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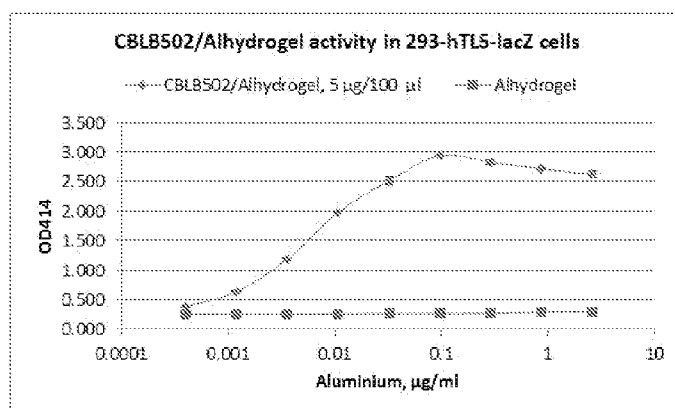
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(54) Title: FLAGELLIN-BASED AGENTS AND USES INCLUDING EFFECTIVE VACCINATION

FIG. 5



(57) Abstract: The present invention relates to, in part, compositions comprising improved flagellin derived constructs and methods of using for vaccination, including adjuvants comprising flagellin-based agents.

FLAGELLIN-BASED AGENTS AND USES INCLUDING EFFECTIVE VACCINATION**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 62/031,116, filed July 30, 2014; 62/110,744, filed February 2, 2015; and 62/117,366, filed February 17, 2015 the entire contents of which are herein incorporated by reference.

FIELD OF THE INVENTION

5 [0002] The present invention relates to, *inter alia*, new compositions and methods for vaccination, including adjuvants comprising flagellin-based agents.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0003] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: CLE019PC SequenceListing.txt; date recorded: July 24, 2015; file size: 261 KB).

BACKGROUND

10 [0004] Vaccines are one of the most effective preventative health tools available against infectious diseases, cancers, and allergies. Vaccination aims to generate a strong immune response to the administered antigen and provide long-term protection against a disorder. Often, however, an antigen alone is insufficient to stimulate protective immunity.

15 [0005] Vaccine adjuvants are compounds that enhance the specific immune responses against antigens in vaccines. Currently, several hundred natural and synthetic compounds are known to have adjuvant activity but only alum salts and AS04 are licensed for use in humans in the United States. This limited list of adjuvants is insufficient for meeting the functional needs of effective vaccination. For example, although alum is able to induce a good T_{H2} response, it has little capacity to stimulate cellular (T_{H1}) immune responses which are so
20 important for protection against many pathogens.

[0006] Accordingly, there remains a need for improved vaccines and/or adjuvants that can effectively stimulate a subject's immune response.

SUMMARY OF THE INVENTION

[0007] Accordingly, in some aspects, the present invention provides for improved vaccines and/or adjuvants.

25 [0008] In some aspects, the present invention provides a vaccine composition, comprising an adjuvant comprising a flagellin-based agent, such as, for example, CBLB502 (a/k/a entolimod) as well as any of the flagellin-based agents or derivatives described herein (e.g. Table 1), and an aluminum gel or salt and an antigen and, optionally, an additional adjuvant. In some aspects, the present invention provides an adjuvant composition,

comprising an adjuvant comprising a flagellin-based agent, including CBLB502 as well as any of the flagellin-based agents or derivatives described herein (e.g. Table 1), and an aluminum gel or salt. In various embodiments, the vaccine described herein causes an improvement in adjuvant properties relative to a vaccine comprising the antigen and the aluminum gel or salt alone. In various embodiments, the vaccine and/or adjuvant described herein causes a broader, more diverse, more robust and longer lasting immunostimulatory effect than the vaccine comprising the antigen and the aluminum gel or salt alone and/or the adjuvant comprising the aluminum gel or salt alone. In various embodiments, the vaccine and/or adjuvant described herein causes immunostimulation of one or more of T_{H1} and T_{H2} -mediated immune response (e.g. both of T_{H1} and T_{H2} -mediated immune response) and/or the vaccine and/or adjuvant described herein causes immunostimulation of T_{H1} -mediated immune response at levels greater than a vaccine comprising the antigen and the aluminum gel or salt alone and/or the adjuvant comprising the aluminum gel or salt alone.

[0009] In some embodiments, the flagellin-based agent may be a flagellin molecule or flagellin-based agent, or variants thereof that has TLR5 agonist activity. The flagellin-based agent may comprise one or more of the sequences of Table 1 (SEQ ID Nos.: 1-252), or variants thereof that have TLR5 agonist activity and can include a variant of *Salmonella dublin* wild type flagellin (SEQ ID No: 1), CBLB502 (SEQ ID NO: 2) or variants thereof (including closely-related variants such as S33ML (SEQ ID NO: 35), CBLB502-485CT (CBLB533, SEQ ID NO: 71), and CBLB502-S33MX (SEQ ID NO: 150)) and more distantly related variants such as flagellin derivatives from a thermophilic microorganism or flagellin derivatives from a microorganism well-tolerated by human (e.g. SEQ ID NOs: 243-252). In some embodiments, the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and potassium aluminum sulfate, and ALHYDROGEL.

[0010] In various embodiments, the present vaccine composition is part of the following vaccines (e.g. the antigens of these vaccines may be used as the antigen of the present vaccines): DTP (diphtheria-tetanus-pertussis vaccine), DTaP (diphtheria-tetanus-acellular pertussis vaccine), Hib (Haemophilus influenzae type b) conjugate vaccines, Pneumococcal conjugate vaccine, Hepatitis A vaccines, Poliomyelitis vaccines, Yellow fever vaccines, Hepatitis B vaccines, combination DTaP, Tdap, Hib, Human Papillomavirus (HPV) vaccine, Anthrax vaccine, and Rabies vaccine.

[0011] In various embodiments, the vaccine described herein is formed, in part, by mixing the flagellin-based agent and aluminum gel or salt to form a stable complex, the ratio (w/w) of flagellin-based agent to aluminum gel or salt being about 1:500 or less (e.g. about 1:500, or about 1:600, or about 1:700, or about 1:800, or about 1:900, or about 1:1000, or about 1:2000, or about 1:5000). In some embodiments, the flagellin-based agent and aluminum gel or salt are mixed in a ratio that is substantially below a loading capacity of the aluminum gel or salt. In some embodiments, the flagellin-based agent and antigen are adsorbed to the aluminum gel or salt.

[0012] In another aspect, the present invention provides a method of vaccinating a subject against a disorder, comprising administering an effective amount of a vaccine comprising an adjuvant comprising a

flagellin-based agent and an aluminum gel or salt and an antigen associated with the disorder. In another aspect, the present invention provides a method of immunostimulating a subject in advance of or concurrent with vaccination, comprising administering an effective amount of an adjuvant comprising a flagellin-based agent and an aluminum gel or salt, wherein both T_{H1} and T_{H2} -mediated immune responses are immunostimulated. In various embodiments the disorder is selected from infectious diseases, cancer, allergy, and autoimmune diseases (by way of example, diphtheria, tetanus, pertussis, influenza, pneumonia, hepatitis A, hepatitis B, polio, yellow fever, Human Papillomavirus (HPV) infection, various cancers, anthrax, rabies, Japanese Encephalitis, meningitis, measles, mumps, rubella, gastroenteritis, smallpox, typhoid fever, varicella (chickenpox), rotavirus, and shingles).

[0013] The details of the invention are set forth in the accompanying description below. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, illustrative methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, 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.

BRIEF DESCRIPTION OF THE FIGURES

[0014] **FIGs. 1A and 1B** show the 13 conserved amino acids of flagellin that may be important for TLR5 activity. **FIGS. 1A and 1B** show a comparison of amino acid sequences of the conserved amino (FIG. 1A) and carboxy (FIG. 1B) terminus from 21 species of bacteria. The 13 conserved amino acids important for TLR5 activity are shown with shading. The amino acid sequences are identified by their accession numbers from TrEMBL (first letter = Q) or Swiss-Prot (first letter = P).

[0015] **FIG. 2** shows ELISA data for the average response of five mouse treatment groups at the time point of 1 week post-boost. Panel A is total IgG, Panel B is IgG1, Panel C is IgG2a, Panel D is IgG2b and Panel E is IgG3. Groups 1-5 are defined in **TABLE B** (Example 2): Group 1 is also called "Untreated," Group 2 is also called "OVA," Groups 3 is also called "OVA + Alum," Group 4 is also called "SA 1 μ g," and Group 5 is also called "SA 10 μ g."

[0016] **FIG. 3** shows ELISA data for the average response of five mouse treatment groups at the time point of 2 weeks post-boost. Panel A is total IgG, Panel B is IgG1, Panel C is IgG2a, Panel D is IgG2b and Panel E is IgG3. Groups 1-5 are defined in **TABLE B** (Example 2): Group 1 is also called "Untreated," Group 2 is also called "OVA," Groups 3 is also called "OVA + Alum," Group 4 is also called "SA 1 μ g," and Group 5 is also called "SA 10 μ g."

[0017] **FIG. 4** shows ELISA data for the average response of five mouse treatment groups at the time point of 4 weeks post-boost. Panel A is total IgG, Panel B is IgG1, Panel C is IgG2a, Panel D is IgG2b and Panel E is

IgG3. Groups 1-5 are defined in **TABLE B** (Example 2): Group 1 is also called "Untreated;" Group 2 is also called "OVA," Groups 3 is also called "OVA + Alum," Group 4 is also called "SA 1 µg," and Group 5 is also called "SA 10 µg."

[0018] **FIG. 5** shows a determination of CBLB502/ALHYDROGEL activity using a cell-based NF-κB activation assay. CBLB502/ALHYDROGEL formulation (5 µg/100 µl) and ALHYDROGEL control were serially diluted in assay media 2000- to 13122000-fold (2.6–0.0004 µg/ml aluminium) and incubated with 293-hTLR5-LacZ cells for 20 hr. Activity of β-galactosidase was measured after addition of cell lysis buffer and ONPG substrate and expressed as optical density at 414 nm (OD₄₁₄).

[0019] **FIG. 6** shows NF-κB-inducing activity of CBLB502/ALHYDROGEL formulated with different CBLB502 doses. 293-hTLR5-LacZ cells were incubated with CBLB502/ALHYDROGEL suspensions prepared at CBLB502 doses ranging from 2.5 to 0.00015 µg/100 µl and diluted 200-fold with media. Activity of β-galactosidase reporter enzyme was measured after 20 hr incubation.

[0020] **FIG. 7** shows dose-response titration curves CBLB502/ALHYDROGEL generated with 293-hTLR5-LacZ reporter cells. CBLB502 was adsorbed to ALHYDROGEL at indicated doses (20 µg/100 µl: top panel, 0.31 µg/100 µl: middle panel and 0.078 µg/100 µl: bottom panel), serially diluted with media and assayed together with soluble CBLB502 standards.

[0021] **FIG. 8** shows the activity of CBLB502/ALHYDROGEL (1 µg/100 µl dose) stored at 4°C for 2 and 6 days. NF-κB-inducing activity was measured using 293-hTLR5-LacZ reporter cell assay.

[0022] **FIG. 9** shows pharmacokinetics of CBLB502 in mouse serum after subcutaneous injection of a 1 µg dose of CBLB502 adsorbed to ALHYDROGEL 2%. The graph shows per-group mean serum CBLB502 concentrations and SEM (standard error of the mean) for each time point.

[0023] **FIG. 10** shows induction of KC, G-CSF and IL-6 following s.c. administration of a 1 µg dose of CBLB502 adsorbed to ALHYDROGEL 2%. Serum concentrations of the cytokines were determined using R&D Systems DuoSet kits DY453 (KC, top panel), DY414 (G-CSF, middle panel) and DY406 (IL-6, bottom panel). The graphs show per-group mean serum cytokine concentrations and SEM (standard error of the mean) for each time point.

