential of NEU^{Fce}, mice injected intravenously with 10 µg OVA-specific mIgE the first day were further injected intravenously with PBS, 100 µg NEU^{Fce} or 100 µg mIgE isotype control (clone MEA-36, Biolegend) the next day and challenged with 1 mg OVA (Sigma-Aldrich) the third day. Core temperature was recorded at the baseline and every 10 min after the allergen challenge by a rectal microprobe thermometer (Physitemp). Histamine in the blood was quantified by histamine enzyme immunoassay kit (SPI-Bio) according to the manufacturer's protocol. Briefly, histamine in the blood was derivatized and incubated with plate precoated with monoclonal anti-histamine antibodies and histamine-AChE tracer at 4°C for 24 h. The plate was then washed and developed with Ellman's reagent and the absorbance measured at 405 nm.

Passive Food Anaphylaxis (PFA) was elicited by adapting PSA described above. Briefly, mice injected intravenously with 20 µg monoclonal mIgE specific for TNP (clone MEA-36; Biolegend) in PBS the first day were administered with 20 mg TNP-OVA in PBS (Biosearch Technologies) by oral gavage the next day. Core temperature

was recorded at the baseline and every 10 min after the challenge by a rectal microprobe thermometer (Physitemp).

To determine *in vivo* half-lives of ^{Sia}mIgE or ^{As}mIgE, mice were injected intraperitoneally with 30 µg DNP-specific ^{Sia}mIgE or ^{As}mIgE and the blood collected at
 5 the indicated times after injection into a Microtainer blood collection tube with clot actiator/SST gel (BD Diagnostics). The level of mIgE was quantified by mIgE ELISA described below.

Human LAD2 mast cell culture and degranulation

Human LAD2 mast cell line was a generous gift of Dr. Metcalfe (NIAID, NIH)
 10 and was maintained as previously described^{23,44}. Briefly, LAD2 cells were cultured in StemPro-34 SFM medium (Life Technologies) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 100 ng/ml recombinant human stem cell factor (PeproTech). The cells were hemi-depleted each week with fresh medium and maintained at $2-5 \times 10^5$ cells/ml at 37°C and 5% CO₂.

15 Degranulation assays were performed as previously described (Shade, 2015), LAD2 cells were sensitized overnight with 1 µg/mL OVA-specific hIgE at 1 µg/mL or 50 ng/mL peanut-allergic hIgE. The following day, the cells were pelleted by centrifugation, resuspended in HEPES buffer, plated in 96-well plates, and stimulated with allergen OVA or crude peanut extract at defined concentrations. Upon allergen challenge, mast
 20 cell degranulation was determined by the amount of substrate p-nitrophenyl *N*-acetyl-β-D-glucosamide digested by β-hexosaminidase release from the mast cell granules at the absorbance of 405 nm. To assess the effect of sialic acid removal on IgE-bound mast cells, IgE-sensitized LAD2 cells were treated with NEU^{Fce}, heat-inactivated NEU^{Fce}, mIgE isotype control (clone MEA-36, Biolegend) for 20 min before allergen challenge.
 25 To inactivate NEU^{Fce}, the enzyme was heated at 95°C for 10 min. To determine whether addition of a surrogate asialylated glycoprotein could recapitulate the phenotype of sialic acid removal from IgE, LAD2 cells sensitized with OVA-specific ^{Sia}hIgE were incubate with sialylated fetuin (^{Sia}Fetuin) or asialylated fetuin (^{As}Fetuin) at defined amount for 20 min before allergen challenge.

Crude peanut extract preparation

Unsalted dry-roasted peanuts (Blanched Jumbo Runner cultivar; Planters) were ground to a smooth paste, followed by washing with 20 volumes of cold acetone, filtered using Whatman paper, and dried as previously described²³. Protein was extracted by
5 agitating the peanut flour overnight with PBS containing protease inhibitor cocktail without EDTA (Roche). The peanut protein extracts were collected as the supernatant after centrifugation at 24,000 x g for 30 min.

IgE Glycosylation Engineering

To remove sialic acids on IgE, IgE was digested with Glyko Sialidase A
10 (Prozyme) at 37°C for 72 h according to the manufacturer's instructions. To re-sialylate ^{As}mIgE by in vitro sialylation reaction, ^{As}mIgE was incubated with 5 mM Cytidine-5'-monophospho-N-acetylneuraminic acid (CMP-Neu5Ac2; Nacalai USA) in the sialylation buffer (150 mM NaCl, 20 mM HEPES, pH7.4) overnight at room temperature. Following reactions, OVA-specific ^{Sia}IgE or ^{As}IgE were purified by OVA-coupled beads to remove
15 glycosylation modifying enzymes as described²³. All digestion or sialylation reactions were verified by lectin blotting or HPLC.

Protein gel stain and lectin blotting

Equal amounts of ^{Sia}IgE or ^{As}IgE were resolved on 3–8% Tris-Acetate protein gels (Life Technologies) in SDS-PAGE under nonreducing conditions. For protein stain, gels
20 were incubated in AcquaStain Protein Gel Stain (Bulldog Bio) for 1 h at room temperature and destained in distilled water. For lectin blotting, the protocol was conducted as described²³. Briefly, after resolved proteins on the gel were transferred to Immobilon-PSQ polyvinylidene difluoride membranes (Millipore Sigma), the membranes were blocked with 0.2% BSA in TBS for 1 hour at room temp, washed in TBS, followed
25 by incubation with biotinylated *Sambucus nigra* lectin (SNA; 0.4 µg/ml; Vector Laboratories) in TBS with 0.1 M Ca²⁺ and 0.1 M Mg²⁺ for 1 hour at room temp to determine the level of terminal α2,6 sialic acids on N-linked glycans of proteins. The membrane was then washed in TBS and incubated with alkaline phosphatase conjugated goat anti-biotin (1:5000 dilution; Vector Laboratories) in TBS for 1 hour at room temp.
30 Sialylated proteins on membranes were visualized by incubation with 1-Step NBT/BCIP plus Suppressor Substrate Solution.

Basophil activation tests

Basophil activation was performed as previously described⁴⁵. Buffy coats of human blood from healthy, de-identified, consenting donors were obtained from the MGH Blood Transfusion Service. Peripheral blood mononuclear cells (PBMCs) were separated from buffy coats by a density gradient centrifugation using Ficoll Paque Plus (GE Healthcare) and resuspended in 0.5% BSA in RPMI 1640 Media (GE Healthcare). PBMCs were incubated for 2 min with ice-cold lactic acid buffer (13.4 mM lactate, 140 mM NaCl, 5 mM KCl, pH 3.9) to remove endogenous human IgE on the cell surface prior to neutralization by 12% Tris (pH 8). Cells were then washed and incubated 1 hour at 37°C with 1 µg OVA-specific ^{Sia}hIgE or ^{As}hIgE per 1 × 10⁶ cells in basophil activation buffer (0.5% BSA, 2 mM CaCl₂ and 2 mM MgCl₂ in RPMI 1640 Media). Sensitized cells were washed and resuspended in basophil activation buffer supplemented with 10 ng/mL human IL3 (Peprotech) prior to 30 min OVA activation. Activation was stopped by addition of ice-cold 0.2 M EDTA in FACS buffer. Activated cells were washed and resuspended in FACS buffer to proceed antibody staining for basophil activation markers.

Flow cytometry

Antibodies used for surface allergen staining are listed in Table A. For staining for mouse cells, suspension cells were incubated with anti-mouse CD16/CD32 (clone 2.4G2, BD Biosciences) prior to antibody staining. Cells were incubated in FACS buffer with desired staining antibodies for 20 minutes at 4°C. Cells were then washed in FACS buffer before being acquired by an LSRII flow cytometer (BD Biosciences) or CytoFLEX (Beckman Coulter). Data were analyzed using FlowJo software version 10.4 software (Tree Star). To quantify hIgE loading following sensitization, PBS or 1 µg/mL OVA-specific ^{Sia}hIgE or ^{As}hIgE were incubated with 2.5 × 10⁵ cells/mL LAD2 mast cells overnight before wash with FACS buffer and stained with OVA-A647. To quantify dermal mast cell IgE loading, single cell suspensions were generated from mouse ears as described²³. Ears were intradermally injection of 40 ng OVA-specific ^{Sia}mIgE or ^{As}mIgE. The following day, ears were removed, separated into dorsal and ventral halves, and minced before incubation in DMEM containing 2% FCS, 1% HEPES, 500 u/mL collagenase type 4 (Worthington), 0.5 mg/mL hyaluronidase (Sigma) and Dnase I (Roche) at 37°C for 1 h at 180 RPM. The digested sample was then subjected to

disruption by Gentle MACS and filtered through a 70 μ m cell strainer followed by a 40 μ m cell strainer in FACS buffer (2 mM EDTA and 0.5% Bovine Serum Albumins (BSA) in PBS).

Table A: Antibodies used for in various experiments

FACS assay	Target	Clone	Fluorochrome s	Vendor	Dilution
IgE loading on mouse skin mast cells	Mouse CD45	30-F11	APC/ Cyanine7	Biolegend	1:400
	Mouse/human CD11b	M1/70	PerCP/ Cyanine5.5	Biolegend	1:100
	Mouse CD11c	N418	FITC	Biolegend	1:100
	Mouse Ly-6G/Ly-6C (Gr-1)	RB6-8C5	FITC	Biolegend	1:100
	Mouse CD117 (c-Kit)	2B8	APC	Biolegend	1:100
	Mouse IgE	RME-1	PE	Biolegend	1:100
IgE loading on human LAD2 cells	Human CD117 (c-kit)	104D2	PE	Biolegend	1:400
	Ovalbumin		A647	Invitrogen	1:400 of 2 mg/mL stock
	Human IgE	MHE-18	APC	Biolegend	1:400
Basophil activation tests	Human HLA-DR	L243	PE/Cy7	Biolegend	1:35
	Human CD123	6H6	PE	Biolegend	1:30
	Human CD63	H5C6	FITC	Biolegend	1:30
General	Viability		eFluor 450	eBioscience	1:500
ELISA assay	Target	Catalog #	Conjugation	Vendor	Dilution
Mouse IgE ELISA	mIgE	A90-115A	No	Bethyl	1:200
	mIgE	A90-115P	HRP	Bethyl	1:30,000
Human IgE ELISA	hIgE	A80-108A	No	Bethyl	1:200
	hIgE	A80-108P	HRP	Bethyl	1:30,000
Immunoblotting	Target	Catalog #	Species/ conjugation	Vendor	Dilution
Immunoblotting for Syk	Phospho-Syk	2701	Rabbit	Cell Signaling Technology	1:2000
	Total	2712	Rabbit	Cell Signaling Technology	1:2000
Secondary antibody Immunoblotting for Actin	Rabbit IgG	W4011	HRP	Promega	1:500
	Actin	sc-47778 HRP	HRP	Santa Cruz Biotechnology	1:50,000
Passive Anaphylaxis	Species	Clone	Antigen specificity	Vendor	
IgE	Mouse	SPE-7	dinitrophenyl (DNP)	Sigma-Aldrich	
IgE	Mouse	MEA-36	Trinitrophenyl (TNP)	Biolegend	

Biolayer interferometric assays for binding

Binding kinetics and affinity of protein interaction studies were performed by the Octet K2 system (Molecular Devices) using Octet buffer (PBS with 0.025% Tween and 1% BSA). For measuring hFcεRIα interaction, ligand 0.25 μg/mL His-tagged hFcεRIα (Acro Biosystems) was loaded onto Anti-Penta-HIS (HIS1K) Biosensors (Molecular Devices). For OVA interaction, ligand 100 μg/mL OVA was immobilized onto Amine Reactive Second-Generation (AR2G) Biosensors in 10 mM sodium acetate, pH 5 using EDC/Sulfo-NHS based chemistry. Association of analyte OVA-specific ^{Sia}hIgE or ^{As}hIgE was performed in 3-fold serial dilution from 90 to 1 nM or NEU^{Fce} in 3-fold serial dilution from 24 to 0.3 nM in Octet buffer. Analyte dissociation was measured in Octet buffer. Analysis of binding kinetic parameters were performed by Octet data analysis software 10.0 using interaction of ligand-loaded biosensor with no analyte during association phase as the reference sensor.

Immunoblotting for Syk Signaling

1.5 × 10⁶ LAD2 cells were sensitized with PBS or 1 μg/mL OVA-specific ^{Sia}hIgE or ^{As}hIgE. Sensitized cells were washed and resuspended in HEPES buffer the next day followed by OVA stimulation at 10 μg/mL OVA at 37°C for indicated times. Cells were immediately centrifuged after OVA stimulation and the cell pellets lysed in ice-cold lysis buffer for 30 min on ice (RIPA buffer (Boston BioProducts), 1x Halt Protease Inhibitor Cocktail (Thermo Scientific), 1x HaltTM Phosphatase Inhibitor Cocktail (Thermo Scientific) and 2.5 mM EDTA). After incubation on ice, lysed pellets were passed rapidly through a 27G needle on ice and centrifuged at maximal speed at 4°C for 15 min to clear the membrane and nuclei. The protein concentration was quantified using Pierce BCA Protein Assay kit (Thermo Scientific) and 20 μg of protein lysate was loaded per well on 4–12% Bis-Tris protein gels (Life Technologies) in SDS-PAGE under denaturing and reducing conditions. Briefly, after protein transferred to PVDF membranes described as above, the membranes were blocked with 5% milk in TBS with 0.1% Tween (TBST) for 1 hour at room temp, washed in TBST, followed by incubation with 1:2000 Rabbit anti-Phospho-Syk (Tyr352) Antibody (Cell Signaling Technology) in 5% BSA in TBST overnight at 4°C. The membrane was then washed in TBST before incubating with anti-rabbit-HRP for 1 hour at room temp and washed in TBST again followed by

chemiluminescent detection using Immobilon Western Chemiluminescent HRP Substrate (Millipore Sigma). To detect total Syk on the membrane, after chemiluminescent detection using autoradiography film, the membrane was stripped by incubating in stripping buffer (2% SDS and 0.1 M β -mercaptoethanol in Tris buffer) at 50°C for 30 min.

5 The stripped membranes were then blocked, washed as above and then incubated with 1:2000 Rabbit anti-Syk Antibody (Cell Signaling Technology) for 2 h in 5% BSA in TBST at room temp before incubating with 1:30,000 anti-rabbit-HRP for 1 hour at room temp. To probe for β -Actin, the stripped membranes were incubated with 1:150,000 anti- β -Actin HRP (Santa Cruz Biotechnology) for 1 hour at room temp, washed and signal
10 determined by chemiluminescent detection.

Calcium flux

5 $\times 10^5$ LAD2 cells were sensitized overnight with PBS or 500 ng/mL OVA-specific $S^{ia}hIgE$ or $A^{sh}IgE$. Next day, sensitized cells were washed before loading with 2 μ M Fluo-4-AM (Invitrogen) at 37°C in HEPES buffer for 20 minutes. After loading, the
15 cells were washed and resuspended in HEPES buffer. Fluorescence was filtered through the 530/30 band pass filter and collected in FL-1/FITC. Baseline Ca^{2+} fluorescence levels were recorded for 1 minute on the Accuri C6 (BD Biosciences) before the addition of indicated allergen or buffer to each sample. At the end of allergen stimulation, cells were added 2 μ M Ca^{2+} ionophore A23187 (Sigma) as a positive control.

20 Generation of NEU^{Fce}

The neuraminidase fusion protein was designed by fusing a kappa light chain secretion signal sequence and the sialidase gene from *Arthrobacter urefaciens* (EC 3.2.1.18, gene AU104)⁴⁶. Stop codon of the AU104 was omitted, instead, a short flexible linker peptide (GGGGGG), mouse IgE C ϵ 2, C ϵ 3, C ϵ 4, and His6-tag was inserted to the
25 C-terminus of the sialidase. The gene was codon optimized for human and synthesized by GenScript. The protein of 288 kDa was then produced by WuXi biologics. Sialidase activity of NEU^{Fce} was determined by the level of p-nitrophenol released from 250 μ M 2-O-(p-Nitrophenyl)- α -D-N-acetylneuraminic acid (Sigma) in 100 mM sodium phosphate (pH 5.5) for 10 min at 37°C. The reaction was terminated by adding 0.5 M sodium
30 carbonate and the absorbance quantified at 405 nm.

Statistical analyses

All statistical analyses were performed using Prism 8 (GraphPad Software), and results are shown as means with SEM. Un-paired and paired Student's t test were used for parametric comparisons of two unmatched and matched groups, respectively. For comparisons of two sample groups of multiple non-parametric conditions, a two-way ANOVA with Sidak's multiple comparison test was used. For parametric comparison between three or more groups, one-way or two-way ANOVA with Tukey's multiple comparison test was used. Accuracy of individual IgE glycan moieties capacity to distinguish allergic IgE was analyzed by receiver operating characteristic (ROC) curves. Area under each ROC curve (AUC) was calculated for each glycan moiety. AUC was interpreted as follows, where a maximum AUC of 1 indicates the specific glycan moiety is able to distinguish allergic IgE from non-allergic IgE. An AUC of 0.5 indicates the differentiation capacity of a specific glycan moiety is poor.

Results

The present study examined whether allergic disease-specific glycosylation patterns existed for IgE, and if so, whether those patterns influenced IgE biological activity. Non-atopic adults reported no history of atopy, had low total IgE titers, and had little IgE reactivity to peanut allergen (Ara h 2), birch tree pollen allergen (Bet v 1), house dust mite allergen (Der p 1), or cat allergen (Fel d 1) (Fig. 1b, c, d; Table 1). Peanut allergic adults reported multiple atopies, had approximately two-fold higher total IgE titers, with IgE reactive to peanut allergen (Ara h 2) but not the other tested allergens, and were clinically diagnosed with peanut allergy as confirmed by oral peanut challenge (Fig. 1b, c, d; Table 1)⁹. We sensitized human LAD2 mast cells with similar amounts of total IgE enriched from the sera of these cohorts (Fig. 5a) and activated the cells by anti-IgE crosslinking. Intriguingly, less degranulation, as measured by β -hexosaminidase release, was observed in mast cells sensitized with IgE isolated from sera of non-atopic individuals compared peanut allergic patients (Fig. 5b), despite similar surface IgE loading (Fig. 5c, d). This suggested possible intrinsic functional differences between non-atopic and allergic IgE, independent of allergen specificity.

Next, the N-glycan residues on total IgE enriched from non-atopic and allergic serum was analyzed by mass spectrometry^{22,24,27}. This revealed similar numbers of mannose moieties between total non-atopic and allergic IgE (Fig. 1e). Fucose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid can be attached to complex glycans (Fig. 1a). While total fucose content was similar between non-atopic and allergic IgE (Fig. 1f), significantly increased levels of bisecting GlcNAc (biGlcNAc) and terminal galactose were found on non-atopic IgE (Fig. 1g, h) whereas increased terminal sialylation was detected on allergic IgE (Fig. 1i).

To determine whether the differences in glycan residues on total IgE were predictive of allergic disease, we assessed the variable glycan content on non-atopic and allergic IgE using Receiver Operating Characteristics (ROC) curves (Fig. 2a). The area-under these curves revealed that galactose and sialic acid content of IgE were strong predictors of allergic disease, but not fucose, mannose, nor biGlcNAc. Of note, differences in IgE sialylation were not sex- or age-dependent (Fig. 5e, f). We next asked where on the IgE molecule glycans differed between allergic and non-atopic individuals (Figures 6A-E, 7G; Tables 2-3). Site-specific analysis showed that N140, N168, N265 and N394 of IgE were fully occupied by N-linked glycans, with N218 and N371 partially occupied (75% and 30% respectively), and N383 completely unoccupied (Fig. 2b; Fig. 7a), consistent with previous results^{21,22,24}. The glycans at N394 were exclusively of oligomannose structure, with predominantly five mannose residues (Man-5) (Fig. 2b; Fig. 7b). N140, N168, N265, and N371 were occupied by complex, antennary structures. Fucose and biGlcNAc content was similar at all sites between samples (Fig. 7c, d). However, complex glycans terminating in galactose were enriched at N140 and N265 on non-atopic IgE, while terminal sialic acid moieties, particularly disialylated glycans were significantly enriched at N168 and N265 on allergic IgE (Fig. 2b; Fig. 7e-g). Together, these results reveal the specific glycosylation patterns that distinguish allergic from non-atopic total IgE.

Table 2: Targeted mass list for IgE glycosylation sites N140, N168 and N265 glycopeptides from the chymotryptic digest

Mass [m/z]	CS [z]	Polarity	Start [min]	End [min]	(N)CE	Comment
1197.84000	3	Positive	23.00	30.00	27	IgE N140 G2F
1295.22000	3	Positive	28.00	34.00	27	IgE N140 A1F

Mass [m/z]	CS [z]	Polarity	Start [min]	End [min]	(N)CE	Comment
1265.54000	3	Positive	25.00	33.00	27	IgE N140 G2F+BglcNAc
1362.91000	3	Positive	29.00	34.00	27	IgE N140 A1F+BGlCNAc
1391.91000	3	Positive	32.00	37.00	27	IgE N140 A2F
1459.94000	3	Positive	32.00	37.00	27	IgE N140 A2F+BglcNAc
1416.94000	3	Positive	28.00	34.00	27	IgE N140 A1F+LacNAc
1513.97000	3	Positive	32.00	37.00	27	IgE N140 A2F+LacNAc
1319.57000	3	Positive	23.00	30.00	27	IgE N140 G2F+LacNAc
922.70000		Positive	30.00	34.00	27	IgE N168 G2F
990.40000	3	Positive	30.00	34.00	27	IgE N168 G2F+BGlCNAc
1019.73000	3	Positive	36.00	40.00	27	IgE N168 A1F
1087.42000	3	Positive	36.00	40.00	27	IgE N168 A1F+BGlCNAc
1184.45000	3	Positive	42.00	50.00	27	IgE N168 A2F+BGlCNAc
1116.43000	3	Positive	42.00	50.00	27	IgE N168 A2F
1141.45000	3	Positive	36.00	40.00	27	IgE N168 A1F+LacNAc
1238.48000	3	Positive	42.00	50.00	27	IgE N168 A2F+LacNAc
1044.43000	3	Positive	30.00	34.00	27	IgE N168 G2F+LacNAc
501.80000	2	Positive	45.00	55.00	27	IgE N265 Agly
1337.20000	3	Positive	65.00	75.00	27	IgE N265 A3F
992.10000	3	Positive	35.00	55.00	27	IgE N265 G2F+GlcNAc
1118.50000	3	Positive	60.00	65.00	27	IgE N265 A2F
1186.20000	3	Positive	60.00	65.00	27	IgE N265 A2F+GlcNAc
1089.10000	3	Positive	52.00	58.00	27	IgE N265 A1F+GlcNAc
924.40000	3	Positive	35.00	55.00	27	IgE N265 G2F
1021.40000	3	Positive	52.00	58.00	27	IgE N265 A1F
1143.40000	3	Positive	52.00	58.00	27	IgE N265 A1F+LacNAc
1240.50000	3	Positive	60.00	65.00	27	IgE N265 A2F+LacNAc

**Table 3: Targeted mass list for IgE glycosylation N218, N371 and N394
glycopeptides from the tryptic digest**

Mass [m/z]	CS [z]	Polarity	Start [min]	End [min]	(N)CE	Comment
905.40000	4	Positive	27.00	32.00	27	N218 A1F
883.10000	4	Positive	24.00	30.00	27	N218 G2F+GlcNAc
1231.50000	3	Positive	23.00	30.00	27	N218 G3F
519.60000	3	Positive	39.00	55.00	27	Agly N218
1029.00000	4	Positive	48.00	63.00	27	N218 A2F+GlcNAc
1142.20000	4	Positive	35.00	45.00	27	N218 A3F
1123.15000	3	Positive	24.00	30.00	27	N218 G1F+GlcNAc
1069.10000	4	Positive	30.00	38.00	27	N218 A2F+LacNAc
956.20000	4	Positive	27.00	33.00	27	N218 A1F+GlcNAc
996.70000	4	Positive	27.00	33.00	27	N218 A1F+LacNAc

Mass [m/z]	CS [z]	Polarity	Start [min]	End [min]	(N)CE	Comment
1109.13000	3	Positive	24.00	30.00	27	N218 G2F
978.20000	4	Positive	30.00	38.00	27	N218 A2F
1002.80000	3	Positive	30.00	37.00	27	N371 G2F+GlcNAc
1099.80000	3	Positive	35.00	40.00	27	N371 A1F+GlcNAc
1197.10000	3	Positive	40.00	50.00	27	N371 A2F+GlcNAc
948.80000	3	Positive	30.00	38.00	27	N371 G1F+GlcNAc
1153.80000	3	Positive	35.00	40.00	27	N371 A1F+LacNAc
1032.80000	3	Positive	34.00	39.00	27	N371 A1F
1045.80000	3	Positive	35.00	41.00	27	N371 G1F+GlcNAc+NeuAc
1129.80000	3	Positive	40.00	50.00	27	N371 A2F
935.10000	3	Positive	30.00	35.00	27	N371 G2F+GlcNAc
1056.80000	3	Positive	30.00	35.00	27	N371 G3F
517.30000	2	Positive	30.00	38.00	27	N371 Agly
912.10000	3	Positive	34.00	40.00	27	N394 HM5
966.10000	3	Positive	34.00	40.00	27	N394 HM6
1020.10000	3	Positive	34.00	40.00	27	N394 HM7
1074.20000	3	Positive	34.00	40.00	27	N394 HM8
1128.20000	3	Positive	34.00	40.00	27	N394 HM9
1071.40000	3	Positive	38.00	45.00	27	N394 A1F-LAcNAc
1179.50000	3	Positive	38.00	45.00	27	N394 3,6,1,1,0
1130.50000	3	Positive	38.00	45.00	27	N394 3,6,0,1,0

These findings raised the possibility that sialic acid content modifies IgE effector functions. Indeed, sialylation has been implicated in regulating most other antibody classes, including IgG1 anti-inflammatory activity, IgA neuropathy and influenza neutralization, and IgM-induced inhibitory signaling on B and T cells²⁸⁻³². However, the role for sialylation in modulating IgE function has not been described. Sialic acid was attached in α 2,6 linkages on hIgE and mouse IgE (mIgE) as determined by neuraminidase (NEU) digestion assays and lectin blotting (Fig. 8, Fig. 3a), consistent with previous studies^{7,21,23}. Thus, we treated mIgE with NEU or digestion buffer as a control to generate mIgE of identical allergen-specificity differing only in sialic acid content (Fig. 3a). In a model of passive cutaneous anaphylaxis (PCA), mice were sensitized with PBS, OVA-specific sialylated-mIgE (^{Sia}mIgE), or OVA-specific asialylated-IgE (^{As}mIgE) intradermally in the ears. The next day, the mice were challenged with allergen, OVA, in Evan's blue dye intravenously. Forty minutes after challenge, the amount of blue dye in the ear was quantified as a surrogate of histamine-mediated vascular leakage. PBS-injection elicited little blue dye accumulation in the ear injection site, while significant blue coloration was observed in ^{Sia}mIgE-sensitized ears (Fig. 3b; Fig. 9a). Strikingly,

^{As}mIgE-sensitized ears exhibited markedly reduced blue coloration, indicative of attenuated anaphylaxis (Fig. 3b; Fig. 9a). To confirm sialic acid removal was responsible for reduced PCA reactivity, sialic acid was reattached to ^{As}mIgE by *in vitro* sialylation reaction (^{Re-sia}mIgE)³³. ^{Re-sia}mIgE triggered a robust PCA reaction (Fig. 3b), demonstrating that IgE sialylation impacts the magnitude of anaphylaxis. Flow cytometry analysis of mast cells recovered from the mouse ears revealed no differences in IgE loading following sensitization with ^{Sia}mIgE or ^{As}mIgE (Fig. 3c; Fig. 9b) and ^{Sia}mIgE or ^{As}mIgE bound allergen similarly as determined by ELISA (Fig. 3d). Thus, attenuated local anaphylaxis by ^{As}mIgE was independent of IgE loading on mast cells *in vivo* or allergen recognition.

Next, we systemically sensitized mice with ^{Sia}mIgE, ^{As}mIgE, or PBS and challenged with allergen the following day in a model of passive systemic anaphylaxis (PSA). ^{Sia}mIgE-sensitized mice elicited a robust anaphylactic response underscored by 3°C loss in temperature 20 minutes after allergen challenge (Fig. 3e; Fig. 10a, b). However, minimal temperature loss was observed in ^{As}mIgE- or PBS-sensitized mice (Fig. 3e; Fig. 10a, b). Consistently, a systemic increase in histamine was detected in ^{Sia}mIgE-sensitized animals following challenge, but not in ^{As}mIgE- or PBS-treated mice (Fig. 3f). Asialylated glycoproteins have decreased serum half-life³⁴, and we therefore compared the levels of ^{Sia}mIgE and ^{As}mIgE in circulation following systemic administration. However, sialic acid removal had little effect on IgE half-life (Fig. 3g, Fig. 10c). To extend these findings to a model of passive food allergy, we sensitized mice systemically with PBS, ^{Sia}mIgE or ^{As}mIgE, and challenged with allergen orally the following day. ^{Sia}mIgE sensitization, but not ^{As}mIgE- or PBS-sensitization resulted in a significant temperature loss following oral allergen challenge (Fig. 3h).

We next asked whether sialylation similarly regulated hIgE. We sensitized human LAD2 mast cells with PBS, sialylated or asialylated human IgE (^{Sia}hIgE and ^{As}hIgE, respectively, Fig. 3i). The cells were stimulated with allergen, and degranulation quantified by β-hexosaminidase release assays. ^{As}hIgE-sensitized cells had markedly reduced degranulation following allergen challenge, compared to ^{Sia}hIgE-sensitized cells (Fig. 3j). hIgE loading on LAD2 mast cells after sensitization was examined by flow cytometry and revealed comparable loading following ^{Sia}hIgE or ^{As}hIgE sensitization

(Fig. 11a). Similar findings were observed in human mast cells derived from primary peripheral blood CD34⁺ cell culture, where ^{As}hIgE-sensitized cells had markedly reduced allergen-specific degranulation compared to ^{Sia}hIgE-sensitized cells (Fig. 3k; Fig. 11b). In parallel, primary basophils were sensitized with PBS, ^{Sia}hIgE and ^{As}hIgE and stimulated with allergen (Fig. 11c). ^{As}hIgE-sensitized basophils elicited reduced degranulation after allergen stimulation as measured by surface staining of the granule marker, CD63, compared to basophils sensitized with ^{Sia}hIgE (Fig. 3l). Although mast cell loading was similar between mouse and human ^{Sia}IgE and ^{As}IgE (Fig. 3c, Fig. 11a), we asked whether sialylation altered binding kinetics of hIgE to its receptor, FcεRI. Bio-layer interferometry (BLI) assays revealed no difference in ^{Sia}hIgE and ^{As}hIgE interactions with FcεRI (Fig. 3m). Sialylation also did not alter IgE binding to the allergen (Fig. 3n). Thus, removing sialic acid from IgE attenuates its effector functions *in vivo* and *in vitro*, while binding to allergen, mast cells and FcεRI remained intact.

Because sialylation does not alter IgE interactions to allergen and receptor, we tested whether signaling downstream of FcεRI was affected by IgE sialylation. LAD2 mast cells sensitized with ^{Sia}hIgE or ^{As}hIgE were stimulated with allergen and cellular lysates collected at defined intervals. Western blotting of mast cell lysates for Syk revealed reduced phosphorylation at 5 and 30 minutes after stimulation (Fig. 4a). Similarly, calcium flux was reduced in ^{As}hIgE-sensitized LAD2 mast cells following allergen stimulation compared to ^{Sia}hIgE-sensitized cells (Fig. 4b). We then asked whether a surrogate asialylated glycoprotein could recapitulate the phenotype of attenuating IgE by removing sialic acid. LAD2 mast cells were sensitized with ^{Sia}hIgE, and supplemented with either sialylated fetuin (^{Sia}Fetuin) or asialylated fetuin (^{As}Fetuin; Fig. 8b) during allergen stimulation. Quantifying the resulting degranulation revealed that addition of sialylated fetuin had no effect, while asialylated fetuin inhibited allergen-induced mast cell degranulation (Fig. 4c). Together, these results suggest that sialic acid removal exposes an inhibitory glycan that dampens FcεRI signaling.

The observation that an asialylated glycoprotein could inhibit mast cell degranulation *in vitro* raised the possibility that ^{As}IgE could actively inhibit anaphylaxis *in vivo*. We therefore sensitized mice intradermally in the ears with PBS, OVA-specific ^{Sia}mIgE, a combination of OVA-specific ^{Sia}mIgE and ten-fold more OVA-specific

^{As}mIgE, or a combination of OVA-specific ^{Sia}mIgE and ten-fold more TNP-specific ^{Sia}mIgE isotype control. The next day mice were challenged with OVA and blue coloration of the ears quantified. Extensive vascular leakage occurred in ears sensitized with OVA-specific ^{Sia}mIgE alone (Fig. 4d). However, co-sensitization of OVA-specific ^{Sia}mIgE with either OVA-specific ^{As}mIgE or TNP-specific ^{Sia}mIgE both resulted in significantly reduced vascular leakage (Fig. 4d). Next, mice were systemically sensitized by DNP-specific ^{Sia}mIgE on day 0, and PBS, OVA-specific ^{Sia}mIgE, or OVA-specific ^{As}mIgE on day 1, and challenged with DNP-HSA on day 2. Intriguingly, mice that were sensitized with DNP-specific ^{Sia}mIgE on day 0 and PBS or OVA-specific ^{Sia}mIgE on day 1 exhibited robust temperature loss after allergen challenge. However, DNP-specific ^{Sia}mIgE-sensitized mice that received OVA-specific ^{As}mIgE on day 1 had significantly attenuated temperature loss upon allergen challenge (Fig. 4e). Systemic challenge of these treatment groups with OVA revealed that only mice sensitized with OVA-specific ^{Sia}mIgE resulted in temperature drop, while all other groups were unaffected (Fig. 12). These results suggest that ^{As}mIgE attenuates anaphylaxis by occupying FcεRI, but can actively dampen systemic anaphylaxis.

As sialic acid removal attenuated IgE effector functions, we explored whether targeting sialic acid on IgE-bearing cells is a viable strategy for attenuating allergic inflammation. Thus, we genetically fused a neuraminidase to the N-terminus of IgE Fc Cε2-4 domains (Neu^{Fce}, Fig. 4f; Fig. 13a) to direct sialic acid removal specifically to IgE-bearing cells. This fusion protein retained binding to FcεRI in BLI binding assays (Fig. 13b), could be loaded on mast cells (Fig. 13c), and had neuraminidase activity (Fig. 13d-g). LAD2 mast cells were sensitized with OVA-specific ^{Sia}hIgE, and then incubated briefly with increasing concentrations of Neu^{Fce}, heat-inactivated Neu^{Fce}, or an IgE isotype to control for FcεRI occupancy, and stimulated with OVA. Remarkably, treatment with Neu^{Fce}, but not heat-inactivated Neu^{Fce} nor the isotype control attenuated OVA-induced degranulation in a dose-dependent manner (Fig. 4g). To extend our findings to allergic hIgE from peanut allergic patients, we sensitized LAD2 mast cells with peanut allergic ^{Sia}hIgE and treated with Neu^{Fce}, or an IgE isotype control. Consistently, allergen-induced degranulation was significantly attenuated by Neu^{Fce} treatment of peanut allergic ^{Sia}hIgE-sensitized cells compared to IgE isotype control

treatment (Fig. 4h). Unsensitized LAD2 mast cells treated with Neu^{Fce} did not degranulate (no IgE + Neu^{Fce}, Fig. 4g, h), indicating Neu^{Fce} treatment does not stimulate mast cells. We next explored the therapeutic potential of modulating sialic acid content *in vivo*. Mice were sensitized systemically with Sia^mIgE on day 0, received PBS, Neu^{Fce}, or IgE isotype control treatment on day 1. The following day, the mice were challenged systemically with allergen, and core body temperature measured as described above. Sia^mIgE-sensitized mice that received PBS or isotype control exhibited robust drops in temperature (Fig. 4i). Remarkably, Neu^{Fce} treatment significantly attenuated allergen-induced temperature drop, providing evidence of the therapeutic potential of targeting sialic acid on IgE-bearing cells.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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WHAT IS CLAIMED IS:

1. A fusion polypeptide comprising:
an Immunoglobulin E (IgE) antibody Fc domain region; and
a sialidase or a functional portion thereof, preferably wherein the sialidase or a functional portion thereof can hydrolyze alpha-(2->3)-, alpha-(2->6)-, alpha-(2->8)-glycosidic linkages of terminal sialic residues on IgE.
2. The fusion polypeptide of claim 1, wherein the sialidase is NEU1, NEU2, NEU3, NEU4, or *Vibrio cholerae* serotype O1 sialidase.
3. The fusion polypeptide of claim 1, wherein the sialidase is a human sialidase.
4. The fusion polypeptide of claim 1, wherein the fusion polypeptide comprises an IgE CH2 region, an IgE CH3 region, and an IgE CH4 region.
5. A polynucleotide encoding the fusion polypeptide of any of claims 1-4.
6. A vector comprising a polynucleotide encoding the fusion polypeptide of any of claims 1-4.
7. A cell comprising the vector of claim 6, and optionally expressing the fusion polypeptide of any of claims 1-4.
8. A method of treating a subject having an IgE-mediated disorder, the method comprising:
administering to the subject an effective amount of a composition comprising the fusion protein of any of claims 1-4.
9. The method of claim 8, wherein the IgE-mediated disorder is an allergic disorder.
10. The method of claim 9, wherein the allergic disorder is an anaphylactic allergy.

11. The method of claim 9, wherein the allergic disorder is asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, or urticaria.
12. A method of preparing glycoengineered IgE, the method comprising:
providing a composition comprising IgE, preferably human IgE, obtained from a plurality of subjects,
contacting the IgE with a sialidase under conditions and for a time sufficient to remove sialylation from the IgE;
thereby preparing glycoengineered IgE.
13. The method of claim 12, wherein the method further comprises formulating the glycoengineered IgE for intravenous administration.
14. A composition comprising the glycoengineered IgE prepared by the method of claim 12, and a pharmaceutically acceptable carrier.
15. A method of treating a subject having an IgE-mediated disorder, the method comprising administering to the subject an effective amount of the composition of any of claims 12-14.
16. The method of claim 15, wherein the IgE-mediated disorder is an allergic disorder.
17. The method of claim 16, wherein the allergic disorder is an anaphylactic allergy.
18. The method of claim 16, wherein the allergic disorder is asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, or urticaria. for use in treating a subject having an IgE-mediated disorder.
19. A composition comprising the fusion polypeptide of claims 1-4, and a pharmaceutically acceptable carrier.

20. The composition of claims 1-4, for use in treating a subject having an IgE-mediated disorder.
21. The composition for the use of claim 20, wherein the IgE-mediated disorder is an allergic disorder.
22. The composition for the use of claim 20, wherein the allergic disorder is an anaphylactic allergy.
23. The composition for the use of claim 20, wherein the allergic disorder is asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, or urticaria.
24. The composition of claims 12-14, for use in treating a subject having an IgE-mediated disorder.
25. The composition for the use of claim 24, wherein the IgE-mediated disorder is an allergic disorder.
26. The composition for the use of claim 24, wherein the allergic disorder is an anaphylactic allergy.
27. The composition for the use of claim 24, wherein the allergic disorder is asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, or urticaria.