[0024] **FIG. 11** shows immunopotentiating effects of a 502 adjuvant. Anti-MA IgG (µg/ml) response (panel A) and anti-502 antibody response (panel B) by ELISA to TT-SMA vaccine in the presence and absence of alum or entolimod (a varying range from 0 to 20µg) in pooled sera collected at week 2,4 and 6 post initial vaccination is shown. Data represent the levels of antibody in the pooled samples from each group (n=5/group).

DETAILED DESCRIPTION OF THE INVENTION

[0025] The present invention is also based, in part, on the surprising discovery that flagellin-based agents, including CBLB502 and any of the flagellin-based agents (e.g. agents comprising the sequences of Table 1,

including MX-33) described herein, can be mixed with aluminum gels or salts (and, optionally antigens), in ratios well-below the loading/adsorbing capacity of the aluminum gel or salt (e.g. 1:500), and cause a broader, more diverse, more robust and longer lasting immunostimulatory effect than the vaccine comprising the antigen and the aluminum gel or salt alone and/or the adjuvant comprising the aluminum gel or salt alone. The inventors have also surprisingly discovered that the immunostimulatory effect can influence both the T_{H1} and T_{H2} -mediated arms of an immune response. The inventors have also surprisingly discovered that low amounts of flagellin-based agents, including CBLB502 and any of the flagellin-based agents (e.g. agents comprising the sequences of Table 1, including MX-33) relative to antigen are effective for immunostimulation.

[0026] In one aspect, the invention provides a vaccine composition, comprising an adjuvant comprising a flagellin-based agent and an aluminum gel or salt and an antigen and, optionally, an additional adjuvant. In one aspect, the invention provides an adjuvant comprising a flagellin-based agent and an aluminum gel or salt.

[0027] In some embodiments, the described vaccine causes an improvement in adjuvant properties relative to a vaccine comprising the antigen and the aluminum gel or salt alone. In various embodiments, the vaccine and/or adjuvant described herein causes a broader, more diverse, more robust and longer lasting immunostimulatory effect than the vaccine comprising the antigen and the aluminum gel or salt alone (or as compared to the vaccine comprising the antigen and flagellin-based agent alone) and/or the adjuvant comprising the aluminum gel or salt alone (or as compared to the adjuvant comprising the flagellin-based agent alone).

[0028] In some embodiments, the described vaccine and/or described adjuvant causes immunostimulation of one or more of T_{H1} and T_{H2} -mediated immune response. In some embodiments, the described vaccine and/or described adjuvant causes immunostimulation of both of T_{H1} and T_{H2} -mediated immune response. In some embodiments, the described vaccine and/or described adjuvant causes immunostimulation of T_{H1} -mediated immune response at levels greater than a vaccine comprising the antigen and the aluminum gel or salt alone or an adjuvant comprising the aluminum gel or salt alone.

[0029] T_{H1} -mediated immune response (or "Type 1 response") largely involves interaction with macrophages and CD8⁺ T cells and may be linked to interferon- γ , TNF- β , interleukin-2, and interleukin-10 production. The T_{H1} -mediated immune response promotes cellular immune system and maximizes the killing efficacy of the macrophages and the proliferation of cytotoxic CD8⁺ T cells. The T_{H1} -mediated immune response also promotes the production of opsonizing antibodies (e.g. IgG, IgM and IgA). The Type 1 cytokine IFN- γ increases the production of interleukin-12 by dendritic cells and macrophages, and via positive feedback, IL-12 stimulates the production of IFN- γ in helper T cells, thereby promoting the T_{H1} profile. Interferon- γ also inhibits the production of cytokines such as interleukin-4, a cytokine associated with the Type 2 response, and thus it also acts to preserve its own response.

[0030] T_{H2} -mediated immune response (or "Type 2 response") largely involves interaction with B-cells, eosinophils, and mast cells and may be linked to interleukin-4, interleukin-5, interleukin-6, interleukin-9,

interleukin-10, and interleukin-13. T_{H2}-mediated immune response promotes humoral immune system and may stimulate B-cells into proliferation, induce B-cell antibody class switching, and increase neutralizing antibody production (e.g. IgG, IgM and IgA as well as IgE antibodies). Other functions of the Type 2 response include promoting its own profile using two different cytokines. Interleukin-4 acts on helper T cells to promote the production of T_{H2} cytokines (including itself; it is auto-regulatory), while interleukin-10 (IL-10) inhibits a variety of cytokines including interleukin-2 and IFN- γ in helper T cells and IL-12 in dendritic cells and macrophages. The combined action of these two cytokines suggests that once the T cell has decided to produce these cytokines, that decision is preserved (and also encourages other T cells to do the same).

[0031] The division is medically relevant to, *inter alia* and without wishing to be bound by theory, the fact that alum adjuvants are only effective at inducing a T_{H2}-mediated response and not a T_{H1}-mediated response (see, e.g., Smith Korsholm, *et al.* Immunology. Jan 2010; 129(1): 75–86, the contents of which are hereby incorporated by reference in their entirety).

[0032] In various embodiments, the stimulation of T_{H1} and T_{H2}-mediated immune responses may be measured by assays known in the art, including a number of antibody surrogate assays (e.g. ELISA and the like). For instance, without wishing to be bound by theory, IgG1 is associated with a T_{H2}-like response, while a T_{H1} response is associated with the induction of IgG2a, IgG2b, and IgG3 antibodies.

[0033] In some embodiments, the described vaccine and/or described adjuvant causes an increase in titer of one or more of IgG1, IgG2a, IgG2b, and IgG3 antibodies (e.g. relative to the adjuvant comprising the aluminum gel or salt or flagellin-based agent alone, or relative to the vaccine comprising the antigen and the aluminum gel or salt alone (or flagellin-based agent) alone)). In some embodiments, the described vaccine and/or described adjuvant causes a relative increase in the titer of all of IgG1, IgG2a, IgG2b, and IgG3 antibodies. In some embodiments, the described vaccine and/or described adjuvant causes a relative increase in the titer of more IgG3 antibodies than the described vaccine and/or described adjuvant in the absence of a flagellin-based agent (or the described vaccine and/or described adjuvant in the absence of an aluminum gel or salt alone).

[0034] Accordingly, in some embodiments, the described vaccine and/or described adjuvant causes a diversified immune response. For example, in some embodiments, the total IgG generated by the described vaccines and/or adjuvants is greater than the described vaccines and/or adjuvants without the flagellin-based agent.

[0035] In some embodiments, the flagellin-based agent may be a flagellin molecule or flagellin-related polypeptide, or variants thereof that have TLR5 agonist activity. The flagellin-based agent may be from various sources, including a variety of Gram-positive and Gram-negative bacterial species. In some embodiments, the flagellin-based agents may have an amino acid sequence that is derived from any of the flagellins from bacterial species that are depicted in, for example, FIG. 7 of U.S. Patent Publication No. 2003/0044429, the contents of

which are incorporated herein by reference in their entirety. The flagellin-based agent may have nucleotide sequences related to those encoding the flagellin polypeptides listed in, for example, FIG. 7 of U.S. 2003/0044429, which are publicly available at sources including the NCBI Genbank database.

[0036] The flagellin-based agent may be the major component of bacterial flagellum. The flagellin-based agents may be composed of one, or two, or three, or four, or five, or six, or seven domains or fragments thereof (see, e.g., FIG. 10 of US Patent 8,324,163, the contents of which are incorporated herein by reference in their entirety). The domains may be selected from ND0, ND1, ND2, D3, CD2, CD1, and CD0. Domains 0 (D0), 1 (D1), and 2 (D2) may be discontinuous and may be formed when residues in the amino terminus and carboxy terminus are juxtaposed by the formation of a hairpin structure. The amino and carboxy terminus comprising the D1 and D2 domains may be most conserved, whereas the middle hypervariable domain (D3) may be highly variable. The non-conserved D3 domain may be on the surface of the flagellar filament and may contain the major antigenic epitopes. The potent proinflammatory activity of flagellin may reside in the highly conserved N and CD1 and D2 regions.

[0037] The flagellin-based agents may be from a species of *Salmonella*, representative examples of which are *S. typhimurium* and *S. dublin* (encoded by GenBank Accession Number M84972). The flagellin related-polypeptide may be a fragment, variant, analog, homolog, or derivative of wild type flagellin (SEQ ID NO: 1), or combination thereof. A fragment, variant, analog, homolog, or derivative of flagellin may be obtained by rational-based design based on the domain structure of flagellin and the conserved structure recognized by TLR5.

[0038] The flagellin-based agent may be related to a flagellin polypeptide from any Gram-positive or Gram-negative bacterial species including, but not limited to, the flagellin polypeptides disclosed in U.S. Pat. Pub. 2003/0044429, the contents of which are incorporated herein, and the flagellin peptides corresponding to the Accession numbers listed in the BLAST results shown in FIG. 7 (panels A-F) of U.S. Patent Pub. 2003/0044429, or variants thereof.

[0039] In some embodiments, the flagellin-based agent comprises or consists of any of the polypeptides or nucleic acids encoding said polypeptides listed in Table 1. In some embodiments, the flagellin-based agent is encoded by the nucleotide sequences listed in Table 1. In a further embodiment, the flagellin-based agent comprises the polypeptides listed in Table 1. In some embodiments, the flagellin-based agent comprises one or more of SEQ ID NOs.: 1-252. In some embodiments, the flagellin-based agent comprises a flexible linker. In a further embodiment, the flexible linker comprises SEQ ID NO: 16. In yet a further embodiment, the flexible linker comprises SEQ ID NO: 242.

[0040] In some embodiments, the flagellin-based agent is a variant of SEQ ID NO: 1. In various embodiments, the flagellin-based agent is not SEQ ID NO: 1.

[0041] In some embodiments, the flagellin-based agent comprises or consists of CBLB502 (SEQ ID NO: 2) or is a variant of CBLB502 (SEQ ID NO: 2). In various embodiments, CBLB502 provides the advantage of, *inter*

alia, removal of epitopes that generate neutralizing anti-flagellin antibodies and therefore allow for the surprising adjuvant properties seen here in combination with alum.

[0042] In some embodiments, the flagellin-based agent comprises mutations in epitopes recognized by neutralizing anti-CBLB502 antibodies. The flagellin-based agent may comprise one or more mutations in the epitopes recognized by neutralizing anti-CBLB502 antibodies which inhibit or abrogate the ability of the antibodies to neutralize the composition. In yet a further embodiment, the flagellin-based agent comprises a truncation and mutations in one or more epitopes. In a further embodiment, the mutations comprise replacement of the epitope residues with alanine. In a further embodiment, the mutated epitopes comprise one or more of the following residues: E153, S444, T154, N440, Q142, F131, D443, N68, T447, S110, Q117, R124, D113, E120, N127, and Q128.

[0043] The flagellin-based agent may comprise insertions, deletions, transposon insertions, and changes to any one of the D0, D1, D2, and the variable D3 domains. The D3 domain may be substituted in part, or in whole, with a hinge or linker polypeptide that allows the D1 and D2 domains to properly fold such that the variant stimulates TLR5 activity.

[0044] In some embodiments, the flagellin-based agent may be a minimal functional core of a flagellin, for example, deleting residues relative to the already shortened CBLB502 molecule. In some embodiments, the flagellin-based agent has altered amino acid identity relative to wild type, including deletions, additions and substitutions, that provide for improved activity. In some embodiments, the flagellin-based agent is derived from CBLB502 (SEQ ID NO: 2). In some embodiments, the flagellin-based agent comprises a truncation in one or more domains. In a further embodiment, the flagellin-based agent comprises a deletion in an N-terminal domain. In yet a further embodiment, the flagellin-based agent comprises a deletion in the ND0 domain. In yet a further embodiment, the flagellin-based agent comprises a deletion of the entire ND0 domain. In a further embodiment, the flagellin-based agent comprises a deletion in a C-terminal domain. In yet another embodiment, the flagellin-based agent comprises a deletion in the CD0 domain. In yet another embodiment, the flagellin-based agent retains amino acids 470-485 of the CD0 domain. In yet a further embodiment, the flagellin-based agent is CBLB502-S33 (SEQ ID NO: 17).

[0045] The flagellin-based agent may comprise at least 10, 11, 12, or 13 of the 13 conserved amino acids shown in FIG. 1A and FIG. 1B (positions 89, 90, 91, 95, 98, 101, 115, 422, 423, 426, 431, 436 and 452). The flagellin may be at least 30-99% identical to amino acids 1-174 and 418-505 of SEQ ID NO: 1.

[0046] In some embodiments, the flagellin-based agent comprises a tag. In yet a further embodiment, the tag is attached to the N-terminus of the flagellin-based agent. In yet another embodiment, the tag is attached to the C-terminus of the flagellin-based agent.

[0047] In some embodiments, the flagellin-based agent comprises a flexible linker. In a further embodiment, the flexible linker comprises SEQ ID NO: 16. In yet a further embodiment, the flexible linker comprises SEQ ID NO:242.

[0048] In various embodiments, the flagellin-based agent is one or more of the flagellin related composition derived from SEQ ID NO: 2. In various embodiments, the flagellin-based agent is one or more of CBLB502-S33ML (SEQ ID NO: 35), CBLB502-485CT (CBLB533, SEQ ID NO: 71), and CBLB502-S33MX (SEQ ID NO: 150).

[0049] In various embodiments, the flagellin-based agent is a flagellin derivative from a thermophilic microorganism. In various embodiments, the flagellin-based agent is a flagellin derivative from a microorganism well-tolerated by human. In some embodiments, the flagellin-based agent is one or more of SEQ ID NOs: 243-252.

[0050] In some embodiments, a variant includes molecules that have TLR5 agonist activity. In some embodiments, a variant includes molecules comprising an amino acid sequence having at least about 70% (e.g. about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99%) sequence identity with SEQ ID NO: 1 or SEQ ID NO: 2.

[0051] In some embodiments, the flagellin-based agent comprises or consists of any of the polypeptides or nucleic acids encoding said polypeptides listed in Table 1. In some embodiments, the flagellin-based agent is encoded by the nucleotide sequences listed in Table 1. In a further embodiment, the flagellin-based agent comprises one or more of the polypeptides listed in Table 1. In some embodiments, the flagellin-based agent comprises or consists of polypeptides encoded by either SEQ ID NOs: 69 or 70. In some embodiments, the flagellin-based agent comprises or consists of the polypeptides of SEQ ID NO: 71, "CBLB543". In some embodiments, the flagellin-based agent comprises or consists of polypeptides encoded by either SEQ ID NOs: 149 or 151. In some embodiments, the flagellin-based agent comprises or consists of the polypeptides of SEQ ID NO: 150, "CBLB533".

Table 1: Illustrative Flagellin-Based Agents

SEQ ID	Construct Name	DNA/PRT	Species	Sequence
001	Wild type	PRT	Salmonella dublin	MAQVINTNSLSLLTQNNLNKSSSLSSAIERLSSGLRINSADKDDAAGQAIAIRFTSNIKGLTQASRN ANDGISIAQTTEGALNEINNLRVREL SVQATNGTNSDSLKSIQDEIQRL EEDRVSNQTQFN GVKVLSDQNQMKIQVGANDGETITIDLQKIDVKSGLDGFNVNGPKEATVGDLSKFKNVTGYDTY AAGADKYRVDINS GAVVTDAAAPDKVYVNAANGQLTTDDAENNTAV/DLFTTKSTAGTAEAKAIA GAIKGKEGDTFDYKGVFTFTIDTKTGDGNGKVSTTINGEKV/TLTVADIATGAADVNAATLQSSKN VYTSVVGQFTFDDKTKNESAKLSDLEANNAVKGESKITVNGAEYTANATGDKITLAGKTMFIDKT ASGVSTLINEAAAAAKKSTANPLASIDSALS KVDVAVRSSLGAIQNRFD SAITNLGNTVTNLSARSRI EDADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVLSLLR
002	CBLB502	PRT	Artificial Sequence	MGRSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLSLLTQNNLNKSSSLSSAIE RLSSGLRINSADKDDAAGQAIAIRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSV QATNGTNSDSLKSIQDEIQRL EEDRVSNQTQFNQVKSQDNQMKIQVGANDGETITIDLQKI DVKSLGLDGFNVNSPGISGGGGILDSMGTLINE DAAAAKKSTANPLASIDSALS KVDVAVRSSLGA QNRFD SAITNLGNTVTNLSARSRIEDADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVLSLL R
003	T7 Promoter (forward)	DNA	Artificial Sequence	TAATACGACTCACTATAGGGG
004	FltC AA74-80 (forward)	DNA	Artificial Sequence	ATTGCGCAGACCACCTGAAGG
005	Thrombin cleavage site	PRT	Artificial Sequence	LVPRGS
006	Enterokinase cleavage site		Artificial Sequence	DDDDK
007	NS (N-terminal spoke region; Ser32 – Ala44)	PRT	Artificial Sequence	SSGLRINSADKDA
008	CS (C-terminal spoke region; Glu464 to Ala469)	PRT	Artificial Sequence	EDADYA
009	linker	PRT	Artificial Sequence	AASAGAGQGGGGSG

0010	linker	PRT	Artificial Sequence	EGKSSGSGSESKST
0011	linker	PRT	Artificial Sequence	GGRTSSSAASAGAGQGGGGSG
0012	linker	PRT	Artificial Sequence	GPSG
0013	linker	PRT	Artificial Sequence	GSAGSAGSGEF
0014	linker	PRT	Artificial Sequence	GSPG
0015	linker	PRT	Artificial Sequence	KESGSVSSEQLAQFRSLD
0016	linker	PRT	Artificial Sequence	SPGISGGGGGILDSMG
0017	Mutant 33-485 Mutant S33	PRT	Artificial Sequence	MRGSHHHHHHGMASMTGGQQMGRDLYDLVPRGSAKDPSGLRINSADDAAGQAIANRFTSNIK GLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQQRLEEIDRV SNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKSLGLDGFNVNSPGISGGGGGILDSM GTLINEAAAANKSTANPLASIDSALSKVDVRRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDA DYATEVSNMSKAQILQQAGTSVLAQANVPQNVLSSLR
0018	Mutant 33-485 Forward Primer CBLB485	DNA	Artificial Sequence	GCAGATTCTGCAGCAGGCTGGTTGATAATCTGGCGCAGGCTAACCAGG
0019	Mutant 33-485 502 template sequence	DNA	Artificial Sequence	TCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCCGTTCTGGCGCAGGCTAACCAGGTT
0020	Mutant 33-485 Reverse Primer CBLB485	DNA	Artificial Sequence	CCTGGTAGCCTGGGCCAGATTATCAACCAGCCTGCTGCAGAAATCTGC
0021	Mutant 33-485 DNA sequence of 485 Mutant (T7 Promoter to Stop)	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATCGGGGTTCTCATCATCATCATCATCATCATGATGGCTA GCATGACTGGTGGACAGCAATGGTGGGATCTGTACGACCTGGTTCGCGCGGTAGCGC GAAGGATCCGTCGTGCTCGGTATCAACAGCGCGGAAAGACGATGCGGCGGCGGCGGCGATT GCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGC ATTCTATTGGCGCAGACCACTGAAGTGGCTGAATGAAATCAACAACCACTGCAGCGGTG

0022	Mutant 33-485 Expressed Mutant 33-485	PRT	Artificial Sequence	GCGTGAGTTGTCTGTTCAGGCCACTAACGGGACTAACTCTGATTCGGATCTGAAATCTATCCA GGATGAAATTCAGCAACGTCGGAAGAAATCGATCGGTTTCTAATCAGACTCAATTTAACGG TGTTAAAGTCTGCTCAGGACAACAGATGAAAATCCAGGTGGTGCTAACGATGGTGAAAC CATTACCATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGCCCTTGATGGTTCAATGTTAAT TCCCCGGAAATTCGGTGGTGGTGAATCTAGACTCCATGGGTACATTAATCAATGAA GACGTCGCCGAGCAAGAAAGTACCGCTAACCCACTGGCTCAATGATTCGCAATTGTC AAAGTGGACGCGAGTTCTCTCTCTGGGGCAATTCAAAACCGTTTGTGATTCAGCCATTAC CAACCTGGCAATACGTAACCAATCTGAATCCGCGGTAGCCGTATCGAAGATGCTGACT ATGCAACGGAAATTTCTAATATGCTAAAGCGCAGATTCAGCAGCGGTGGTTGATAA MRGSHHHHHGMSMTGGQMGRLDLYDLVPRGSAKDPGLRINSKDDAAGQAIAFRFTSNIK GLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQRLLEIDRV SNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKSGLDGFNVNSPGISGGGGILDSM GTLINEDAAAANKSTANPLASIDSALS KVDVRRSSLGAIQNRFD SAI TNLGNVTNLNSARSRIEDA DYATEVSNMSKAQILQQAG
0023	Mutant 45CT Mutant 506T	PRT	Artificial Sequence	MAQVINTSLSTQNNLNKSQSSLSAIERLSSGLRINSKDDAAGQAIAFRFTSNIKGLTQASRN ANDGISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQRLLEIDRVSNQTQFN GVKVLSDQNQMKIQVGANDGETITIDLQKIDVKSGLDGFNVNSPGISGGGGILDSMGLINEDA AAKKSTANPLASIDSALS KVDVRRSSLGAIQNRFD SAI TNLGNVTNLNSARSRIEDADYATEVSN MSKAQILQQAGTSVL AQANQVPQNVL SLLVPRGSHHHHHHGMASMTGGQMGRLDLYDDDDKD P
0024	Mutant 45CT Mutant 506T	DNA	Artificial Sequence	ATGGCACAAAGTCATTAACAAACAGCCTGTCGCTGTTGACCCAGAAATAACCTGAACAAATCT CAGTCCTCACTGAGTCCGCTATTGAGCGTCTGCTCTGGTCTGCGTATCAACAGCGCGAA AGACGATGCGGAGCCAGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTC AGGCTCCCGTAACGCTAACGACGCGCATTTCTATTGCGCAGACCCTGAAGGTGCGCTGAAT GAAATCAACAACAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAAGGCCACTAACGGGACTAA CTCTGATTCGGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATCG CGTTTCTAATCAGACTCAATTAACGGTGTAAAGTCTGTCTCAGGACAACCCAGATGAAAAATC CAGGTGGTGTAAACGATGGTGAACCATTAACATCGATCTGCAAAAAATTTGATGTGAAAAGC CTTGGCCTTGATGGGTCAATGTTAATTCCTCGGGAAATTCCTGGTGGTGGTGGAAATCTA GACTCCATGGGTACATTAATCAATGAAGACGCTGCCGAGCCCAAGAAAAGTACCGCTAACCC ACTGGCTTCAATGATTTCTGCTGATTGCAAAAGTGACGCGAGTTCGTTCTCTCTGGGGCAAT TCAAAACCGTTTTGATTCAGCCATTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCG GCGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTAATATGCTAAAGCGCAGAT TCTGCAGCAGGCTGGTACTTCCGTTCTGGCGCAGGCTAACCCAGGTTCCGCAAAACGTCCTCT