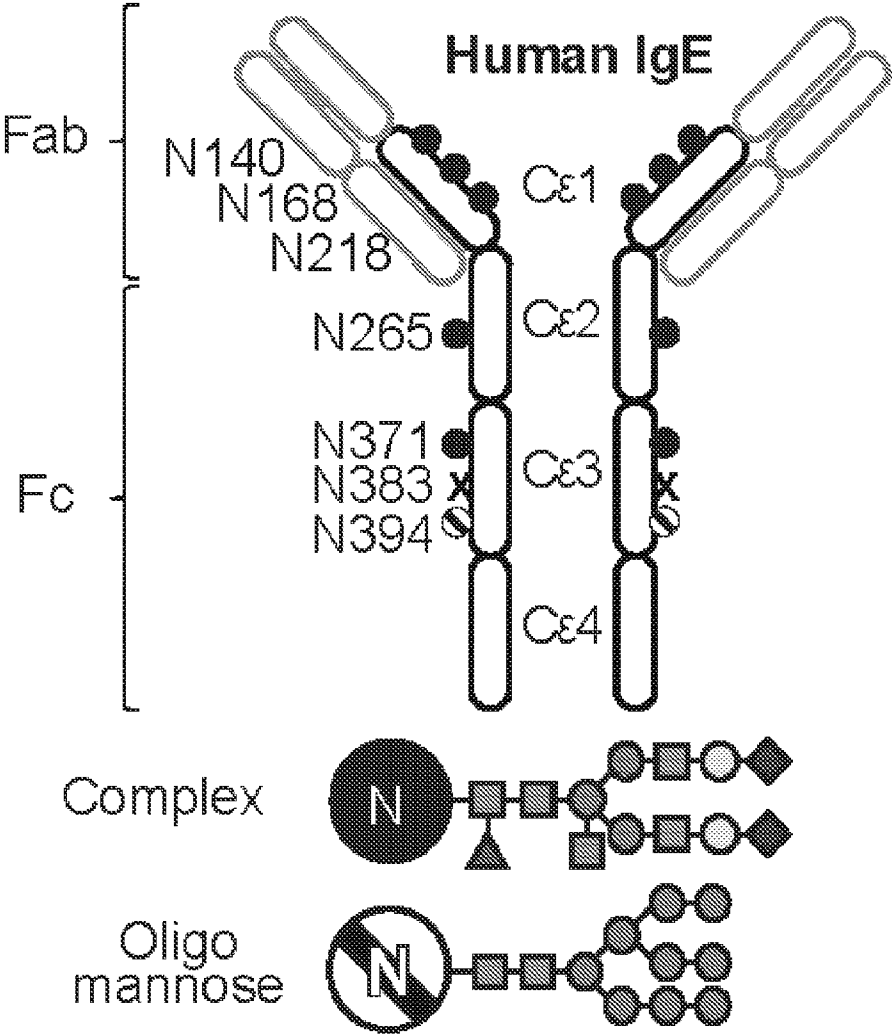


FIG. 1A

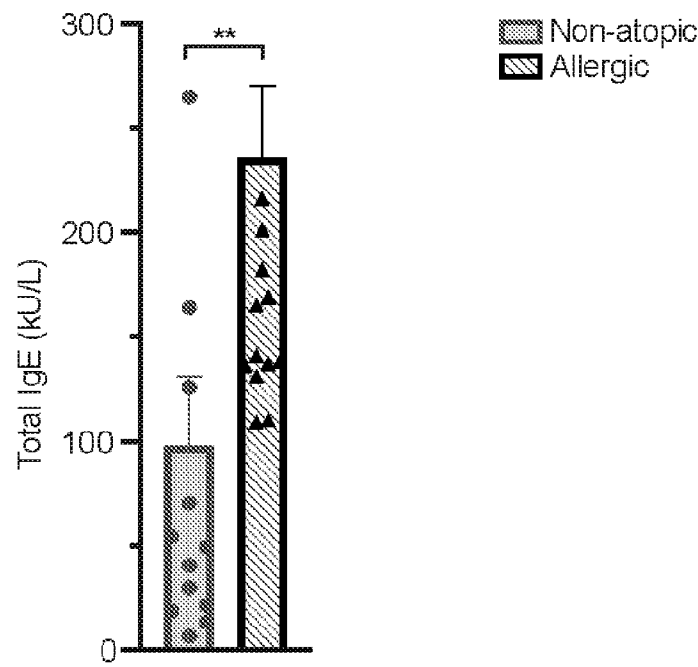


FIG. 1B

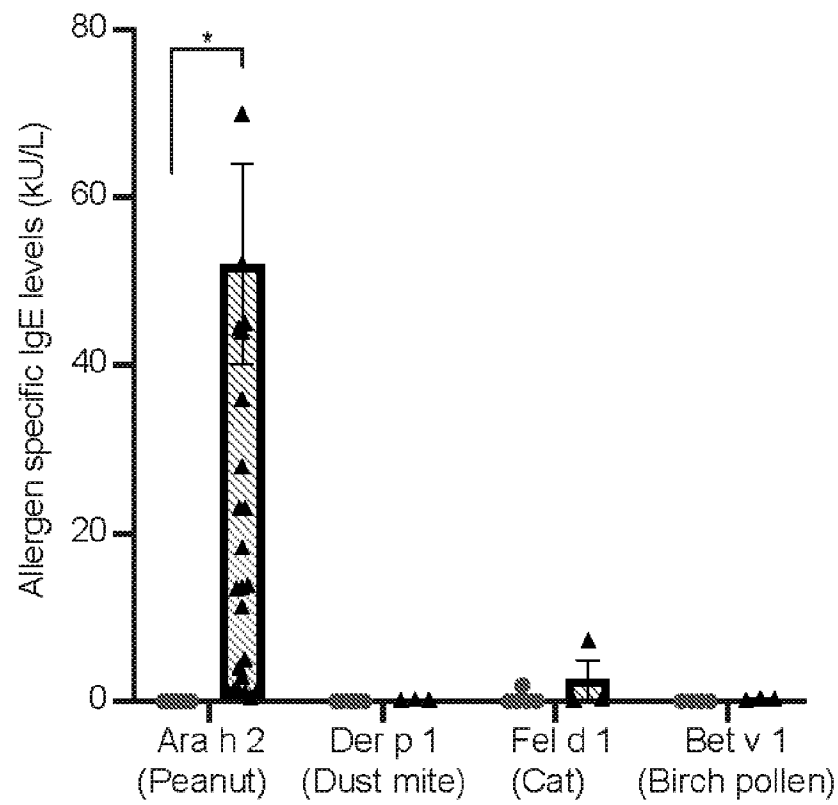


FIG. 1C

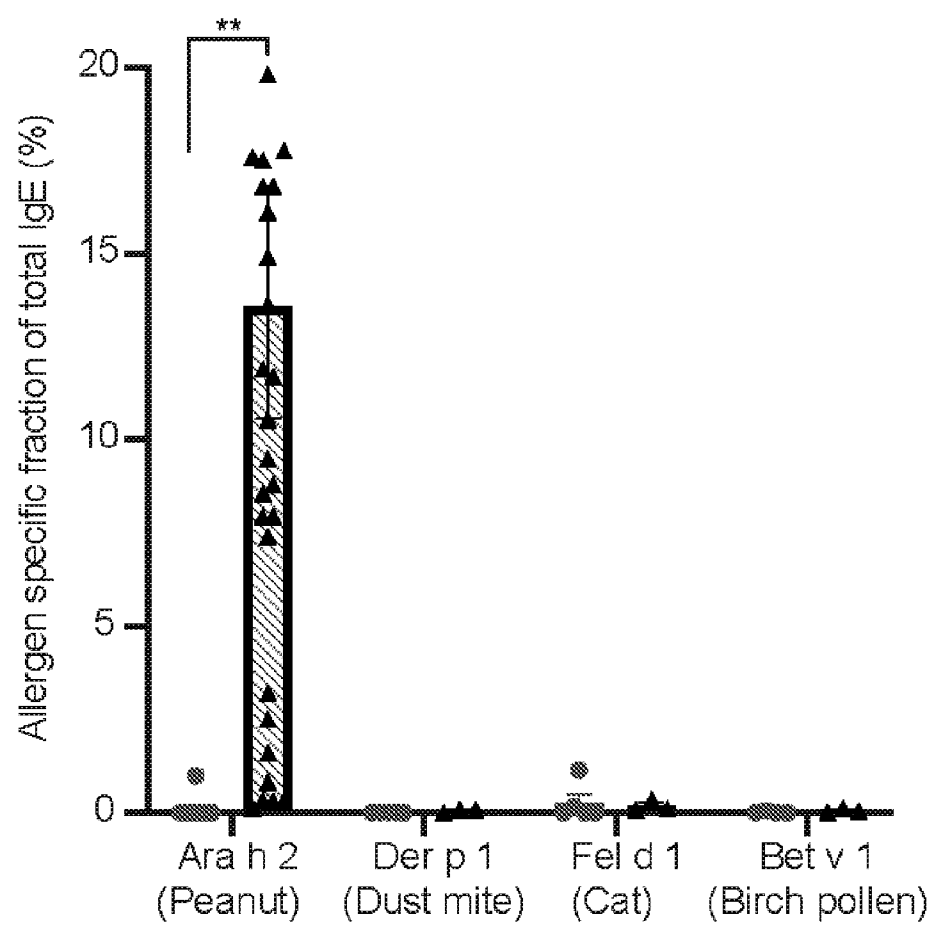


FIG. 1D

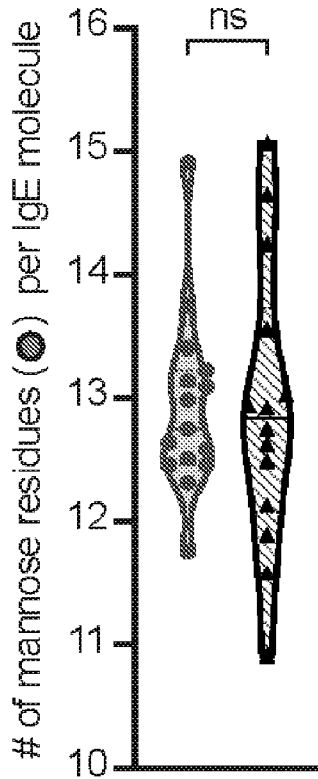


FIG. 1E

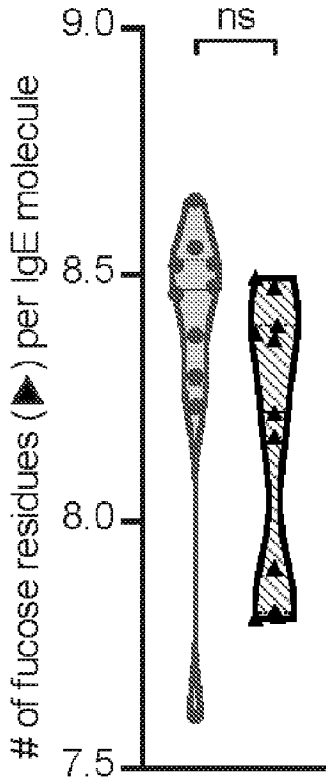


FIG. 1F

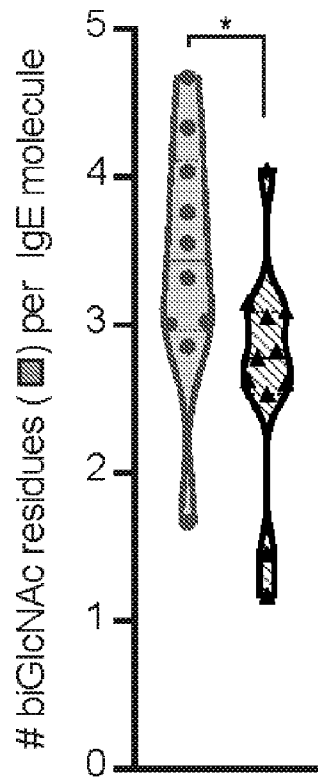


FIG. 1G

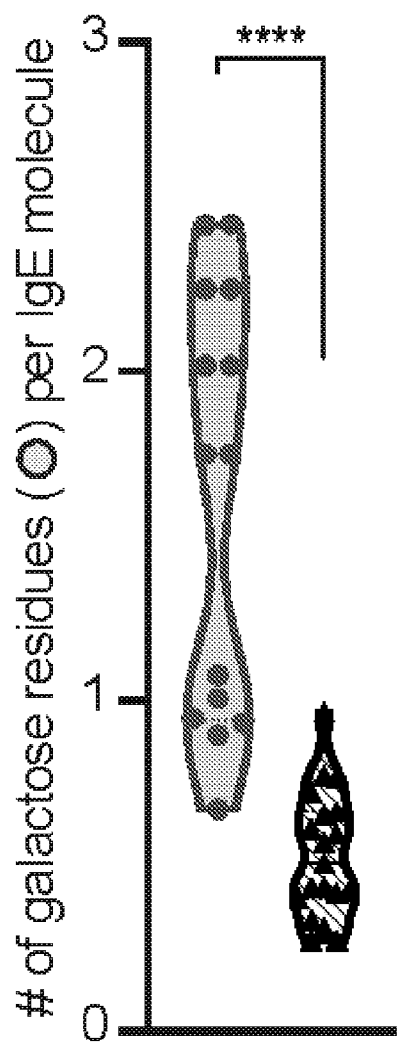


FIG. 1H

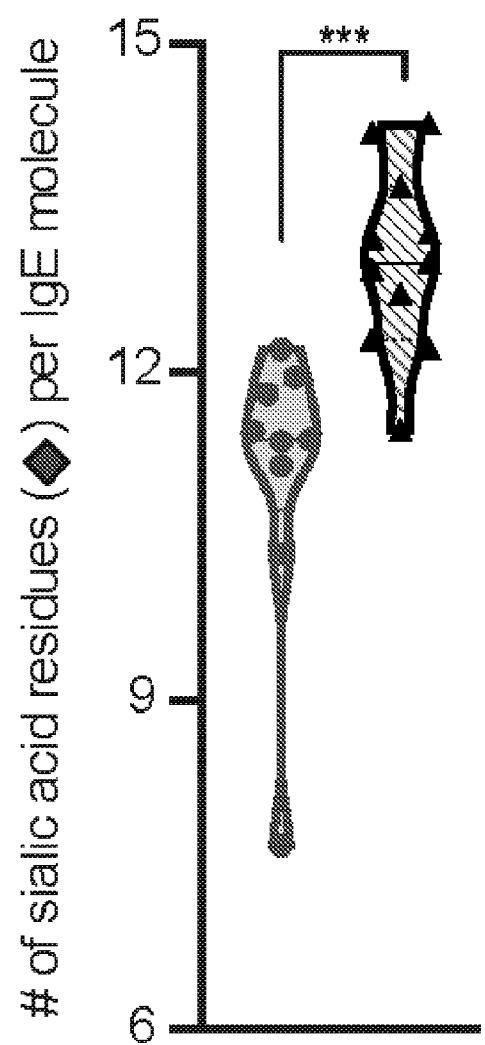


FIG. 1I

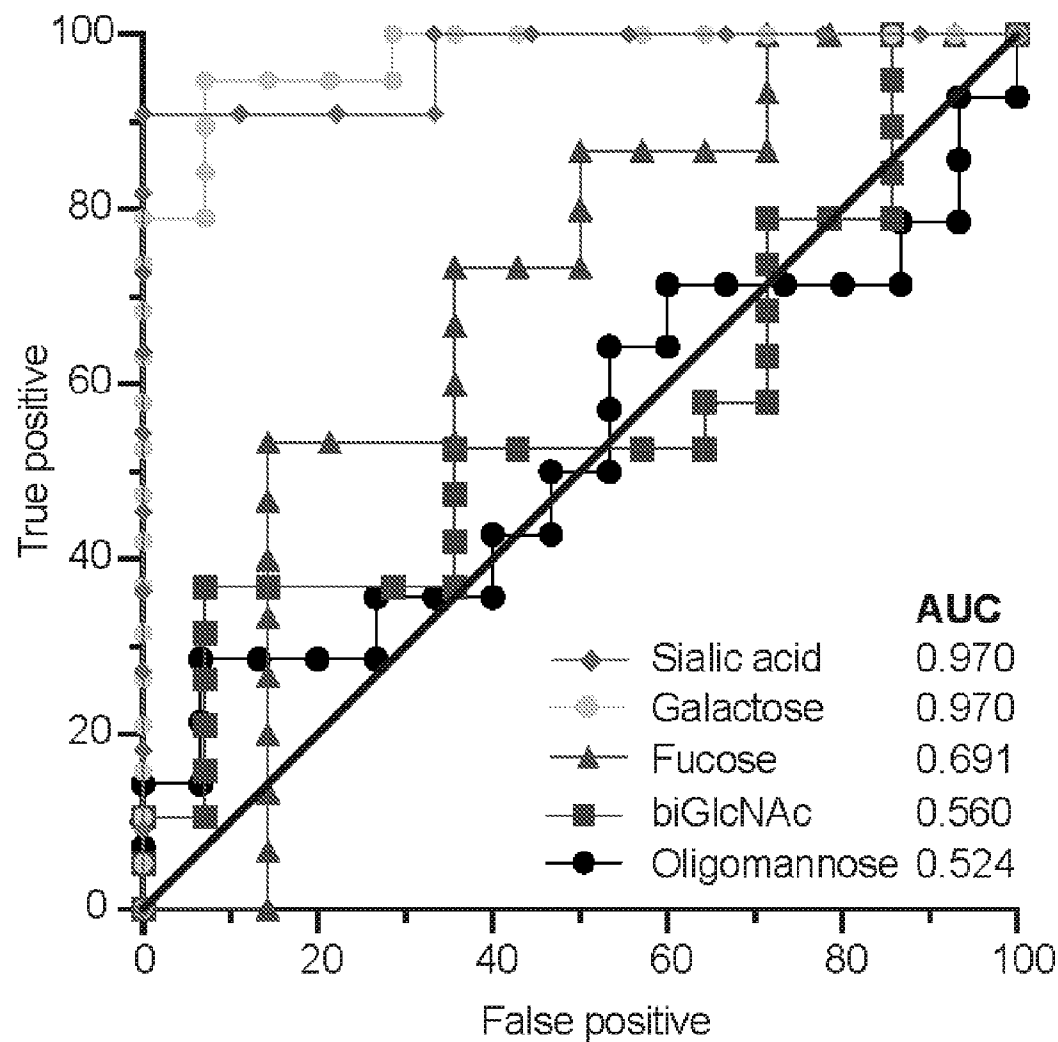


FIG. 2A

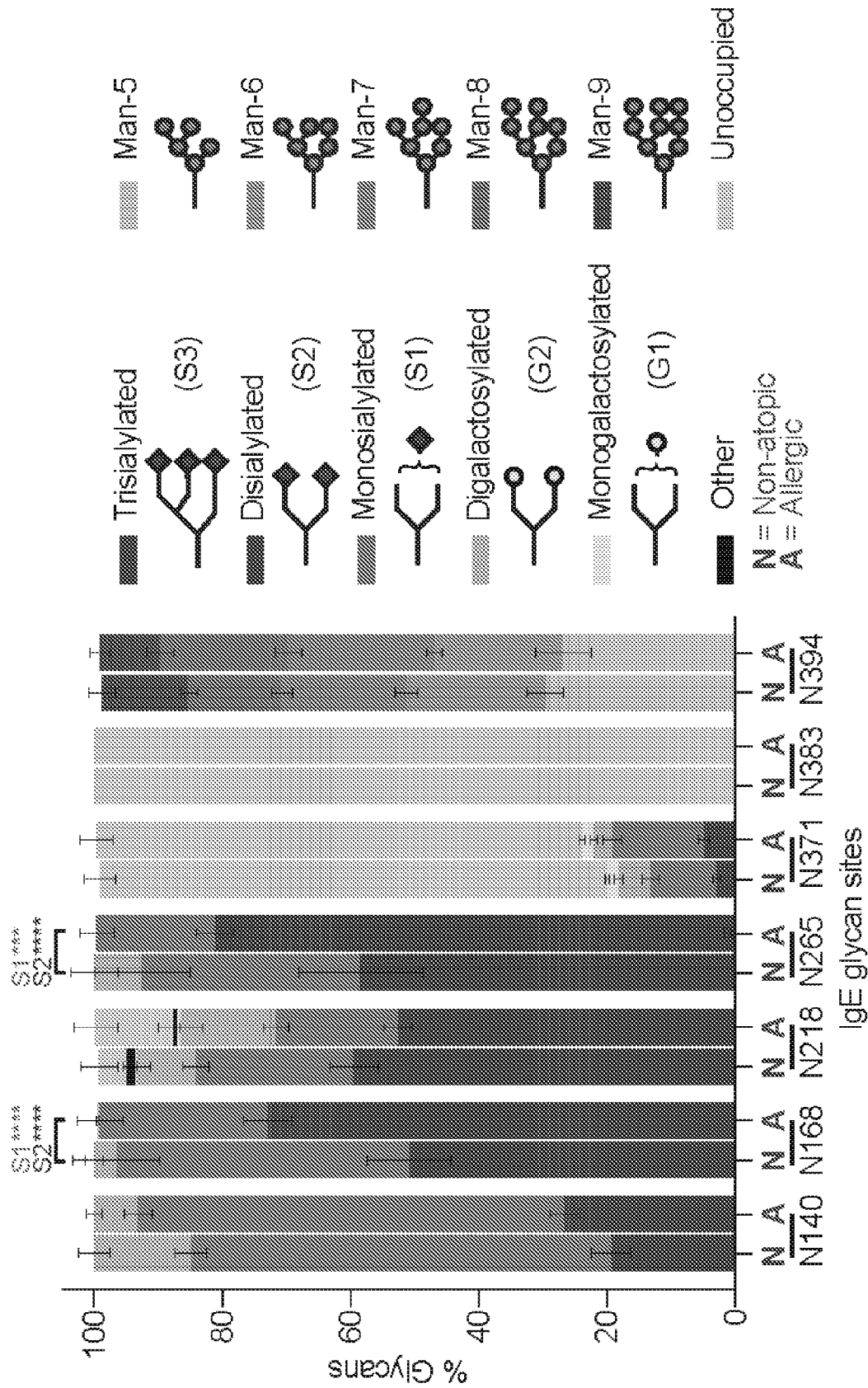


FIG. 2B

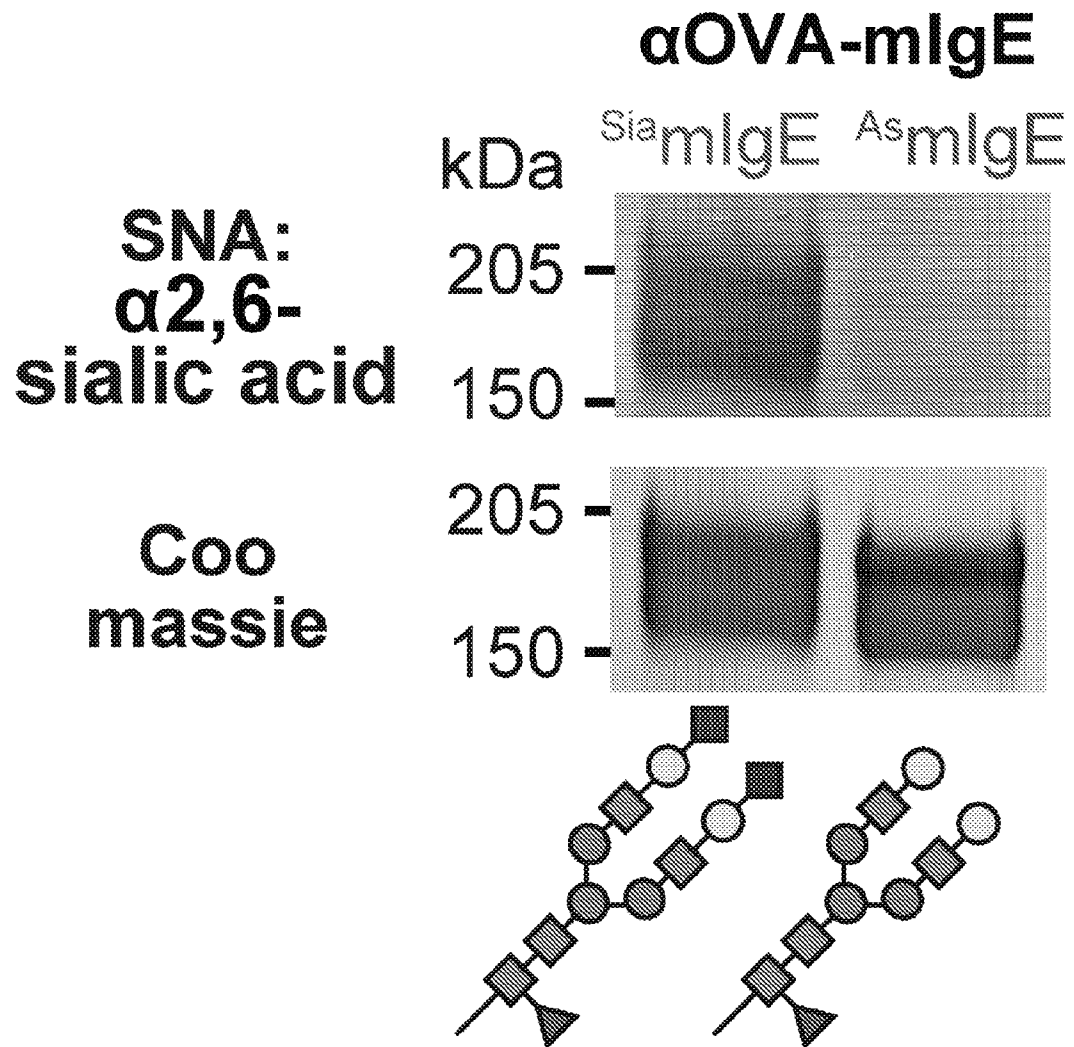


FIG. 3A

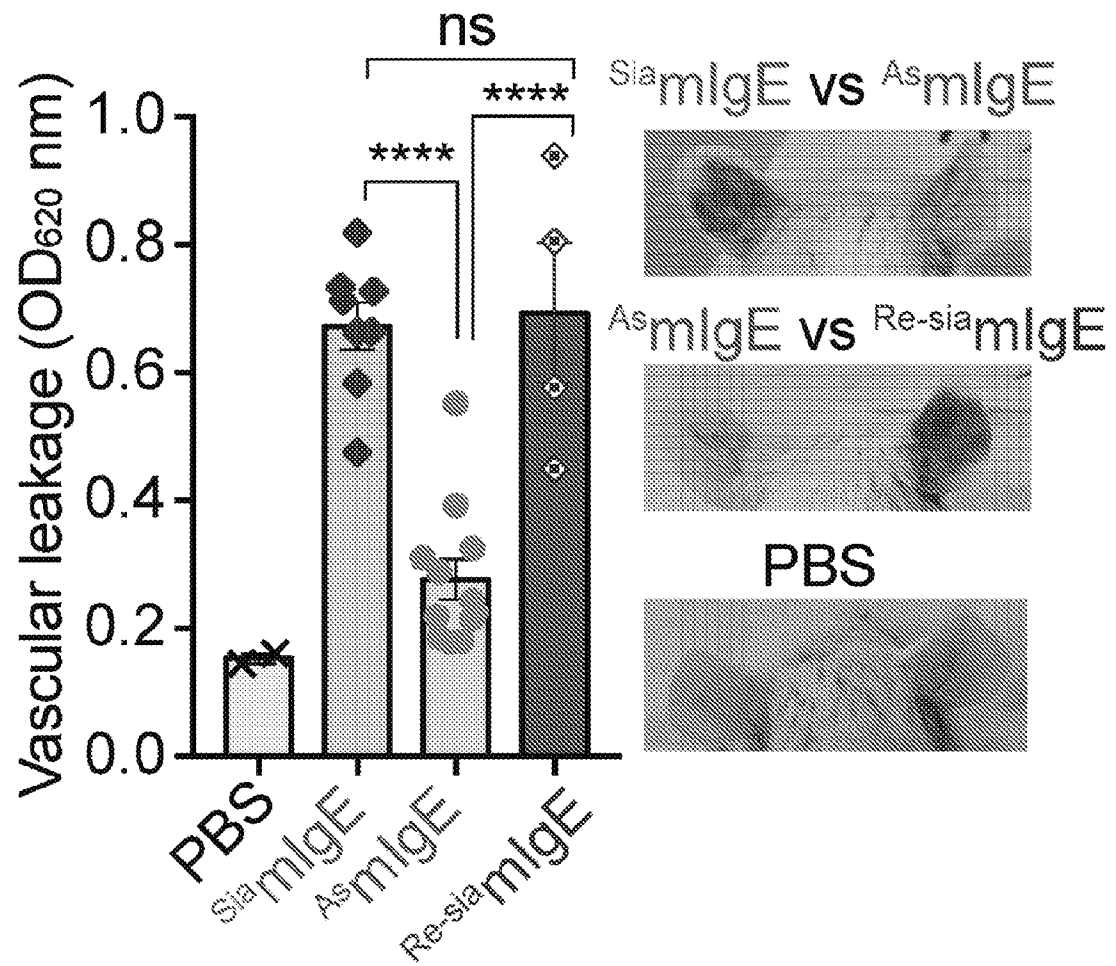


FIG. 3B

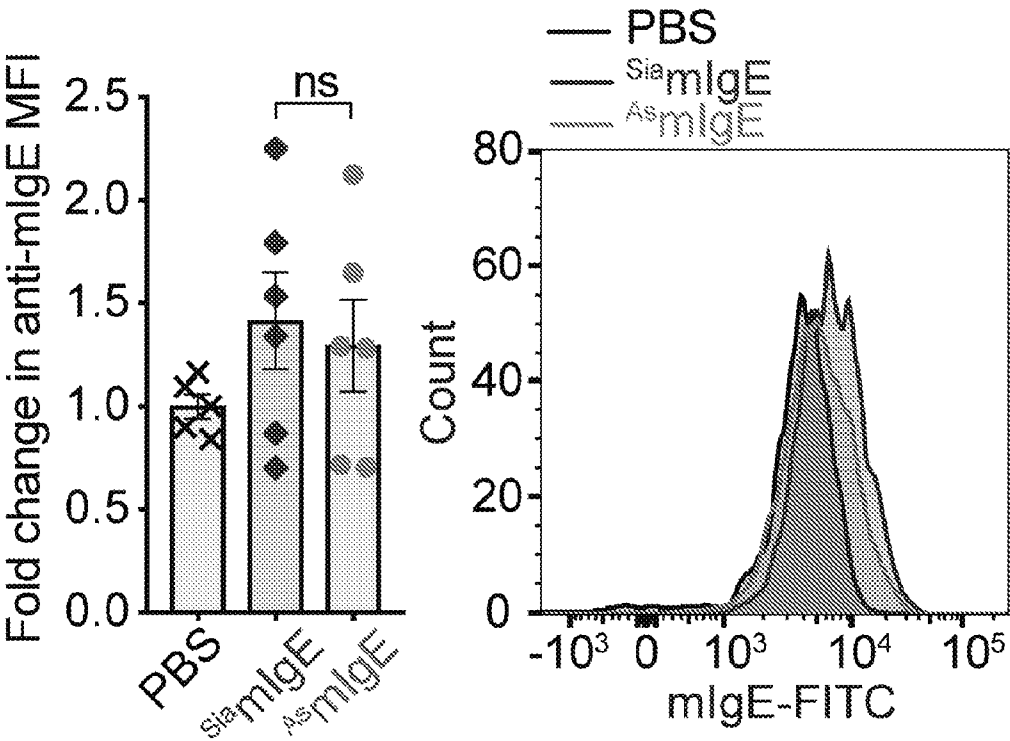


FIG. 3C

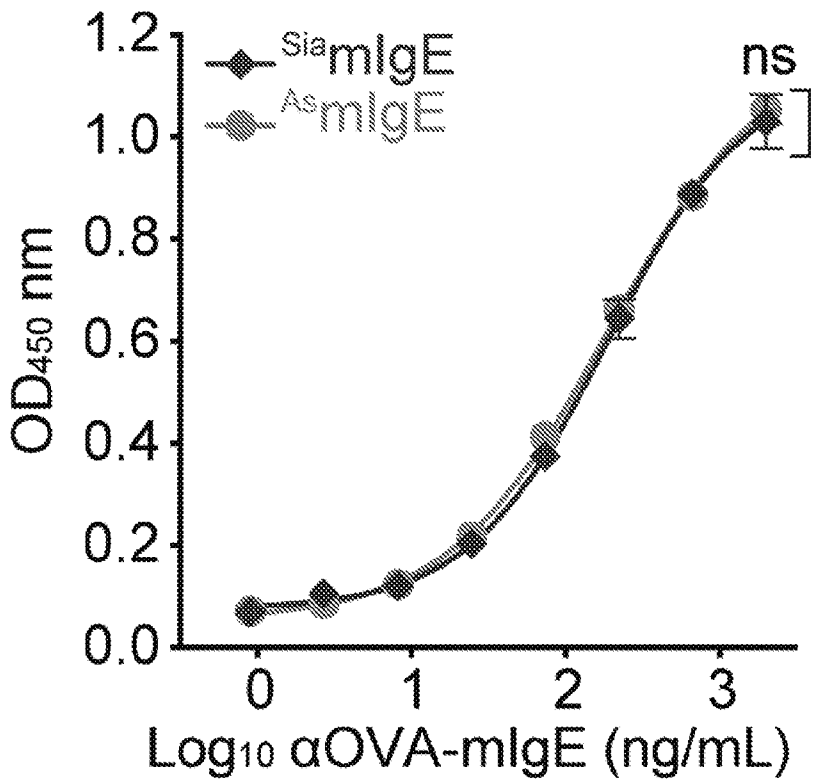


FIG. 3D

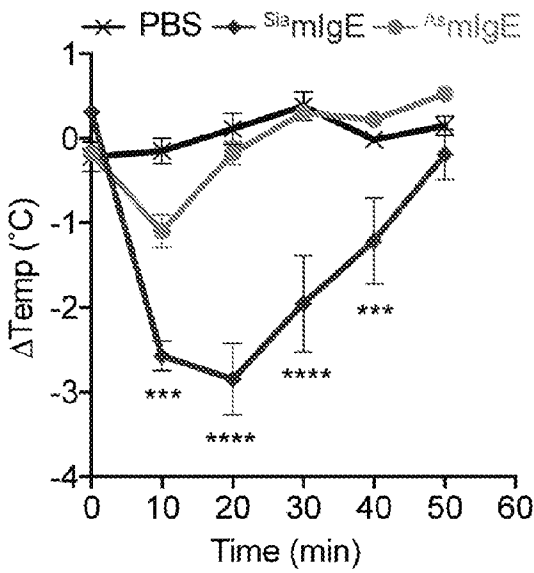


FIG. 3E

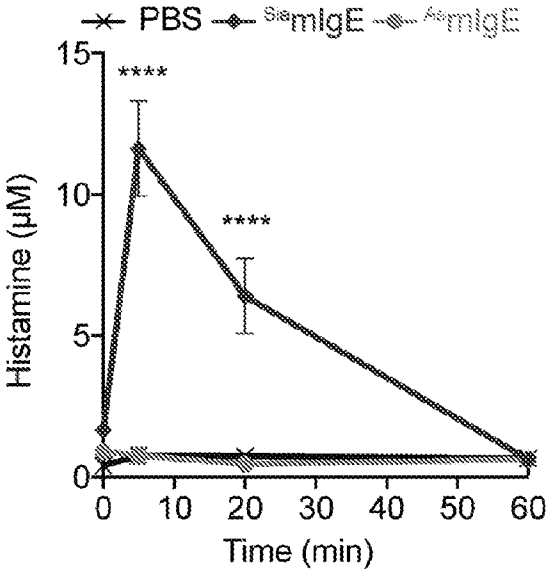


FIG. 3F

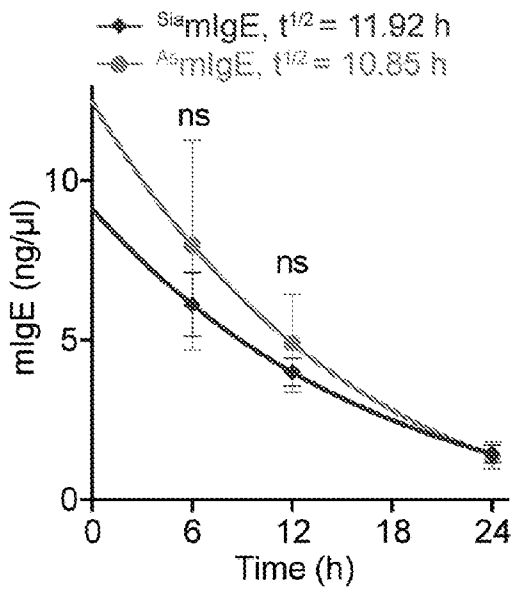


FIG. 3G

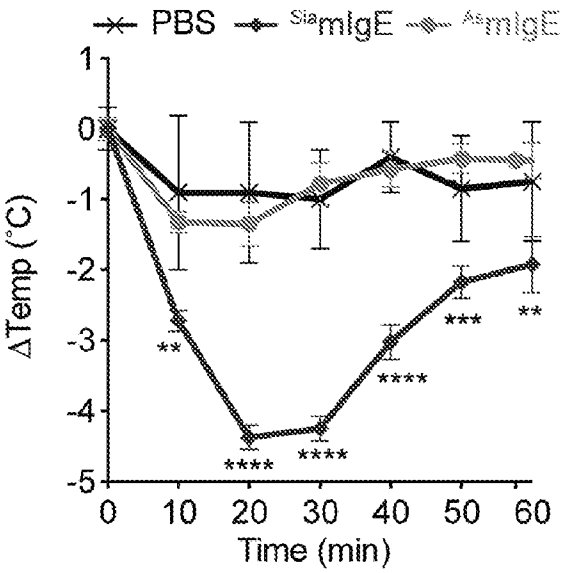
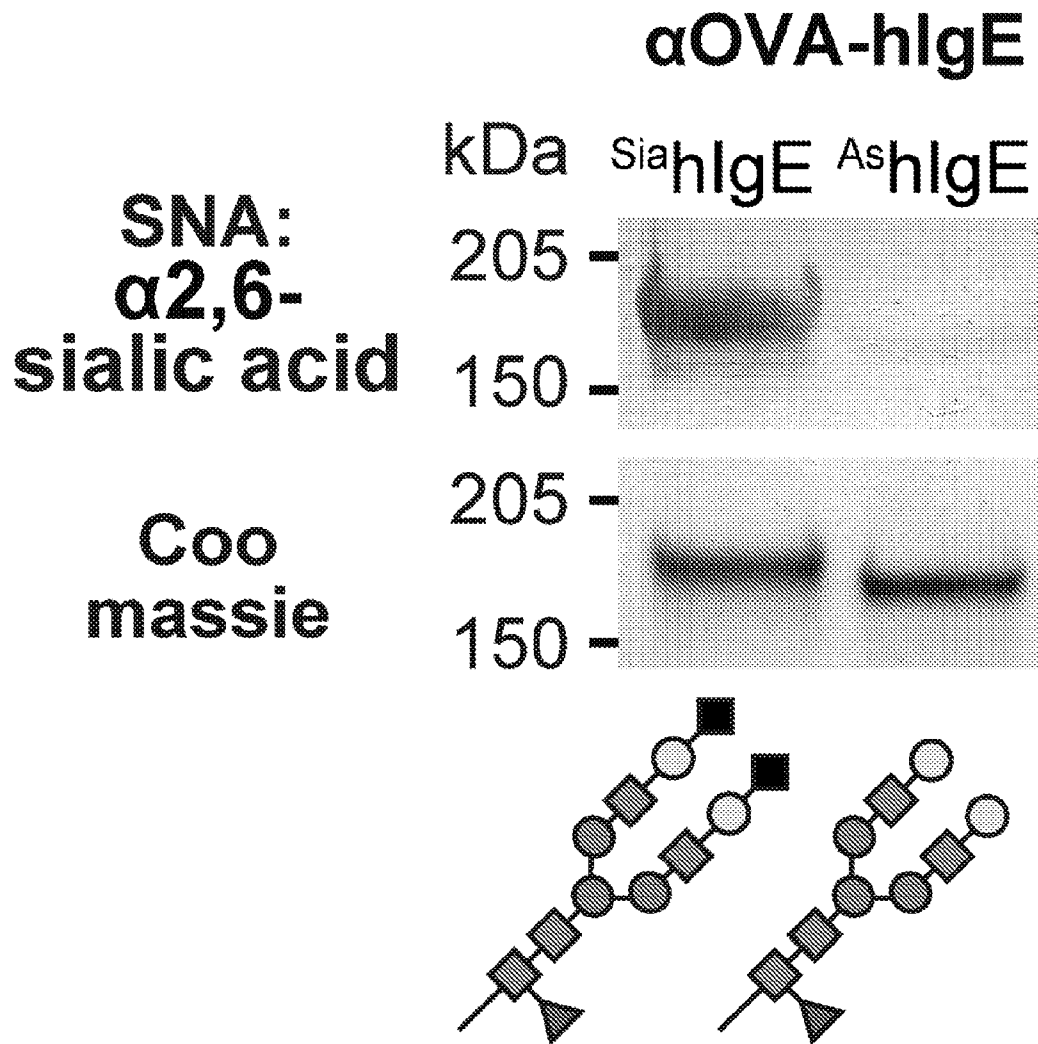