				CTTTACTGGTCCGCGGGTTCTCATCATCATCATGGTATGGTAGCATGACTGGTG GACAGCAAAATGGGTGCGGATCTGTACGACGATGACGATAAGGATCCGTAAGTCGACAAGCTT GCG
0025	Mutant 45CT Forward F45CT	DNA	Artificial Sequence	CGAAAGACCATATGGCAGGCCAGGCGGATTGC
0026	Mutant 45CT Reverse R45CT	DNA	Artificial Sequence	CGCAAGCTTGTGCGACTTACGGGATCCTTATCGTC
0027	Mutant 45CT Sequence of 45CT construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCTCTAGAAAATAATTTTGT TAACCTTTAAGAAGGAGATATACATATGCGAGGCCAGCGATTGCTAACCGCTTCACTTCTAAT ATCAAAGGTCTGACTCAGGCTCCCGTAACGCTAACGACGCGATTCTATTGCGCAGACCACCT GAAGGTCCGCTGAATGAAATCAACAACAACCTGCAGCGTGTGCGTGAGTTGCTGTTTCAGGC CACTAACGGGACTAACTCTGATTCCGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCT GGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGA CAACCAGATGAAAATCCAGGTGGTGTGCTAACGATGGTGAACCAATACCATCGATCTGCAAAA AATTGATGTGAAAAGCCTTGGCCTTGATGGGTCAATGTTAATCCCGGGAATTTCCGGTGG TGGTGGTGAATTCTAGACTCCATGGGTACATTAATCAATGAAGACGCTGCCGACGCCAAGA AAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAATGTCAAAAGTGACGCGAGTTCTGTT CTTCTCTGGGGCAATTCAAAAACCGCTTTGATTACGCCATTACCAACCTTGGCAATACGGTAA CCAATCTGAACCTCCGCGGTAGCGGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTAATA TGCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTCCGTTCTGGCGCAGGCTAACCCAGGTT CCGCAAAACGTCCTCTTTACTGGTTCCGCGGGGTTCTCATCATCATCATCATCATGATG GCTAGCATGACTGGTGGACAGCAAAATGGTCTGGGATCTGTACGACGATGACGATAAGGATCC GTAAGTCGAC
0028	Mutant 45CT Expressed Mutant 45CT	PRT	Artificial Sequence	MAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELVSQATNGTNSDSLK SIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQV/GANDGETITIDLQKIDVKSGLDGFNVNS PGISGGGGILDMSGTLINEDAAAAKKSTANPLASIDSALSKVDAVRSSLGAIQNRFDSAITNLGNT VTNLNSARSRIEDADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVLSSLVPRGSHHHHHHGM ASMTGGQQMGRDLYDDDDKDP
0029	Mutant 33GPS Expressed Mutant 33ML	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATGCGGCAGGCCAGGCGATTGCTAACCC GCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTCCCGTAACGCTAACGACGCGCATTTCTA TTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGTGCGTGAG TTGTCTGTTTCAGGCCACTAACGGGACTAACTCTGATTCGCGATCTGAAATCTATCGGACCATCA GGTCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTT AACGGTGTAAAGTCCTGTCTCAGGACAACCCAGATGAAAATCCAGGTTGGTGTCTAACGATGG

				TGAACCATACCATCGATCTGCAAAAAATTGATGTGAAAAAGCCTTGCCCTTGATGGGTCAA TGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTCAATTGATTCTGCAATTGTCAAAAGT GGACGCAGTTCTTCTCTGCGGCAATTCAAAACCGCTTGATTACGCCATTACCAACCT TGGCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAA CGGAAGTTTCTAATATGCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCCGTTCTGGCG CAGGCTAACCAAGTTCCGCAAAACGCTCTCTTTACTGGTCCGCGGGGTTCTCATCATCAT CATCATCATGGTTAA
0030	Mutant 33GPS Expressed Mutant 33ML	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQPSGGDEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQV/GANDGETITIDL QKIDVKSGLDGFNVNPSGSTANPLASIDSALSKV/DVRRSLGAIQNRFDSAITNLGNTVTLNLSAR SRIEDADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVLSLLVPRGSHHHHHHG GATATACATATGAGCGGGTTACGGATCAACAG
0031	Mutant 33GPS Forward primer FSY3CT	DNA	Artificial Sequence	AGATCTCCCCGGGAATTAAACATTGAACCC
0032	Mutant 33GPS Reverse primer RMIMxN	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATGCG GCAGGCCAGGCGATTGTAACCGCTTCACTTCTAATATCAAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGCATTTCTATTGCGCAGACCACTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGGTGCGTGAGTTGCTGTTCAGGCCACTGGACCATCAGGTGAAATTCAGC AACGCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCCCTGT CTCAGGACAACCAAGATGAAAATCCAGGTGGTGCTAACGATGGTGAACCATTAACCATCGATC TGCAAAAAATTGATGTGAAAAGCCTTGCGCTTGATGGTTCATGTTAATCCCCGGGAAGTA CCGCTAACCCACTGGCTTCAATTGATTCTGCATTGTCAAAAGTGGACGCGAGTTCGTTCTTCTC TGGGGGCAATTCAAAACCGCTTTGATTACGCCATTACCAACCTTGGCAATACGGTAACCAATC TGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTAATATGCTA AAGCGCAGATTCTGCAGCAGGCTGGTACTTCCGTTCTGGCGCAGGCTAACCAAGTCCGCAA AACGTCTCTCTTACTGTTCCGCGGGGTTCTCATCATCATCATCATCATGTTAAGTCGAC
0034	Mutant 33GPS Expressed Mutant 33GPS	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATGPSGEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQV/GANDGETITIDLQKIDVKSGLDGFN VNSPGSTANPLASIDSALSKV/DVRRSLGAIQNRFDSAITNLGNTVTLNLSARSRIEDADYATEVSN MSKAQILQQAGTSVLAQANQVPQNVLSLLVPRGSHHHHHHG
0035	Mutant 33ML Mutant 33CT (Fixed A)	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQV/GANDGETITIDLQKIDV

0036	Mutant 33ML Mutant 33CT (Fixed A)	DNA	Artificial Sequence	KSLGLDGFVNSPGISGGGGILDSMGLINEDAAAAKKSTANPLASIDSALSKVDVRRSLGAIQ NRFDSAITNLGNTVTLNLSARSRIEDADYATEVSNMSKAQILQQAGTSVLAQANQV/PQNVLSLLVP RGSHHHHHG ATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATCGGGCAGGCCAGGGCGATTGCTAACC GCTTCACTTCTAATATCAAAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTCTTA TTGCGCAGACCACCTGAAGGTGCGTGAATGAATCAACAACAACCTGCAGCGTGTGCGGTGAG TTGCTGTTTACGGCCACTAACGGGACTAATCTGATCCGATCTGAATCTATCCAGGATGAA ATTACGCAACGCTCTGGAAGAAATCGATCGGTTTCTAATCAGACTCAATTTAACGGTGTAA GTCCTGTCTCAGGACAAACAGATGAAATCCAGGTGGTGGTCAACGATGGTGAACCATTAAC CATCGATCTGCAAAAAATTGATGTGAAAGCCTTGGCCTTGATGGTTCAATGTTAATTCCTCC GGGAATTCGGTGGTGGTGAATTTAGACTCCATGGGTACATTAATCAATGAAGACG CTGCCGACGCCAAGAAAGTACCGCTAACCCACTGGCTTCAATTGATTCGATTTGTCAAAAG TGGACGCAGTTCGTTCTCTGCGGGCAATTCAAAACCGCTTTGATTACGCCATTACCAACC TTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGATCGAAGATGCTGACTATGCA ACGGAAGTTTCTAATATGCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTCCGTTCTGGCG CAGGCTAACGAGTTCCGCAAAACGTCCTCTCTTTACTGGTTCGCGGGGTCTCATCATCAT CATCATCATGGTTAA TCTAGACCCGGGAAGTACCGCTAACCCACTGGCTTCAATTG CCAGTCATGTCGACTTAACCATGATGATGATGATGAG CTCATCATCATCATCATGGTTAAGTCGACAAGCTTGCAGCGCCGACAGGCTCGC TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGGCATTCTATTGGCAGACCCACTGAAGGTGGCTGAATGAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAATCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAAACAGATGAAATCCAGGTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTGATGTGAAAGCCCTGGCCTTG ATGGGTTCAATGTTAATTCCTCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGCAT TGTCAAAAGTGGACGCGAGTTCGTTCTCTCTGGGGCAATTCAAAACCGCTTGTGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACTCCGCGGTAGCCGTATCGAAGATGCT
0037	Mutant 33ML Forward primer F502ML	DNA	Artificial Sequence	TCTAGACCCGGGAAGTACCGCTAACCCACTGGCTTCAATTG
0038	Mutant 33ML Reverse primer R33CT	DNA	Artificial Sequence	CCAGTCATGTCGACTTAACCATGATGATGATGATGAG
0039	Mutant 33ML 502 template sequence	DNA	Artificial Sequence	CTCATCATCATCATCATGGTTAAGTCGACAAGCTTGCAGCGCCGACAGGCTCGC
0040	Mutant 33ML 33ML construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGGCATTCTATTGGCAGACCCACTGAAGGTGGCTGAATGAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAATCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAAACAGATGAAATCCAGGTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTGATGTGAAAGCCCTGGCCTTG ATGGGTTCAATGTTAATTCCTCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGCAT TGTCAAAAGTGGACGCGAGTTCGTTCTCTCTGGGGCAATTCAAAACCGCTTGTGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACTCCGCGGTAGCCGTATCGAAGATGCT

0041	Mutant 33ML Expressed Mutant 33ML	DNA	Artificial Sequence	GACTATGCAACGGAGTTTCTAATATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGCTAACCGGTTCCGAAAAAGTCTCTCTTTACTGGTTCGCGGGGTTCTC TCATCATCATCATCATGGTTAAGTCGAC ATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATCGGCGAGGCCGCGATTGCTAACCC GCTTCACTTCTAATATCAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTCTTA TTGGCAGACCACTGAAGGTGCGTGAATGAATCAACAACAACCTGCAGCGTGTGCGTGAG TTGCTGTTTCCAGGCCACTAACGGGACTAATCTGATTCGATCTGAATCTATCCAGGATGAA ATTGAGCAACGCTCTGGAAGAAATCGATCGGTTTCTAATCAGACTCAATTTAACGGTGTAAA GTCCTGTCTCAGGACAAACAGATGAAATCCAGGTGGTGCTAACGATGGTGAACCATTAAC CATCGATCTGCAAAAAATTGATGTGAAAAAGCCTTGGCCTTGATGGTTCAATGTTAATTCCTCC GGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCATTGTCAAAAGTGGACGCAGTTC GTTCTTCTGCGGGCAATTCAAAACCGCTTTGATTTCAGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAATCCGCGGTAGCGGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTTA ATATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTCCGTTCTGGCGCAGGCTAACCCAG GTTCCGCAAAACGTCTCTTTACTGGTTCCGCGGGGTCTCATCATCATCATCATGATGATGAT TAA
0042	Mutant 33ML Mutant 33ML	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTOASRNANDIGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRIEEDIRVSNQTFNGVKVLSQDNQMKIVGANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDVVRSSLGAIQNRFDISATNLGNTVNLNSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANVPQNVLSLLVPRGSHHHHHHG
0043	Mutant 37CT delta ND0 mutant based on CBLB506T	DNA	Artificial Sequence	ATGGCACAAGTCATTAAACAACAGCGCTGTCGCTGTTGACCCAGAAATAACCTGAACAAATCT CAGTCCTCACTGAGTTCGGCTATTGAGCGTCTGCTCTGGTCTGCGTATCAACGCGCGGAA AGACGATGCGGCAGGCCAGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTC AGGCTTCCCGTAACGCTAACGACGCGCATTTCTATTGCGCAGACCACTGAAGGTGCGCTGAAT GAAATCAACAACAACCTGCAGCGTGTGCGTGAGTTGCTGTTTCAGGCCACTAACGGGACTAA CTCTGATTCCGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATCG CGTTTCTAATCAGACTCAATTAACGGTGTAAAGTCTGCTCAGGACAACCCAGATGAAATC CAGGTGGTGTAAACGATGGTGAACCATTAACATCGATCTGCAAAAAATTGATGTGAAAAAGC CTTGGCCTTGATGGGTCAATGTTAATTCCTCCGGGAAATTCCTGGTGGTGGTGGAAATCTTA GACTCCATGGGTACATTAAATCAATGAAGACGCTGCCGAGCCCAAGAAAGTACCGCTAACCC ACTGGCTTCAATTGATTCTGCATTGTCAAAAGTGACGCGAGTTCGTTCTTCTCTGGGGCAAT TCAAAACCGCTTTGATTTCAGCCATTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCG GCGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTAATATGTTAAAGCGCAGAT TCTGCAGCAGGCTGGTACTTCCGTTCTGCGCGCAGGCTAACCCAGGTTCCGCAAAACGTCCTCT