FIG. 3H

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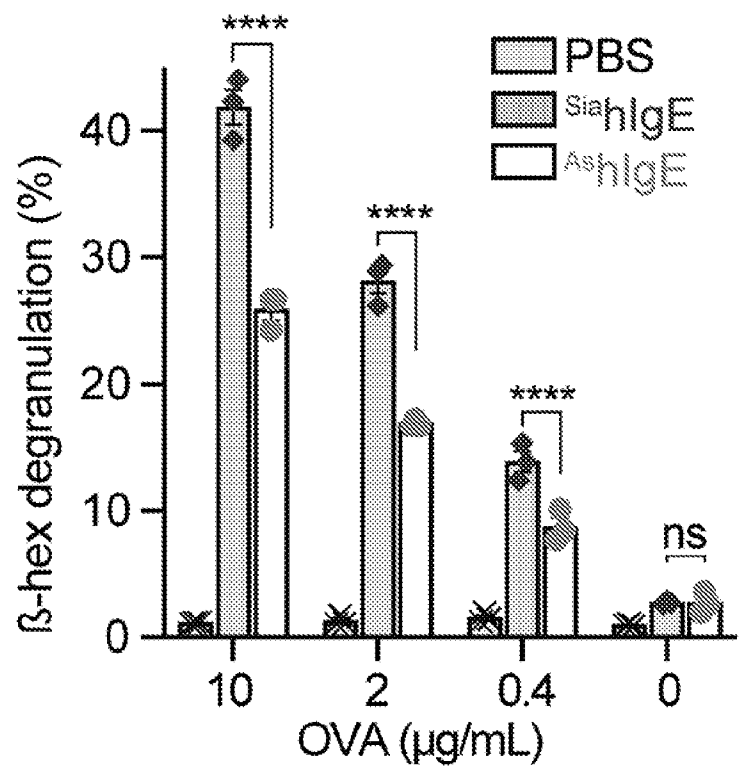


FIG. 3J

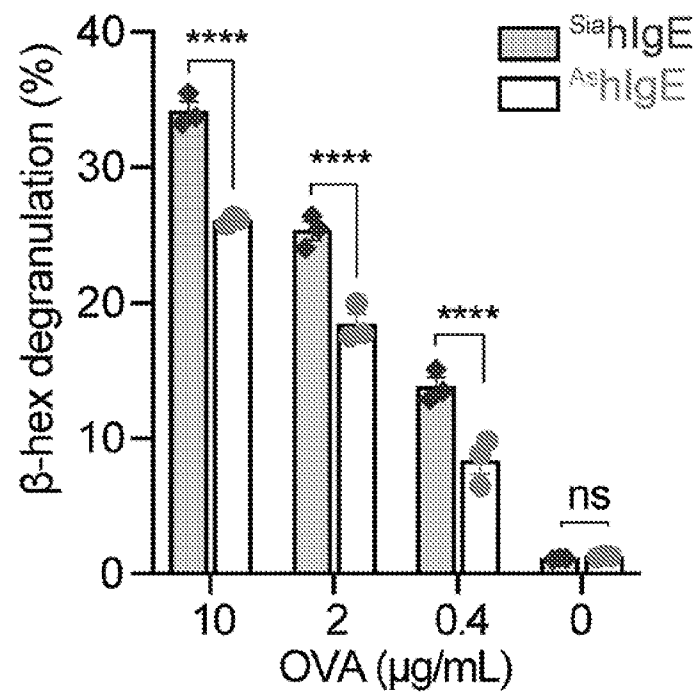


FIG. 3K

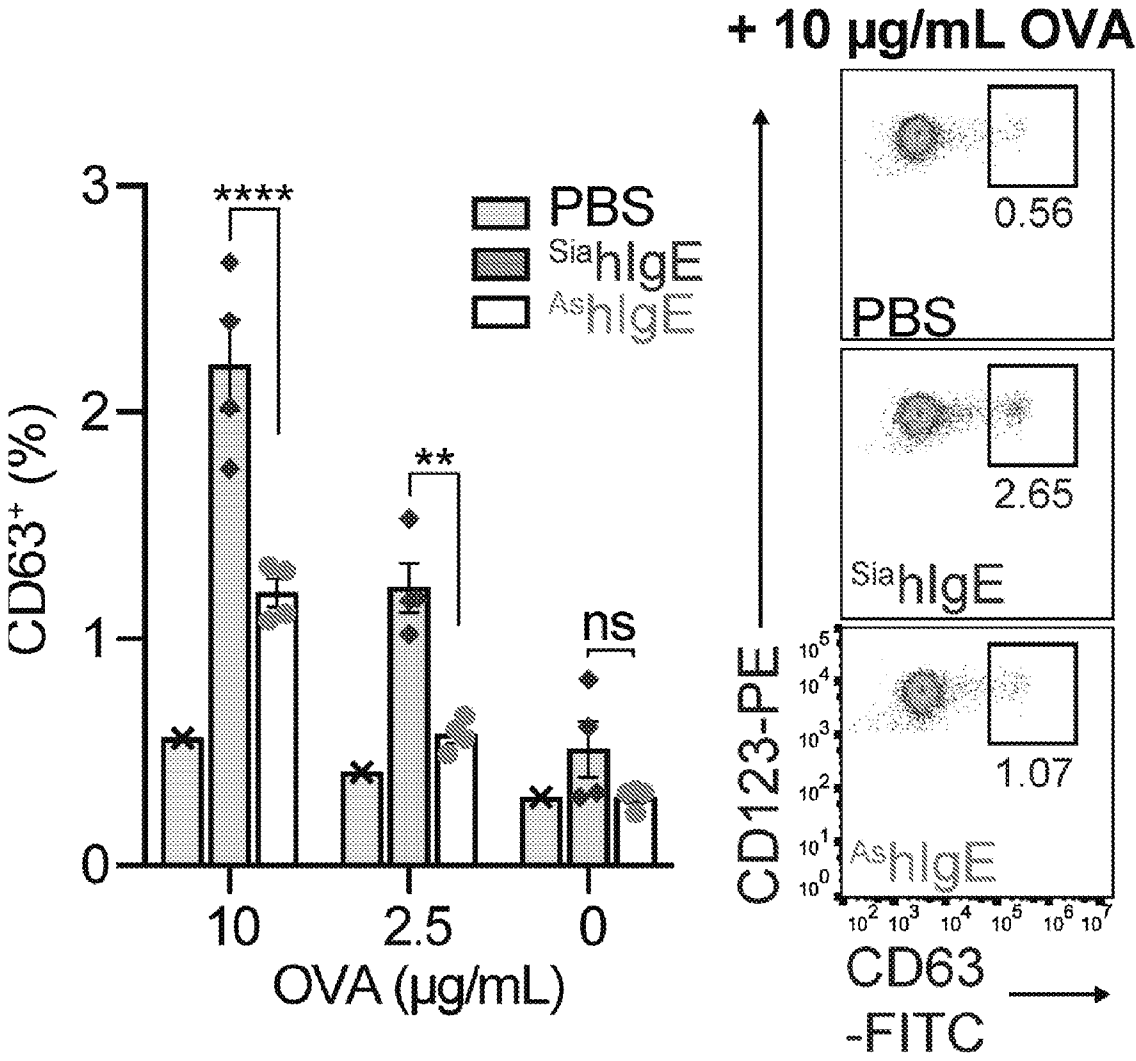


FIG. 3L

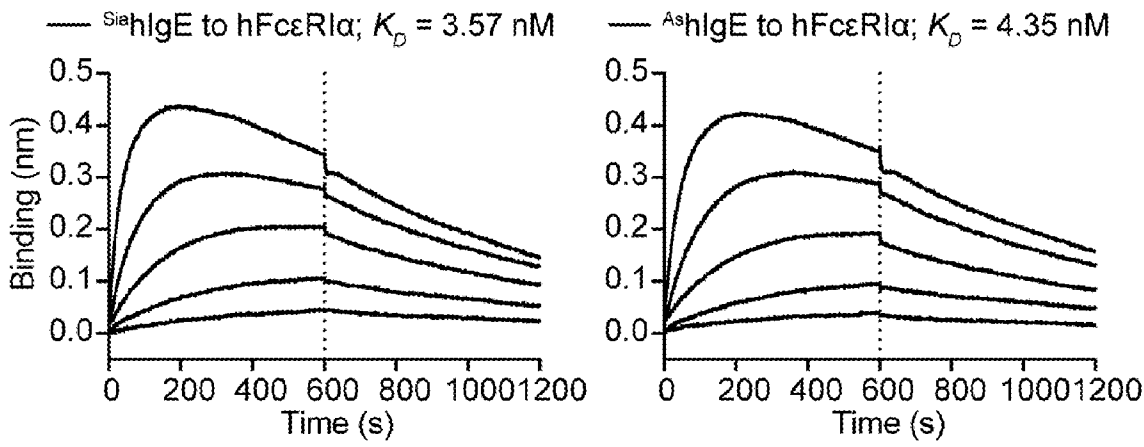


FIG. 3M

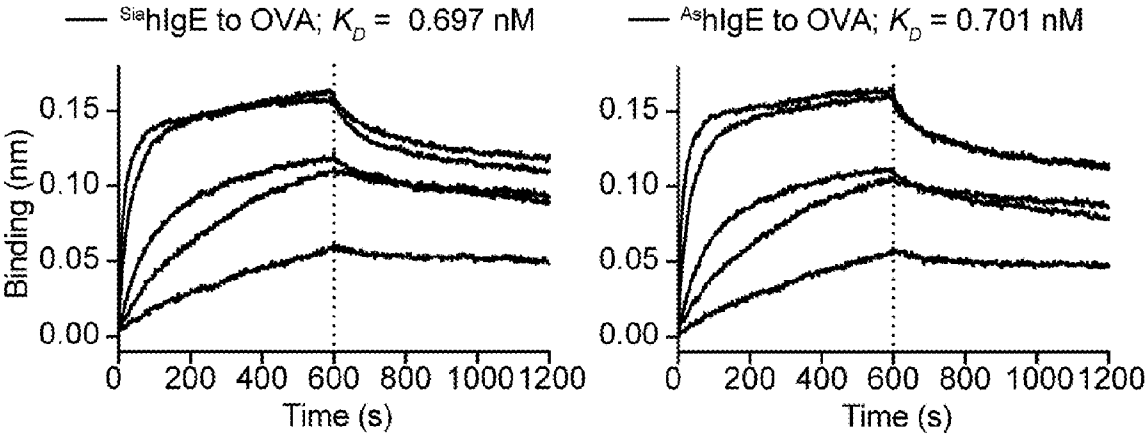


FIG. 3N

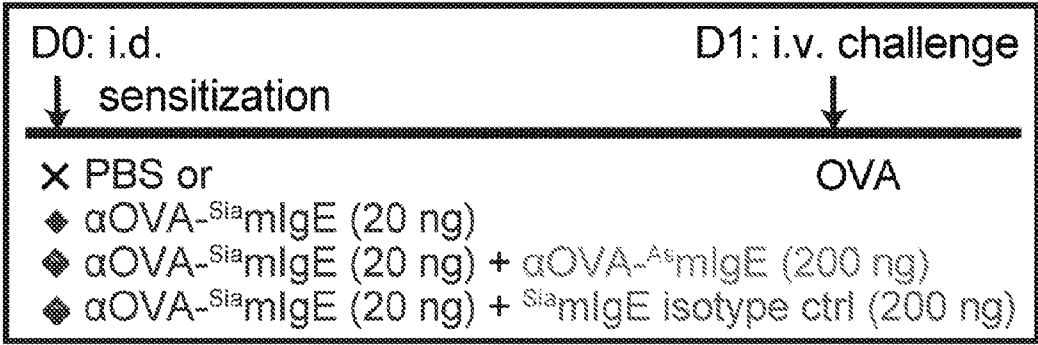
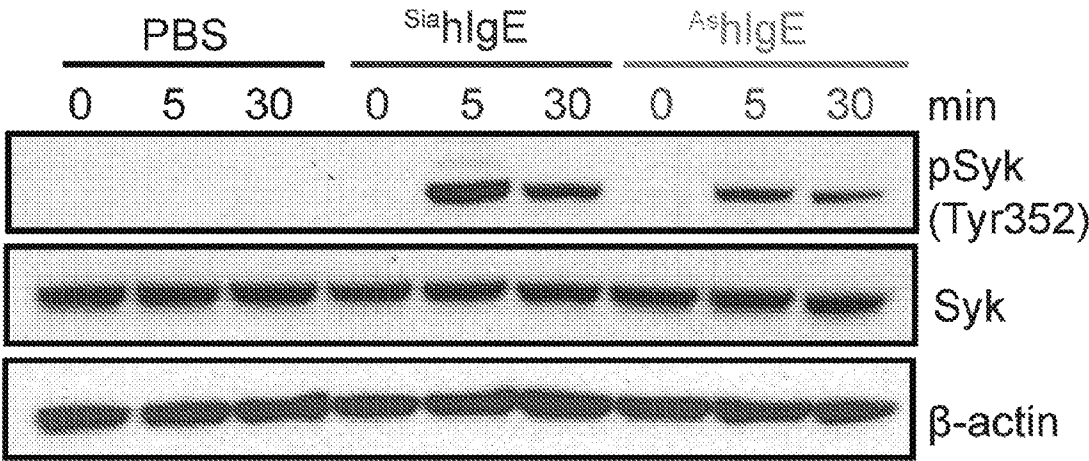
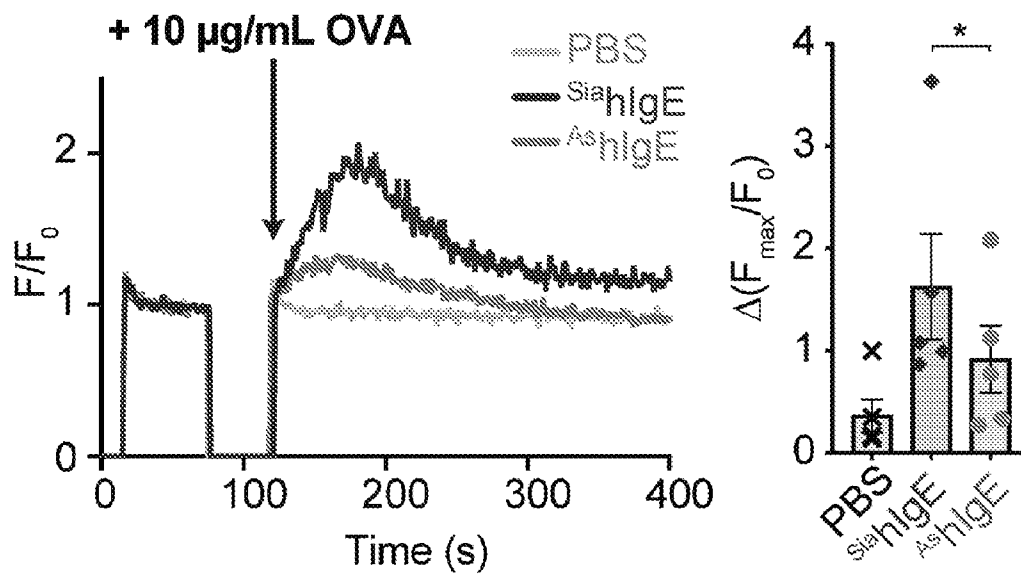


FIG. 4A



D0: i.v. ↓ sensitization	D1: i.v. ↓ treatment	D2: i.v. challenge ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓
✕ αDNP-Sia mlgE	+ PBS	DNP Measure
◊ αDNP-Sia mlgE	+ Sia mlgE isotype ctrl	-HSA Temp
◻ αDNP-Sia mlgE	+ As mlgE isotype ctrl	

FIG. 4B

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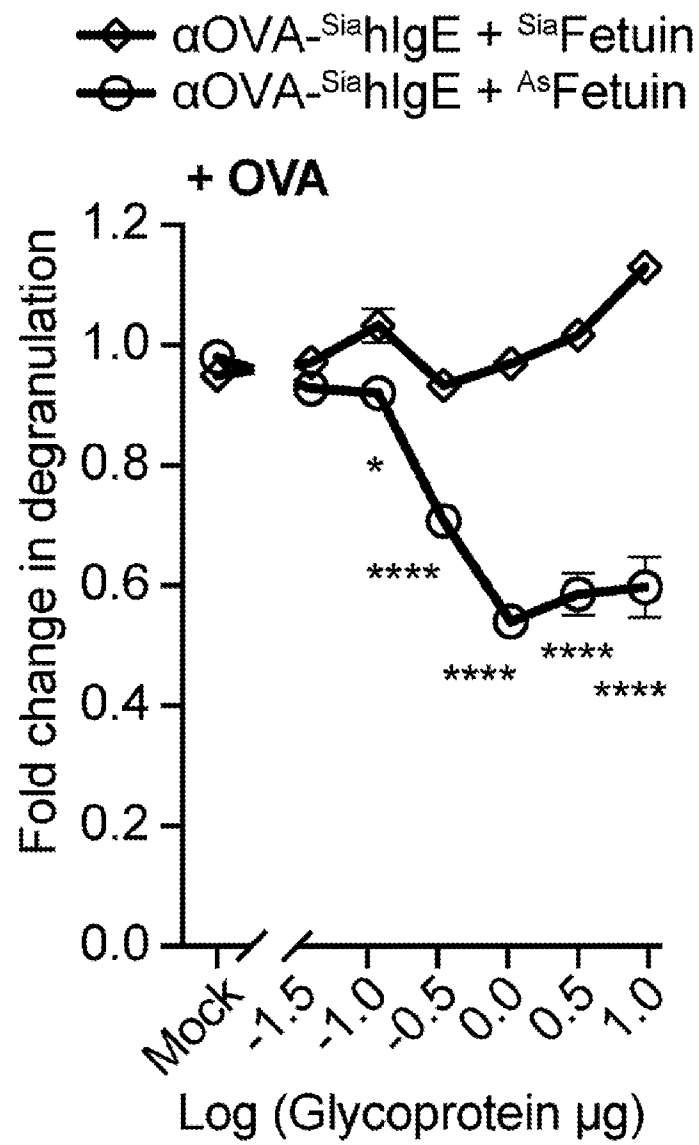


FIG. 4C

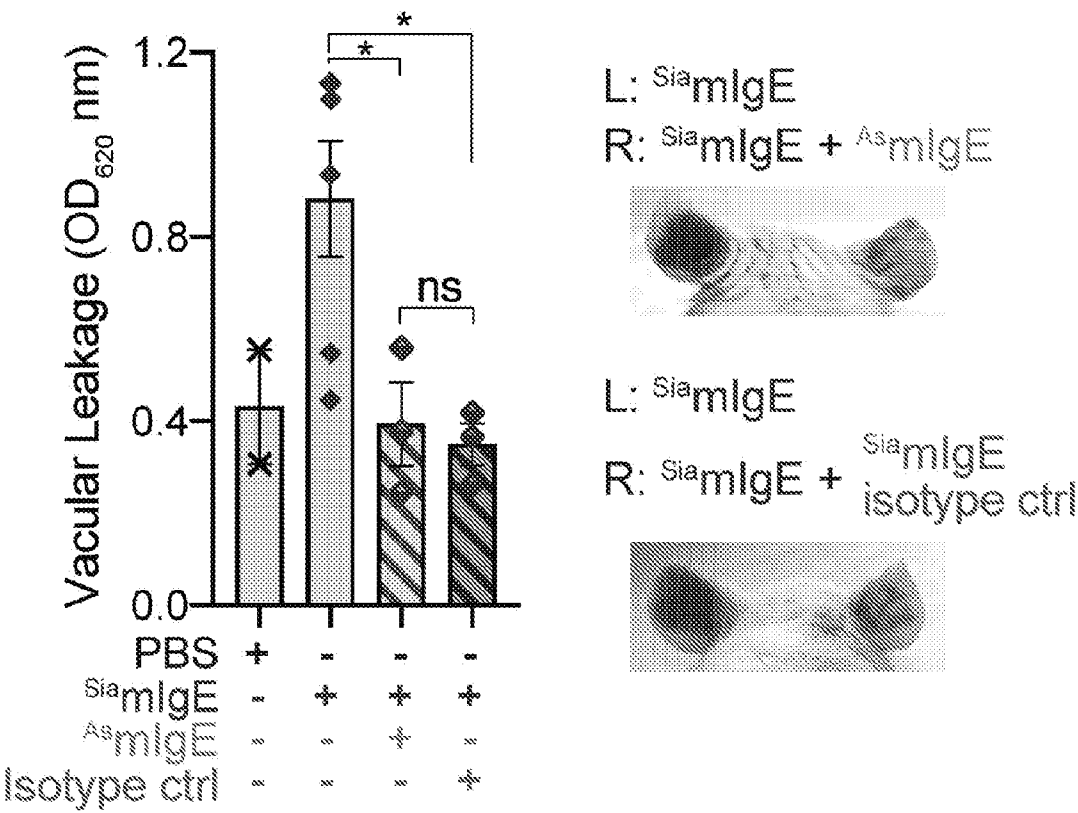


FIG. 4D

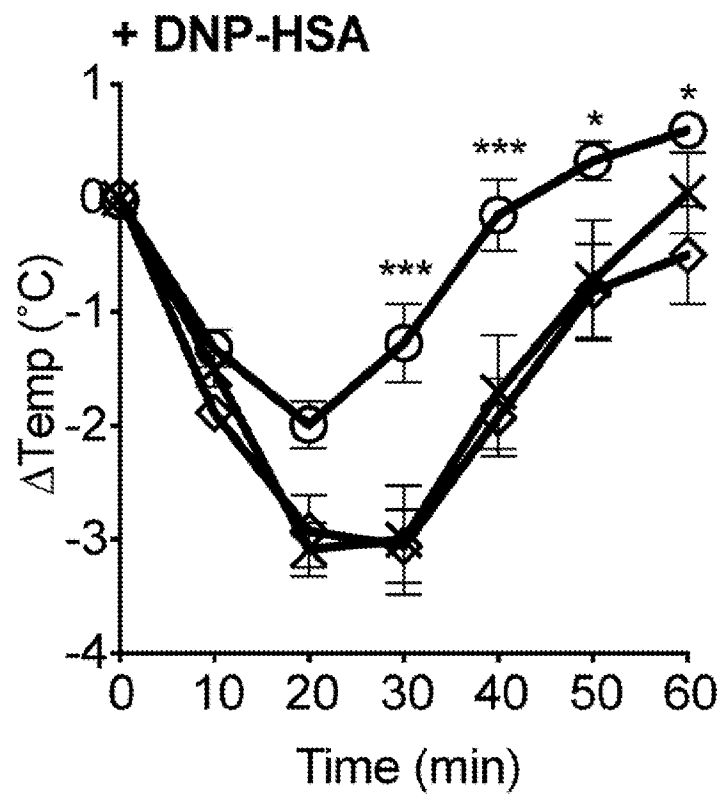


FIG. 4E

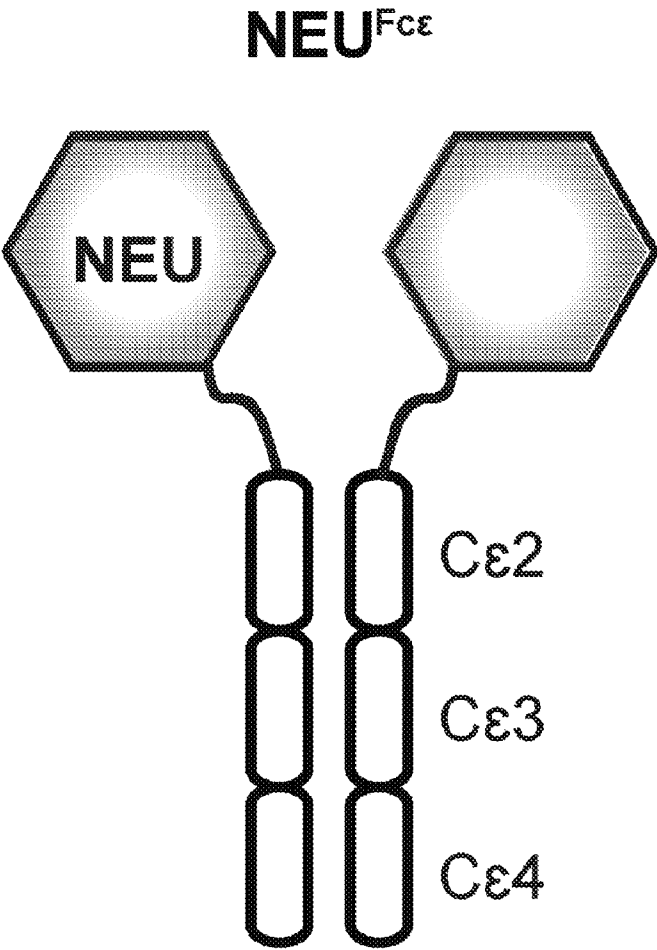


FIG. 4F

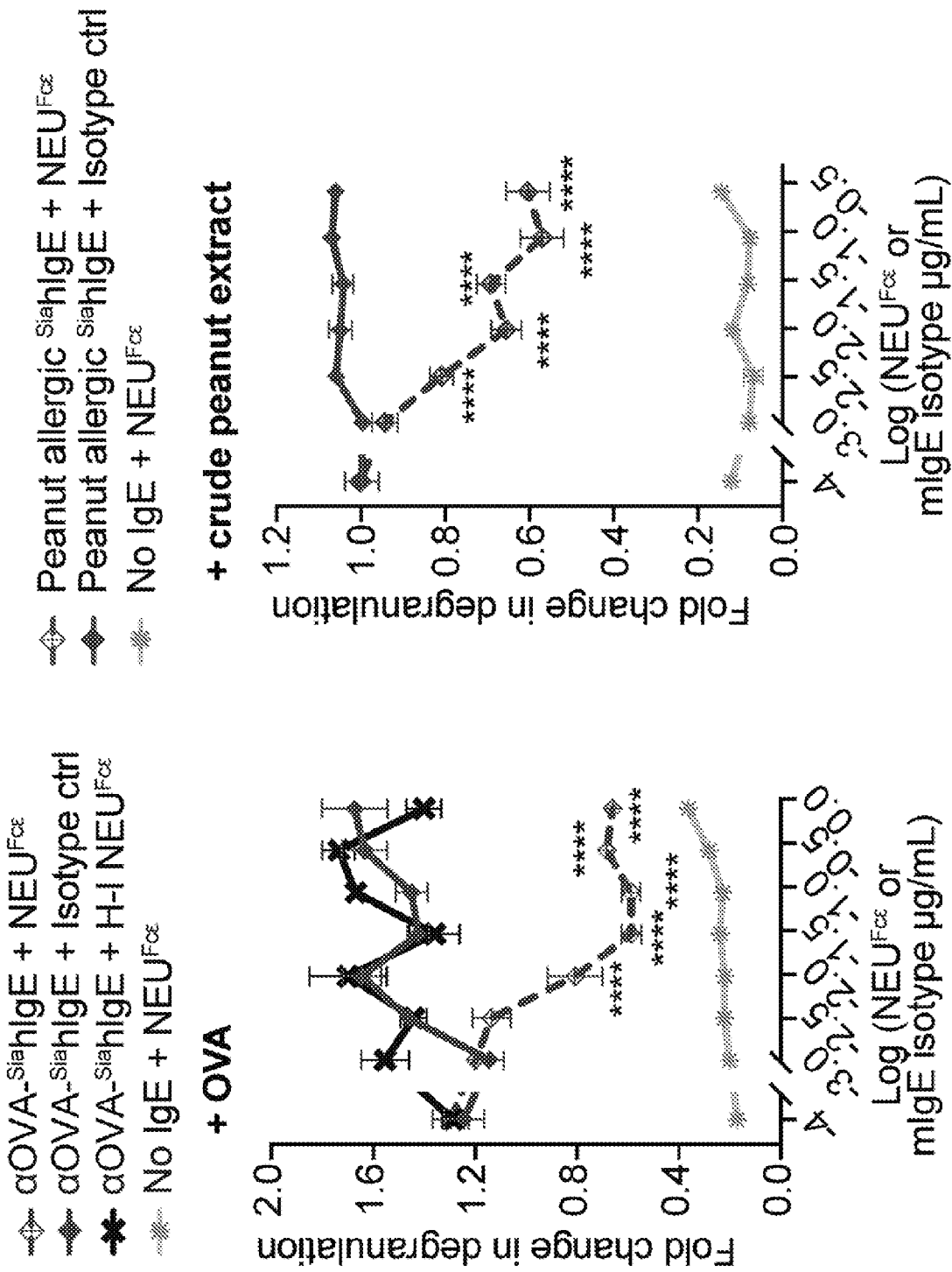


FIG. 4H

FIG. 4G

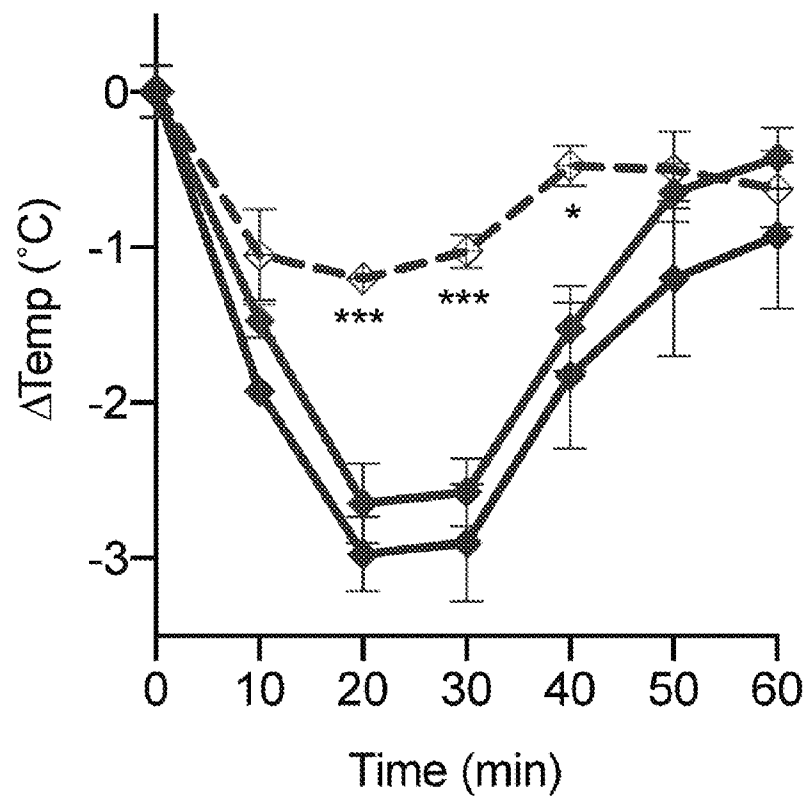
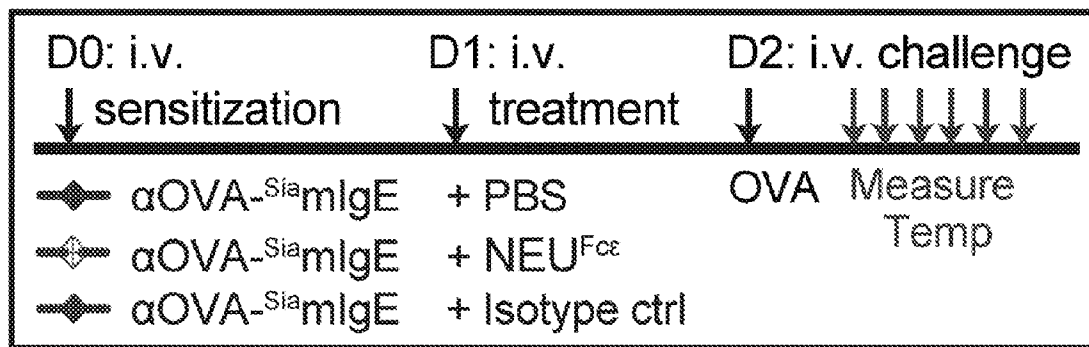


FIG. 4I

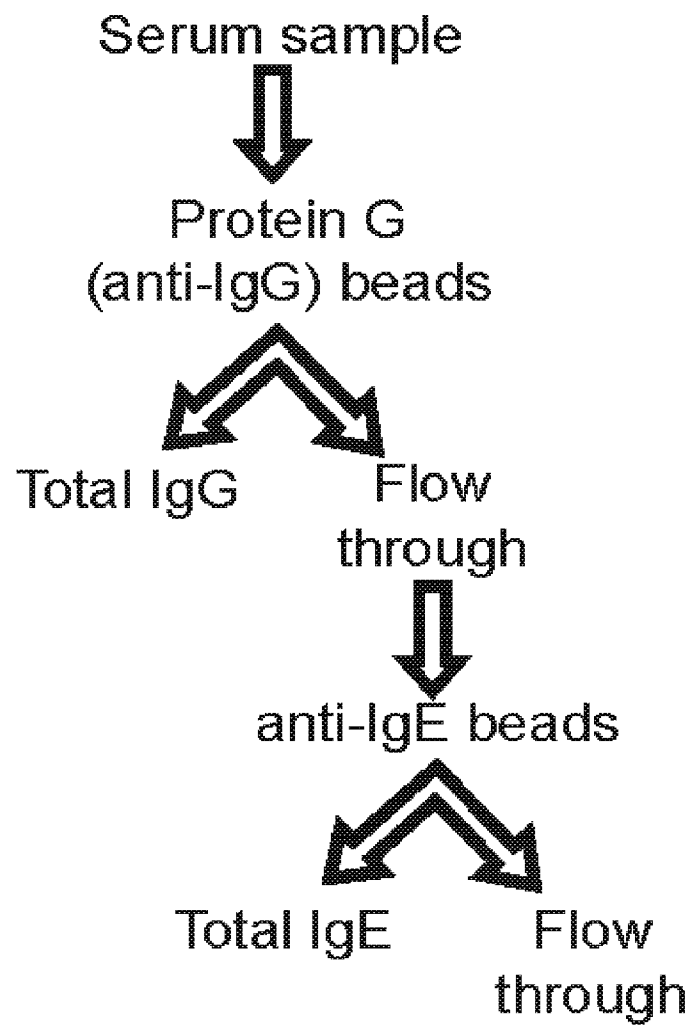


FIG. 5A

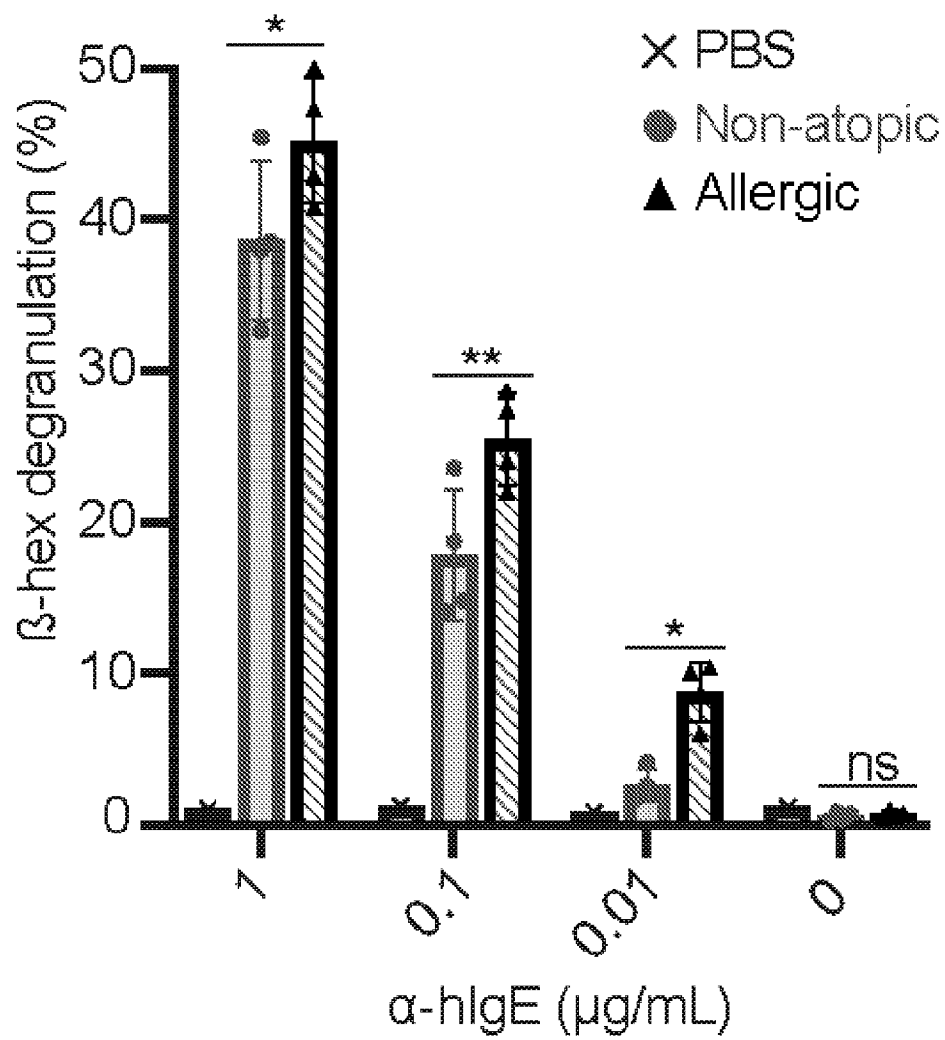


FIG. 5B

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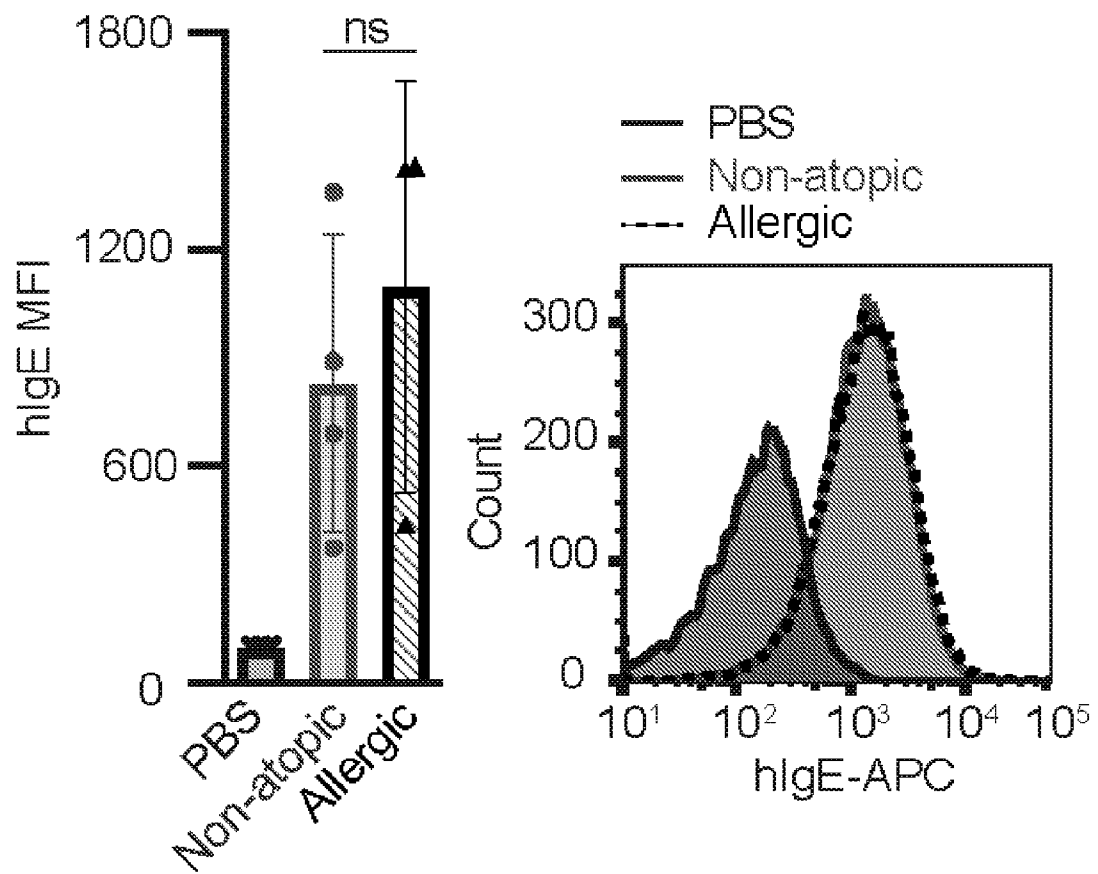