0044	Mutant 37CT delta ND0 mutant based on CBLB506T	PRT	Artificial Sequence	CTTTACTGGTCCGCGGGTTCTCATCATCATCATCATGGTAGCTGGTAGCTAGCTGGTG GACAGCAAAATGGGTGCGGATCTGTACGACGATGACGATAAGGATCCGTAAGTCGACAAGCTT GCG
0045	Mutant 37CT Forward F37CT	DNA	Artificial Sequence	MAQVINTNSLSLL TQNNLNKSQSSLS SAIERLSSGLRINGAKDDAAGQAIAINRFTSNIKGLTQASRN ANDGISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQRL EEDRVSNQTQFN GVKVLSDQNQMKIQVGANDGETITIDLQKIDVKSGLDGFNVNPSGSGGGGILDSMGTLINEA AAKKSTANPLASIDSALSKVDVRSLSLGAIONRFD SAITNLGNTVNLNSARSRIEDADYATEVSN MSKAQILQQAGTSVLAQANQVPQNVL SLLVPRGSHHHHHHGHGMSMTGGQQMGRDLYDDDDKD P
0046	Mutant 37CT Reverse R37CT	DNA	Artificial Sequence	CTCTGGTCATATGATCAACAGCGCGGAAAGACGATGC
0047	Mutant 37CT 37CT construct	DNA	Artificial Sequence	TCTAGAGTCGACTATTAAAGCCATACCATGATGATGATGATGAG TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCTCTAGAAAATAATTTTGT TAACTTTAAAGAGGAGATATACATATGATCAACAGCGCGAAGACGATGCGGAGGCCAGGC GATTGCTAACCGCTTCACCTTAATATCAAAAGGTCTGACTCAGGCTTCCCGTAACGCTAAACGA CGGCATTTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGC GTGCGTGAGTTGTCTTTCAGGCCACTAACGGGACTAATCTGATCCGATCTGAAATCTA TCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTA ACGGTTAAAGTCTGCTCAGGACAACCATGAAATCCAGTTGGTGGTACGATGGT GAAACCATACCATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGGCCTTGATGGGTCAAT GTTAATCCCCGGGAATTTCCGGTGGTGGTGGTGAATTTAGACTCCATGGGTACATTAATC AATGAAGACGCTGCCGAGCCAGAAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGC ATTGTCAAAAGTGGACGAGTTCTGTTCTTCTGCGGGCAATTCAAAACCGCTTTGATTACGC CATTACCAACCTTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGTATCGAAGATG CTGACTATGCAACGGAAATTTCTAATATGCTAAAGCGCAGATTTCTGAGCAGGCTGGTACTT CCGTTCTGGCGCAGGCTAACCGGTTCCGCAAAACGTCCTCTCTTACTGGTTCGCGGGGT TCTCATCATCATCATCATGGTATGGCTTAATAGTCGAC
0048	Mutant 37CT Mutant 37CT	PRT	Artificial Sequence	MINSAKDDAAGQAIAINRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQATNG TNSDSLKSIQDEIQRL EEDRVSNQTQFNQVKSQDNQMKIQVGANDGETITIDLQKIDVKSGL LDGFNVNPSGSGGGGILDSMGTLINEA AAKKSTANPLASIDSALSKVDVRSLSLGAIONRFD SAITNLGNTVNLNSARSRIEDADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVL SLLVPRGSH HHHHHGHMA
0049	Mutant 445	PRT	Artificial	MRGSHHHHHHGHGMSMTGGQQMGRDLYDLVPRGSAKDPMAQVINTNSLSLL TQNNLNKSQSSLS

502-SY1	Sequence		SAIERLSSGLRINSKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRQVR ELSVQATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQV/GANDGETITD LQKIDV/KSLGLDGFVNSPGISGGGGILDMSMGLINEDAAAAKKSTANPLASIDSALSKVDVRSS LGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYATEVSNMSKAQILQQAGTSVLAQANQV/PQNVL SLLR
0050	Artificial Sequence	DNA	ATGCGGGTTCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGAGCAGCAAAATG GGTCGGGATCTGTACGACCTGGTCCGGCGGTAGCGGAAGGATCCGATGGCACAAGTCA TTAATACAAACAGCCTGTCGTGTGACCCAGAATAACCTGAACAAATCTCAGTCTCTACTGA GTTCCGCTATTGAGCGTCTGCTCTGCTGCTGCTATCAACAGCGCGAAAGACGATGCGGCA GGCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCGTAAC GCTAACGACGGCATTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAA CCTGCAGCGTGTGCGTGAGTTGCTGTTGAGGCCACTAACGGGACTAACTCTGATTCGGATC TGAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTCTAATCAGA CTCAATTTAACGGGTAAAGTCTGCTCAGGACAACAGATGAAATCCAGGTGGTGCTA ACGATGGTGAACCAATTACCATGATCTGCAAAAATTTGATGTAAAGCCCTGGCCCTTGATG GGTCAATGTAAATCCCGGGAAATTCGGTGGTGGTGGTAAATCTAGACTCCATGGGT ACATTAATCAATGAAGACGCTGCCGAGCCAAGAAAGTACCGCTAACCCACTGGCTTCAATT GATCTGCATTGTCAAAAGTGACGCGAGTTCTCTCTCTGGGGCAATTCAAAACCGTTTT GATTCAGCCATTACCAACCTTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGTATC GAAGATGCTGACTATGCAACGGAAGTTCTAATATGCTAAAGCGCAGATCTGACGACGAGCT GGTACTCCGTTCTGGCGCAGGCTAACGAGTTCCGCAAAACGTCCTCTCTTACTGCGTTAA GGCAATTCAAAACCGTTTTGATTAGCCATTACCAACCTTGG
0051	Artificial Sequence	DNA	CCAAGGTTGGTAATGGCTTAATCAAAAACGGTTTTGAATTGCC
0052	Artificial Sequence	DNA	TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGCGGGGTCTCATCATCATCATCATGATGGTATGGCTA GCATGACTGGTGGACAGCAAAATGGTGGGATCTGTACGACCTGGTTCCGCGGGTAGCGC GAAGGATCCGATGGCACAAGTCATTAATACAAACAGCCTGTGCTGTGACCCAGAAATAACCT GAACAAATCTCAGTCTCAGTCTGAGTTCCGCTATTGAGCGTCTGTCTCTGCTGCGTATCAA CAGCGGAAAGACGATGCGGACGGCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAG GTCGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTATTGCGCAGACCCTGAAGGT
0053	Artificial Sequence	DNA	

				GGCTGAATGAAATCAACAACCTGCAGCGTGTGCGTGAGTTGTCTGTTCAGGCCACTAA CGGACTAACTCTGATTCGATCTGAAATCTATCCAGGATGAAATTCAGCAACGTCTGGAAGA AATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCTCTGCTCAGGACAACCA GATGAAATCCAGGTTGGTCTAACGATGGTGAACCATTAACCATGATCTGCAAAAAATTGA TGTGAAAGCCTTGGCCTTGATGGTTCAATGTTAATCCCGGGAATTCGCGTGGTGGTG GTGGAATCTAGACTCCATGGGTACATTAAATCAATGAAGACGCTGCCGAGCCAAAGAAAGTA CCGCTAACCCACTGGCTTCAATTGATCTGCATTGTCAAAAGTGGACGCGAGTTCGTTCTCTC TGGGGCAATTCAAAACCGTTTTGATTAA
0054	Mutant 445 mutant 445	PRT	Artificial Sequence	MGRSHHHHHGMSMTGGQQMGRDLYDLVPRGSAKDPMAQVINTNSLL TQNNLNKSQSSL SAIERLSSGLRINSKDDAAGQAIANRFTSNIKGL TOASRNANDGISAQTTGALNEINNLRVR ELSVQATNGTNSDSLKSIQDEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITD LQKIDVKSGLDGFNVNSPGISGGGGILDMSMGLINEDAAAAKKSTANPLASIDSALSKVDAVRSS LGAIQNRFD
0055	Mutant 461 Forward Primer CBLB461	DNA	Artificial Sequence	CAATCTGAACCTCCGCGGTTGACGTATCTAAGATGCTGACTATGC
0056	Mutant 461 Reverse Primer CBLB461	DNA	Artificial Sequence	GCATAGTCAGCATCTTAGATACGTCAACGCGCGGAGTTCAGATTG
0057	Mutant 461 Mutant 461	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCTCTAGATAATTTTGT AACTTTAAGAGGAGATATACATATGCGGGGTTCTCATCATCATCATCATCATGATGGCTA GCATGACTGGTGGACAGCAATGGTGGGATCTGTACGACCTGGTTCCGCGCGGTAGCGC GAAGGATCCGATGGCACAAGTCATTAATAACAACAGCCTGTGCTGTTGACCCAGAAATACCT GAACAAATCTCAGTCTCACTGAGTTCCGCTATTGAGCGTCTGTCTCTGCTGCGTATCAA CAGCGGAAAAGACGATCGGCAAGCGGCGGATTGCTAACCGCTTCACTTCTAATATCAAAG GTCTGACTCAGGCTTCCCGTAACGCTAACGACGCGATTCTATTGCGCAGACCCACTGAAAGT GCGTGAATGAAATCAACAACAACCTGCAGCGTGTGCGTGAGTTGTCTGTTAGGCCACTAA CGGGACTAATCTGATTCGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGA AATCGATCGGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCTGCTCAGGACAACCA GATGAAATCCAGGTTGGTGTACCGATGGTGAACCATTAACCATGATCTGCAAAAAATTGA TGTGAAAGCCTTGGCCTTGATGGTTCAATGTTAATCCCGGGAATTCGCGTGGTGGTG GTGGAATCTAGACTCCATGGGTACATTAAATCAATGAAGACGCTGCCGAGCCAAAGAAAGTA CCGCTAACCCACTGGCTTCAATTGATCTGCATTGTCAAAAGTGGACGCGAGTTCGTTCTCTC TGGGGCAATTCAAAACCGTTTTGATTGAGCCATTACCAACCTTGGCAATACGGTAACCAATC

0058	Mutant 461 Mutant 461	PRT	Artificial Sequence	TGAACTCCGCGGTTGACGTATCTAA MRGSHHHHHGMSMTGGQMGRLDYLVPRGSAKDPMAQVINTNSLSLLTQNNLNKSQSSLS SAIERLSSGLRINSKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVVR ELSVQATNGTNSDSLKSIQDEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQV/GANDGETITID LQKIDVKSGLDGFNVNSPGISGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALS/SKVDVAVRSS LGAIQNRFDSAITNLGNTVTNLNSAR
0059	Mutant 467 Forward Primer CBLB467	DNA	Artificial Sequence	CGTAGCCGTCGAAAGATGCTTAATAGGCAACGGAAAGTTTCTAATATG
0060	Mutant 467 Reverse Primer CBLB467	DNA	Artificial Sequence	CATATTAGAAACTCCGTTGCCTATTAAAGCATCTTCGATACGGCTACG
0061	Mutant 467 Mutant 467	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAAAGGAGATATACATATGCGGGGTTCTCATCATCATCATCATCATCATCATCATCATCAT GCATGACTGGTGACAGCAAAATGGTCGGGATCTGTACGACCTGGTTCCGCGCGGTAGCGC GAAGGATCCGATGGCACAAGTCATTAATACAAACAGCCTGTGCTGTTGACCCAGAAATAACCT GAACAAATCTCAGTCTCAGTCTCAGTTCGCTGAGTTCGCTATTGAGCGTCTGTCTCTGCTCGGTATCAA CAGCGGAAAGACGATCGGCAGCGGCGGAGTTCGTAACCGCTTCACTTCTAATATCAAAAG GTCGACTCAGGCTCCCGTAACGCTAACGACGCGCATTTCTATTGCGCAGACCATGAAAGT GCGTGAATGAATCAACAACCAACCTGCAGCGTGCCTGAGTTGCTGTTGAGGCGCACTAA CGGACTAACTCTGATTCGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGA AATCGATCGGTTTCTAATCAGACTCAATTAACGGTGTAAAGTCTGCTCAGGACAACCA GATGAAATCCAGGTTGGTGCTAACGATGGTGAACCAATACCATCGATCTGCAAAAAATTGA TGTGAAAAGCCTTGGCCTTGATGGTTCAATGTTAATCCCGGGAATTCGGGTGGTGTG GTGGAATTCAGACTCCATGGGTACATTAATCAATGAAGACGCTGCCGACGCAAGAAAAAGTA CCGCTAACCCCACTGGCTTCAATTGATTCGATTTGCAAAAGTGGACGCGAGTTCTGTTCTCTC TGGGGGCAATTCAAAAACCGTTTGTTCAGCCATTACCAACCTTGGCAATACGGTAACCAATC TGAACCTCCGCGCGTAGCCGTATCGAAGATGCTTAATAG
0062	Mutant 467 Mutant 467	PRT	Artificial Sequence	MRGSHHHHHGMSMTGGQMGRLDYLVPRGSAKDPMAQVINTNSLSLLTQNNLNKSQSSLS SAIERLSSGLRINSKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVVR ELSVQATNGTNSDSLKSIQDEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQV/GANDGETITID LQKIDVKSGLDGFNVNSPGISGGGGILDSMGTLINEDAAAAKKSTANPLASIDSALS/SKVDVAVRSS LGAIQNRFDSAITNLGNTVTNLNSARSIEDA
0063	Mutant 470CT	DNA	Artificial	ATCGCGGGTTCATCATCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAAATG

	CBLB502		Sequence	GGTCGGGATCTGTACGACGATGACGATAAGGATCCGATGGCACAAGTCATTAATACAAACAG CCTGTCGCTGTTGACCCAGAATAACCTGAACAAATCTCAGTCCTCACTGAGTTCGCTATTGA GCGTCTGCTCTGCTGCGTATCAACAGCGGAAAGACGATCGGCGAGGCCAGCGGATT GCTAACCCGCTTCACTTCTAATATCAAAAGGTCTGACTCAGGCTCCCGTAACGCTAACGACGGC ATTCTATTGCGCAGACCACTGAAGGTGCGCTGAATGAAATCAACAACAACTGCAGCGTGT GCGTGAGTTGCTGTTCAAGGCCACTAACGGGACTAACTCTGATCCGATCTGAAATCTATCCA GGATGAAATTCAGCAACGCTGGAAGAAATCGATCGGTTTCTAATCAGACTCAATTTAACGG TGTTAAAGTCTCTGCTCAGGACAACAGATGAAATCCAGGTTGGTGTACCGATGGTGAAC CATTACCATCGATCTGCAAAAAATTGATGTGAAAGCCTTGCCCTTGATGGTTCAATGTTAAT TCCCCGGGAATTCGCGTGGTGGTGGAATTTCTAGACTCCATGGGTACATTAATCAATGAA GACGCTGCCGACGCAAGAAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCATTGTC AAAGTGGACGCGAGTTCTTCTCTGGGGCAATTCAAAACCGTTTGTGATTGAGCCATTAC CAACCTTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGTATCGAAGATGCTGACT ATGCAACGGGAAGTTTCTAATATGCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCCGTTT TGGCGCAGGCTAACCAAGTTCCGCAAAACGTCCTTCTTACTGCGTTAA CGATAAGGATCATATGGCACAAGTCATTAATAC
0064	Mutant 470CT Forward Primer F470CT	DNA	Artificial Sequence	
0065	Mutant 470CT Reverse Primer R470CT	DNA	Artificial Sequence	AGATCTGTCGACTTAACCATGATGATGATGATGATGAGAACCCCGCGGAACCAAGTCATAGT CAGCATCTTCGATACG
0066	Mutant 470CT Mutant 470CT	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATGTGAGCGGATAACAAATCCCTCTAGATAATTTTGTTT AACTTTAAGAAGGAGATATACATATGGCACAAGTCATTAAACAAACAGCCCTGTCGCTGTGA CCCAGAAATAACCTGAACAAATCTCAGTCCCTCACTGAGTCCGCTATTGAGCGTCTGTCCTCTG GTCTGCGTATCAACAGCGCGAAAGACGATGCGGCGAGCCAGGCGATTGCTAACCCGCTTAC TTCTAATATCAAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTATTGGCA GACCACTGAAGGTGCGCTGAATGAAATCAACAACCAACCTGCAGCGTGTGCGTGAGTTGCTG TTCAGGCCACTAACGGGACTAACTCTGATTCGATCTGAAATCTATCCAGGATGAAATTCAGC AACGCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCCCTGT CTCAGGACAACCAAGATGAAATCCAGGTGGTGTACGATGGTGAACCAATACCATCGATC TGCAAAAAATTGATGTGAAAGCCCTTGGCCTTGATGGTCAATGTTAATCCCCGGGAATTT CCGGTGGTGGTGGAAATTCAGACTCCATGGGTACATTAAATCAATGAAGACGCTGCCGCA GCCAAGAAAAGTACCGCTAACCCACTGGCTTCAATGATTTGCTGCAATGTCAAAAAGTGGACGCA GTTGCTTCTTCTCTGGGGCAATTCAAAACCGTTTGTATTGAGCCATTACCAACCTTGGCAAT ACGGTAACCAATCTGAACCTCCGCGGTAGCGGTATCGAAGATGCTGACTATGCACTGCTGTTCC GCGGGTTCTCATCATCATCATCATGTTAAGTCGAC

0067	Mutant 470CT Mutant 470CT	PRT	Artificial Sequence	MAQVINTNSLSLLTQNNLNKSSSLSSAIERLSSGLRINSKADDAAGQAIAIRFTSNIKGLTQASRN ANDGISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTQFN GVKVLSDQNQMKIQV/GANDGETITIDLQKIDV/KSLGLDFNVNSPGISGGGGILDSMGTLINEDA AAAKKSTANPLASIDSALSVDVRSLSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYALVPRG SHHHHHHG
0068	Mutant 485CT Reverse primer R485MC	DNA	Artificial Sequence	AGATCTCCGCGGAACACAGACCAGCCTGCTGCAGAAATCTGC
0069	Mutant 485CT DNA Sequence of 485CT	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCCG TAACGCTAACGACGCGATTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAACAGATGAAATCCAGGTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTG ATGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTTCTGCAT TGTCAAAAGTGGACGAGTTCGTTCTTCTCTGGGGCAATTCAAAACCGCTTTGATTCAGCCA TTACCAACCTTGGCAATACGGTAAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAGTTTCTAATATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTCTGGTT CCGCGGGTCTCATCATCATCATCATCATGTTAAGTCGAC
0070	Mutant 485CT Mutant 485CT	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATGCGGCAGGCCAGGGGATTGCTAACC GCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTA TTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGTGCGTGAG TTGTCTGTTCAGGCCACTAACGGGACTAACTCTGATTCGGATCTGAAATCTATCCAGGATGAA ATTAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGGTGTTAAA GTCCTGTCTCAGGACAACAGATGAAATCCAGGTGGTGTACGATGGTGAACCCATTAC CATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGGCCTTGATGGTTCAATGTTAATCCCC GGGAAGTACCGCTAACCCACTGGCTTCAATGATTTCTGCAATTGCAAAAGTGGACGCAGTTC GTTCTTCTCTGGGGCAATTCAAAACCGCTTTGATTCAGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTA ATATGCTAAAGCGCAGATTCTGCAGCAGGCTGGTCTGGTTCCGCGGGTCTCATCATCAT CATCATCATGGTTAA
0071	Mutant 485CT Mutant 485CT	PRT	Artificial Sequence	MSGLRINSKADDAAGQAIAIRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTQFNQV/KVLSQDNQMKIQV/GANDGETITIDLQKIDV

				KSLGLDGFVNSPGSTANPLASIDSALSKVDVAVRSSLGAIQNRFDNSAITNLGNTVTVNLNSARSRIED ADYATEVSNMSKAQILQQAGLVPRGSHHHHHHG
0072	Mutant 485D DNA template for deletion mutations from Mutant 485CT variant	DNA	Artificial Sequence	AACCCACTGGCTTCAATTGATTCTGCATTGTCAAAAAGTGGACGCAGTTGTTCTTCTCTGCGG GCAATTCAAAACCGTTTTGATTACGCCATTACCGCCCTTGGCAATACGGTAACCAAT
0073	Mutant 485D PRT sequence for deletion mutations from Mutant 485CT variant	PRT	Artificial Sequence	NPLASIDSALSKVDVAVRSSLGAIQNRFDNSAITALGNTVTN
0074	Mutant 485D Forward Primer F485D	DNA	Artificial Sequence	GTCGTTCTTCTCTGGGGCAATTGATTCAGCCATTACCGCCCTTG
0075	Mutant 485D Reverse Primer R485D	DNA	Artificial Sequence	CAAGGGCGGTAAATGGCTGAATCAATTGCCCCCAGAGAAGAACGAAC
0076	Mutant 485D DNA Sequence of 485CT_Delta construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGTAACCGCTTCACTTCTAATATCAAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAAACAGATGAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCTCAGGACAAACAGATGAAAGCCCTTGGCCTTG ATGGTTCAATGTTAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAAGTGGACGCGAGTTGTTCTTCTCTGGGGCAATTGATTACGCCATTACCGCCCTTG GCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACG GAAGTTTCTAATATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTCTGGTTCGCGGGGTTCTC TCATCATCATCATCATCATGTTAAGTCGAC
0077	Mutant 485D Mutant 485D (CT_Delta 439-442)	PRT	Artificial Sequence	MSGLRINSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQV/GANDGETITDLQKIDV KSLGLDGFVNSPGSTANPLASIDSALSKVDVAVRSSLGAIDSALTALGNTVTVNLNSARSRIEDADYA TEVSNMSKAQILQQAGLVPRGSHHHHHHG
0078	Mutant CGD1 Mutant SY3CT	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATCGGCGAGGCCAGGCGGATTGCTAACCC GCTTCACTTCTAATATCAAAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTCTTA

0079	Mutant CGD1 Mutant SY3CT	PRT	Artificial Sequence	TTGGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGTGCGTGAG TTGTCTGTTCAAGCCACTAACGGACTAACTCTGATCCGATCTGAAATCTATCCAGGATGAA ATTAGCAACGCTCTGGAAGAAATCGATCGGTTTCTAATCAGACTCAATTTAACGGTGTAA GTCCTGTCTCAGGACAACCCAGATGAAATCCAGGTGGTCTAACGATGGTGAACCATTA CATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGCCCTTGATGGTTCAATGTTAATCCCC GGGAAGTACCGCTAACCCACTGGCTTCAATTGATCTGCATTGTCAAAAGTGGACGCAGTTC GTTCTCTCTGGGGCAATTCAAAACCGCTTTGATTCAGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAACCTCCGCGGTAGCGTATCGAAGATGCTGACTATGCACTGCTCCGCGG GGTCTCATCATCATCATCATGTTAA
0080	Mutant CGD1 GFP _{uv4}	DNA	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTOASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQVANDGETITIDLKIDV KSLGLDGFNVNSPGSTANPLASIDSALS SKVDAVRSSLGAIQNRFD SAI NLGNTVTNLNSARSRIED ADYALVPRGSHHHHHHG ATGAGTAAAGGAGAGAAGACTTTTCACTGGAGTTGCCCCAATCTTGTGAATTAGATGGTGAT GTTAATGGGCACAAATTTTCTGTCACTGGAGAGGTGAAGGTGATGCAACATACGGAAACATT ACCTTAAATTTATTTGCACTACTGGAACACTACCTGTCCATGGCCAACTTGTCACTACTC TGACGTATGGTGTCAATGCTTTTCCCGTTATCCGGATCATATAAAGCGCATGACTTTTTTCAA GAGTGCCATGCCCGAAGTTATGTACAGGAACGCACATATATCTTTCAAAGATGACGGGAAC ACAAGACGCGTGTGCTGAAGTCAAGTTGAAGGTGATACCTTGTAAATCGTATCGAGTTAAAG GTATTGATTTAAAGAAGATGGAACATCTCGACACAACTCGAGTACAACTATACTCACA CAATGTATACATCAGGCAGACAAACAAAGAAATGGAATCAAAGCTAACTCAAAATTCGCCA CAACATTGAAGATGGATCCGTTCAACTAGCAGACCATTAACAACAAATACTCCAATTGGCGA TGCCCTGTCTTTACCAGACAACCATACCTGTGACACACAATCTGCCC TTTTGAAGAATCC CAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACCTGCTGCTGGGATTACACATGG CATGGATGAACATATACAAA
0081	Mutant CGD1 GFP _{uv4}	PRT	Artificial Sequence	MSKGEELFTGWVPIVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVPWPVLVTTLTY GVQCFSRYPDHMKRHDFKSAWPEGYVQERTISFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK EDGNILGHKLEYNYNSHNVITADKQKNGIKANFKIRHNIEDGSVQLADHYQQNTPIGDPVLLPD NHYLSTQSALLKDPNEKRDHMLLEFVTAAGITHGMDELYK
0082	Mutant CGD1 GFP _{uv4} mutation of wt NdeI site	DNA	Artificial Sequence	ATGAGTAAAGGAGAGAAGACTTTTCACTGGAGTTGCCCCAATCTTGTGAATTAGATGGTGAT GTTAATGGGCACAAATTTTCTGTCACTGGAGAGGTGAAGGTGATGCAACATACGGAAACATT ACCTTAAATTTATTTGCACTACTGGAACACTACCTGTCCATGGCCAACTTGTCACTACTC TGACGTATGGTGTCAATGCTTTTCCCGTTATCCGGATCATATAAAGCGCATGACTTTTTTCAA GAGTGCCATGCCCGAAGTTATGTACAGGAACGCACATATATCTTTCAAAGATGACGGGAAC

				ACAAGACGCGTGCTGAAGTCAAGTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAAG GTATTGATTTTAAAGAAGATGGAACATTCTCGACACAAACTCGAGTACAACTATAACTCACA CAATGTATACATCACGGCAGACAAAACAAAAGAAATGGAATCAAAGCTAACTTCAAAAATTCGCCA CAACATTGAAGATGGATCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGA TGGCCCTGTCTTTTACCAGACAACCATTTACCTGTGACACAACTGCCCCCTTTTGAAGAATCC CAACGAAAAGCGTGACCACATGGTCTTCTTGAGTTTGTAACCTGCTGCTGGGATTACACATGG CATGGATGAACATATACAAATAA
0083	Mutant CGD1 Forward primer FCGFP	DNA	Artificial Sequence	TCTAGACGGCCGATCTCAGGTAAGAATGGAATCAAAGCTAACTTCAAAAATTCGC
0084	Mutant CGD1 PRT altered GFPuv4 sequence	PRT	Artificial Sequence	NVYIPISGKNIGIKANFKIRH
0085	Mutant CGD1 Reverse RCGFP	DNA	Artificial Sequence	AGATCTCCGCGGTTTGTTATAGTTTCATCCATGCCCATGTGTAATCCC
0086	Mutant CGD1 DNA Sequence of CGD1 construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGAAATGTGAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACCTTAATATCAAAGGTCTGACTCAGGCTTCCCCG TAACGCTAACGACGCGATTTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAAACCAGATGAAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAAATTGATGTGAAAAGCCTTGCCCTTG ATGGGTTCAATGTTAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGTCAT TGTCAAAAGTGGACGCAAGTTCTGTTCTCTGCGGGCAATTCAAAACCGCTTTGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGTATCGAAGATGCT GACTATGCATGGTCCGCGATCTCAGGTAAGATGGAATCAAAGCTAACTTCAAAAATTCGC CACAACATTGAAGATGGATCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGC GATGGCCCTGTCTTTTACCAGACAACCAATTACCTGTCGACACAACTGCCCCCTTTGAAAGAT CCCAACGAAAAGCGTGACCACATGGTCTTCTTGAGTTTGTAACCTGCTGCTGGGATTACACAT GGCATGGATGAACATATACAAACCGCGGGTTCTCATCATCATCATGTTAAGTCGAC
0087	Mutant CGD1 Expressed Mutant CGD1	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGAAAAGACGATCGGCAGGCCAGGCGATTGCTAACCC GCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTA TTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAAACACCTGCAGCGGTGCGGTGAG TTGTCTGTTCAGGCCACTAACGGGACTAACTCTGATTCGGATCTGAAATCTATCCAGGATGAA

0088	Mutant CGD1 Expressed Mutant CGD1	PRT	Artificial Sequence	ATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAG GTCCTGTCTCAGGACAAACAGATGAAATCCAGGTGGTGGTACCATGATGGTGAACCATTAC CATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTGATGGTTCAATGTTAATTCCTCC GGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGCAATGTCAAAAGTGGACGCAGTTC GTTCTTCTGGGGCAATTCAAAACCGCTTGTGATTCAGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAATCCGCGGTAGCGTATCGAAGATGCTGACTATGCACTGGTCCGCCG ATCTCAGGTAGAAATGGAATCAAGCTAATTCAAAATTCGCCACAACATTTGAAGATGGATCC GTTCAACTAGCAGACCATATCAACAAAATACCTCAAATGGCGATGGCCCTGTCTTTTACCA GACAACCATACCTGTCGACACAATTCGCCCTTTTGAAGATCCCAACGAAAAGCGTGACCAC ATGGTCTTCTTGAGTTTGAATGCTGCTGGGATTACACATGGCATGGATGAATATACAAA CCGCGGGTCTCATCATCATCATCATCATGTTAA
0089	Mutant CPM194 Mutant CPM194	DNA	Artificial Sequence	MSGLRNSAKDDAAGQAIAANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITIDLKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIED ADYALVPPISGKNGIKANFKIRHNIEDGSVQLADHYQQNTPIGDPVLLPDNHYLSTQSALLKDPNE KRDHMLLEFVTAAGITHGMDLYKPRGSHHHHHH
0090	Mutant CPM194 Mutant CPM194	PRT	Artificial Sequence	ATGAGTACCGCTAACCCACTGGCTTCAATTTGATTTCTGCATTTGTCAAAGTGGACGCAGTTTCGT TCTTCTCTGGGGCAATTCAAAACCGCTTTGATTCAGCCATTACCAACCTTGGCAATACGGTA ACCAATCTGAATCCGCGGTAGCGGTATCGAAGATGCTGACTATGCATCCCGGGGAAAGCGG GTTACGGATCAACAGCGCGAAAGACGATGCGGAGCGGCGAGGCGATTGCTAACCGCTTCACTT CTAATATCAAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTATTGCGCAGA CCACTGAAGGTGCGCTGAATGAAATCAACAACACCTGCAGCGTGTGCGTGAGTTGCTGTT CAGGCCACTAACGGGACTAATCTGATCCGATCTGAAATCTATCCAGGATGAAATTCAGCAA CGTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCTCTGCT CAGGACAACCAAGATGAAAATCCAGGTGGTGGTCAACGATGGTCTGGTCCGCGGGTCTCTCA TCATCATCATCATCATGTTAA
0091	Mutant CPM194 Mutant CPM194	DNA	Artificial Sequence	MSTANPLASIDSALSKVDVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYASPGSLRIN SAKDDAAGQAIAANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQATNGTNS DSDLKSIQDEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGLVPRGSHHHHHH

					GGCGATTGCTAAACCGCTTCACTTCTAAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAA CGACGGCATTTCTATTGCGCAGACCCACTGAAGGTGCGCTGAATGAAATCAACAACACCTGC AGCGTGTGCGTGAGTTGTCTGTTCAGGCCACTAACCGGACTAACTCTGATTCGGATCTGAAAT CTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAAT TTAACGGTGTAAAGTCTGTCTCAGGACAACCCAGATGAAATCCAGGTTGGTCTAACGATG GTCTGTTCCGCGGGGTTCTCATCATCATCATCATGGTTAAGTCGAC
0092	Mutant CPM194 Forward primer FCD1	DNA	Artificial Sequence		TC TAGACATATGAGTACCGCTAACCCCACTGGCTTCAATTG
0093	Mutant CPM194 Reverse primer RCD1J	DNA	Artificial Sequence		GCTTCCCGGGGATGCATAGTCAGCATCTTCGATACGGC
0094	Mutant CPM194 Forward primer FND1J	DNA	Artificial Sequence		GCATCCCCGGGAAGCGGGTTACGGATCAACAGCG
0095	Mutant CPM194 Reverse primer RND1	DNA	Artificial Sequence		AGATCTCCGCGGAACCAAGACCATCGTTAGCACCAACCTGGATTTTCATCT
0096	Mutant CPM217 Mutant CPM217	DNA	Artificial Sequence		ATGAGTACCGCTAACCCCACTGGCTTCAATTGATTCGATTGTCAAAAAGTGACGCAGTTCGT TCITCTCTGGGGCAATTCAAAACCGCTTTGATTCAGCCATTACCAACCTTGCCAATACGGTA ACCAATCTGAACCTCGCGCTAGCCGTATCGAAGATGCTGACTATGCATCCCCGGGAAGCGG GTTACGGATCAACAGCGCGAAAGACGATGCGGAGGCCAGGCGATTGCTAACCGCTTCACCT CTAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTATTGCGCAGA CCACTGAAGGTGCGCTGAATGAAATCAACAACACCTGCAGCGTGTGCGTGAGTTGCTGTT CAGGCCACTAACGGGACTAACTCTGATCCGATCTGAAATCTATCCAGGATGAAATTCAGCAA CGTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCTGCT CAGGACAACCCAGATGAAATCCAGGTGGTGTAAACGATGGTGAACCATTACCATCGATCT GCAAAAAATTGATGTGAAAAGCCTTGCCCTTGATGGGTCAATGTTAATCTGTTCCGCGGGG TTCTCATCATCATCATCATGGTTAA
0097	Mutant CPM217 Mutant CPM217	PRT	Artificial Sequence		MS TANPLASIDSALSKVDVAVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYASPGSLRIN SAKDDAAGQAIA NRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQATNGTNS DSDLKSIQDEIQRL EIDRVSNQTQFNGVKVLSQDNQM KIVGANDGETITIDLQKIDV/KSLGLDG FNVNLVPRGSHHHHHHG
0098	Mutant CPM217 Mutant CPM217	DNA	Artificial Sequence		TAA TACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTCTAGATAATTTTGT AACTTTAAGAAGGAGATA TACATATGAGTACCGCTAACCCACTGGCTTCAATTGATCTGCATT GTCAAAAGTGACGCGAGTTCGTTCTCTCTGGGGCAATTCAAAACCGCTTGTGATTCAGCCAT TACCAACCTTGGCAA TACGGTAAACCAATCTGAAC TCCGCGCGTACGCCGTATCGAAGATGCTG