FIG. 5C

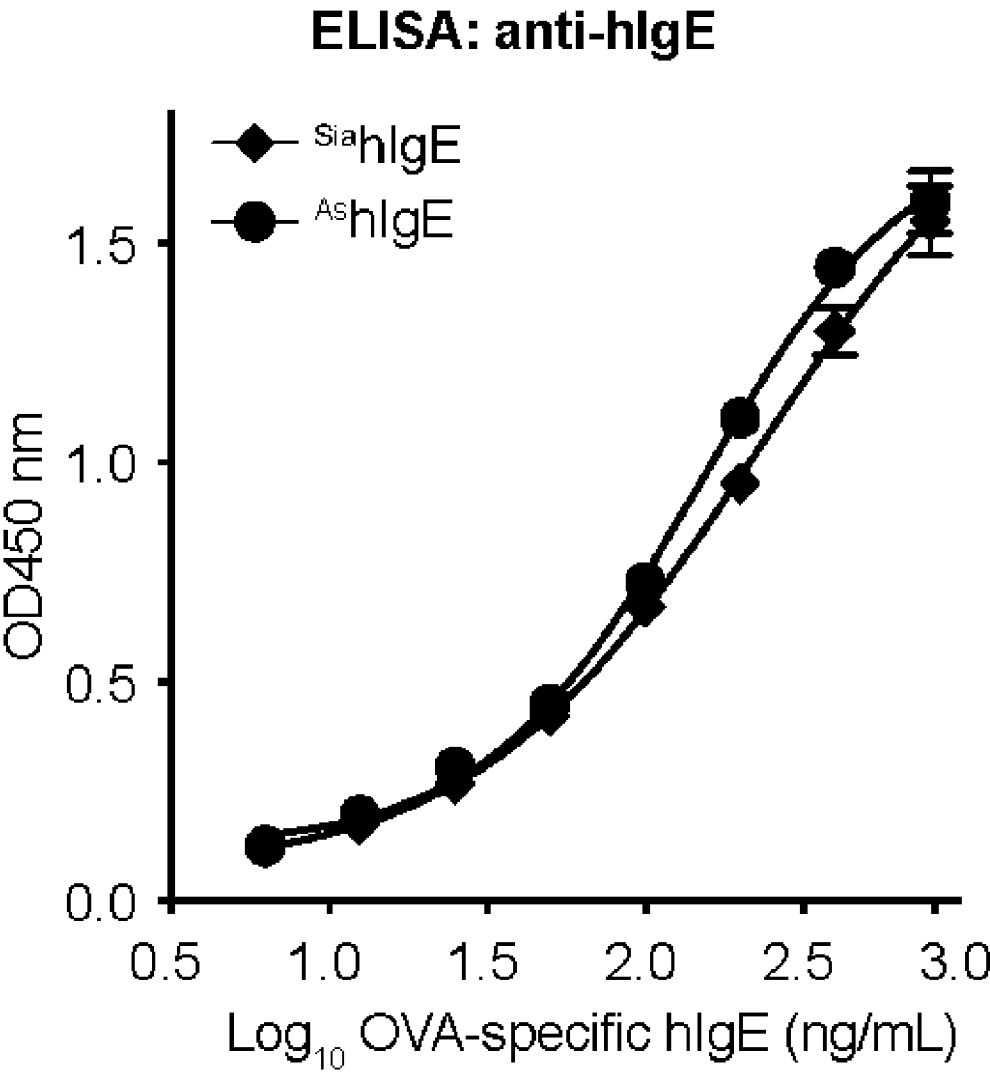


FIG. 5D

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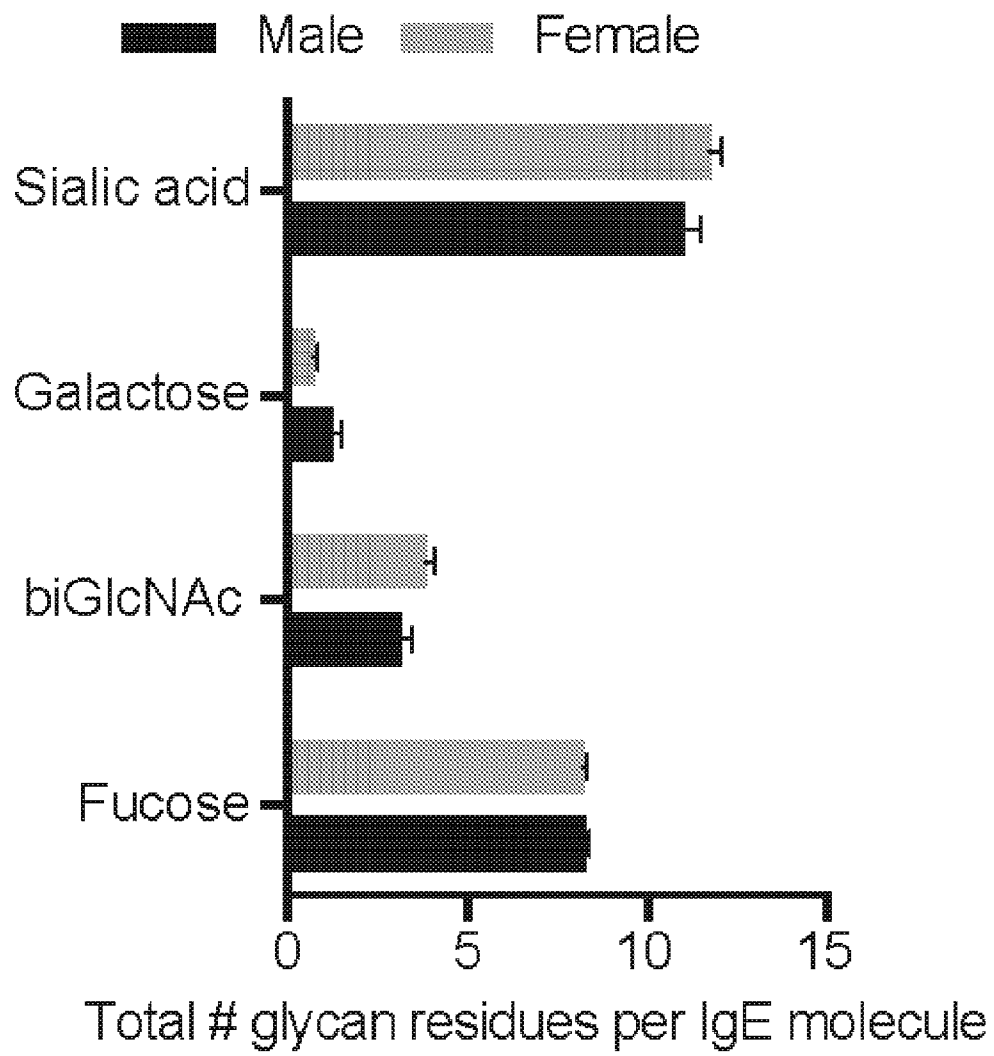


FIG. 5E

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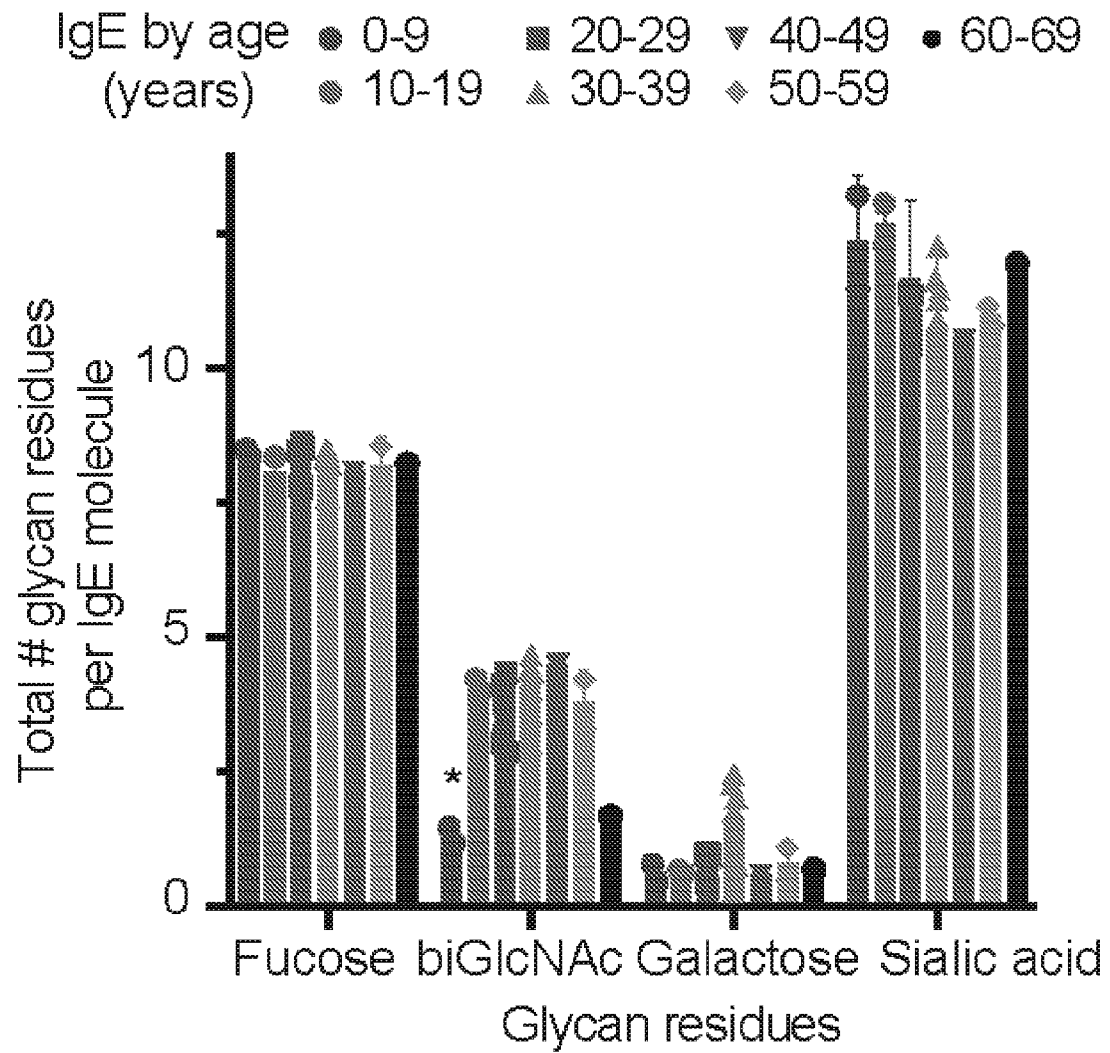


FIG. 5F



FIG. 6A

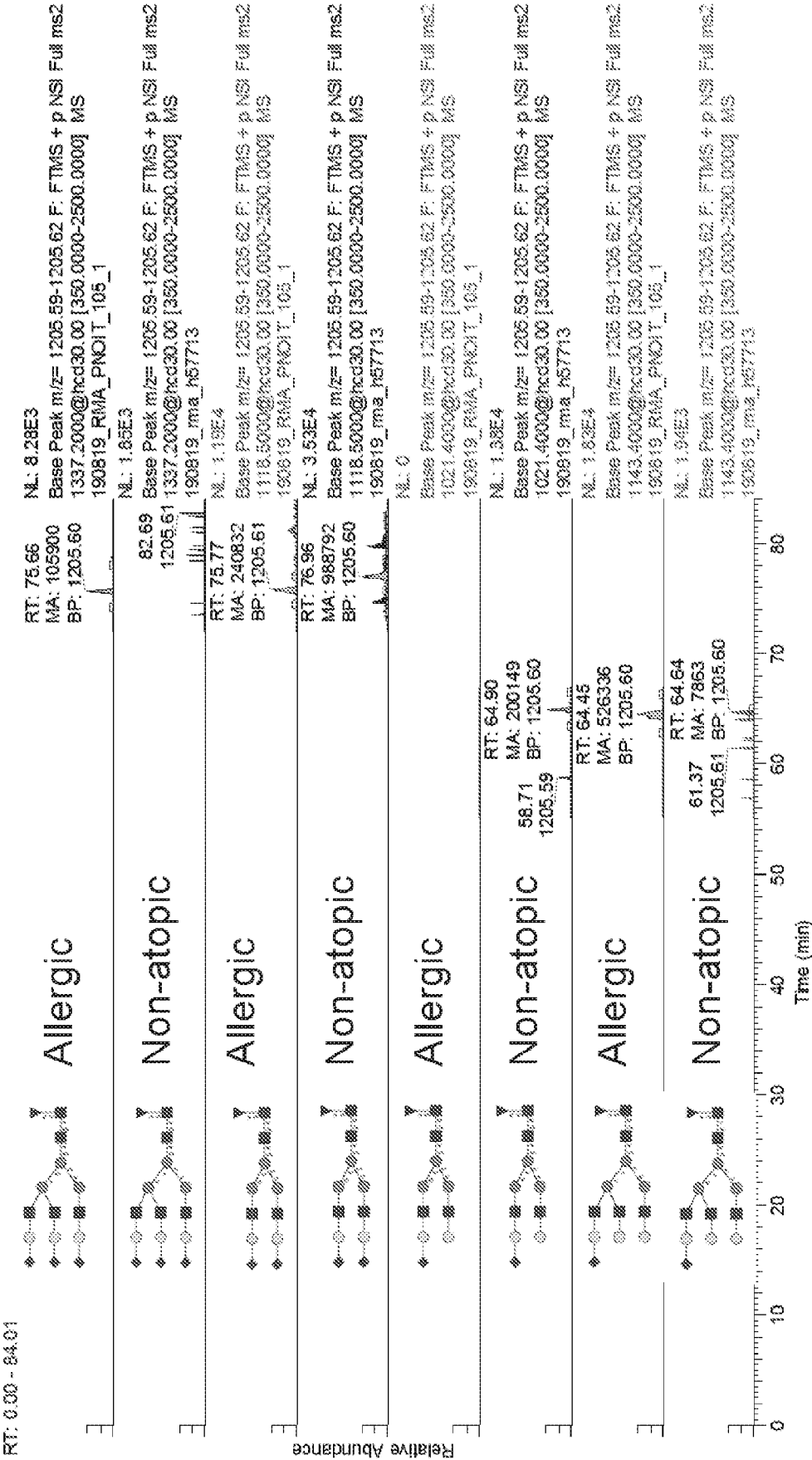


FIG. 6B

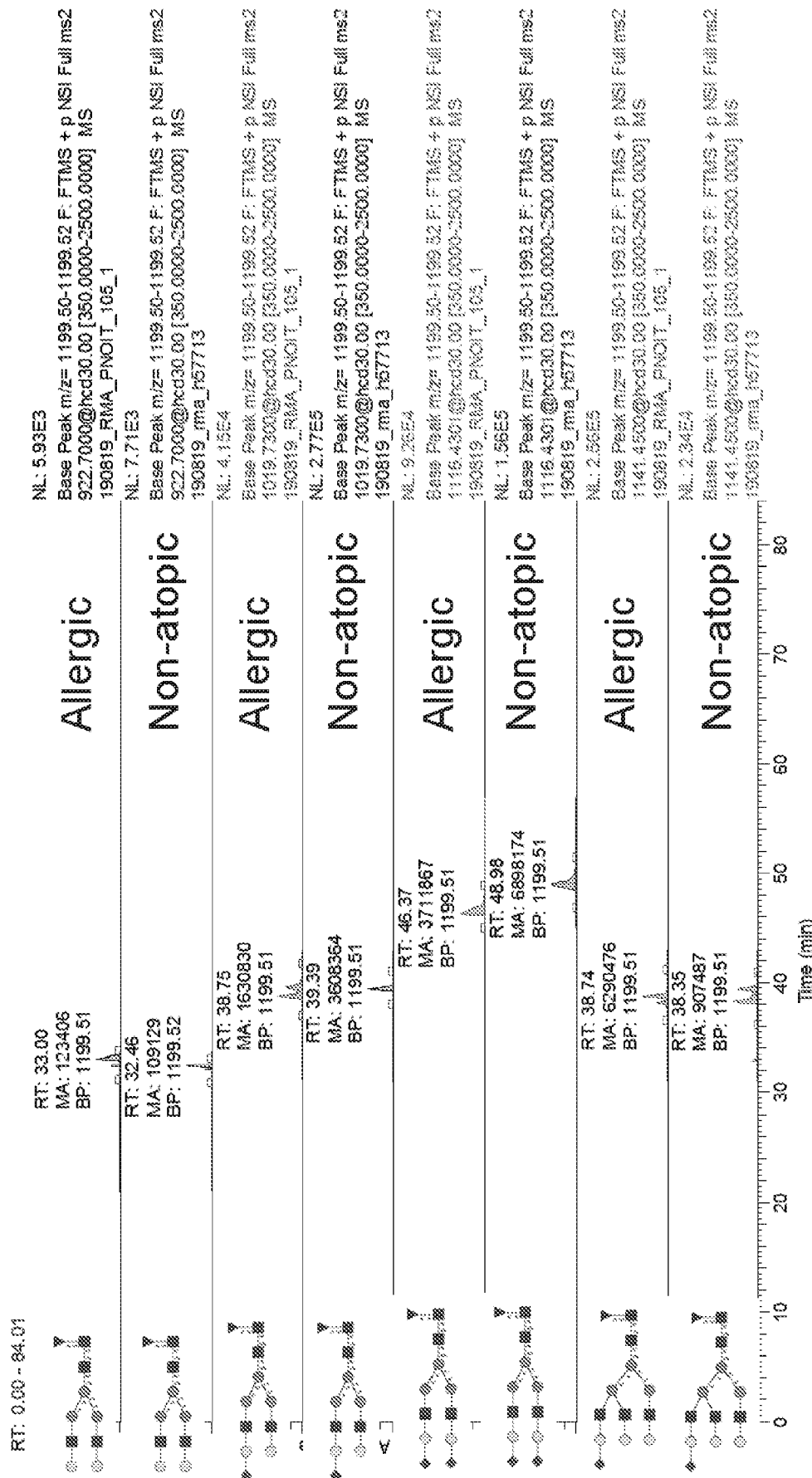


FIG. 6C

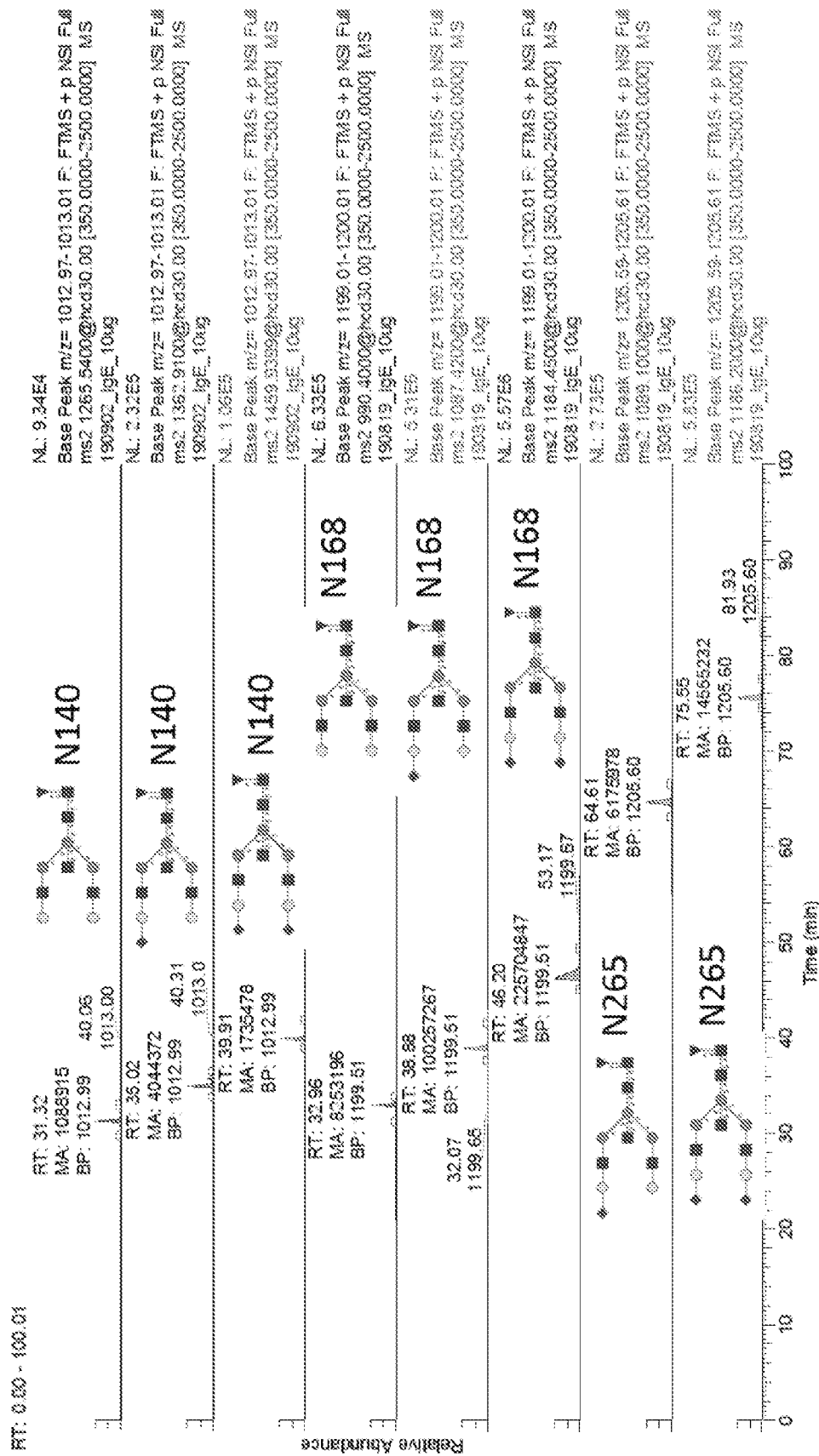


FIG. 6D

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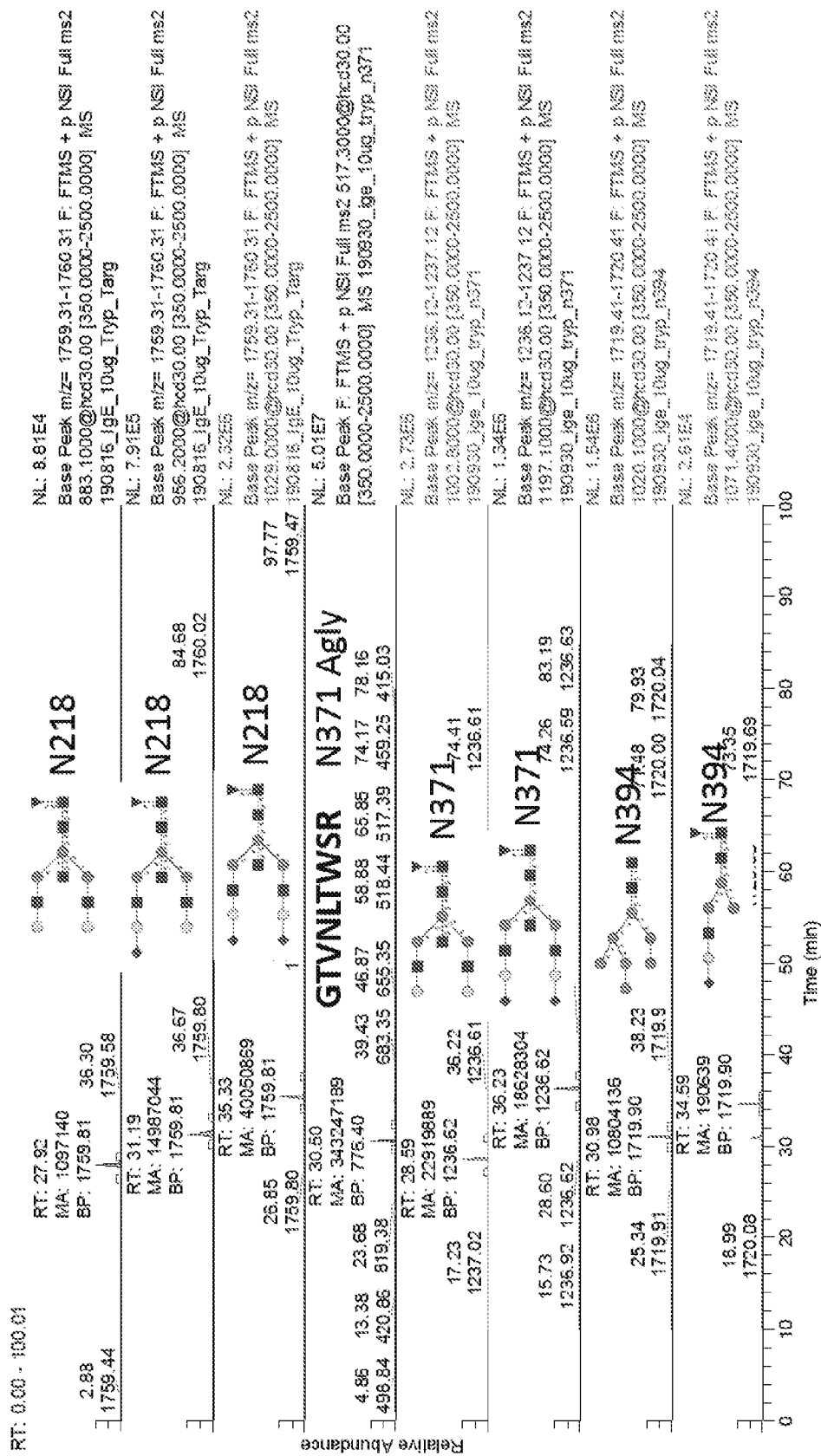


FIG. 6E

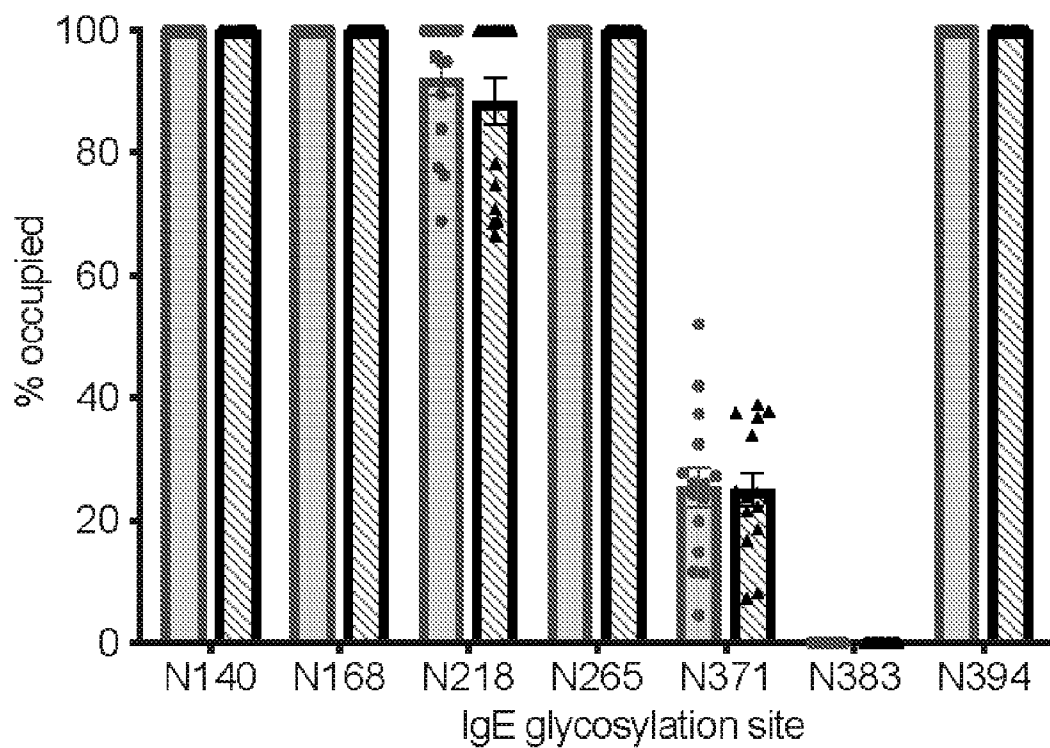


FIG. 7A

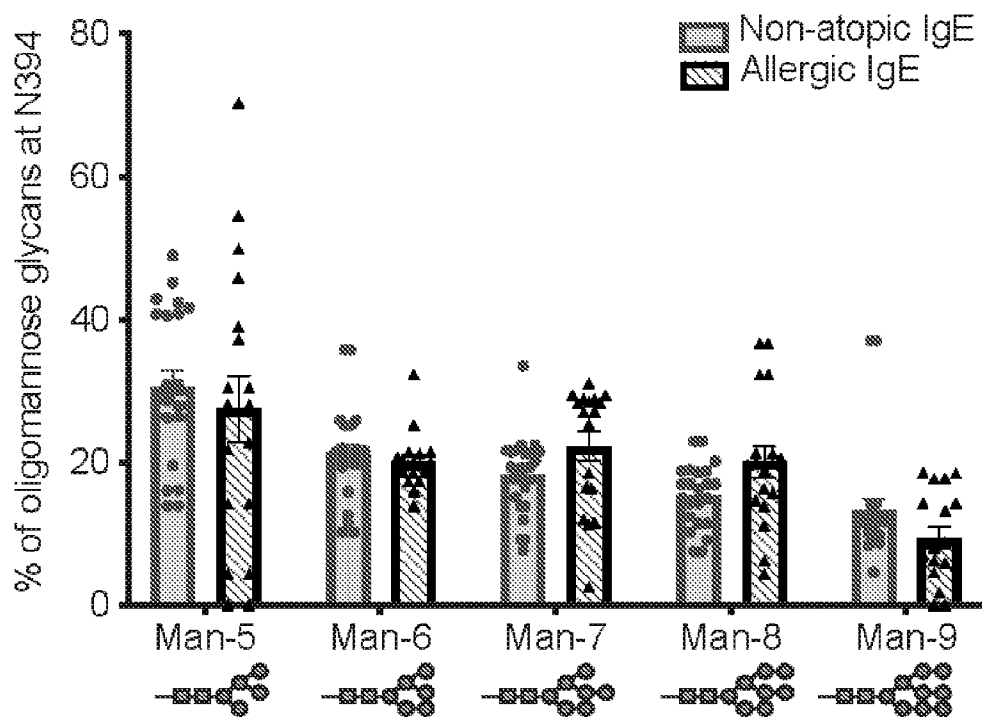


FIG. 7B

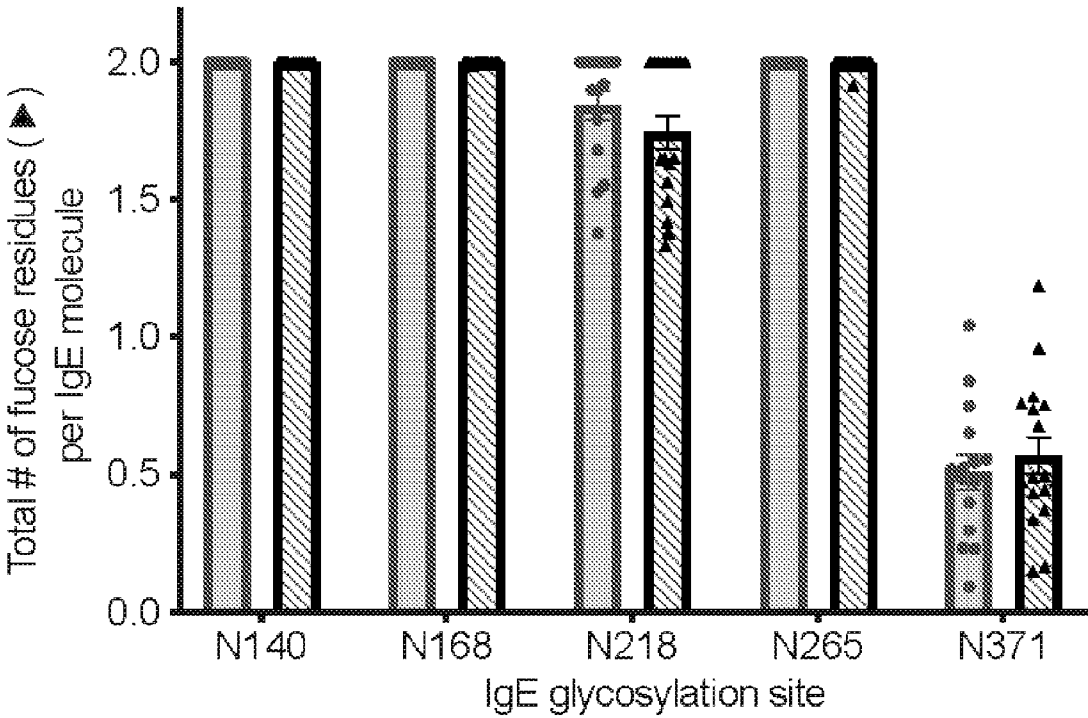


FIG. 7C

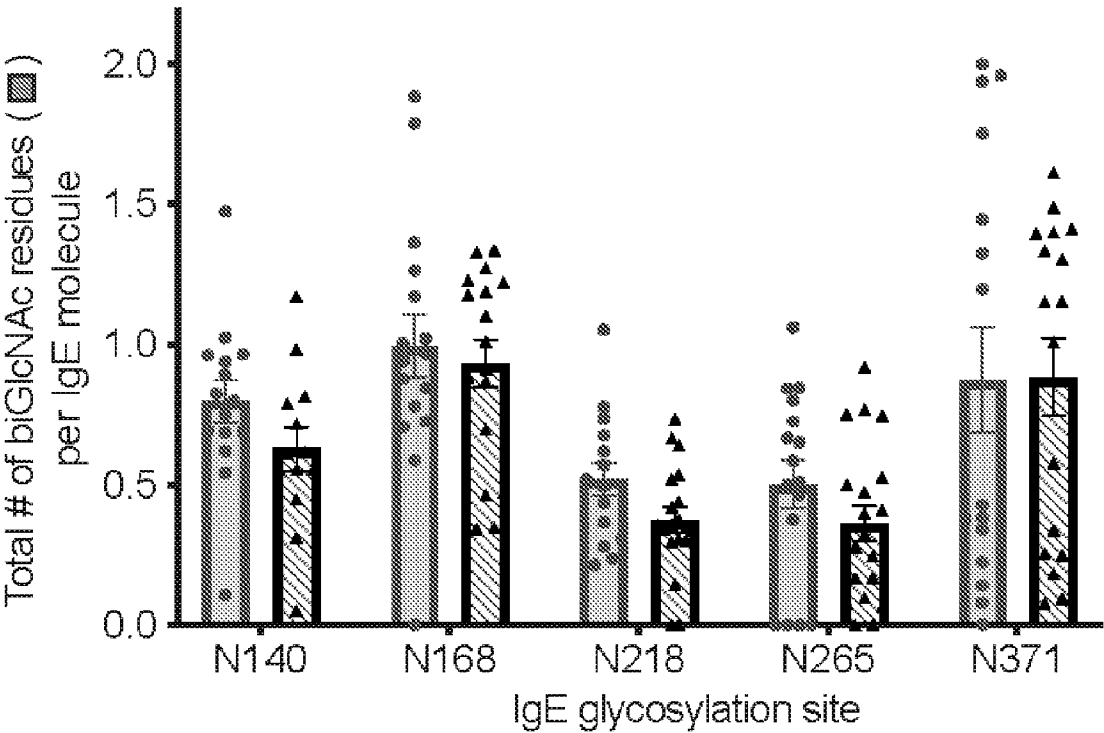


FIG. 7D

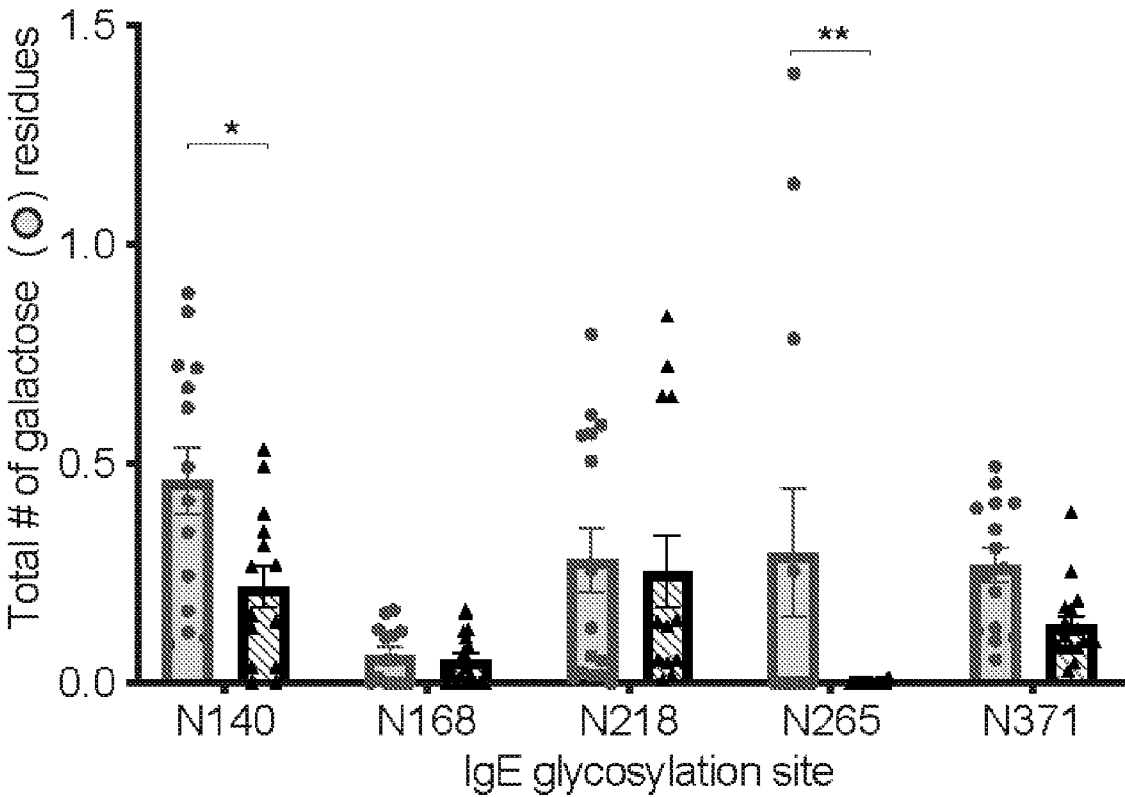


FIG. 7E

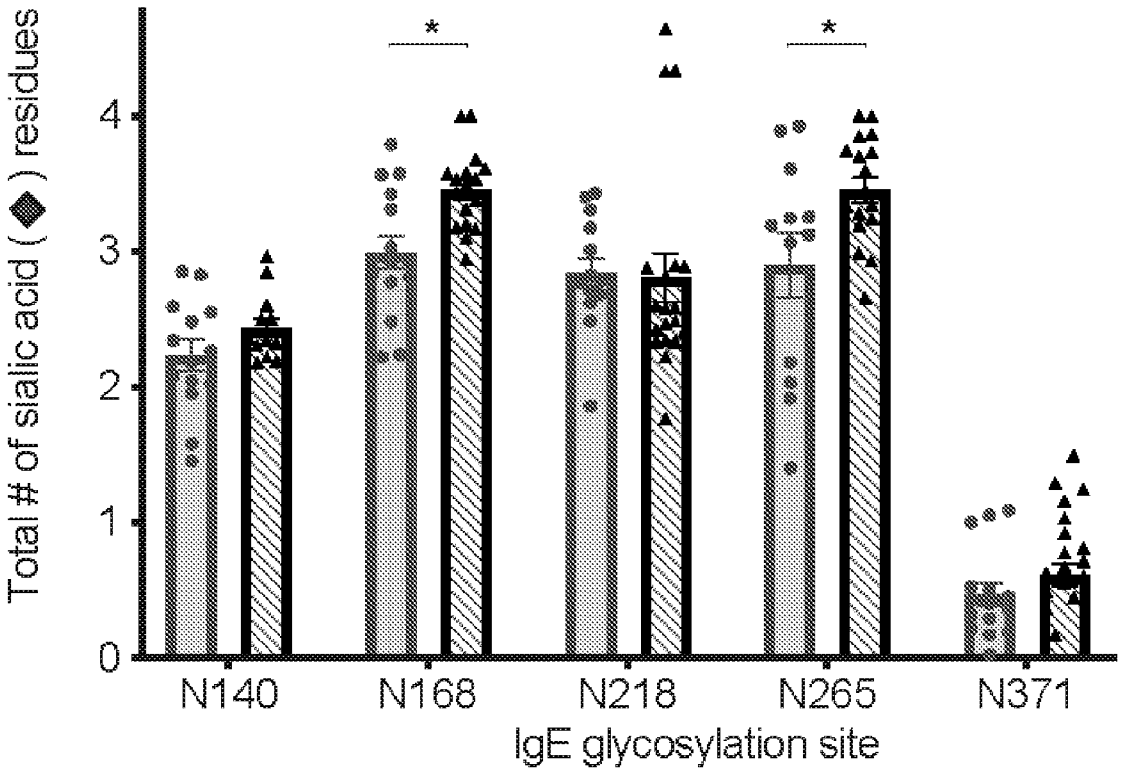


FIG. 7F

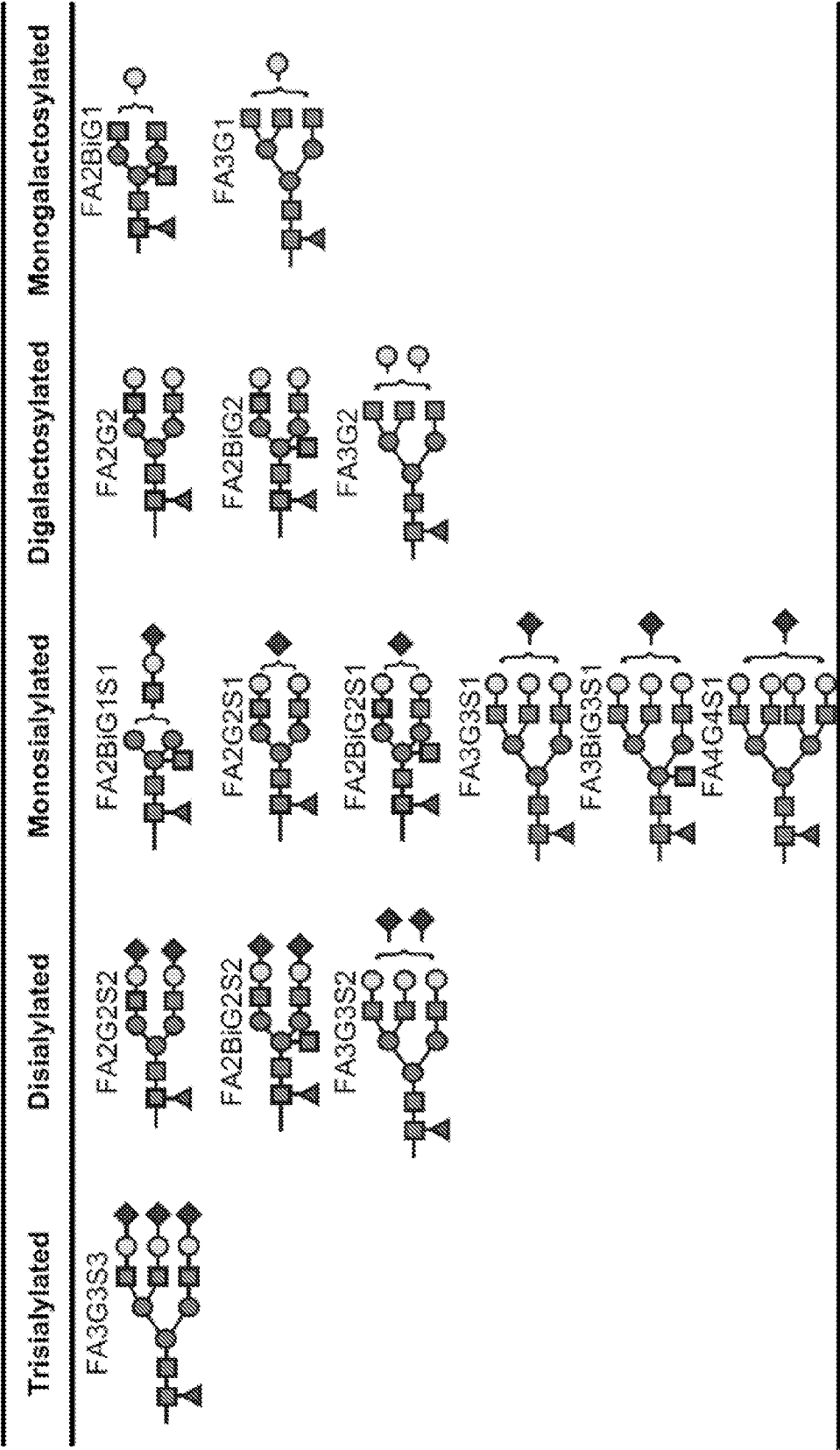


FIG. 7G

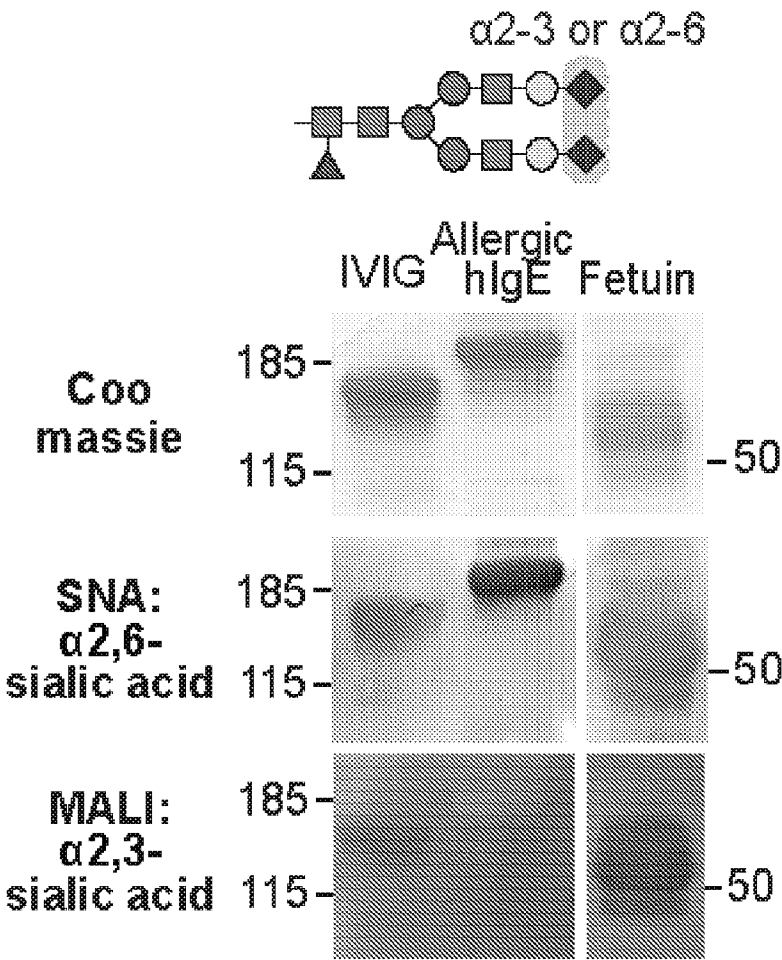


FIG. 8A

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FIG. 8B

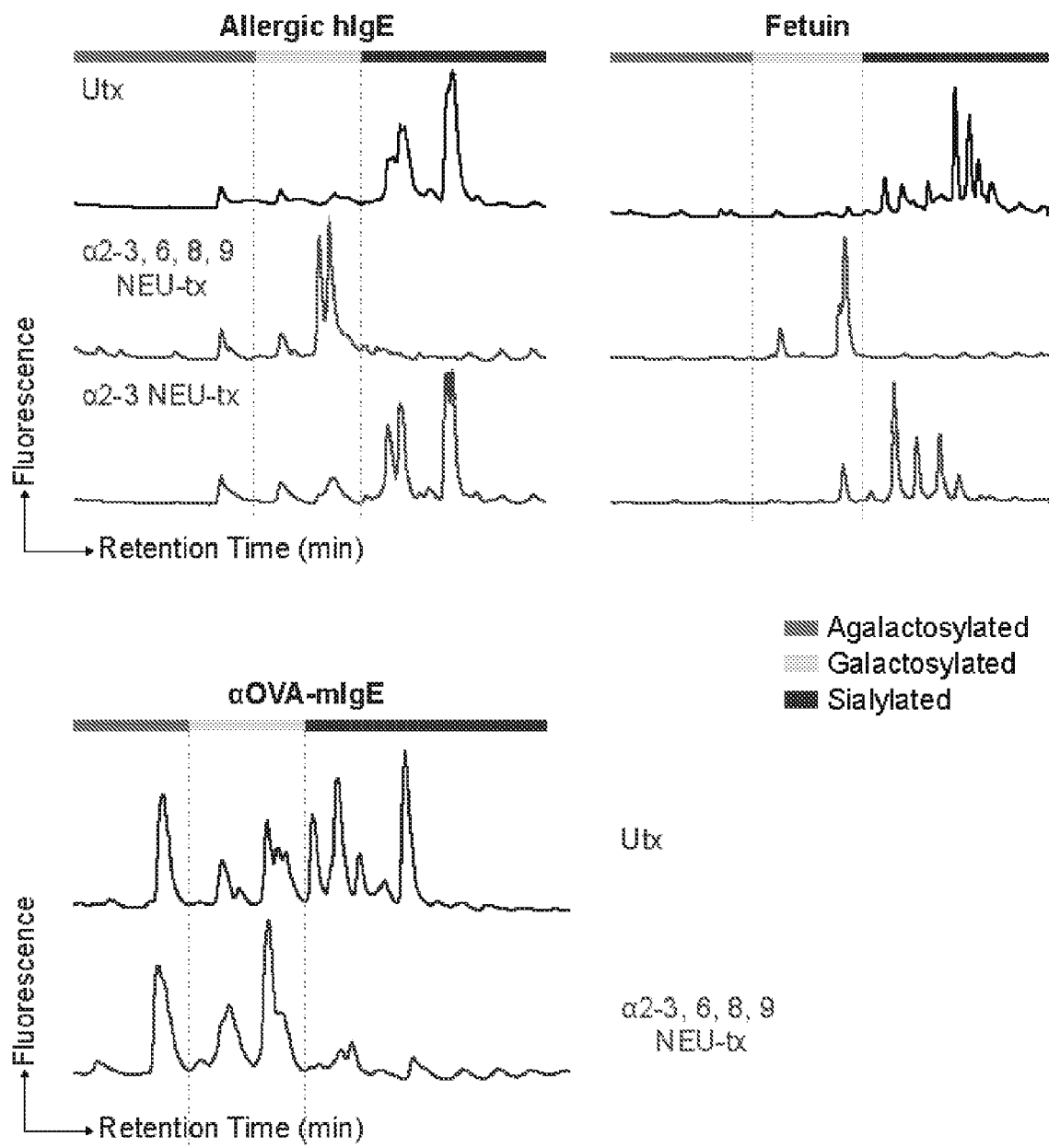
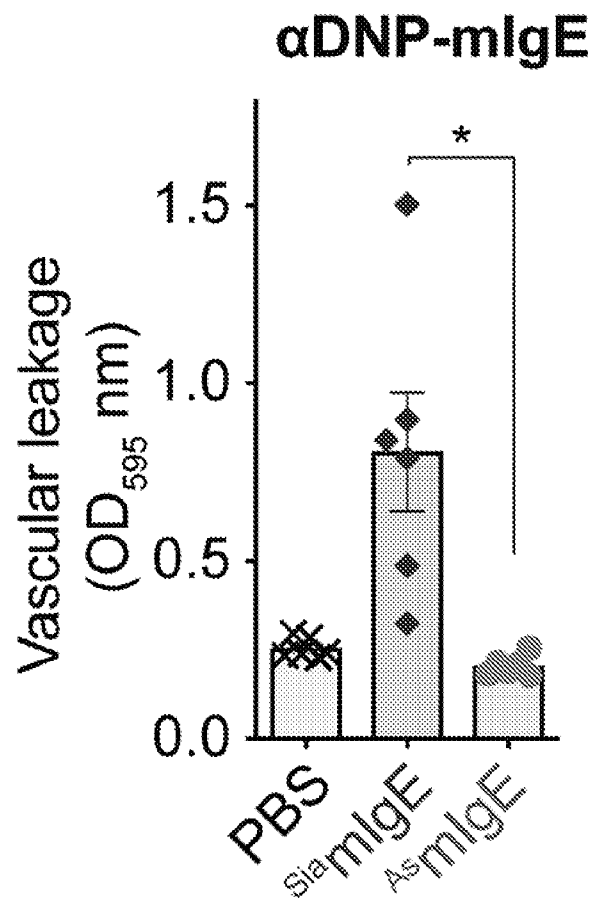
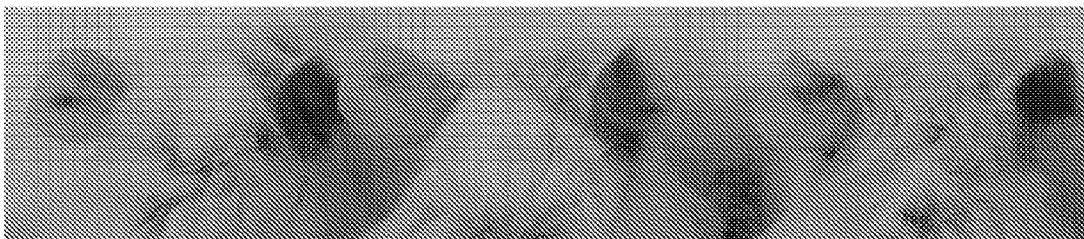


FIG. 8C



ID injection: ^{As}mIgE (Left) vs ^{Sia}mIgE (Right)



ID injection: PBS on both ears

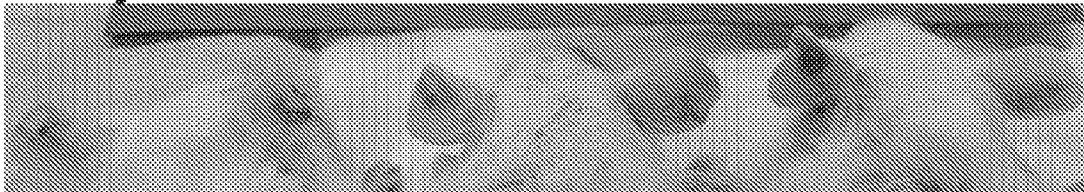


FIG. 9A

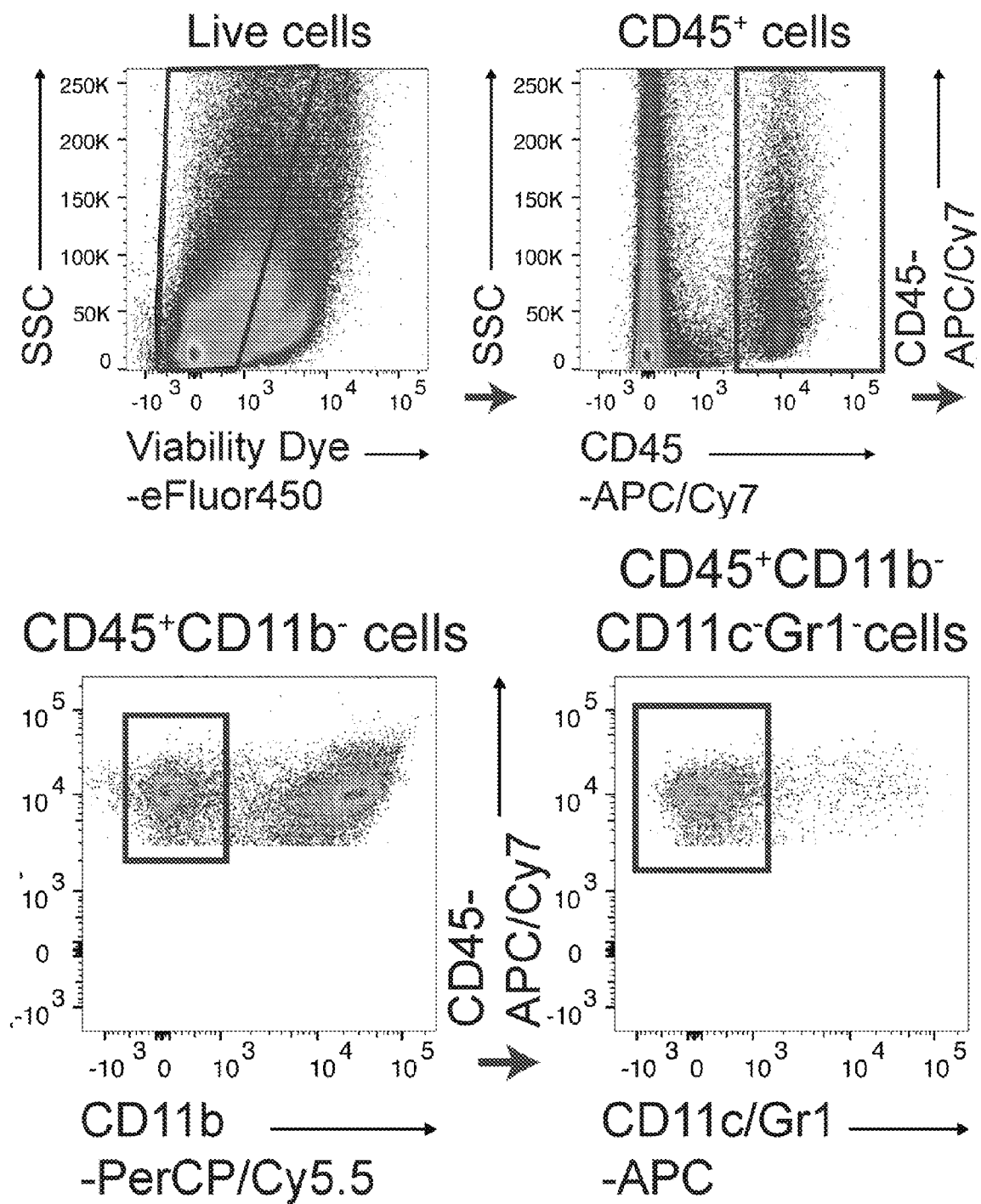
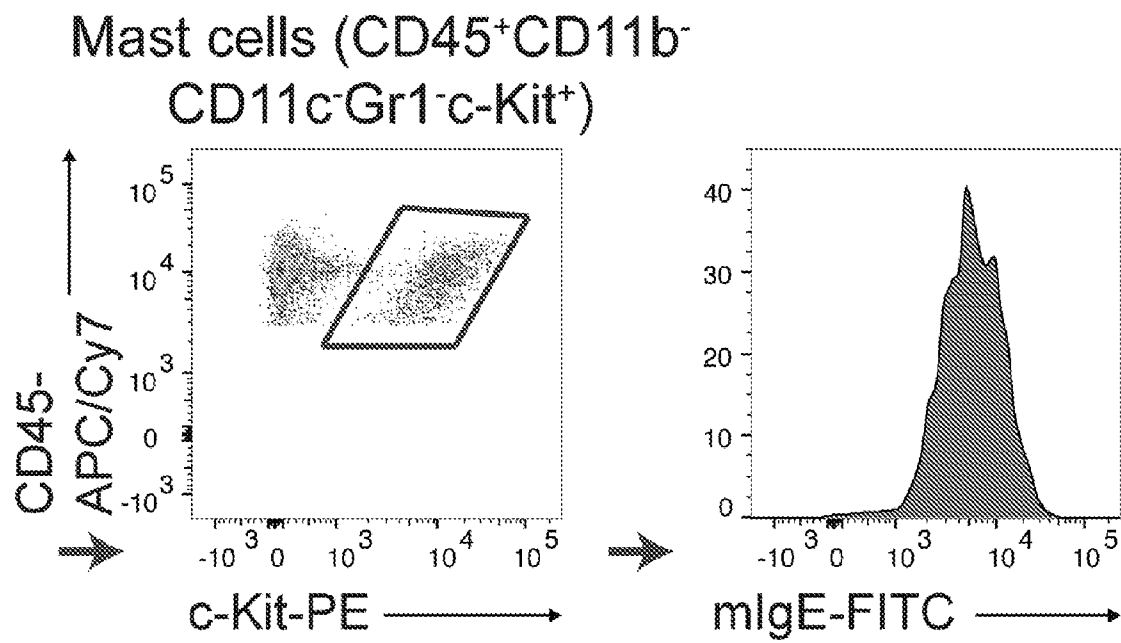


FIG. 9B

*FIG. 9B, continued*

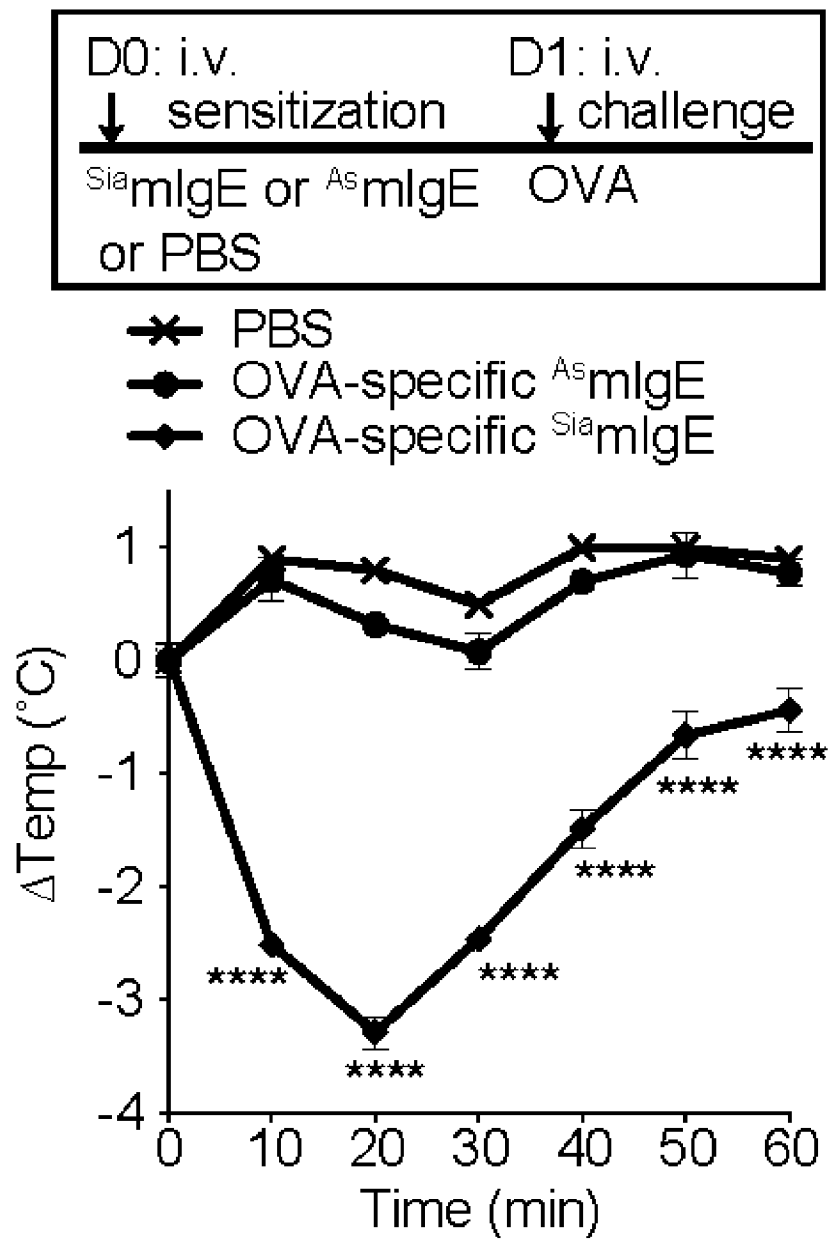


FIG. 10A

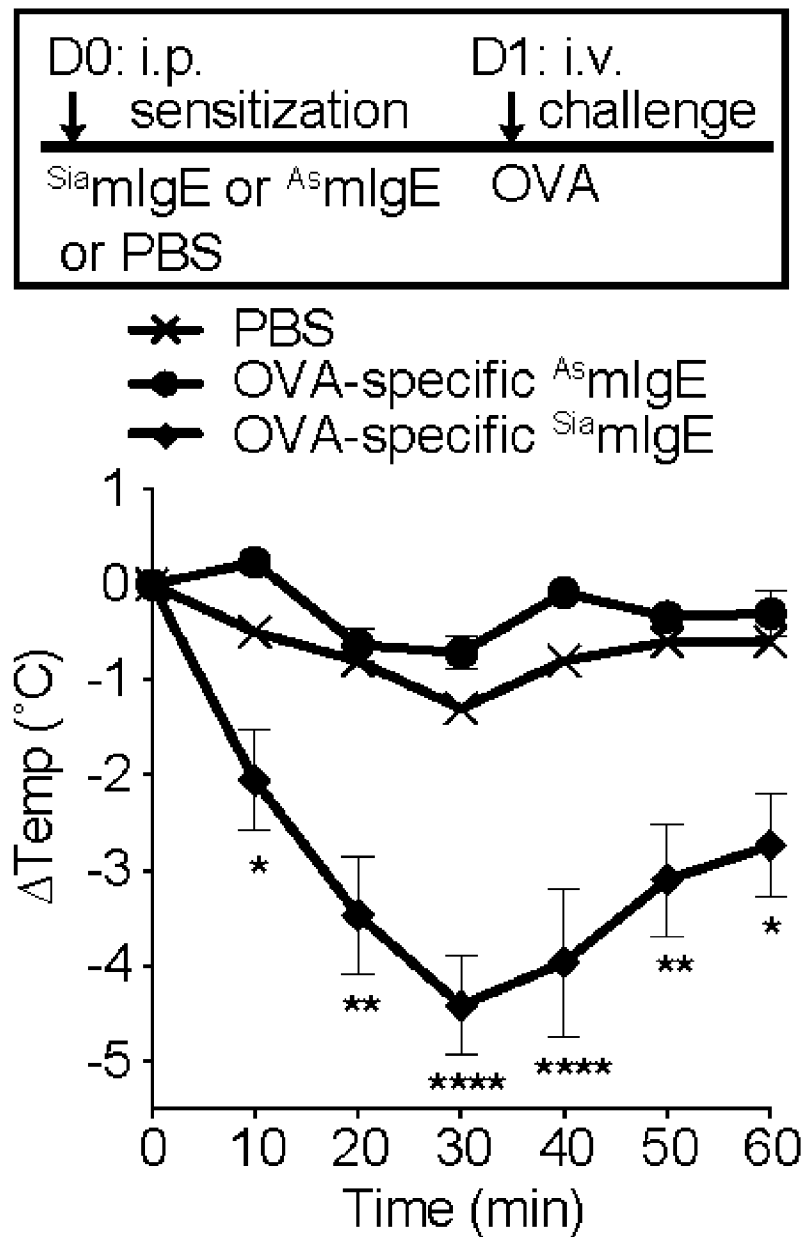


FIG. 10B

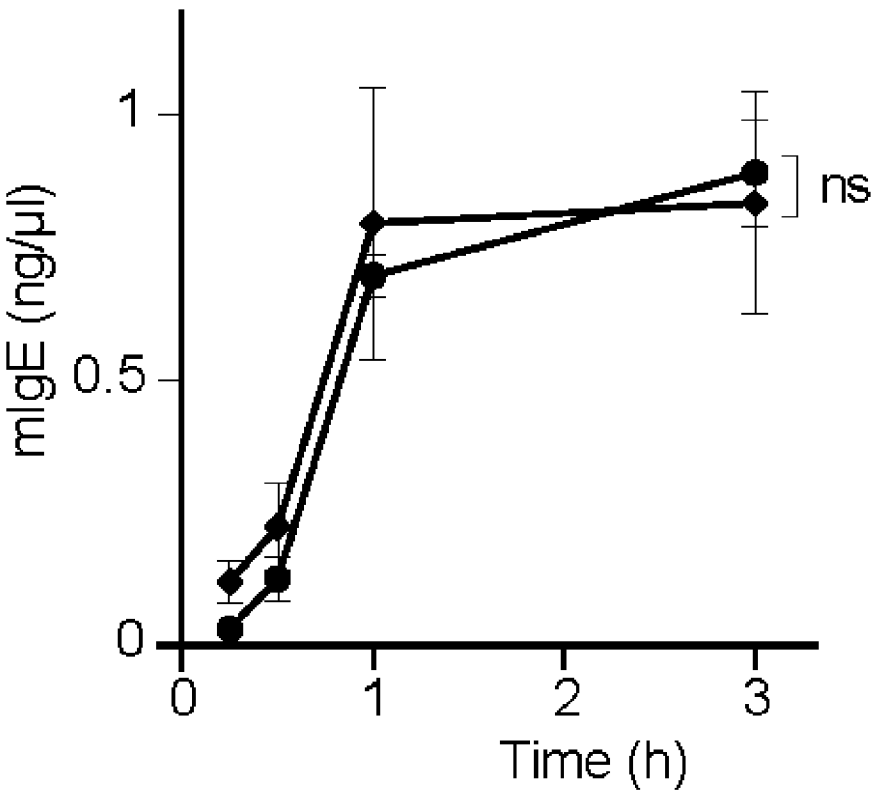


FIG. 10C

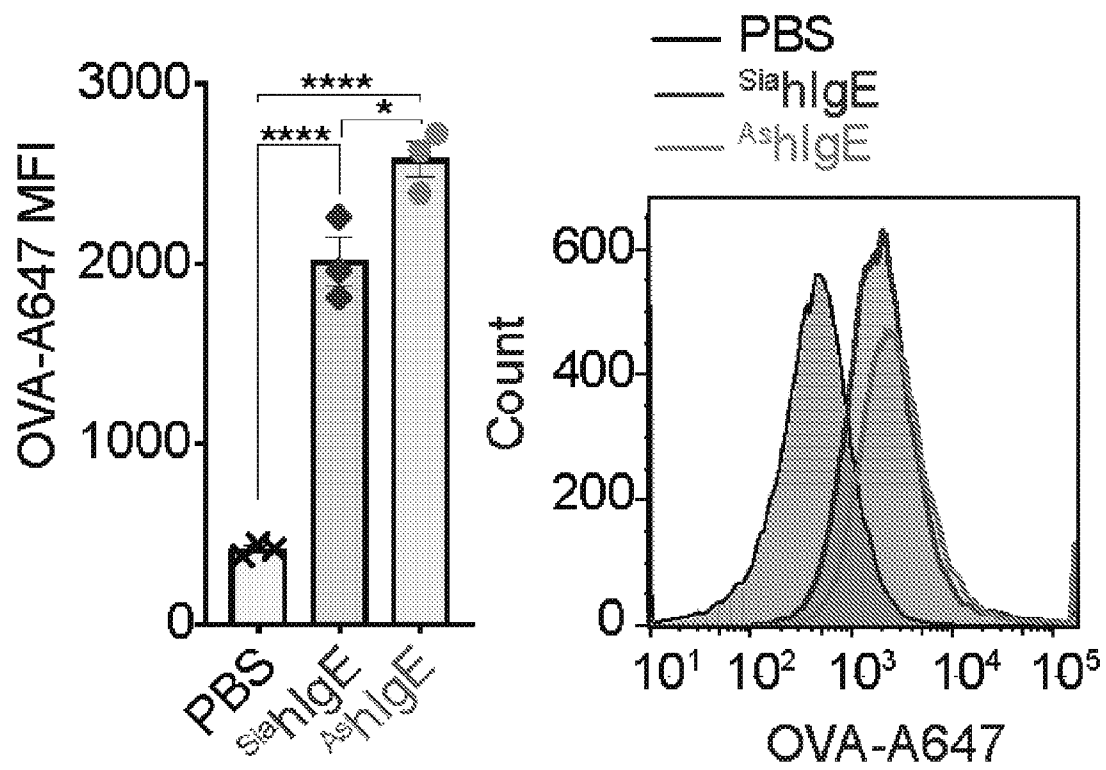


FIG. 11A

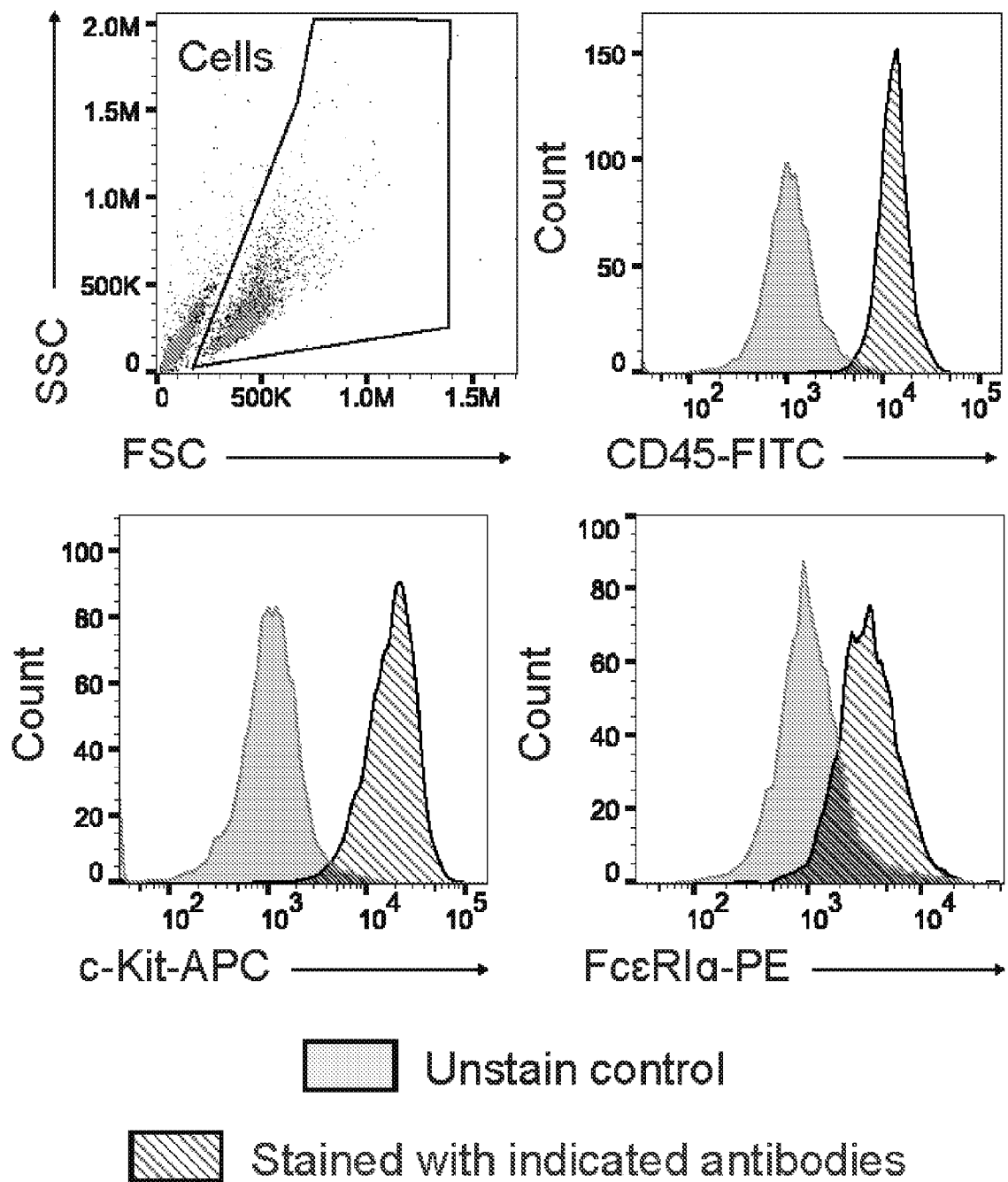


FIG. 11B

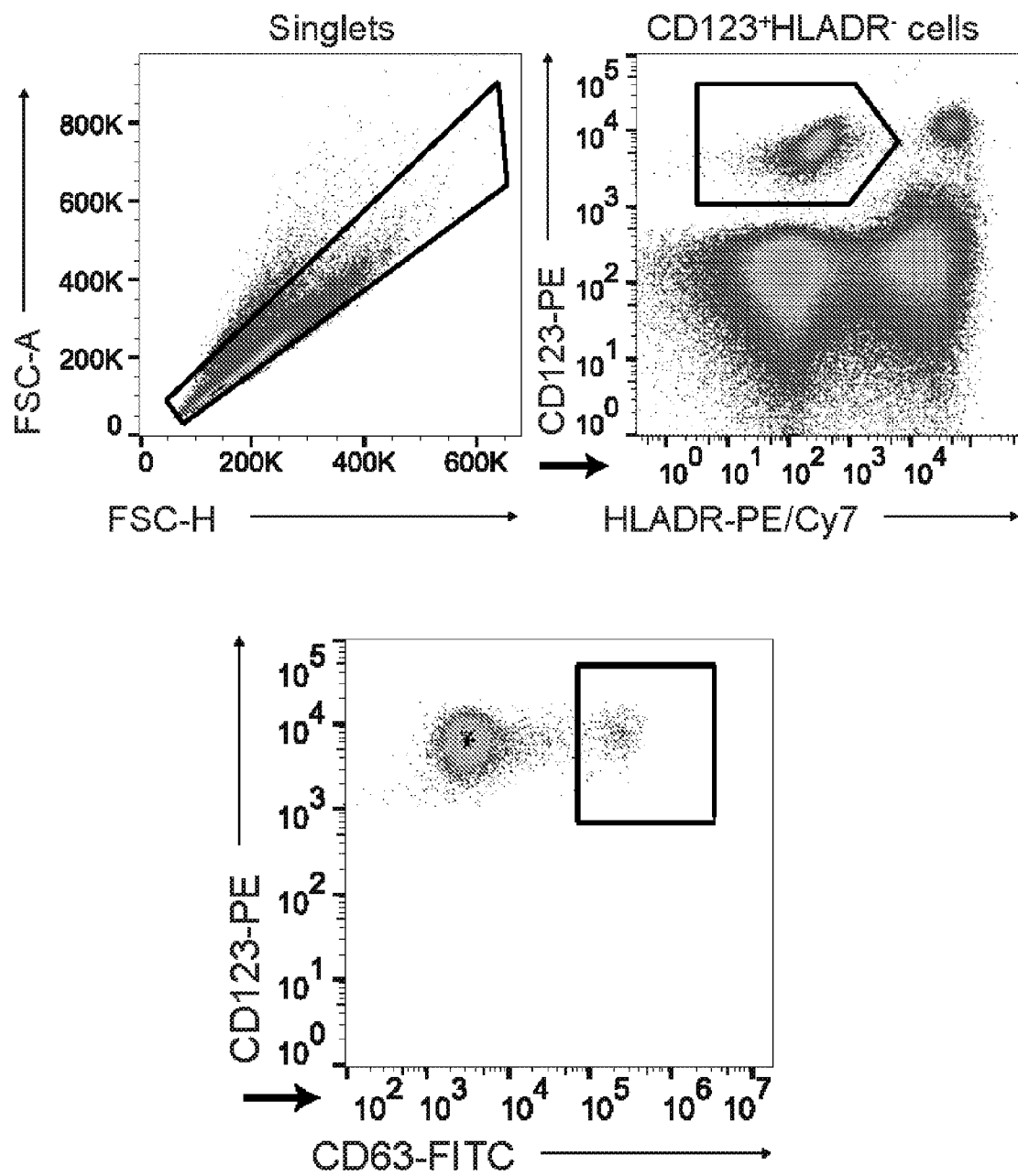


FIG. 11C

D0: i.v. ↓ sensitization	D1: i.v. ↓ treatment	D2: i.v. challenge ↓ OVA	Measure Temp
✕ α DNP- ^{Sia} mIgE (10 μ g)	+PBS		
◊ α DNP- ^{Sia} mIgE (10 μ g)	+ α OVA- ^{Sia} mIgE (20 μ g)		
○ α DNP- ^{Sia} mIgE (10 μ g)	+ α OVA- ^{As} mIgE (20 μ g)		

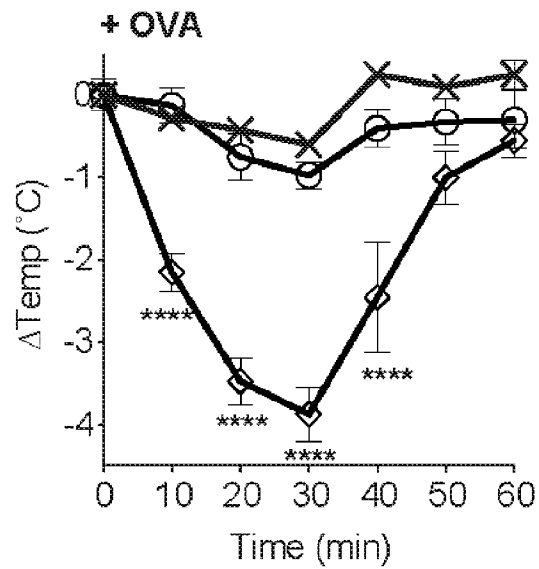


FIG. 12

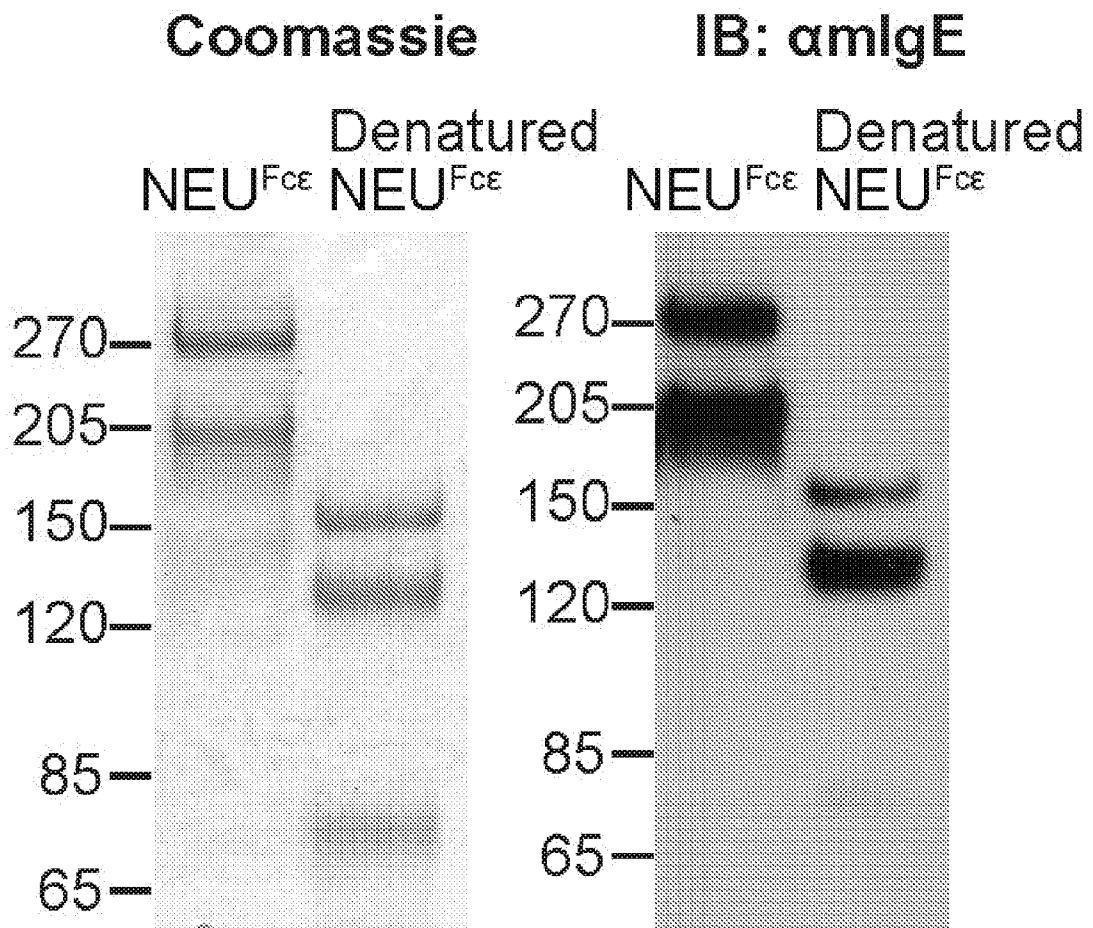


FIG. 13A

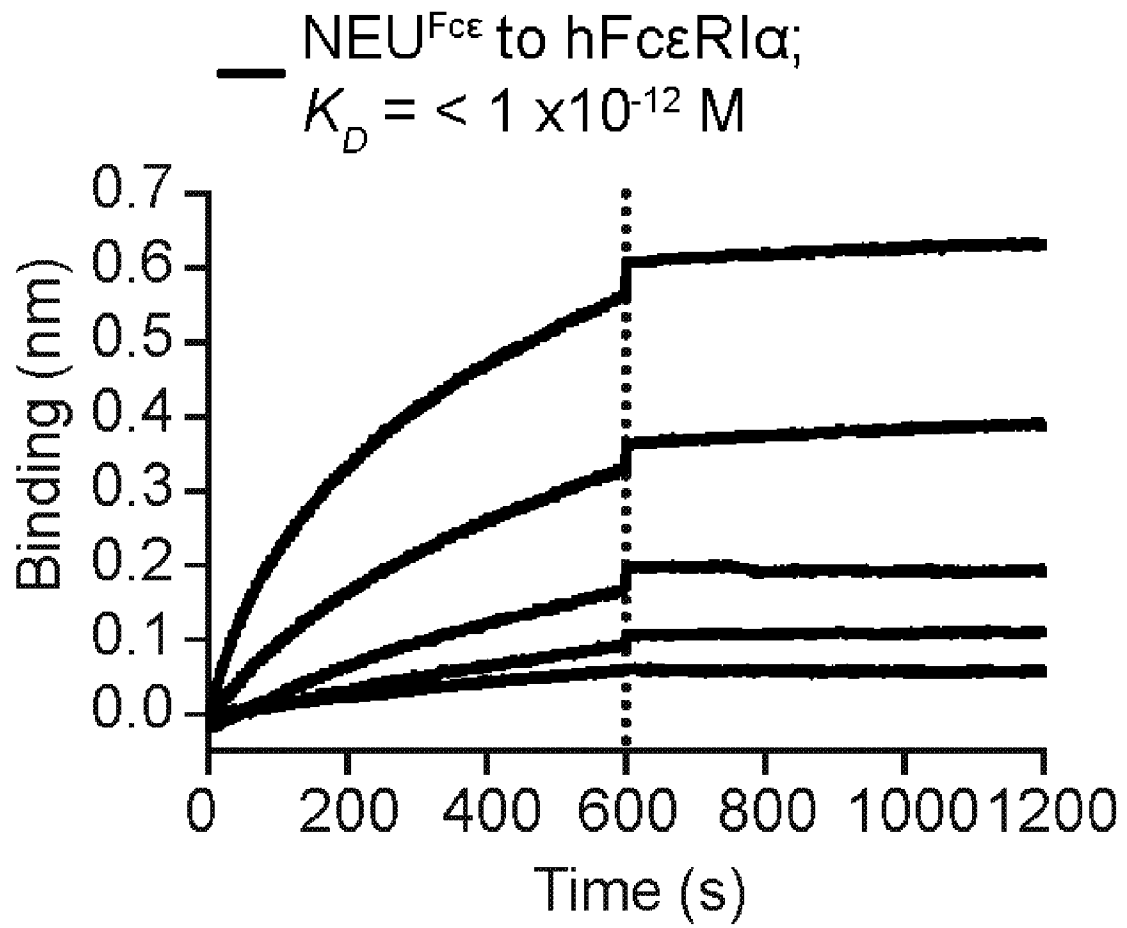


FIG. 13B

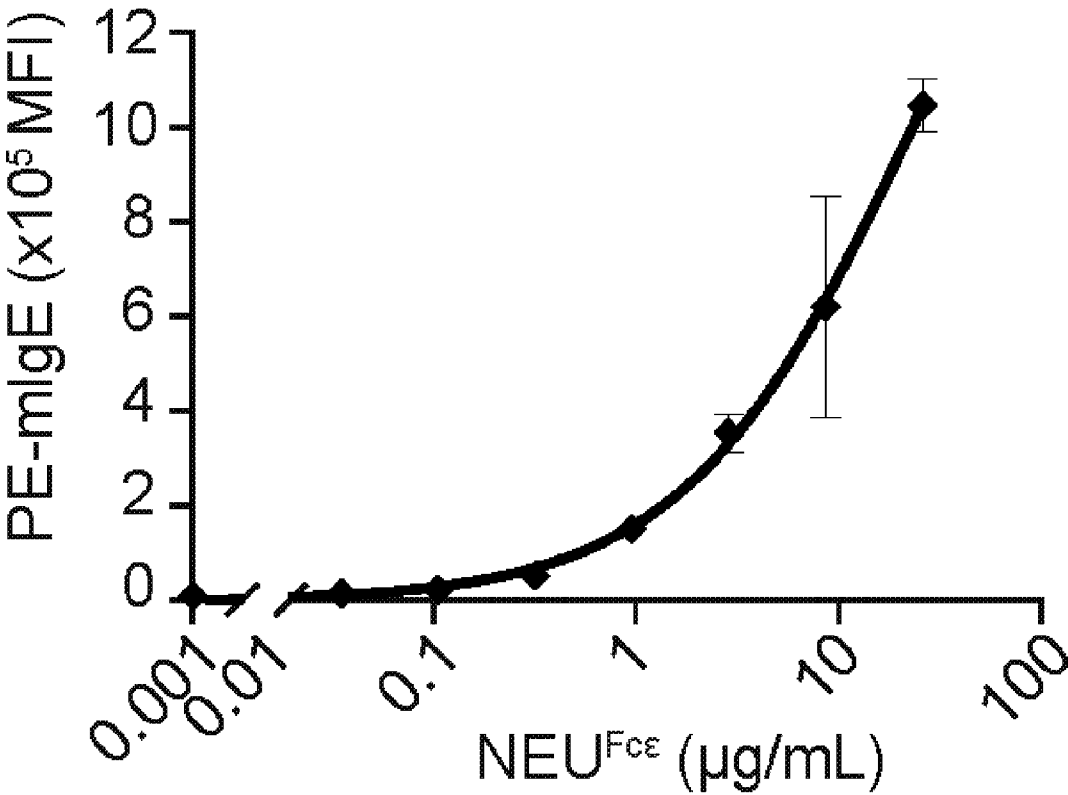


FIG. 13C

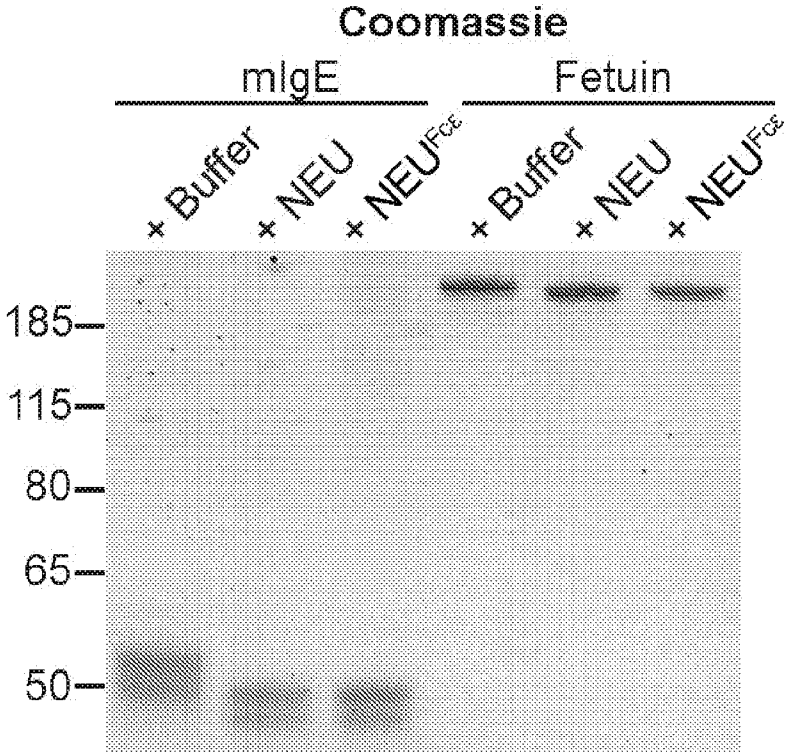


FIG. 13D
SNA

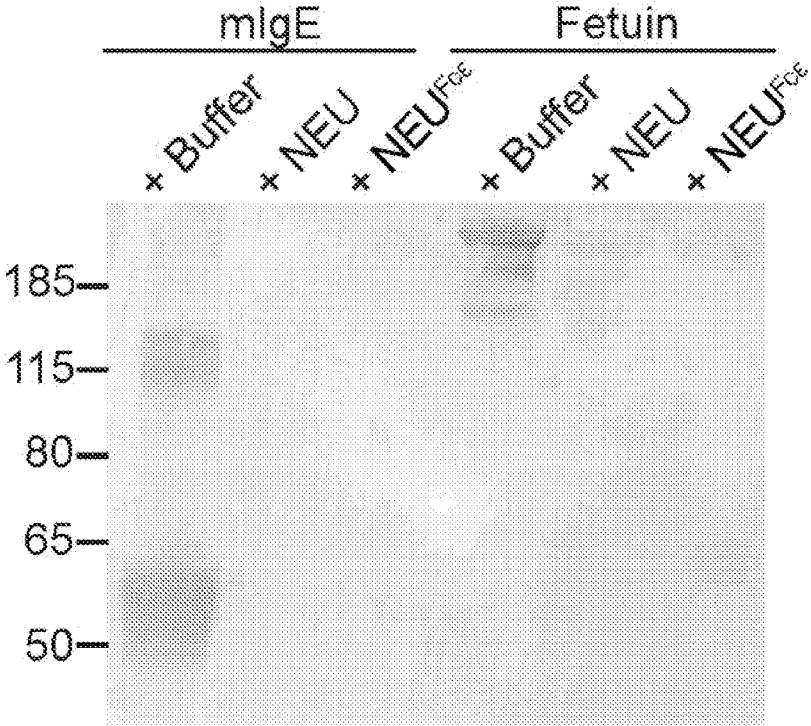


FIG. 13E

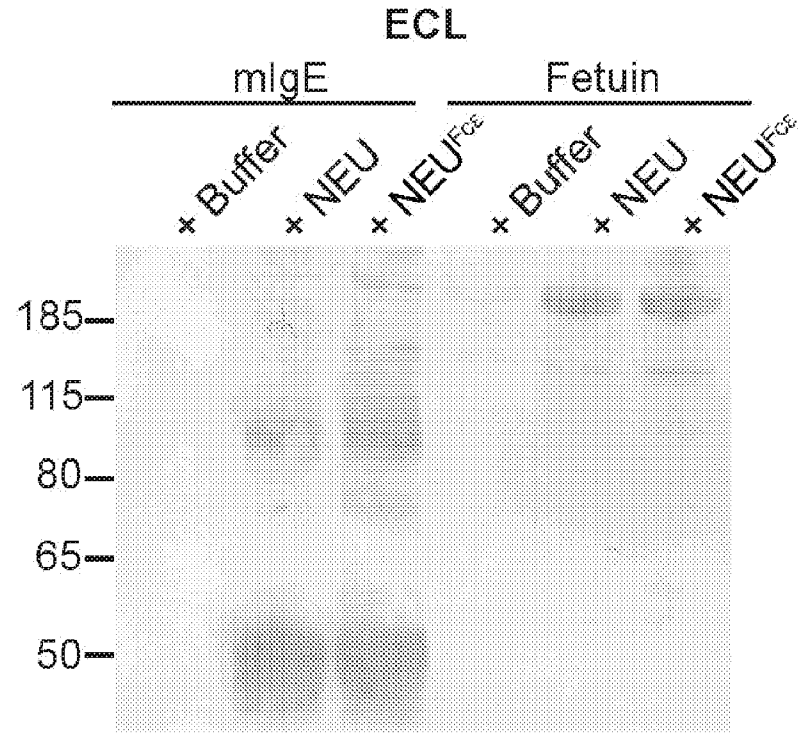


FIG. 13F

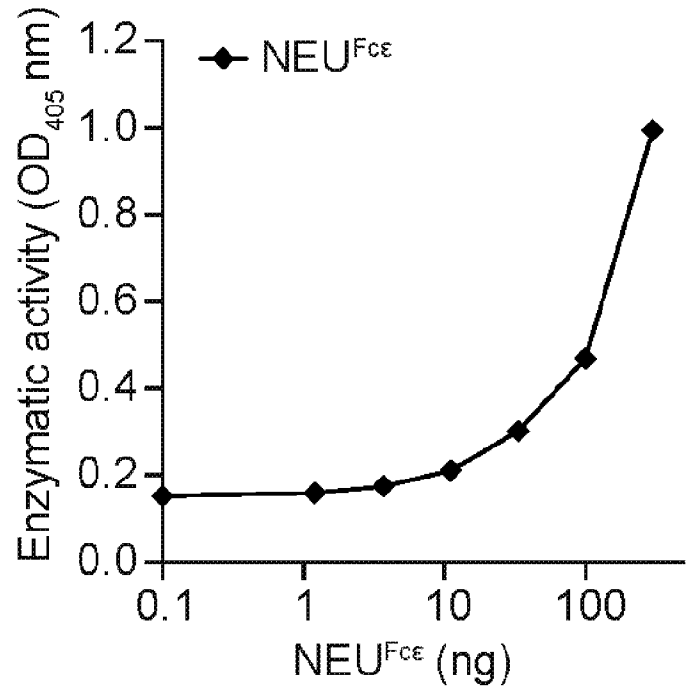


FIG. 13G

FIG. 14

SEQ ID NO: 1

hNEU1 (Sialidase-1)

MTGERPSTALPDRRWGPRILGFWGGCRVWVFAAIFLLLSLAASWSKAENDFGLVQPLVTMEQLLWVSG
 RQIGSVDTFRIPLITATPRGTLTAFAEARKMSSSDEGAKFIALRRSMDQGSTWSPTAFIVNDGDVPDG
 LNLGAVVSDVETGVVFLFYSLCAHKAGCQVASTMLVWSKDDGVSWSTPRNLSLDIGTEVFAPGPGSGI
 QKQREPRKGRLIVCGHGTLELDGVFCLLSDDHGASWRYGSGVSGIPYGQPKQENDFNDECQPYELPD
 GSVVINARNQNNYHCHCRIVLRSYDACDTLRPRDVTFDPELVDPVVAAGAVVTSSGIVFFSNPAHPEF
 RVNLTLRWSFSNGTWSRKETVQLWPGPSGYSSLATLEGSMDGEEQAPQLYVLYEKGRNHYTESISVAK
 ISVYGT

SEQ ID NO: 2

hNEU2 (Sialidase-2)

MASLPVLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAPTHQVQW
 QAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLLFFIAIPGQVTEQQQLQTRANVTRLCQVTSTDHGRW
 SSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPIQRPIPSAFCFLSHDHGR
 TWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQESQLVKKLVPPFQGC
 QGSVISFSPSPRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEAWSEPVLLAKGSCAYSIDLQSM
 GTGPDGSPLEFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQ

SEQ ID NO: 3

hNEU3 (Sialidase-3)

MRPADLPFRPMEESSPASSAPTETEEPGSSAEVMEEVTTCSFNSPLFRQEDDRGITYRIPALLYIPPT
 HTFLAFAEKRSTRRDEDALHLVLRRLRIGQLVQWGPKPLMEATLPGHRTMNPSPVWEQKSGCVFLF
 FICVRGHVTERQQIVSGRNAARLCFTYSQDAGCSWSEVRDLTEEVIKSELKHWATFAVGPGHGIQLQS
 GRLVIPAYTYIIPSWFFCFQLPCKTRPHSLMIYSDDLGVTHHGRILRPMVTVECEVAEVTGRAGHPV
 LYCSARTPNRCRAEALSTDHGEFQRLALSRQLCEPPHGCQGSVVSFRPLEIPHRCDSSSKDAPTIQ
 QSSPGSSRLLEEAGTPSESWLLYSHPTSRKQVRDLGIYLNQTPLEAACWSRPWILHCGPCGYSDLAA
 LEEGLFGCLFECGKQCEQIAFRLFTHREILSHLQGDCTSPGRNPSQFKSN

SEQ ID NO: 4

hNEU4 (Sialidase-4)

MGVPRTPSRTVLFERERTGLTYRVPSSLVPVPGPTLLAFVEQRLSPDDSHAHRLVLRRTLAGGSRVW
 GALHVLGTAALAEHRSMNPCPVHDAGTGTVFLFFIAVLGHTPEAVQIATGRNAARLCCVASRDAGLSW
 GSARDLTEEAIGGAVQDWATFAVGPGHGVQLPSGRLLVPAYTYRVDRRECFCIKIRTSFHSFAFYSD
 HGRTWRCGGLVPNLRSGECQLAAVDGGQAGSFLYCNARSPLGSRVQALSTDEGTSFLPAERVASLPET
 AWGCQGSIVGFAPAPNRPDDSWSVGPGSPLQPPLGPGVHEPPEAAVDPRGGQVPVGGPFPSRLQPR
 GDGPRQPGPRPGVSGDVGSWTALALPMPFAAPPQSPTWLLYSHPVGRRARLHMGIRLSQSPLDPRSWTE
 PWVIYEGPSGYSDLASIGPAPEGGLVFACLYESGARTSYDEISFCTFSLREVLNVPASPKPPNLGDK
 PRGCCWPS

SEQ ID NO: 5

Sialidase (*Vibrio cholerae* serotype O1)

MRFKNVKKTALMLAMFGMATSSNAALFDYNATGDTEFDSAPAKQGWMDNTNNGSGVLTNADGMPAWLV
 QGIGGRAQWYSLSTNQHAQASSFGWRMTTEMKVLSGGMITNYANGTQRVLPISLDSGSLVVEFE
 GQTGRTVLATGTAATEYHKFELVFLPGSNPSASFYFDGKLIRDNIQPTASKQNMIVWNGSSNTDQVA
 AYRDIKFEIQGDVIFRGPDRIPSIVASSVTPGVVTAFAEKRVGGDPGALSNTNDIITRTSRDGGITW
 DTELNLTEQINVSDEFDFSDPRPIYDPSNTVLVSARWPTDAAQNGDRIKPPMNGIFYSVYDVASG
 NWQAPIDVTDQVKERSFQIAGWGGSELYRRNTSLNSQDQWQSNAKIRIVDGAANQIQVADGSRKYVVT
 LSIDESGGLVANLNGVSAPIILQSEHAKVHSFHDYELQYSALNHTTTLFVDGQQITTWAGEVSQENNI
 QFGNADAQIDGRLHVQKIVLTQQGHNLVEFDAFYLAQQTPEVEKDLEKLGWTKIKTGNTMSLYGNASV
 NPGPGHGITLTRQQNISGSQNGRLIYPAILVDRFFLNVMISIYSDDGGSNWQTGSTLPIPFWRKSSSIL
 ETLEPSEADMVELQNGDLLLTARLDNFQIVNGVNSPRQQFLSKDGGITWSLLEANNANVFSNISTGT
 VDASITRFEQSDGSHFLFTNPQGNPAGTNGRQNLGLWFSFDEGVTWKGPIQLVNGASAYSIDIYQLDS
 ENAIVIVETDNSNMRIILRMPITLLKQLTSLQN

FIG. 15A

SEQ ID NO: 6**Human IgE-Fc**

CSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELA
STQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSFDLFIKRSPTITCL
VVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSALPVGTRDWIEGETYQCRVTHPHLPR
ALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNEMPEDISVQWLHNEVQLPDARHSTTQP
RKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGK

hIgE-Fc (Amino Acids: 21-344)

SEQ ID NO: 7**Mouse IgE-Fc**

VRPVNITEPTLELLHSSCDPNAFHSTIQLYCFIYGHILNDVSVSWLMDDREITDTLAQTVLIKEEGKL
ASTCSKLNITEQQWMSESTFTCKVTSQGVLDYLAHTRCPDHEPRGVITYLIPPSPLDLYQNGAPKLTCL
LVVDLESEKNVNVTWNQEKTSVSASQWYTKHHNNATTSITSILPVVAKDWIEGYGYQCIVDHPDFPK
PIVRSITKTGQRSAPPEVYVFPPEEESEDKRTLTLCLIQNFFPEDISVQWLGDKLISNSQHSTTTPL
KSNQSNQGGFFIFSRLEVAKTLWTQRKQFTCQVIHEALQKPRKLEKTISTSLGNTSLRPS

mIgE-Fc (Amino Acids: 21-351)

SEQ ID NO: 8**Canine IgE-Fc**

CALNFIPPTVKLFHSSCNPVGDTHTTIQLLCLISGYVPGDMEVIWLVDGQKATNIFPYTAPGTKEGNV
TSTHSELNITQGEWVSQKTYTCQVTYQGFTFKDEARKCSESDPRGVTSYLSPPSPLDLYVHKAPKITC
LVVDLATMEGMNLTWYRESKEPVNPGFLNKKDHFNGTITVTSTLPVNTNDWIEGETYYCRVTHPHLPR
DIVRSIAKAPGKRAPPDVYLFLLPPEEEQGTKDRVTLTLCLIQNFFPADISVQWLNRDNDSPVTEQQATTG
PHKVSGSRPAFFIFSRLEVSRVDWEQKNKFTCQVVHEALSGSRILQKWVSKTPGK

SEQ ID NO: 9**Feline IgE-Fc**

CTMNFIPPTVKLFHSSCNPLGDTGSTIQLLCLISGYVPGDMEVTWLVDGQKATNIFPYTAPGKQEGKV
TSTHSELNITQGEWVSQKTYTCQVTYQGFTFEDHARKCTESDPRGVSTYLSPPSPLDLYVHKSPKITC
LVVDLANTDGMILTWSRENGESVHPDPMVKKTYNGTITVTSTLPVDATDWVEGETYQCKVTHPDLPR
DIVRSIAKAPGRRFPPEVYVFLPPEGEPKTKDKVTLTLCLIQNFFPPDISVQWLHNDSPVRTEQQATTW
PHKATGPSPAFFVFSRLEVSRADWEQRDVFTCQVVHEALPGFRTLKKS SVSKNPGK

FIG. 15B

SEQ ID NO: 10**Human IgG1-Fc**

PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMEALHNHYTQKSLSLSPGK

SEQ ID NO: 11**Human IgG2-Fc**

PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMEALHNHY
TQKSLSLSPGK

SEQ ID NO: 12**hIgG3-Fc**

LKTPLGDTHTCPRCPEPKSCDTPPFCPRCPEPKSCDTPPFCPRCPEPKSCDTPPFCPRCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVS
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESSGQPENNYNTTTPMLDSDGSFFLYSKLTVDKSRWQQGNI FSCSVMEALHNHFTQKSL
SLSPGK

SEQ ID NO: 13**hIgG4-Fc**

PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNH
YTQKSL

SEQ ID NO: 14**Mouse IgG1-Fc**

VPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITITLTPKVTCTVVDISKDDPEVQFSWFVDDVEVHTAQ
TKPREEQINSTRSVSELPIMHQDWLNGKEFKCRVNSAAPPAPIEKTISKTKGRPKAPQVYTI PPPKE
QMAKDKVSLTCMITNFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLVNQKSNWEAGNTFT
CSVLHEGLHNHHTKSLSHSPGK

SEQ ID NO: 15**Mouse IgG2a-Fc**

MPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMI SLSPIVTCVVDVSEDDPDVQISW FVNNV
EVHTAQ TQTHREDYNSTLRVVSALPIQH QDWMSGKEFKCKVNNKDL PAPIERTISKPKGSVRAPQVYV
LPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWV
ERNYSYSCSVVHEGLHNHHTTKSFSRTPGK

FIG. 15B (Cont'd)

SEQ ID NO: 16

Mouse IgG2b-Fc

EPSPGPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIKDVLMI SLTPKVTCVVVDVSEDDPDVVRIS
WVNNNEVHTAQTQTHREDYNSTIRVVSALPIQHQDWMGKEFKCKVNNKDLPSPIERTISKIKGLVR
APQVYILPPPAEQLSRKDVSLTCLVVGFNPGDISVEWTSNGHTEENYKDTAPVLSDGSYFIYSKLDI
KTSKWEKTDSFSCNVRHEGLKNYYLKKTISRSPGK

SEQ ID NO: 17

Mouse IgG3-Fc

EPRIPKPSTPPGSSCPPGNILGGPSVFIFPPKPKDALMISLTPKVTCVVVDVSEDDPDVHVSWFVDNK
EVHTAWTQPREAQYNSTFRVVSALPIQHQDWMRGKEFKCKVNNKALPAPIERTISKPKGRAQTPQVYT
IPPPREQMSKKKVS LTCLVTNFFSEAISVEWERNGELEQDYKNTPPILSDGTYFLYSKLTVDTDSWL
QGEIFTCSVVHEALHNHHTQKNLSRSPGK

SEQ ID NO: 18

Canine IgG-A (IgG1)-Fc

PPCPVPEPLGGPSVLIFPPKPKDILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKTQSREQ
QFNGTYRVVSVLP IEHQDWLTGKEFKCRVNHIDLPSP IERTISKARGRAHKPSVYVLPSPKELSSSD
TVSITCLIKDFYPPDIDVEWQ SNGQQEPERKHRMT PPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCAV
MHETLQNHYTDL SL SHSPGK

SEQ ID NO: 19

Feline IgG1Fc

PKCPPPEMLGGPSIFIFPPKPKDTLSISRTPEVTCLVVDLGPDDSDVQITWFVDNTQVYTAKTSPREE
QFNSTYRVVSVLPILHQDWLKGKEFKCKVNSKSLPSPIERTISKAKGQPHEPQVYVLPQAQEELSRNK
VSVTCLIKSFHPPDIAVEWEITGQPEPENNYRTTPQLDSDGTYFVYSKLSVDRSHWQRGNTYTCSVS
HEALHSHHTQKSLTQSPGK

FIG. 16

SEQ ID NO: 20

hNEU1_hIgE_{FC}

MYRMQLLSICIALSLALVTNSGSPAQWKLENDGFLVQPLVTMEQLLWVSGRQIGSVDTFRIPLITATPR
 GTLLAFAEARKMSSSDEGAKFIALRRSMDQGSTWSPTAFIVNDGDVPDGLNLGAVVSDVETGVVFLFY
 SLCAHKAGCQVASTMLVWSKDDGVSWSTPRNLSLDIGTEVFAPGPGSGIQKQREPRKGRLIVCGHGT
 ERDGVFCLLSDDHGASWRYGSGVSGIPYGQPKQENDFNPDQCQPYELPDGSVINARNQNNYHCHCRI
 VLRSDACDTRLRPRDVTFDPELVDPVVAAGAVVTSSGIVFFSNPAHPEFRVNLTLRWSFSNGTSWRKE
 TVQLWPGPSGYSSLATLEGSMGEEQAPQLYVLYEKGRNHYTESISVAKISVYGTLLGGGGGGCSRDT
 PPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSEL
 TLSQKHWSLDRITYTCQVTYQGHTFEDSTKKCADSNPRGVSAAYLSRPSPFDLFIRKSPTITCLVVDLAP
 SKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTTSALPVGTRDWIEGETYQCRVTHPHLPRALMRST
 TKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTGSG
 GFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKHHHHHH

IL2 Secretion signal 1-20
 Human Sialidase-1 (NEU1) (No signal peptide) 29-396
 Flexible linker 397-402
 Human IgE-Fc 403-726
 Hisx6 727-733

SEQ ID NO: 21

hIgE_{FC}-NEU2

MYRMQLLSICIALSLALVTNSCSRDTFPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLED
 GQVMDVDLSTASTTQEGELASTQSELTLSQKHWSLDRITYTCQVTYQGHTFEDSTKKCADSNPRGVSA
 YLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTTSALPVGT
 RDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDIS
 VQWLHNEVQLPDARHSTTQPRKTGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSV
 NPGKMASLPVLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRGDYDAPTH
 QVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRLCQVTSTDH
 GRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPIQRPIPSAFCFLSH
 DHGRTWARGHFVAQDTLEQCVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQESQLVKKLVEPP
 PQGCQGSVISFSPSPRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEAWSEPVLLAKGSCAYSD
 LQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQ

IL2-signal sequence (Amino Acids: 1-20)
 hIgE-Fc (Amino Acids: 21-344)
 NEU2 (Amino Acids: 355-724)
 CH2 (Amino Acids: 21-126)
 CH3 (Amino Acids: 128-234)
 CH4 (Amino Acids: 236-344)

FIG. 16 (Cont'd)

SEQ ID NO: 22

hNEU2-hIgEFc

MPLLLLLLPLLWAGALAASLPVLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIV
 LRRGDYDAPTHQVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANV
 TRLCQVTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPIQR
 PIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDFQE
 SQLVKKLVEPPPQGCQGSVISFPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEAWSEPV
 LLAKGSCAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQGGGGGGCSRDFTP
 PTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELT
 LSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPS
 KGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSALPVGTRDWIEGETYQCRVTHPHLPRALMRSTT
 KTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSG
 FFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKHHHHHH

Secretion signal 1-16

Human Sialidase-2 (NEU2) 17-395

Flexible linker 396-401

Human IgE-Fc 402-725

Hisx6 726-731

SEQ ID NO:23

hNEU3-hIgEFc

MPLLLLLLPLLWAGALAMRPADLPPRMEESPASSSAPTETEEPGSSAEVMEEVTTCSFNSPLFRQEDD
 RGITYRIPALLYIPPTHFLAFAEKRSTRDEDALHLVLRRLRIGQLVQWGPKPLMEATLPGHRTM
 NPCPVWEQKSGCVLFFICVRGHVTERQQIVSGRNAARLCFIYSQDAGCSWSEVRDLTEEVIGSELKH
 WATFAVGPGHGIQLQSGRLVIPAYTTYIPSWFFCFQLPCKTRPHSLMIYSDDLGVTHHHGRLIRPMVT
 VECEVAEVTGRAGHPVLYCSARTPNRCRAEALSTDHGEQFQRLALSRQLCEPPHGCQGSVVSFRPLEI
 PHRCQDSSSKDAPTIQQSSPGSSLRLEEEAGTPSESWLLYSHPTSRKQVRDLGIYLNQTPLEAACWSR
 PWILHCGPCGYSDLAALEEEGLFGCLFECGKQCEQIAFRLFTHREILSHLQGDCTSPGRNPSQFKS
 NGGGGGGCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTAST
 TQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPSPFDLFIRK
 SPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSALPVGTRDWIEGETYQCRV
 THPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDA
 RHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKHHHHHH

Secretion signal 1-16

Human Sialidase-3 (NEU3) 17-477

Flexible linker 478-483

Human IgE-Fc 484-807

Hisx6 808-813

FIG. 16 (Cont'd)

SEQ ID NO:24

hNEU4-hIgE_{FC}

MPLLLLLLPLWAGALAMGVPRTPSRTVLFERERTGLTYRVPSLLPVPPGPTLLAFVEQRLSPDDSHAH
 RLVLRRGTLAGGSVRWGALHVLGTAALAEHRSMNPCPVHDAGTGTVFLFFIAVLGHTPEAVQIATGRN
 AARLCCVASRDAGLSWGSARDLTEEAIGGAVQDWATFAVGPGHGVQLPSGRLLVPAYTYRVDRRECFG
 KICRTSPHSFAFYSDDHGRTWRCGGLVPNLRSGECQLAAVDGGQAGSFLYCNARSPLGSRVQALSTDE
 GTSFLPAERVASLPETAWGCQGSIVGFPAAPAPNRPRDDSWSVGPGSPLQPPLLGPVHEPPEEAAVDP
 RGGQVPGGPFSRLQPRGDGPRQPGPRPGVSGDVGSWTLALPMPFAAPPQSPTWLLYSHPVGRRARLHM
 GIRLSQSPLDPRSWTEPWVIYEGPSGYSDLASIGPAPEGGLVFACLYESGARTSYDEISFCTFSLREV
 LENVPASEFKPPNLGDKPRGCCWPSGGGGGGCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTP
 GTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLSQKHWSLDRITYTCQVTYQGHTFEDSTKKCA
 DSNPRGVSAYLSRPSFDFLIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTL
 TVTSALPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACL
 IQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASP
 SQTVQRAVSVNPGKHHHHH

Secretion signal 1-16
 Human Sialidase-4 (NEU4) 17-500
 Flexible linker 501-506
 Human IgE-Fc 507-830
 Hisx6 831-836

SEQ ID NO: 25

hIgE_{FC}-Vibrio Sialidase

MYRMQLLSICIALSLALVTNSCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLED
 GQVMDVDLSTASTTQEGELASTQSELTLSQKHWSLDRITYTCQVTYQGHTFEDSTKKCADSNPRGVSAY
 LSRPSFDFLIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSALPVGT
 RDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLINFMPEDIS
 VQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSV
 NPGKMRFKNVKKTALMLAMFGMATSSNAALFDYNATGDTEFDSQAKQGWMDNTNNGSGVLTNADGMP
 AWLVQIGGQRAQWYSLSTNQHAQASSFGWRMTTEMKVLSGGMITNYANGTQRVLPISLSSGNLV
 VEFEGQTGRVTLATGTAATEYHKFELVFLPGSNPSASFYFDGKLIRDNIQPTASKQNMIVWNGSSNT
 DGVAAYRDIKFEIQGDVIFRGPDRIPSIIVASSVTPGVVTAFAEKRVGGDPGALSNTNDIITRTSRDG
 GITWDTLNLTEQINVSDEFDFSDPRPIYDPSNTVLVSYARWPTDAAQNGDRIKPWMPNGIFYSVYD
 VASGNWQAPIDVTDQVKERSFQIAGWGGSELYRRNTSLNSQDQWQSNAKIRIVDGAANQIQVADGSRK
 YVVTLSIDESGGLVANLNGVSAPIILQSEHAKVHSFHDYELQYSALNHTTTLFVDGQQITTWAGEVSQ
 ENNIQFGNADAQIDGRLHVQKIVLTQQGHNLVEFDAFYLAQQTPEVEKDLEKLGWTKIKTGNTMSLYG
 NASVNPFGHGITLTRQQNISGSQNGRLIYPAIVLDRFFLNVMISIYSDDGGSNWQTGSTLPIPFWRKS
 SSILETLEPSEADMVELQNGDLLLTARLDFNQIVNGVNYSPRQQFLSKDGGITWSLLEANNANVFSNI
 STGTVDASITRFEQSDGSHFLLFTNPQGNPAGTNGRQNLGLWFSFDEGVTWKGPQLVNGASAYSIDIY
 QLDSENAIVIVETDNSNMRILRMPITLLKQKLTLSQN

IL2-signal sequence (Amino Acids: 1-20)
 hIgE-Fc (Amino Acids: 21-344)
 Sialidase (Amino Acids: 345-1125)
 CH2 (Amino Acids: 21-126)
 CH3 (Amino Acids: 128-234)
 CH4 (Amino Acids: 236-344)

FIG. 16 (Cont'd)

SEQ ID NO:26

hNEU1_mIgE_{FC}

MYRMQLLSICIALSLALVTNSGSPAQWKLENDGFLVQPLVTMEQLLWVSGRQIGSVDTFRIPLITATPR
 GTLLAFAEARKMSSSDEGAKFIALRRSMDQGSTWSPTAFIVNDGDVPDGLNLGAVVSDVETGVVFLFY
 SLCAHKAGCQVASTMLVWSKDDGVSWSTPRNLSLDIGTEVFAPGPGSGIQKQREPRKGRLIVCGHGTL
 ERDGVFCLLSDDHGASWRYGSGVSGIPYGQPKQENDFNPDECQPYELPDGSVVINARNQNNYHCHCRI
 VLRSYDACDTLRPRDVTFDPELVDPVVAAGAVVTSSGIVFFSNPAHPEFRVNLTLRWSFSNGTSWRKE
 TVQLWPGPSGYSSLATLEGSMGEEQAPQLYVLYEKGRNHYTESISVAKISVYGTLLGGGGGGVREPVNI
 TEPTLELLHSSCDPNAFHSTIQLYCFIYGHILNDVSVSWLMDDREITDTLAQTVLKEEGKLASTCSK
 LNITEQQWMSESTFTCKVTSQGVLYLAHTRRCPDHEPRGVITYLIPPSPLDLYQNGAPKLTCLVVDLE
 SEKNVNVVTWNQEKKTSVSASQWYTKHHNNATTSITSILPVVAKDWIEGYGYQCIVDHPDFPKPIVRSI
 TKTPGQRSAPENVYVFPPEEESEDKRTLTLCLIQNFFPEDISVQWLGDKLISNSQHSTTTPLKSNGSN
 QGFFIFSRLEVAKTLWTQRKQFTCQVIHEALQKPRKLEKTISTSLGNTSLRPSHHHHHH

Secretion signal 1-20

Linker 21-28

Human Sialidase-1 (NEU1) 29-396

Flexible linker 397-402

Mouse IgE-Fc 403-733

Hisx6 734-739

SEQ ID NO:27

hNEU2_mIgE_{FC}

MPLLLLLLPLWAGALADMASLPVLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAEL
 IVLRRGDYDAPTHQVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRA
 NVTRLQCQVTSTDHGRWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHPI
 QRPISAFCFLSHDHGRWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQSTNDGLDF
 QESQLVKKLVEPPPQGCQGSVISFSPSRSGPGSPAQWLLYTHPTHSWQRADLGAYLNPRPPAPEAWSE
 PVLLAKGSCAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQAFPAEYLPQGGGGGGVREPVN
 ITEPTLELLHSSCDPNAFHSTIQLYCFIYGHILNDVSVSWLMDDREITDTLAQTVLKEEGKLASTCS
 KLNITEQQWMSESTFTCKVTSQGVLYLAHTRRCPDHEPRGVITYLIPPSPLDLYQNGAPKLTCLVVDL
 ESEKNVNVVTWNQEKKTSVSASQWYTKHHNNATTSITSILPVVAKDWIEGYGYQCIVDHPDFPKPIVRS
 ITKTPGQRSAPENVYVFPPEEESEDKRTLTLCLIQNFFPEDISVQWLGDKLISNSQHSTTTPLKSNGS
 NQGFFIFSRLEVAKTLWTQRKQFTCQVIHEALQKPRKLEKTISTSLGNTSLRPSHHHHHH

Secretion signal 1-17

Human Sialidase-2 (NEU2) 18-397

Flexible linker 398-403

Mouse IgE-Fc 404-734

Hisx6 735-740

FIG. 16 (Cont'd)

SEQ ID NO:28

hNEU3_mIgEfc

MPLLLLLLPLWAGALAMRPADLPPRPMEESSPASSAPTETEEPGSSAEVMEEVTTCSFNSPLFRQEDD
 RGITYRIPALLYIPPTHFLAFAEKSTRRDEDALHLVLRRLRIGQLVQWGPKPLMEATLPGHRTM
 NPCPVWEQKSGCVLFFICVRGHVTERQQIVSGRNAARLCFIYSQDAGCSWSEVRDLTEEVIGSELKH
 WATFAVGPGHGIQLQSGRLVIPAYTTYIPSWFFCFQLPCKTRPHSLMIYSDDLGVTHHHGRLIRPMVT
 VECEVAEVTGRAGHPVLYCSARTPNRCRAEALSTDHGEGFQRLALSRQLCEPPHGCQGSVVSFRPLEI
 PHRCQDSSSKDAPTIQQSSPGSSLRLEEEAGTPSESWLLYSHPTSRKQRVDLGIYLNQTPLEAACWSR
 PWILHCGPCGYSDLAALEEEGLFGCLFECGTKQECEQIAFRLFTHREILSHLQGDCTSPGRNPSQFKS
 NGGGGGGVRPNITEPTLELLHSSCDPNAFHSTIQLYCFIYGHILNDVSVSWLMDDREITDTLAQTVL
 IKEEGKLASTCSKLNITEQQWMSESTFTCKVTSQGVVDYLAHTRCPDHEPRGVITYLIPPSPLDLYQN
 GAPKLTCLVVDLESEKNVNVNTWNQEKKTSVSASQWYTKHHNNATTSITSILPVVAKDWIEGYGYQCIV
 DHPDFPKPIVRSITKTPGQSAPEVYVFPPEEESEDKRTLTLCLIQNFFPEDISVQWLGDGKLISNSQ
 HSTTTPLKSNQSGFFIFSRLEVAKTLWTQRKQFTCQVIHEALQKPRKLEKTISTSLGNTSLRPSHH
 HHHH

Secretion signal 1-16
 Human Sialidase-3 (NEU3) 17-477
 Flexible linker 478-483
 Mouse IgE-Fc 484-814
 Hisx6 815-820

SEQ ID NO:29

hNEU4_mIgEfc

MPLLLLLLPLWAGALAMGVPRTPSRTVLFERERTGLTYRVPSLLPVPPGPTLLAFVEQRLSPDDSHAH
 RLVLRRGTLAGGSVRWGALHVLGTAALAEHRSMNCPVHDAGTGTVFLFFIAVLGHTPEAVQIATGRN
 AARLCCVASRDAGLSWGSARDLTEEAIGGAVQDWATFAVGPGHGVQLPSGRLLVPAYTYRVDRRECFCG
 KICRTSPHSFAFYSDDHGRTWRCGGLVPNLRSGECQLAAVDGGQAGSFLYCNARSPLGSRVQALSTDE
 GTSFLPAERVASLPETAWGCQGSIVGFAPAPAPNRPRDSSWSVGPSPQLQPPLLGPGVHEPPEEAAVDP
 RGGQVPGGPFPSRLQPRGDGPRQPGPRPGVSGDVGSWTALALPMPFAAPPQSPTWLLYSHPVGRRARLHM
 GIRLSQSPLDPRSWTEPWVIYEGPSGYSDLASIGPAPEGGLVFACLYESGARTSYDEISFCTFSLREV
 LENVPASPKPPNLGDKPRGCCWPSGGGGGGVRPNITEPTLELLHSSCDPNAFHSTIQLYCFIYGHIL
 NDVSVSWLMDDREITDTLAQTVLKEEGKLASTCSKLNITEQQWMSESTFTCKVTSQGVVDYLAHTRC
 PDHEPRGVITYLIPPSPLDLYQNGAPKLTCLVVDLESEKNVNVNTWNQEKKTSVSASQWYTKHHNNATT
 SITSILPVVAKDWIEGYGYQCIVDHPDFPKPIVRSITKTPGQSAPEVYVFPPEEESEDKRTLTLCLI
 QNFFPEDISVQWLGDGKLISNSQHSTTTPLKSNQSGFFIFSRLEVAKTLWTQRKQFTCQVIHEALQ
 KPRKLEKTISTSLGNTSLRPSHHHHHH

Secretion signal 1-16
 Human Sialidase-4 (NEU4) 17-500
 Flexible linker 501-506
 Mouse IgE-Fc 507-837
 Hisx6 838-843

FIG. 16 (Cont'd)

SEQ ID NO: 30

Canine IgE Fc-NEU2

MYRMQLLSICIALSLALVTNSCALNFIPPTVKLFHSSCNPVGDTHTTIQLLCLISGYVPGDMEVIWLVD
GQKATNIFPYTAPGTKEGNVTSTHSELNITQGEWVSQKTYTCQVTYQGFTFKDEARKCESDPRGVTS
YLSPPSPDLPLYVHKAPKITCLVVDLATMEGMNLTWYRESKEPVNPGPLNKKDHFNGTITVTSTLQVNT
NDWIEGETYYCRVTHPHLPKDIVRSIAKAPGKRAPPDVYLFLLPPEEEQGTKDRVTLTCLIQNFFPADI
SVQWLRNDSPIQTDQYTTTGPHKVSRSRPAFFIFSRLEVSRVDWEQKNKFTCQVVHEALSGSRILQKW
VSKTPGKMASCPLQKEKVFQSGAHAYRIPALLYLPQOKTLLAFAEKRTSKKDEHAELIVLRRGSYDA
STHHVQWHAQEVAQAQLEGHRSMPNPSPLYDEKTGTLFLFFIAIPGQVSEHHQLHTRVNVTRLCQVTS
TDHGRSWSPARDLTGPAIGAAHKEWATFAVGPGHCLQLHNKAQSLVVPAYAYRNHSLPRPSPFAFCF
VSHDHGHTWERNFVAQDTLEQVAEVGGAERRVYLNARSPRRARVQAESTNEGLDFTEAQVVKELV
EPPHGCQGSVISFPNPHAGSGACDRWLLYTHPTDPQRRALGAYLHRRRPGREAWSEPVPLAAGGCAY
SDLQAMGAGPDGSPQFGCLYESGHYEEVVFMLFTLKQAFPAEFVS

IL2-signal sequence (Amino Acids: 1-20)

dogIgE Fc (Amino Acids: 21-347)

NEU2 (Amino Acids: 348-725)

SEQ ID NO: 31

Feline IgE1 Fc-NEU2

MYRMQLLSICIALSLALVTNSCTMNFIPPTVKLFHSSCNPLGDTGSTIQLLCLISGYVPGDMEVTWLVD
GQKATNIFPYTAPGKQEGKVTSTHSELNITQGEWVSQKTYTCQVTYQGFTFEDHARKCTESDPRGVST
YLSPPSPDLPLYVHKSPKITCLVVDLANTDGMILTWSRENGESVHPDPMVKKTYNGTITVTSTLPVDA
TDWVEGETYQCKVTHPDLPKDIVRSIAKAPGRRFPPEVYVFLPPEGEPKTKDKVTLTCLIQNFFPPDI
SVQWLHNDSPVRTEQQATTWPHKATGSPAPFFVFSRLEVSRADWEQRDVFTCQVVHEALPGFRTLKKS
VSKNPGKMESCPILQKERVQSGAHAYRIPALLYLPEQKTVLAFAEQRISKKDEHAELIVLRRGSYDA
STHQVQWHAQEVAQAQLEGHRSMPNPSPLYDEKTGTLFLFFIAIPGQVSEHHQLHTRVNVTRLCQVTS
PDHGRSWSPARDLTDPAIGTAHKEWATFAVGPGHCLQLGNKTRSLVLPAYAYRNHLPHRRPSPFAFCF
VSHDHGHTWQRGNFVAQDTVECQVAEVGGAEQRVYLNARSPRLRARVQAESTNEGLDFKEAQLVEELV
EPPHGCQGSIVSFPGPGAGADASDRWLLYTHPTDPRQRAHLGAYLSKPRPGPAWSEPALLATGSCAY
SDLQSMGTGPDGSPQFGCLYESDDYEAVMFLFTLKQAFPAEFLSL

IL2-signal sequence (Amino Acids: 1-20)

catIgE Fc (Amino Acids: 21-347)

NEU2 (Amino Acids: 348-726)

FIG. 17

SEQ ID NO: 45**Canine NEU1**

>tr|F1P9W6|F1P9W6_CANLF Uncharacterized protein OS=Canis lupus familiaris GN=NEU1 PE=4 SV=2
MAGRPGAALPGRPGGPRVPGCWGGWRVRARAATFLLLWPLAGLGAGIKNDFSLVQPLVTM
EQLLWVSGRQIGNVHTFRIPLITATPRGTLTLLAFAEARKMSTSDEGAKFIALRRSTDEGST
WSPTAFIVNDGETPDGLNLGAVVSDMETGVVFLFYSLCAHKAGCQVASTMLVWSKDDGVS
WSPPRNLSLDIGTEMFAPGPGSGIQKRHDPRKGRLIVCGHGTLERDGVFCLLSDDHGASW
RYGSGVSGIPYGQPKREND FNPDECQPYELPDGSVVINARNQNNYHCHCRIVLRSYDACD
TIRPRDVTFDPELVDPVVAAGAVATGSGLVFFSNPAHPEFRVNLTLRWSFSNGTSWRKET
VQLWPGPSGYSSLAALEGSVGGKDQAPQLYVLYEKGRNQYTESISLAKVSVYGT

SEQ ID NO: 46**Canine NEU2**

>tr|J9P7D3|J9P7D3_CANLF Uncharacterized protein OS=Canis lupus familiaris GN=NEU2 PE=4 SV=1
MASCPVLQKEKVFQSGAHAYRIPALLYLPQQKTLLAFAEKRTSKKDEHAELIVLRGSDYD
ASTHHVQWHAQEVVAQAQLEGHRSMNPSPLYDEKTGTLFLFFIAIPGQVSEHHQLHTRVN
VTRLQCQVTSTDHGRSWSPARDLTGPAIGAAHKEWATFAVGPGHCLQLHNKAQSLVVPAYA
YRNLHSLPRPSPFAFCFVSHDHGHTWERGNFVAQDTLECQVAEVGGAERRVVYLNARSPR
RARVQAESTNEGLDFTEAQVVKELVEPPHGCQGSVISFPNPHAGSGACDRWLLYTHPTDF
QRRRAHLGAYLHRRRPGREAWSEPVPPLAAGGCAYSIDLQAMGAGPDGSPQFGCLYESGHYEE
VVFLMFTLKQAFPAEFVS

SEQ ID NO: 47**Canine NEU3**

>tr|F1PN90|F1PN90_CANLF Uncharacterized protein OS=Canis lupus familiaris GN=NEU3 PE=4 SV=2
MEEPESRGEVMEEVTSCSFNSPLFQQEDKRGITYRIPALLYIPPTHTFLAFAEKRSTSRD
EDALHLVLRRLRTGHSVQWEPLKPLMEAMLPGHRTMNPCPVWEQKSGCVYLFFICVRGH
VTERQQVMSGRNARLCFICSQDAGCSWSEVRDLTEEVI GA EVKRWATFAVGPGHGIQLQ
SGRLIIPAYAYYIPYWFFCFRLPCKAKPHSLMIYSDDL GATW HHGR LIKPTATVECEVAE
VTGRAGDPVLYCSARTPNRCRAEAFSINNGECFQRPALSNQLCEPPHGCQGSVVSFRPLE
ILHGYQDLNGKDSPTIQQSPLLDSSLWPEQEGGILSKSWLLYSHPTSKKRRVNLGIYLNQ
TPLDAACWSRPWILHCGPCGYSDLAAL EKEGLFGCLFECG TKRECEQ IAFRLFTDREILS
HMHEDSTGPGKNSEPTES

FIG. 18

Fc

SEQ ID NO: 48**Feline NEU1**

>tr|M3X3Z7|M3X3Z7_FELCA Uncharacterized protein OS=Felis catus
GN=NEU1 PE=4 SV=1

MMADRAGQVLLGRPGDPRMPGFWGGCRVQVLATIIIFLLLLSLAESGARAKNDFSLVQPLV
TMEQLLWVSGRQIGSVHTFRIPLITTTTPQGTLLAFAEARKMSTSDEGAKFIALRRSLDQG
STWSPTAFIVDDGDTPDGLNLGAVVNDMETGVVFLFYSLCAHKAGCQVASTMLVWSKDDG
VSWSPPRNLSLDIGTEMFAPGPGSGIQKQREPRKGR LIVCGHGTLE RDGVFCLLSDDHGA
TWRYGSGVSGIPYGQPKRENDFNPDECQPYELPDG SVVINARNQNNYHCHCRIVLRSYDA
CDTIRPRDVTFDPELVDPVVAAGAVATSSGIVFFSNPAHPEFRVNLTLRWSFSNGTSWRK
ETVQLWPGPSGYSSLAALLEGNVGKDKQAPQLYVLYEKGRNQYTESISLAKVSVYGTL

SEQ ID NO: 49**Feline NEU2**

>tr|M3WEW5|M3WEW5_FELCA Uncharacterized protein OS=Felis catus
GN=NEU2 PE=4 SV=1

MESCPILQKERVFQSGAHAYRIPALLYLPEQKTVLAFAEQRISKKDEHAELIVLRGSYD
ASTHQVQWHAQEVVAQAQLEGHRSMNPSPLYDEKTGTLFLFFIAIPGQVSEHHQLHTRVN
VTRL CQVTSPDHGRSWSPARDLTDPAIGTAHKEWATFAVGPGHCLQLGNKTRSLLPAYA
YRNLHPHRRPSPFAFCFVSHDHGHTWQRGNFVAQDTEVCQVAEVGGAEQRVVYLNARSPL
RARVQAESTNEGLDFKEAQLVEELVEPPHGCQGSIVSFPGPGAGADASDRWLLYTHPTDF
RQRAHLGAYLSKPRPGPAAWSEPALLATGSCAYSDLQSMGTGPDGSPQFGCLYESDDYEA
VMFLTFTLTKQAFPAEFLSL

SEQ ID NO: 50**Feline NEU3**

>tr|M3WG19|M3WG19_FELCA Uncharacterized protein OS=Felis catus
GN=NEU3 PE=4 SV=1

MEELGSREEVMEEVTSCSFSSPLFQQEDKRGITYRIPALLYIPPTHTFLAFAEKRSTSRD
EDALHLVLRGLRTGHSVQWGPLKPLMEATLPGHRTMNPCPVWEQKSGCVYLFFICVRGH
VTERQQIMSGRNAARLCFICSRDTGCSWSEVRDLTEEVI GA EVKRWATFAVGPGHGIQLQ
SGRLVIPAYAYYIPYWFFCFRLPCKAKPHSLMIYSDDL GATWHHGRLIQPMATVECEVAE
VTGRGGNPVLYCSARTPNRCRAEAFSDDRGE CFQRLALSRELCEPPHGCQGSVVSFRPPE
ILRGCDPENGKDASTIQQSPLLDRLSLRLEQEAGTLSESWLLYSHPTCKKRRVNLGIYLNQ
TPLDAACWSPWILHCGPCGYSDLAAL EKEGLFGCLFECG TKRECEQIAFRLFTDREILS
HVHGDFTS PDKNPEPIES

SEQ ID NO: 51**Feline NEU4**

>tr|M3W397|M3W397_FELCA Uncharacterized protein OS=Felis catus
GN=NEU4 PE=4 SV=1

TWXAARDLTEEAVGGAEQDWATFAVGPGHGIQLRSGRLLVPAYTYHVDRECFGKICRTS
PHAFTFYSDDHGRTWRHGGGLVPNLRS GECQLAAVDRGQAGHILYCNARSPLGSRVQALSA
DEGTSFLPGELVPALAEARGCQGSVLGFPAPLFSRPRNGRWPVGAGSHSYSPHLCLGVQ
EPSEEGTGDTQGAQGPSGMEGCRDSP EEPGLGPGLSGIMAAWTSKVPGPPATLSQSPTWL
LYSHPVGRRARLHMGIYLSRSPLDPHSWTEPWVIYEGPSGYSDLASVGPTVKGALTFACL
FESGARVSYEEISFCMFSLG EVLQNLPPGPKPPGLGDKPLERCRPS

FIG. 19

SEQ ID NO: 52

Bovine IgE heavy chain constant region

>AAB09546.2 IgE heavy chain constant region, partial [Bos taurus]
SIQAPSIYPLRLCCTEEARVRLGCLVKDYLPGSVTVTWDTVPLDGSTLTFPSIQMASSSLYITTSQLT
ISGEQSKEFTCRVLHPETNSNLNKTISTECAKNFSDPSVKLFYSSCNPNGDTQTTIHLLCRISGYTPG
KIKVTWLVDGLQSEELYARSGPEIQEGNLTSTYSEVNITQGQWVSEKTYTCRVNYYGYNFESHHRCT
AESEPRGVSAYLSPPTPLELYVNKSPKITCLVVDLANEKNLSLTWSRANGKPVHAGPPEIKRQFNGTV
TVTSTLPVDVTDWVEGETYYCKVSHSDLPTDIQRSISKDVGKRLAPKAYVFLPDGKELENEEELTLTC
MIQKFFPRDIFVRWLHNKELMRADQHTTTQPHRADNNTPAFFAYSRLAVPRANWKRGDFTCQVIHEA
LPRTRTLEKSVFINSKG

SEQ ID NO: 52

Bovine Sialidase-1 (NEU1) -lysosomal

>sp|A6BMK7|NEUR1_BOVIN Sialidase-1 OS=Bos taurus GN=NEU1 PE=2 SV=2
MTEEGPGIVSLGKLRPRMLRLWGICRVQIFSAIFMLMSPAGVGAGAKDDFSLVHPLVTM
EQLLWVSGKQIGSVDTFRIPLITTTTPRGTLTLLAFEAARKMSTSDKGAKFIALRRSMDQGST
WSPTAFIVDDGETPDGLNLGAVVSDTTTGVVFLFYSLCAHKAGCQVASTMLVWSKDDGVS
WSSPRNLSLDIGTEMFAPGPGSGIQKQREPRKGR LIVCGHGTLE RDGVFCLLSDDHGVSW
RYGGGVSGIPYGQPKRENDFNDECQPYELPDGSSVINARNQNNYHCHCRIILRSYDACD
TLRPRDVTFTDELVDPVVAAGAVATSSGIVFFSNPAHPEFRVNLTLRWSFSNGTSWRKET
VQLWPGPSGYSSLTTLLEGNVDGKDEAPQLYVLYEKGRNQYMESISLVKVS VYGTL
Signal Peptide aa 1-47

SEQ ID NO: 53

Bovine Sialidase-3 (NEU3) - Plasma membrane

>sp|O97859|NEUR3_BOVIN Sialidase-3 OS=Bos taurus GN=NEU3 PE=1 SV=1
MEEVTSCSFSSPLFQQEDKRGVTYRIPALIYVPPAHTFLAFAEKRSSSKDE DALHLVLR
GLRTGQSVQWEPLKSLMKATLPGHRTMNPCPVWERKSGYVYLFFICVQGHVTERQQIMSG
RNPARLCFICSQDAGYSWSDVRDLTEEVI GPEVTHWATFAVGPGHGIQLQSGRLIIPAYA
YYIPFWFFCFRLPYRAPHSLMIYSDDL GATWHHGRLIKPMVTVECEVAEVIGKAGHPVL
YCSARTPNRHRAEALSIDHGECFQKPVL SHQLCEPPHGCQGSVVSFCPLEIPGGCQDLA
G EDAPAIQQSPLLCSSVRPEPEAGT LSESWLLYSHPTNKKRRVDLGIYLNQSPLEAACWSR
PWILHCGPCGYSDLAALENEGLFGCLFECG TKQECEQIAFRLFTDREILSHVQGD CSTPG
MNSEPSKK

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FIG. 20

SEQ ID NO: 55**Equine IgE heavy chain constant region**

>CAC44497.1 immunoglobulin epsilon heavy chain constant region,
allotype a, partial [Equus caballus]
VSKQAPLILPLAACCKDTKTTNITLGCLVKGYFPEPVTVTWDAGSLNRSTMTFPAVFDQTSGLYTTIS
RVVASGKWAKQKFTCNVVSQETFNKTFNACIVTFTPPTVKLFHSSCDPGGDSHTTIQLLCLISDYTP
GDIDIVWLIDGQKVDEQFPQHGLVKQEGKLASTHSELNITQGQWASENTYTCQVTYKDMIFKDQARKC
TESDPRGVSVYLSPPSPLDLYVSKSPKITCLVVDLANVQGLSLNWSRESGEPLQKHTLATSEQFNKTF
SVTSTLPVDTTDWIEGETYKCTVSHPDLPREVVRSAKAPGKRLSPEVYVFLPPEEDQSSKDKVTITC
LIQNFPPADISVQWLRNNVLIQTDQQATTREPQKANGPNPAFFVFSRLEVSRAEWEQKNKFACKVVHEA
LSQRTLQKEVSKDPGK

SEQ ID NO: 56**Equine Neuraminidase (NEU1) - lysosomal**

>tr|F6V6F7|F6V6F7_HORSE Neuraminidase 1 OS=Equus caballus GN=NEU1
PE=4 SV=1
MMAERSGTVLRDRPGGPRTLGFWGGCRVQVLAIFLLLLSLARFGTRAGNDFDLVQPLVT
MEQLLWVSGKQIGSVDTFRIPLITVTPQGTLTLLAFEAARKMSSSDLGAKFIALRRSMDQGS
TWSPTAFIVDDGETPDGLNLGAVVSDRETGIVFLFYSLCAHKAGCQVASTMLVWSKDDGV
SWSSPRNLSLDIGTEVFAPGPGSGIQKQRQPRKGR LIVCGHGTLE RDGVFCLLSDDHGAS
WRYGNGIRGIPYGQPKQKYDFNPDECQPYELPDGSSVINARNQNNYHCHCRIILRSYDAC
DTLRPRDVTFDPELVDPVVAAGAVATNSGIVFFSNPAHPEFRVNLTLRWSFSNGTSWRKE
TVQLWPGPSGYSSMAALEGSLGGEDQAPQLYVLYEKGLSQYTESISLAKVSVYGTLL

SEQ ID NO: 57**Equine Neuraminidase (NEU2) -cytosolic**

>tr|F6ZZS9|F6ZZS9_HORSE Neuraminidase 2 OS=Equus caballus GN=NEU2
PE=4 SV=1
MASCPILQKEKVFQSGAHAYRIPALLYLPQQKTLLAFAEKRRDKRDEHAELIALRKGSYD
VATHRVLWHAEEVVTQAQLEGHRSMNPSPLYDKKTGTLELFFISIPGQVSE RHQLHTRVN
VTRLCQVTSTDHGRSWSPARDVTDSTIGSAYKEWATFALGPGHCLQLHNESQSLVVPAYA
YRNLHPRHRPAPFAVCFISHDHGHTWQRGNFVAQDTLECQVAEVGSTKQRVVYLNARSPL
RARVQAESNNDGLDFKEIQLVTKLAEP PQGCQGSIVSFPNPTVQSDFSKWLLYTHPTDS
RQRSNLGVYLNKWPLAPTAWSEPTLLAKGSCAYSDLQSMGAGPDGSPQFACLYESNNYEE
IIFLMFTLKQAFPAEFLPQ

SEQ ID NO: 58**Equine Neuraminidase (NEU3) - membrane**

>tr|F7ADI2|F7ADI2_HORSE Neuraminidase 3 OS=Equus caballus GN=NEU3
PE=4 SV=1
MEEVTSCSFNSPLFQQEDKRGV TYRIPALLYVPPTHTFLAFAEKRSTCRDEDALHLVLR
GVRTEHSVQWGPKPLMEATLP GHRTMNPCPVWEQKSGCVYLFFICVRDHVTERQQIVSG
RNAARLCFICSQDAGCSWSELRLDTEEVIGPEMKRWATFAVGPBGHGIQLQSGRLVIPAYA
YYIPYLFFCFRLPCKARPHSLMIYSDDL GATWHHGR LIKPMVTVECEVAEVERTERTGYPVL
YCSARTPNRCRAEALSTDHGECEQRPALSQQLCPEPHGCQGSVVSFQPLEIPHGYQDP
SGQDAPTIQQNPLDSSLRLEQEAGTLSQSWLLYSHPTSKKRRVNLGIYLNQTPLEATCWSR
PWILHCGPCGYSDLAALKEGLFACLFECGIKRECEQIAFRLFTVQEIVSHVQGDCTSPG
RISEPIQN

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FIG. 21

Sequence encoding hNEU1_hIgEFC (SEQ ID NO: 59)

atgtacaggatgcaactcctgtccttgcatgtgactaagtcttgcaacttgtcacgaattcgggatcccc
agcacagtggagccttgagAACGACTTCGGTCTGGTGACCGCTGGTGACCATGGAGCAACTGCTGT
GGGTGAGCGGGAGACAGATCGGCTCAGTGGACACCTCCGCATCCCGCTCATCACAGCCACTCCGCGG
GGCACTCTTCTCGCCTTTGCTGAGGCGAGGAAAATGTCCTCATCCGATGAGGGGGCCAAGTTCATCGC
CCTGCGGAGGTCCATGGACCAGGGCAGCACATGGTCTCCTACAGCGTTCATTGTCAATGATGGGGATG
TCCCCGATGGGCTGAACCTTGGGGCAGTAGTGAGCGATGTTGAGACAGGAGTAGTATTTCTTTCTAC
TCCCTTTGTGCTCACAAGGCCGGCTGCCAGGTGGCCTCTACCATGTTGGTAIGGAGCAAGGATGATGG
TGTTTCCTGGAGCACACCCCGGAATCTCTCCCTGGATATTGGCACTGAAGTGTTTGCCCCCTGGACCGG
GCTCTGGTATTTCAGAAACAGCGGGAGCCACGGAAGGGCCGCTCATCGTGTGTGGCCATGGGACGCTG
GAGCGGGACGGAGTCTTCTGTCTCCTCAGCGATGATCATGGTGCCTCCTGGCGCTACGGAAGTGGGGT
CAGCGGCATCCCCCTACGGTCAGCCCCAAGCAGGAAAATGATTTCAATCCTGATGAATGCCAGCCCTATG
AGCTCCCAGATGGCTCAGTCGTTCATCAATGCCCCGAAACCAGAACAACTACCCTGCCACTGCCGAATT
GTCCTCCGCAGCTATGATGCCTGTGATACACTAAGGCCCGGTGATGTGACCTTCGACCCTGAGCTCGT
GGACCCTGTGGTAGCTGCAGGAGCTGTAGTCACCAGCTCCGGCATTTGTCTTCTTCTCCAACCCAGCAC
ATCCAGAGTTCCGAGTGAACCTGACCCTGCGATGGAGCTTCAGCAATGGTACCTCATGGCGGAAAAGAG
ACAGTCCAGCTATGGCCAGGCCCCAGTGGCTATTCATCCCTGGCAACCCTGGAGGGCAGCATGGATGG
AGAGGAGCAGGCCCCCAGCTCTACGTCTGTATGAGAAAGGCCGGAACCACTACACAGAGAGCATCT
CCGTGGCCAAAATCAGTGTCTATGGGACACTCGGAGGAGGAGGAGGAGGATGCTCCAGGGACTTCACC
CCGCCCCACCGTGAAGATCTTACAGTCGTCTGCGACGGCGGGCACTTCCCCCCGACCATCCAGCT
CCTGTGCCTCGTCTCTGGGTACACCCCAGGGACTATCAACATCACCTGGCTGGAGGACGGGCAGGTCA
TGGACGTGGACTTGTCCACCGCCTCTACCACGCAGGAGGGTGAGCTGGCCTCCACACAAAGCGAGCTC
ACCCTCAGCCAGAAGCACTGGCTGTGACACGCACCTACACCTGCCAGGTCACCTATCAAGGTCACAC
CTTTGAGGACAGCACCAAGAAGTGTGCAGATTCCAACCCGAGAGGGGTGAGCGCTACCTAAGCCGGC
CCAGCCCCGTTCGACCTGTTTCATCCGCAAGTCGCCCACGATCACCTGTCTGGTGGTGGACCTGGCACC
AGCAAGGGGACCGTGAACCTGACCTGGTCCCGGGCCAGTGGGAAGCCTGTGAACCACTCCACCAGAAA
GGAGGAGAAGCAGCGCAATGGCACGTTAACCCTCACGTCCGCCCTGCCGGTGGGCACCCGAGACTGGA
TCGAGGGGGAGACCTACCAGTGCAGGGTGACCCACCCCCACCTGCCAGGGCCCTCATGCGGTCCACG
ACCAAGACCAGCGGCCCGCGTGTGCCCCGGAAGTCTATGCGTTTTCGACGCCGGAGTGCCCGGGGAG
CCGGGACAAGCGCACCCCTCGCCTGCCTGATCCAGAACTTCATGCCTGAGGACATCTCGGTGCAGTGGC
TGCACAACGAGGTGCAGCTCCCGGACGCCCCGGCACAGCACGACGACGCCCCGCAAGACCAAGGGCTCC
GGCTTCTTCTGCTTTCAGCCGCCTGGAGGTGACCAGGGCCGAATGGGAGCAGAAAGATGAGTTCATCTG
CCGTGCAGTCCATGAGGCAGCGAGCCCCCTCACAGACCGTCCAGCGAGCGGTGTCTGTAAATCCCGGTA
AACACCATCACCATCACCATTGA

Secretion signal 1-60

Human Sialidase-1 (NEU1) (No signal peptide) 85-1188

Flexible linker 1189-1206

Human IgE-Fc 1207-2178

Hisx6 2179-2199

FIG. 22

Sequence encoding hNEU2_hIgEFC (codon optimized) (SEQ ID NO: 60)

atgcctctgctgctgctgctgctgccactgctgtgggctggcgctctggctGCTTCTCTGCCAGTGTGCA
GAAGGAGTCCGTGTTTCAGAGCGGAGCTCACGCTTACAGGATCCCCGCCCTGCTGTATCTGCCTGGAC
AGCAGTCCCTGCTGGCCTTCGCTGAGCAGAGGGCCAGCAAGAAGGACGAGCACGCTGAGCTGATCGTG
CTGAGGAGGGGCGACTACGATGCTCCTACCCATCAGGTGCAGTGGCAGGCTCAGGAGGTGGTGGCTCA
GGCTAGGCTGGACGGACACCGGAGCATGAACCCATGCCCTCTGTATGATGCCAGACCGGCACACTGT
TTCTGTTCTTTATCGCTATCCAGGCCAGGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAATGTG
ACCCGCCCTGTGCCAGGTGACCTCTACAGACCATGGAAGAACCTGGTCCAGCCCAAGGGACCTGACAGA
TGCTGCTATCGGCCCTGCCTACAGAGAGTGGAGCACCTTTGCTGTGGGACCAGGACACTGCCTGCAGC
TGCATGATAGAGCCCGCTCTCTGGTGGTGCCAGCCTACGCTTATAGAAAGCTGCACCCAATCCAGAGG
CCAATCCCATCCGCCCTTCTGCTTTCTGTCTCACGACCATGGAAGGACCTGGGCTAGGGGACATTTCTG
GGCTCAGGATACACTGGAGTGTGAGGTGGCTGAGGTGGAGACCGGAGAGCAGAGGGTGGTGACACTGA
ACGCTCGGTCTCACCTGAGGGCTAGGGTGCAGGCTCAGTCCACCAATGACGGCCTGGATTTTTCAGGAG
AGCCAGCTGGTGAAGAAGCTGGTGGAGCCACCTCCACAGGGATGTGAGGGATCCGTGATCAGCTTCCC
TTCTCCACGCTCCGGACAGGATCTCCAGCTCAGTGGCTGCTGTACACACACCCAACCCATTCTCTGGC
AGAGAGCCGACCTGGGAGCTTATCTGAACCCCCGCCCCCTGCTCCTGAGGCTTGGTCCGAGCCCGTG
CTGCTGGCTAAGGGATCTTGCGCTTACTCCGACCTGCAGAGCATGGGAACCGGACCAGATGGAAGCCC
ACTGTTTGGCTGTCTGTACGAGGCTAATGATTATGAGGAGATCGTGTTCCTGATGTTTACACTGAAGC
AGGCCTTCCCAGCTGAGTATCTGCCACAGGGAGGAGGAGGAGGAGGATGCTCTAGGGACTTTACACCA
CCCACCGTGAAGATCCTGCAGTCTTCTGCGATGGCGGCGGCCACTTCCCTCCAACCATCCAGCTGCT
GTGCCTGGTGTCCGGCTACACACCCGGCACCATCAACATCACCTGGCTGGAGGACGGCCAGGTGATGG
ACGTGGATCTGAGCACAGCCTCTACCACACAGGAGGGCGAGCTGGCTTCCACACAGAGCGAGCTGACC
CTGTCCCAGAAGCACTGGCTGAGCGATCGGACCTACACATGCCAGGTGACATATCAGGGCCATACCTT
CGAGGACAGCACAAAGAAGTGTGCCGATTCTAACCTAGGGGCGTGTCCGCTTATCTGTCTCGGCCCT
CCCCTTTCGACCTGTTTATCAGAAAGAGCCCAACCATCACATGTCTGGTGGTGGATCTGGCCCCCTCT
AAGGGCACAGTGAATCTGACCTGGAGCCGCGCTTCTGGCAAGCCCGTGAACCATTCACACAGGAAGGA
GGAGAAGCAGCGGAATGGCACCCCTGACAGTGACCAGCGCCCTGCCTGTGGGCACAAGGGACTGGATCG
AGGGCGAGACCTACCAGTGCCGGGTGACACACCCTCATCTGCCAAGAGCTCTGATGCGCTCTACCACA
AAGACCTCCGGCCCTAGAGCCGCTCCAGAGGTGTATGCCTTTGCTACCCCCGAGTGGCCTGGCTCCAG
AGATAAGCGCACACTGGCCTGTCTGATCCAGAACTTCATGCCTGAGGACATCTCTGTGCACTGGCTGC
ACAATGAGGTGCAGCTGCCTGATGCTAGACATTCCACCACACAGCCACGCAAGACCAAGGGCAGCGGC
TTCTTCGTGTTCTCCAGGCTGGAGGTGACAAGGGCTGAGTGGGAGCAGAAGGACGAGTTTATCTGCAG
AGCTGTGCACGAGGCCGCTAGCCCTTCTCAGACCGTGCAGCGCGCCGTGAGCGTGAATCCAGGCAAGC
ACCATCACCATCACCATTGA

Secretion signal 1-48

Human Sialidase-2 (NEU2) (Codon optimized for mammalian expression)

49-1185

Flexible linker 1186-1203

Human IgE-Fc 1204-2175

Hisx6 2176-2193

FIG. 23

Sequence encoding hNEU3_hIgEFc (SEQ ID NO:61)

atgcctctgctgctgctgctgctgccactgctgtgggctggcgctctggctATGAGACCTGCGGACCTGCC
CCCGCGCCCCATGGAAGAATCCCCGGCGTCCAGCTCTGCCCCGACAGAGACGGAGGAGCCGGGGTCCA
GTGCAGAGGTCATGGAAGAAGTGACAACATGCTCCTTCAACAGCCCTCTGTTCCGGCAGGAAGATGAC
AGAGGGATTACCTACCGGATCCCAGCCCTGCTCTACATACCCCCACCCACACCTTCCTGGCCTTTGC
AGAGAAGCGTTCTACGAGGAGAGATGAGGATGCTCTCCACCTGGTGCTGAGGCGAGGGTTGAGGATTG
GGCAGTTGGTACAGTGGGGGCCCTGAAGCCACTGATGGAAGCCACACTACCGGGGCATCGGACCATG
AACCCCTGTCTGTATGGGAGCAGAAGAGTGGTTGTGTGTTTCTGTTCTTCATCTGTGTGCGGGGCCA
TGTCACAGAGCGTCAACAGATTGTGTGTCAGGCAGGAATGCTGCCCCCTTTGCTTCATCTACAGTCAGG
ATGCTGGATGTTTCATGGAGTGAGGTGAGGGACTTGACTGAGGAGGTCATTGGCTCAGAGCTGAAGCAC
TGGGGCCACATTTGCTGTGGGCCAGGTTCATGGCATCCAGCTGCAGTCAGGGAGACTGGTCATCCCTGC
GTATACCTACTACATCCCTTCCTGGTTCTTTTGCTTCCAGCTACCATGTAAAACCAGGCCTCATTCTC
TGATGATCTACAGTGATGACCTAGGGGTCACATGGCACCATGGTAGACTCATTAGGCCCATGGTTACA
GTAGAATGTGAAGTGGCAGAGGTGACTGGGAGGGCTGGCCACCCTGTGCTATATTGCAGTGCCCGGAC
ACCAAACAGGTGCCGGGCAGAGGCGCTCAGCACTGACCATGGTGAAGGCTTTCAGAGACTGGCCCTGA
GTCGACAGCTCTGTGAGCCCCACATGGTTGCCAAGGGAGTGTGGTAAGTTTCCGGCCCCCTGGAGATC
CCACATAGGTGCCAGGACTCTAGCAGCAAAGATGCACCCACCATTTCAGCAGAGCTCTCCAGGCAGTTC
ACTGAGGCTGGAGGAGGAAGCTGGAACACCGTCAGAATCATGGCTCTGTACTCACACCCAACAGTA
GGAAACAGAGGGTTGACCTAGGTATCTATCTCAACCAGACCCCTTGGAGGCTGCCTGCTGGTCCCGC
CCCTGGATCTTGCCTGTGGGCCCTGTGGCTACTCTGATCTGGCTGCTCTGGAGGAGGAGGGCTTGTT
TGGGTGTTTGTGTTGAATGTGGGACCAAGCAAGAGTGTGAGCAGATTGCCTTCCGCCTGTTTACACACC
GGGAGATCCTGAGTCACCTGCAGGGGGACTGCACCAGCCCTGGTAGGAACCCAAGCCAATTCAAAAGC
AATGGAGGAGGAGGAGGAGGATGCTCTAGGGACTTTACACCACCCACCGTGAAGATCCTGCAGTCTTC
CTGCGATGGCGGCGGCCACTTCCCTCCAACCATCCAGCTGCTGTGCCTGGTGTCCGGCTACACACCCG
GCACCATCAACATCACCTGGCTGGAGGACGGCCAGGTTCATGGACGTGGATCTGAGCACAGCCTCTACC
ACACAGGAGGGCGAGCTGGCTTCCACACAGAGCGAGCTGACCCTGTCCCAGAAGCACTGGCTGAGCGA
TCGGACCTACACATGCCAGGTGACATATCAGGGCCATACCTTCGAGGACAGCACAAAGAAGTGTGCCG
ATTCTAACCCCTAGGGGCGTGTCCGCTTATCTGTCTCGGCCCTCCCCTTTCGACCTGTTTATCAGAAAG
AGCCCAACCATCACATGTCTGGTGGTGGATCTGGCCCCCTCTAAGGGCACAGTGAATCTGACCTGGAG
CCGCGCTTCTGGCAAGCCCGTGAACCATTCACCAGGAAGGAGGAGAAGCAGCGGAATGGCACCCCTGA
CAGTGACCAGCGCCCTGCCTGTGGGCACAAGGGACTGGATCGAGGGCGAGACCTACCAGTGCCGGGTG
ACACACCCCTCATCTGCCAAGAGCTCTGATGCGCTCTACCACAAAGACCTCCGGCCCTAGAGCCGCTCC
AGAGGTGTATGCCTTTGCTACCCCCGAGTGGCCTGGCTCCAGAGATAAGCGCACACTGGCCTGTCTGA
TCCAGAACTTCATGCCTGAGGACATCTCTGTGCAGTGGCTGCACAATGAGGTGCAGCTGCCTGATGCT
AGACATTCCACCACACAGCCACGCAAGACCAAGGGCAGCGGCTTCTTCGTGTTCTCCAGGCTGGAGGT
GACAAGGGCTGAGTGGGAGCAGAAGGACGAGTTTATCTGCAGAGCTGTGCACGAGGCCGCTAGCCCTT
CTCAGACCGTGCAGCGCGCCGTGAGCGTGAATCCAGGCAAGCACCATCACCATCACCATTGA

Secretion signal 1-48

Human Sialidase-3 (NEU3) 49-1431

Flexible linker 1432-1449

Human IgE-Fc 1450-2421

Hisx6 2422-2439

FIG. 24

Sequence encoding hNEU4_hIgEFc (SEQ ID NO: 62)

atgcctctgctgctgctgctgctgccactgctgtgggctggcgctctggctATGGGGGTCCCTCGTACCCC
TTCACGGACAGTGCTCTTCGAGCGGGAGAGGACGGGCTGACCTACCGCGTGCCCTCGCTGCTCCCCG
TGCCCCCGGGCCACCCTGCTGGCCTTTGTGGAGCAGCGGCTCAGCCCTGACGACTCCACGCCCCAC
CGCCTGGTGCTGAGGAGGGGCACGCTGGCCGGGGGCTCCGTGCGGTGGGGTGCCCTGCACGTGCTGGG
GACAGCAGCCCTGGCGGAGCACCGGTCCATGAACCCCTGCCCTGTGCACGATGCTGGCACGGGCACCG
TCTTCCTCTTCTTCATCGCGGTGCTGGGCCACACGCCTGAGGCCGTGCAGATCGCCACGGGAAGGAAC
GCCGCGCGCCTCTGCTGTGTGGCCAGCCGTGACGCCGGCCTCTCGTGGGGCAGCGCCCGGGACCTCAC
CGAGGAGGCCATCGGTGGTGCCGTGCAGGACTGGGCCACATTGCTGTGGGTCCCGGCCACGGTGTC
AGCTGCCCTCAGGCCGCCTGCTGGTACCCGCCTACACCTACCGCGTGACCGCCGAGAGTGTTTTGGC
AAGATCTGCCGACCAGCCCTCACTCCTTCGCCTTCTACAGCGATGACCACGGCCGCACCTGGCGCTG
TGGAGGCCTCGTGCCCAACCTGCGCTCAGGCGAGTGCCAGCTGGCAGCGGTGGACGGTGGGCAGGCCG
GCAGCTTCCTCTACTGCAATGCCCGGAGCCCACTGGGCAGCCGTGTGCAGGCGCTCAGCACTGACGAG
GGCACCTCCTTCCTGCCCCGAGAGCGCGTGGCTTCCCTGCCCGAGACTGCCTGGGGCTGCCAGGGCAG
CATCGTGGGCTTCCCAGCCCCCGCCCCAACAGGCCACGGGATGACAGTTGGTCAGTGGGCCCCGGGA
GTCCCCCTCCAGCCTCCACTCCTCGGTCTTGGAGTCCACGAACCCCCAGAGGAGGCTGCTGTAGACCCC
CGTGGAGGCCAGGTGCCTGGTGGGCCCTTCAGCCGTCTGCAGCCTCGGGGGGATGGCCCCAGGCAGCC
TGGCCCCAGGCCTGGGGTCAGTGGGGATGTGGGGTCTTGGACCCTGGCACTCCCCATGCCCTTTGCTG
CCCCGCCCCAGAGCCCCACGTGGCTGCTGTACTCCCACCCAGTGGGGCGCAGGGCTCGGCTACACATG
GGTATCCGCTGAGCCAGTCCCCGCTGGACCCGCGCAGCTGGACAGAGCCCTGGGTGATCTACGAGGG
CCCCAGCGCTACTCCGACCTGGCGTCCATCGGGCCGGCCCTGAGGGGGGCTGGTTTTTGCCTGCC
TGTACGAGAGCGGGGCCAGGACCTCCTATGATGAGATTTCTTTGTACATTCTCCCTGCGTGAGGTC
CTGGAGAACGTGCCCGCCAGCCCCAAACCGCCCAACCTTGGGGACAAGCCTCGGGGGTGCTGCTGGCC
CTCCGGAGGAGGAGGAGGAGGATGCTCTAGGGACTTTACACCACCCACCGTGAAGATCCTGCAGTCTT
CCTGCGATGGCGGCGGCCACTTCCCTCCAACCATCCAGCTGCTGTGCCTGGTGTCCGGCTACACACCC
GGCACCATCAACATCACCTGGCTGGAGGACGGCCAGGTGATGGACGTGGATCTGAGCACAGCCTCTAC
CACACAGGAGGGCGAGCTGGCTTCCACACAGAGCGAGCTGACCCTGTCCAGAAGCACTGGCTGAGCG
ATCGGACCTACACATGCCAGGTGACATATCAGGGCCATACCTTCGAGGACAGCACAAAGAAGTGTGCC
GATTCTAACCCCTAGGGGCGTGTCGCTTATCTGTCTCGGCCCTCCCCTTTCGACCTGTTTATCAGAAA
GAGCCCAACCATCACATGTCTGGTGGTGGATCTGGCCCCCTCTAAGGGCACAGTGAATCTGACCTGGA
GCCGCGCTTCTGGCAAGCCCGTGAACCATTCACCAGGAAGGAGGAGAAGCAGCGGAATGGCACCCCTG
ACAGTGACCAGCGCCCTGCCTGTGGGCACAAGGGACTGGATCGAGGGCGAGACCTACCAGTGCCGGGT
GACACACCCCTCATCTGCCAAGAGCTCTGATGCGCTCTACCACAAAGACCTCCGGCCCTAGAGCCGCTC
CAGAGGTGTATGCCTTTGCTACCCCCGAGTGGCCTGGCTCCAGAGATAAGCGCACACTGGCCTGTCTG
ATCCAGAACTTCATGCCTGAGGACATCTCTGTGCACTGGCTGCACAATGAGGTGCAGCTGCCTGATGC
TAGACATTCCACCACACAGCCACGCAAGACCAAGGGCAGCGGCTTCTTCGTGTTCTCCAGGCTGGAGG
TGACAAGGGCTGAGTGGGAGCAGAAGGACGAGTTTATCTGCAGAGCTGTGCACGAGGCCGCTAGCCCT
TCTCAGACCGTGCAGCGCGCCGTGAGCGTGAATCCAGGCAAGCACCATCACCATCACCATTTGA

Secretion signal 1-48

Human Sialidase-4 (NEU4) 49-1500

Flexible linker 1501-1518

Human IgE-Fc 1519-2490

Hisx6 2491-2508

FIG. 25

Sequence encoding hNEU1_mIgEFc (SEQ ID NO:63)

atgtacaggatgcaactcctgtcttgcattgcactaagtcttgcacttgtcacgaattcgggatcccc
agcacagtggaagcttGAGAACGACTTCGGTCTGGTGACCGCTGGTGACCATGGAGCAACTGCTGT
GGGTGAGCGGGAGACAGATCGGCTCAGTGGACACCTCCGCATCCCGCTCATCACAGCCACTCCGCGG
GGCACTCTTCTCGCCTTTGCTGAGGCGAGGAAAATGTCCTCATCCGATGAGGGGGCCAAGTTCATCGC
CCTGCGGAGGTCCATGGACCAGGGCAGCACATGGTCTCCTACAGCGTTCATTGTCAATGATGGGGATG
TCCCCGATGGGCTGAACCTTGGGGCAGTAGTGAGCGATGTTGAGACAGGAGTAGTATTTCTTTTCTAC
TCCCTTTGTGCTCACAAAGGCCGGCTGCCAGGTGGCCTCTACCATGTTGGTATGGAGCAAGGATGATGG
TGTTTCCTGGAGCACACCCCGGAATCTCTCCCTGGATATTGGCACTGAAGTGTTTGCCCCCTGGACCGG
GCTCTGGTATTTCAGAAACAGCGGGAGCCACGGAAGGGCCGCTCATCGTGTGTGGCCATGGGACGCTG
GAGCGGGACGGAGTCTTCTGTCTCCTCAGCGATGATCATGGTGCCTCCTGGCGCTACGGAAGTGGGGT
CAGCGGCATCCCCCTACGGTCAGCCCCAAGCAGGAAAATGATTTCAATCCTGATGAATGCCAGCCCTATG
AGCTCCCAGATGGCTCAGTCGTCAATGCCCCGAAACCAGAACAACTACCACTGCCACTGCCGAATT
GTCCTCCGCAGCTATGATGCCTGTGATACACTAAGGCCCCCGTGATGTGACCTTCGACCCTGAGCTCGT
GGACCCTGTGGTAGCTGCAGGAGCTGTAGTCACCAGCTCCGGCATTGTCTTCTTCTCCAACCCAGCAC
ATCCAGAGTTCCGAGTGAACCTGACCCTGCGATGGAGCTTCAGCAATGGTACCTCATGGCGGAAAAGAG
ACAGTCCAGCTATGGCCAGGCCCCAGTGGCTATTCATCCCTGGCAACCCTGGAGGGCAGCATGGATGG
AGAGGAGCAGGCCCCCAGCTCTACGTCTGTATGAGAAAGGCCGGAACCACTACACAGAGAGCATCT
CCGTGGCCAAAATCAGTGTCTATGGGACACTCGGAGGAGGAGGAGGAGGCGTGAGACCAGTGAACATC
ACAGAGCCCACCCTGGAGCTGCTGCACTCTTCTGCGATCCCAATGCCTTCCATAGCACAAATCCAGCT
GTACTGTTTTATCTATGGCCACATCCTGAACGACGTGAGCGTGTCTTGGCTGATGGACGATCGCGAGA
TCACAGATAACCCTGGCTCAGACCGTGCTGATCAAGGAGGAGGGCAAGCTGGCCAGCACATGCTCTAAG
CTGAATATCACCGAGCAGCAGTGGATGTCCGAGTCCACCTTCACCTGCAAGGTGACATCTCAGGGCGT
GGACTACCTGGCTCACACCAGACGCTGTCCTGATCATGAGCCAAGGGGCGTGATCACATACTGATCC
CACCTCTCCACTGGACCTGTATCAGAACGGCGCCCCCAAGCTGACCTGTCTGGTGGTGGATCTGGAG
TCCGAGAAGAACGTGAATGTGACATGGAATCAGGAGAAGAAGACCTCCGTGTCCGCCAGCCAGTGGTA
CACAAAGCACCATAACAATGCCACCACATCCATCACCAGCATCCTGCCAGTGGTGGCTAAGGACTGGA
TCGAGGGCTACGGCTATCAGTGCATCGTGGACCATCCCGATTTCCCTAAGCCAATCGTGAGGAGCATC
ACAAAGACCCCTGGACAGCGGTCTGCCCCAGAGGTGTACGTGTTCCCTCCACCCGAGGAGGAGTCTGA
GGATAAGCGGACACTGACCTGTCTGATCCAGAACTTCTTTCTGAGGACATCAGCGTGCAGTGGCTGG
GCGATGGCAAGCTGATCTCTAATTTCCAGCACTCTACCACAACCCCACTGAAGTCTAACGGCTCCAAT
CAGGGCTTCTTTATCTTCTCCAGGCTGGAGGTGGCTAAGACACTGTGGACCCAGCGGAAGCAGTTTAC
ATGCCAGGTCATCCATGAGGCCCTGCAGAAGCCCAGAAAGCTGGAGAAGACAATCAGCACCTCTCTGG
GCAACACCTCCCTGCGCCCTAGCCACCATCACCATCACCATTGA

Secretion signal 1-60

Linker 61-84

Human Sialidase-1 (NEU1) 85-1188

Flexible linker1189-1206

Mouse IgE-Fc 1207-2199

Hisx6 2200-2217

FIG. 26

Sequence encoding hNEU2_mIgEFc (SEQ ID NO:64)

atgcctctgctgctgctgctgctgccactgctgtgggctggcgctctggctgacATGGCTAGCCTGCCTGT
GCTGCAGAAGGAGAGCGTGTTCAGTCTGGAGCTCACGCTTACAGGATCCCCGCCCTGCTGTATCTGC
CTGGCCAGCAGTCCCTGCTGGCTTTTGCCGAGCAGCGGGCTAGCAAGAAGGATGAGCACGCTGAGCTG
ATCGTGCTGAGGAGGGGCGACTACGATGCTCCAACCCATCAGGTGCAGTGGCAGGCTCAGGAGGTGGT
GGCTCAGGCTAGACTGGACGGACACCGCTCCATGAACCCTTGCCCACTGTATGATGCTCAGACAGGCA
CCCTGTTCTGTTCCTTTATCGCTATCCAGGACAGGTGACCGAGCAGCAGCAGCTGCAGACAAGGGCC
AATGTGACCCGGCTGTGCCAGGTGACAAGCACCGACCATGGCAGAACATGGTCCAGCCCTAGGGACCT
GACCGATGCTGCTATCGGCCAGCTTACAGAGAGTGGTCCACCTTCGCCGTGGGACCAGGACACTGCC
TGCAGCTGCATGATAGGGCTCGGAGCCTGGTGGTGCCAGCTTACGCCTATAGGAAGCTGCACCCCTATC
CAGCGGCCCATCCCTTCTGCCTTCTGCTTTCTGTCCACGACCATGGCAGGACATGGGCTCGGGGCCA
TTTTGTGGCCAGGATACCCTGGAGTGTCAGGTGGCTGAGGTGGAGACAGGCGAGCAGAGAGTGGTGA
CCCTGAACGCCCGCTCTCACCTGAGAGCTCGCGTGCAGGCCAGTCCACCAATGACGGCCTGGATTTTC
CAGGAGAGCCAGCTGGTGAAGAAGCTGGTGGAGCCACCTCCACAGGGATGTCAGGGATCCGTGATCAG
CTTTCCATCTCCACGCTCCGGACCAGGATCTCCTGCTCAGTGGCTGCTGTACACACACCCCCACCCATT
CCTGGCAGAGAGCTGACCTGGGAGCTTATCTGAACCCCCGCCCTGCTCCTGAGGCTTGGAGCGAG
CCTGTGCTGCTGGCTAAGGGCTCCTGCGCCTACAGCGACCTGCAGTCTATGGGCACAGGCCAGATGG
CTCTCCCCGTTCGGCTGTCTGTACGAGGCTAATGACTATGAGGAGATCGTGTTCCTGATGTTTACCC
TGAAGCAGGCTTTTCCAGCCGAGTATCTGCCACAGGGAGGAGGAGGAGGCGTGAAGACAGTGAAC
ATCACAGAGCCCACCCTGGAGCTGCTGCACTCTTCCTGCGATCCCAATGCCTTCCATAGCACAATCCA
GCTGTACTGTTTTATCTATGGCCACATCCTGAACGACGTGAGCGTGTCTTGGCTGATGGACGATCGCG
AGATCACAGATACCCTGGCTCAGACCGTGCTGATCAAGGAGGAGGGCAAGCTGGCCAGCACATGCTCT
AAGCTGAATATCACCGAGCAGCAGTGGATGTCCGAGTCCACCTTCACCTGCAAGGTGACATCTCAGGG
CGTGGACTACCTGGCTCACACCAGACGCTGTCCTGATCATGAGCCAAGGGGCGTGATCACATACCTGA
TCCCACCCTCTCCACTGGACCTGTATCAGAACGGCGCCCCAAGCTGACCTGTCTGGTGGTGGATCTG
GAGTCCGAGAAGAACGTGAATGTGACATGGAATCAGGAGAAGAAGACCTCCGTGTCCGCCAGCCAGTG
GTACACAAAGCACCATAACAATGCCACCACATCCATCACCAGCATCCTGCCAGTGGTGGCTAAGGACT
GGATCGAGGGCTACGGCTATCAGTGCATCGTGGACCATCCCGATTTCCCTAAGCCAATCGTGAGGAGC
ATCACAAAGACCCCTGGACAGCGGTCTGCCCCAGAGGTGTACGTGTTCCCTCCACCCGAGGAGGAGTC
TGAGGATAAGCGGACACTGACCTGTCTGATCCAGAACTTCTTTCTGAGGACATCAGCGTGCAGTGGC
TGGGCGATGGCAAGCTGATCTCTAATTCCCAGCACTCTACCACAACCCCACTGAAGTCTAACGGCTCC
AATCAGGGCTTCTTTATCTTCTCCAGGCTGGAGGTGGCTAAGACACTGTGGACCCAGCGGAAGCAGTT
TACATGCCAGGTCATCCATGAGGCCCTGCAGAAGCCAGAAAGCTGGAGAAGACAATCAGCACCTCTC
TGGGCAACACCTCCCTGCGCCCTAGCCACCATCACCATCACCATTGA

Human Sialidase-2 (NEU2) (Codon optimized for mammalian expression)
Secretion signal 1-51
52 - 1191
Flexible linker 1192-1209
Mouse IgE-Fc 1210-2202
Hisx6 2203-2220

FIG. 27

Sequence encoding hNEU3_mIgEFc (SEQ ID NO:65)

atgcctctgctgctgctgctgctgccactgctgtgggctggcgctctggctATGAGACCTGCGGACCTGCC
CCCGCGCCCCATGGAAGAATCCCCGGCGTCCAGCTCTGCCCCGACAGAGACGGAGGAGCCGGGGTCCA
GTGCAGAGGTCATGGAAGAAGTGACAACATGCTCCTTCAACAGCCCTCTGTTCCGGCAGGAAGATGAC
AGAGGGATTACCTACCGGATCCCAGCCCTGCTCTACATACCCCCACCCACACCTTCTGGCCTTTGC
AGAGAAGCGTTCTACGAGGAGAGATGAGGATGCTCTCCACCTGGTGCTGAGGCGAGGGTTGAGGATTG
GGCAGTTGGTACAGTGGGGGCCCTGAAGCCACTGATGGAAGCCACACTACCGGGGCATCGGACCATG
AACCCCTGTCTGTATGGGAGCAGAAGAGTGGTTGTGTGTTTCTGTCTTTCATCTGTGTGCGGGGCCA
TGTCACAGAGCGTCAACAGATTGTGTTCAGGCAGGAATGCTGCCCCCTTTGCTTCATCTACAGTCAGG
ATGCTGGATGTTTCATGGAGTGAGGTGAGGGACTTGACTGAGGAGGTCATTGGCTCAGAGCTGAAGCAC
TGGGCCACATTTGCTGTGGGCCCAGGTTCATGGCATCCAGCTGCAGTCAGGGAGACTGGTCATCCCTGC
GTATACCTACTACATCCCTTCTGGTTCTTTTGCTTCCAGCTACCATGTAAAACCAGGCCTCATTTCTC
TGATGATCTACAGTGATGACCTAGGGGTCACATGGCACCATGGTAGACTCATTAGGCCCATGGTTACA
GTAGAATGTGAAGTGGCAGAGGTGACTGGGAGGGCTGGCCACCCTGTGCTATATTGCAGTGCCCGGAC
ACCAAACAGGTGCCGGGCAGAGGCGCTCAGCACTGACCATGGTGAAGGCTTTCAGAGACTGGCCCTGA
GTCGACAGCTCTGTGAGCCCCACATGGTTGCCAAGGGAGTGTGGTAAGTTTCCGGCCCCCTGGAGATC
CCACATAGGTGCCAGGACTCTAGCAGCAAAGATGCACCCACCATTTCAGCAGAGCTCTCCAGGCAGTTC
ACTGAGGCTGGAGGAGGAAGCTGGAACACCGTCAGAATCATGGCTCTTGTACTIONCACACCCAACAGTA
GGAAACAGAGGGTTGACCTAGGTATCTATCTCAACCAGACCCCTTGGAGGCTGCCTGCTGGTCCCGC
CCCTGGATCTTGCACTGTGGGCCCTGTGGCTACTCTGATCTGGCTGCTCTGGAGGAGGAGGGCTTGTT
TGGGTGTTTGTGTTGAATGTGGGACCAAGCAAGAGTGTGAGCAGATTGCCTTCCGCCTGTTTACACACC
GGGAGATCCTGAGTCACCTGCAGGGGGACTGCACCAGCCCTGGTAGGAACCCAAGCCAATTCAAAAGC
AATGGAGGAGGAGGAGGAGGAGTGAACATCAGAGAGCCACCCTGGAGCTGCTGCACTC
TTCCTGCGATCCCAATGCCTTCCATAGCACAAATCCAGCTGTACTGTTTTATCTATGGCCACATCCTGA
ACGACGTGAGCGTGTCTTGCTGATGGACGATCGCGAGATCAGAGATACCCTGGCTCAGACCGTGCTG
ATCAAGGAGGAGGGCAAGCTGGCCAGCACATGCTCTAAGCTGAATATCACCGAGCAGCAGTGGATGTC
CGAGTCCACCTTCACCTGCAAGGTGACATCTCAGGGCGTGGACTACCTGGCTCACACCAGACGCTGTC
CTGATCATGAGCCAAGGGGCGTGATCACATACCTGATCCCACCCTCTCCACTGGACCTGTATCAGAAC
GGCGCCCCCAAGCTGACCTGTCTGGTGGTGGATCTGGAGTCCGAGAAGAACGTGAATGTGACATGGAA
TCAGGAGAAGAAGACCTCCGTGTCCGCCAGCCAGTGGTACACAAAGCACCATAACAATGCCACCACAT
CCATCACCAGCATCCTGCCAGTGGTGGCTAAGGACTGGATCGAGGGCTACGGCTATCAGTGCATCGTG
GACCATCCCGATTTCCCTAAGCCAATCGTGAGGAGCATCACAAAGACCCCTGGACAGCGGTCTGCCCC
AGAGGTGTACGTGTTCCCTCCACCCGAGGAGGAGTCTGAGGATAAGCGGACACTGACCTGTCTGATCC
AGAACTTCTTCCCTGAGGACATCAGCGTGAGTGGCTGGGCGATGGCAAGCTGATCTCTAATTCCCAG
CACTCTACCACAACCCCACTGAAGTCTAACGGCTCCAATCAGGGCTTCTTTATCTTCTCCAGGCTGGA
GGTGGCTAAGACACTGTGGACCCAGCGGAAGCAGTTTACATGCCAGGTCATCCATGAGGCCCTGCAGA
AGCCCAGAAAGCTGGAGAAGACAATCAGCACCTCTCTGGGCAACACCTCCCTGCGCCCTAGCCACCAT
CACCATCACCATTGA

Secretion signal 1-48
Human Sialidase-3 (NEU3) 49-1431
Flexible linker 1432-1449
Mouse IgE-Fc 1450-2442
Hisx6 2443-2460

FIG. 28

Sequence encoding hNEU4_mIgEFC (SEQ ID NO:66)

atgcctctgctgctgctgctgccactgctgtgggctggcgctctggctATGGGGGTCCCTCGTACCCC
TTCACGGACAGTGCTCTTCGAGCGGGAGAGGACGGGCCTGACCTACCGCGTGCCCTCGCTGCTCCCCG
TGCCCCCGGGCCACCCTGCTGGCCTTTGTGGAGCAGCGGCTCAGCCCTGACGACTCCCACGCCCCAC
CGCCTGGTGCTGAGGAGGGGCACGCTGGCCGGGGGCTCCGTGCGGTGGGGTGCCCTGCACGTGCTGGG
GACAGCAGCCCTGGCGGAGCACCGGTCCATGAACCCCTGCCCTGTGCACGATGCTGGCACGGGCACCG
TCTTCCTCTTCTTCATCGCGGTGCTGGGCCACACGCCTGAGGCCGTGCAGATCGCCACGGAAGGAAC
GCCGCGCGCCTCTGCTGTGTGGCCAGCCGTGACGCCGGCCTCTCGTGGGGCAGCGCCCCGGACCTCAC
CGAGGAGGCCATCGGTGGTGCCGTGCAGGACTGGGCCACATTCGCTGTGGGTCCCGGCCACGGTGTGC
AGCTGCCCTCAGGCCGCCTGCTGGTACCCGCCTACACCTACCGCGTGACCGCCGAGAGTGTTCCTGGC
AAGATCTGCCGGACCAGCCCTCACTCCTTCGCTTCTACAGCGATGACCACGGCCGCACCTGGCGCTG
TGGAGGCCTCGTGCCCAACCTGCGCTCAGGCGAGTGCCAGCTGGCAGCGGTGGACGGTGGGCAGGCCG
GCAGCTTCCTCTACTGCAATGCCCGGAGCCCACTGGGCAGCCGTGTGCAGGCGCTCAGCACTGACGAG
GGCACCTCCTTCCTGCCCGCAGAGCGCGTGGCTTCCCTGCCCAGACTGCCCTGGGGCTGCCAGGGCAG
CATCGTGGGCTTCCCAGCCCCCGCCCCAACAGGCCACGGGATGACAGTTGGTCAGTGGGCCCCGGGA
GTCCCCCTCCAGCTCCACTCCTCGGTCTTGGAGTCCACGAACCCCCAGAGGAGGCTGCTGTAGACCCC
CGTGGAGGCCAGGTGCCTGGTGGGCCCTTCAGCCGTCTGCAGCCTCGGGGGGATGGCCCCAGGCAGCC
TGGCCCCAGGCCTGGGGTCAGTGGGGATGTGGGGTCTTGGACCCTGGCACTCCCCATGCCCTTTGCTG
CCCCGCCCCAGAGCCCCACGTGGCTGCTGTACTCCCACCCAGTGGGGCGCAGGGCTCGGCTACACATG
GGTATCCGCCTGAGCCAGTCCCCGCTGGACCCGCGCAGCTGGACAGAGCCCTGGGTGATCTACGAGGG
CCCCAGCGGCTACTCCGACCTGGCGTCCATCGGGCCGGCCCCCTGAGGGGGGCCTGGTTCCTTGCCTGCC
TGTACGAGAGCGGGGCCAGGACCTCCTATGATGAGATTTCTTTTGTACATTCTCCCTGCGTGAGGTC
CTGGAGAACGTGCCCGCCAGCCCCAACCCGCCAACCTTGGGGACAAGCCTCGGGGGTGCTGCTGGCC
CTCCGAGGAGGAGGAGGAGGAGTGAAGACAGTGAACATCACAGAGCCCACCCTGGAGCTGCTGCACT
CTTCCTGCGATCCCAATGCCTTCCATAGCACAAATCCAGCTGTACTGTTTTATCTATGGCCACATCCTG
AACGACGTGAGCGTGTCTTGGCTGATGGACGATCGCGAGATCACAGATAACCCTGGCTCAGACCGTGCT
GATCAAGGAGGAGGGCAAGCTGGCCAGCACATGCTCTAAGCTGAATATCACCGAGCAGCAGTGGATGT
CCGAGTCCACCTTCACCTGCAAGGTGACATCTCAGGGCGTGGACTACCTGGCTCACACCAGACGCTGT
CCTGATCATGAGCCAAGGGGCGTGATCACATACCTGATCCCACCCTCTCCACTGGACCTGTATCAGAA
CGGCGCCCCCAAGCTGACCTGTCTGGTGGTGGATCTGGAGTCCGAGAAGAAGCTGAATGTGACATGGA
ATCAGGAGAAGAAGACCTCCGTGTCCGCCAGCCAGTGGTACACAAAGCACCATAACAATGCCACCACA
TCCATCACAGCATCCTGCCAGTGGTGGCTAAGGACTGGATCGAGGGCTACGGCTATCAGTGCATCGT
GGACCATCCCGATTTCCCTAAGCCAATCGTGAGGAGCATCACAAAGACCCCTGGACAGCGGTCTGCC
CAGAGGTGTACGTGTTCCCTCCACCCGAGGAGGAGTCTGAGGATAAGCGGACACTGACCTGTCTGATC
CAGAACTTCTTTCCTGAGGACATCAGCGTGCAGTGGCTGGGCGATGGCAAGCTGATCTCTAATTCCCA
GCACTCTACCACAACCCCACTGAAGTCTAACGGCTCCAATCAGGGCTTCTTTATCTTCTCCAGGCTGG
AGGTGGCTAAGACACTGTGGACCCAGCGGAAGCAGTTTACATGCCAGGTCATCCATGAGGCCCTGCAG
AAGCCCAGAAAGCTGGAGAAGACAATCAGCACCTCTCTGGGCAACACCTCCCTGCGCCCTAGCCACCA
TCACCATCACCATTTGA

Secretion signal 1-48
Human Sialidase-4 (NEU4) 49-1500
Flexible linker 1501-1518
Mouse IgE-Fc 1519-2511
Hisx6 2512-2529

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/018380

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395; A61P 37/08; C07H 21/04; C07K 5/08; C07K 7/02; C07K 16/00 (2020.01)

CPC - A61K 39/395; A61K 47/68; A61K 47/6811; A61P 29/00; A61P 35/00; C07K 2317/732 (2020.02)

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)

See Search History document

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

USPC - 424/133.1; 435/68.1; 530/387.3 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2018/006034 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY et al) 04 January 2018 (04.01.2018) entire document	1-6 ----- 8-11
X -- Y	US 2017/0051328 A1 (UNIVERSITY OF MANITOBA) 23 February 2017 (23.02.2017) entire document	12-16, 18 ----- 17
Y	US 2013/0177553 A1 (DESJARLAIS et al) 11 July 2013 (11.07.2013) entire document	8-11, 17
P, X	WO 2019/136167 A1 (PALLEON PHARMACEUTICALS INC) 11 July 2019 (11.07.2019) entire document	1-6, 8-18
A	US 2014/0051834 A1 (HOFFMAN-LA ROCHE, INC et al) 20 February 2014 (20.02.2014) entire document	1-6, 8-18
A	NASO et al. "Engineering Host Cell Lines to Reduce Terminal Sialylation of Secreted Antibodies," mAbs, 01 September 2010 (01.09.2010), Vol. 2, No. 5, Pgs. 519-527. entire document	1-6, 8-18
P, A	WO 2019/126041 A1 (THE GENERAL HOSPITAL CORPORATION) 27 June 2019 (27.06.2019) entire document	1-6, 8-18

☐ 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

28 April 2020

Date of mailing of the international search report

20 MAY 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*. 1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*. 1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

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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.: 7, 19-27
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:

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 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.:

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.