				ACTATGCATCCCCGGGAAGCGGTTACGGATCAACAGCGCGAAAGACGATCGCGCAGGCCA GGCGATTGCTAACCGCTTCACTTCTAATAATCAAAAGTCTGACTCAGGCTCCCGTAACGCTAA CGACGGCATTCTATTGCGCAGACCACTGAAGGTGCGCTGAATGAAATCAACAACAACCTGC AGCGTGTGCGTGAGTTGTCTGTTACGGCCACTAACGGGACTAACTCTGATCCGATCTGAAAT CTATCCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAAT TTAACGGTGTAAAGTCTGCTCAGGACAACCAAGATGAAAATCCAGGTGGTGCTAACGATG GTGAAACCATACCATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGGCCTTGATGGGTTCA ATGTTAATCTGGTCCGCGGGTTCATCATCATCATCATCATGTTAAGTCGAC
0099	Mutant CPM217 Reverse primer RCPM217	DNA	Artificial Sequence	AGATCTCCGCGGAACCAGATTAAACATTGAACCCCATCAAGGCCAAG
0100	Mutant GD1G Forward Primer FGFP77	DNA	Artificial Sequence	CCCGTTATCCGGATCACATGAAACGGCATGACTTTTTTC
0101	Mutant GD1G Reverse Primer RGFP77	DNA	Artificial Sequence	GAAAAAGTCATGCCGTTTCATGTGATCCGGATAACGGG
0102	Mutant GD1G FGFP54	DNA	Artificial Sequence	CTGTTCCATGGCCAAACACTTG
0103	Mutant GD1G Forward primer FNGFP	DNA	Artificial Sequence	TCTAGACATATGAGTAAAGGAGAGAAGAACTTTTCACTGGAGTTGTCC
0104	Mutant GD1G altered GFP DNA sequence	DNA	Artificial Sequence	GGCCTATGCGGCGCCAGTAAAGGAGAGAAGAACTTTTCACTGGAGTTGCCCAATCTTGTGAA
0105	Mutant GD1G Reverse RNF6P	DNA	Artificial Sequence	AGATCTATTAATGCGGCTGATAGGCCCTTGTGTTGCTGCGGTGATGTATACATTGTG
0106	Mutant GD1G altered GFP PRT sequence	PRT	Artificial Sequence	SHNVYITADKQGLSGRNM
0107	Mutant GD1G DNA Sequence of GD1G construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGTAAAGGAGAGAAGAACTTTTCACTGGAGTTGTCCCA TTCTTGTGTAATTAGATGGTGTGATGTTAATGGGCACAAAATTTCTGTCAGTGGAGAGGGTGAAG GTGATGCAACATACGGAAAACTTACCCCTAAATTTATTTGCACTACTGGAAGAACTACCTGTTC ATGGCCAACACTTGTCACTACTCTGACGTATGGTGTCAATGCTTTTCCCGTTATCCGGATCA CATGAAACGGCATGACTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTAT

0108	Mutant GD1G Expressed Mutant GD1G	DNA	Artificial Sequence	ATCTTTCAAAGATACGGGAACTACAAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCT TGTTAATCGTATCGAGTTAAAAGGTATTGATTTTAAAGAAAGATGAAACATTTCTCGGACACAAA CTCGAGTACAACATAACTACACAATGTATACATACGGCAGACAAAACAAAGGCCATATCAGGC CGCATTATGAGCGGTTACGGATCAACAGCGCGAAAGACGATGCGGCAGGCCAGCGGATG CTAACCGCTTCACTTCTAATATCAAAGGCTGACTCAGGCTTCCGTAAACGTAACGTAACGACGGCA TTTCTATTGCGCAGACCCTGAAGTGCCTGAATGAAATCAACAACAACCTGCAGCGTG CGTGAGTTGCTGTTACGGCCACTAACGGGACTAACTCTGATCCGATCTGAAATCTATCCAG GATGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAAACGGT GTTAAAGTCTCTCAGGACAAACCCAGATGAAAATCCAGGTTGGTGCTAACGATGGTGAAAC CATTACCATCGATCTGCAAAAAAATGATGTGAAAAGCCTTGGCCTTGATGGGTTCAATGTTAAT TCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTTCTGCATTTGCAAAAGTGGACGC AGTTCGTTCTTCTCTGGGGCAATTCAAAACCGCTTGTATTCAGCCATTACCAACCTTGGCAA TACGGTAACCAATCTGAATCCGCGCGTAGCGGTATCGAAGATGCTGACTATGCATGCACTGGTTC CGCCGATCTCAGGTAAAGATGGAATCAAAGCTAACTTCAAAATTCGCCACAACATTTGAAGATG GATCCGTTCAACTAGCAGACCATTATCAACAAAAATACTCCAATGGCGATGGCCCTGTCTTT TACCAGACAACCATTAACCTGTGCACACAATCTGCCCTTTTGAAGATCCCAACGAAAAAGCGTG ACACATGGTCTTCTTGAGTTTGTAACCTGCTGGGATTACACATGGCATGGATGAACATAT ACAAACCGCGGGTCTCATCATCATCATCATCATGTTAAGTCGAC ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGCCCCAATCTTGTGAATTAGATGGTGAT GTTAATGGGCACAAATTTCTGTCACTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTT ACCTTAAATTTATTGCACACTACTGAAAACACTACCTGTCCATGGCCAAACACTGTCTACTCTC TGACGTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAA GAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACATATCTTTCAAAGATGACGGGAACT ACAAGACGCTGCTGAAGTCAAGTTTGAAGGTGATACCCCTTGTAACTCGATCGAGTTAAAAAG GTATTGATTTTAAAGAAGATGAAACATTTCTCGACACAACACTCGAGTACAACATAAATCACA CAATGTATACATCACGGCAGACAAAACAAGGCCATATCAGGCCGCTTATGAGCGGTTACGGGA TCAACAGCGCGAAAAGACGATCGGGCAGGCCAGCGATTGCTAACCCGCTTCACTTCTAATATC AAAGGCTCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTATTGCGCAGACCCTGA AGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCCA CTAACGGGACTAACTCTGATTCGGATCTGAAATCTATCCAGGATGAAATCAGCAACGCTCTGG AAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACA ACCAGATGAAAAATCCAGGTTGGTCTAACGATGGTGAACCACTTACCATCGATCTGCAAAAAA TTGATGTAAAAAGCCTTGGCCTTGATGGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCC CACTGGCTTCAATTGATTTCTGCAATTTGCAAAAGTGGACGCGAGTTTCGTTCTTCTCTGGGGCAA

				TTCAAAACCGCTTTGATTAGCCATTACCAACCTTGCCAATACGGTAACCAATCTGAACTCCG CGGTAGCCGTATGAAGATGCTGACTATGCACTGGTTCGCCGATCTCAGGTAAGAATGGA ATCAAAGCTAACTTCAAAATTCGCCACAACATTGAAGATGGATCGCTTCAACTAGCAGACCAT TATCAACAAAATACTCCAATTGGCGATGCCCTGTCTTTTACCAGACAACCATTAACCTGTCCG ACACAATCTGCCCTTTTGAAGATCCCAACGAAAGCGTGACCACATGGTCTCTTGTAGTTT GTAACCTGCTGGGATTACACATGGCATGGATGAACTATACAAACCGCGGGGTCTCATCAT CATCATCATCATGGTTAA
0109	Mutant GD1G Expressed Mutant GD1G	PRT	Artificial Sequence	MSKGEELFTGVVPIVLVDGDNVNGHKFSVSGEGEDATYKGLTLKFICTTGKLPVPWPTLVTLTY GVQCFSRYPDHMKRHHFFKSPAMPEGVYQERTISFKDDGNKTRAEVKFEGDITLVNRIELKGIDFK EDGNILGHKLEYNNSHNVIYADKQGLSGRIMSGLRINSKDDAAGQAIAANRFTSNIKGLTQASR NANDGISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQRLLEEDRVSNQTQF NGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKSGLDGFNVNSPGSTANPLASIDSALSKVDVAV RSSLGAIGNRFDSAITNLGNTVTNLNSARSRIEDADYALVPPISGKNGIKANFKIRHNIEDGSVQLAD HYQQNTPIGDGPVLLPDNHYLSTQSALLKDPNEKRDHMLLEFVTAAGITHGMDELKPRGSHHH HHHG
0110	Mutant MF227C mutant 470CT template	DNA	Artificial Sequence	CTTGGCCTTGATGGTTCAATGTTAATCCCCGGGAATTTCCGGTGGTGGTGGGAATTACA TTAATCAATGAAGACCGTGCCGACGCCAAGAAAGTACCGCTAACCCACTGGCTTCAATTG
0111	Mutant MF227C mutant 470CT template	PRT	Artificial Sequence	LGLDGFNVNSPGISGGGGITLINEDAAAAAKKSTANPLASI
0112	Mutant MF227C Reverse Primer R2YY	DNA	Artificial Sequence	AGATCTCCGCGGAACCACTAAAGAGAGGACGCTTTTGCAGAACCTGGTTTGCATAGTCAGCAT CTTCGATACG
0113	Mutant MF227C DNA sequence of Mutant MF227C	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAATCCCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAATCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAACCCAGATGAAATCCAGGTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAAATTGATGTGAAAAGCCTTGGCCTTG ATGGGTCAATGTAAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATCTGCAT TGTCAAAAGTGGACGAGTTCTGTTCTCTCTGGGGCAATTCAAAACCGTTTGTATTGATCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAAAACCAAGTTCCGCAAAACGTCCTCTCTTACTGGTTCGCGGGGTCTCATCAT CATCATCATCATGGTTAAGTCGAC

0114	Mutant MF227C Mutant MF227C	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCGGCAGGCCAGCGGATTGCTAACC GCTTCACTTCTAATATCAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTCTTA TTGCGCAGACCACTGAAGGTGCGCTGAATGAAATCAACAACACCTGCAGCGTGTGCGTGAG TTGCTGTTTCAGGCCACTAACCGGACTAACTCTGATTCGATCTGAAATCTATCCAGGATGAA ATTGAGCAACGCTGGAAGAAATCGATCGGTTTCTAATCAGACTCAATTTAACGGTGTAA GTCCTGCTCAGGACAACCCAGATGAAATCCAGTTGGTGTGCTAACGATGGTGAACCATTA CATCGATCTGCAAAAAATTGATGTAAAAGCCCTGGCCCTGATGGGTTCAATGTAAATCCCC GGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGATTTGCAAAAGTGGACGCAGTTC GTTCTTCTGGGGCAATTCAAAACCGTTTGTATTCAGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAACCTCCGCGTAGCCGTATCGAAGATGCTGACTATGCAAAACAGGTTCCG CAAAACGTCCTCTCTTACTGGTCCGCGGGTCTCATCATCATCATCATCATCATCATCATCAT MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLIEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDV KSLGLDGFNVNPSGSTANPLASIDSALS/KDAVRSSLGAIQNRFDASITNLGNTVTNLSARSRIED ADYANQVPQNVL SLLVPRGSHHHHHHG
0115	Mutant MF227C Mutant MF227C	PRT	Artificial Sequence	AGATCTCCCGGGAACCATCGTTAGCACCAACCTGGATTTC
0116	Mutant MF227N Reverse Primer RMF227N	DNA	Artificial Sequence	TAATACGACTCACATAGGGGAATTGTAGCGGATAACAAATCCCCCTCTAGAATAATTTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCCG TAACGCTAACGACGCGATTTCATTGGCAGACCCACTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCCTGTCTCAGGACAACCCAGATGAAATCCAGGTTGGTG CTAACGATGGTCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGCATTGTCAA AAGTGGACGCGAGTTCTTCTCTCTGGGGCAATTCAAAACCGCTTGTGATTCAGCCATTACCA ACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGATCGAAGATGCTGACTAT GCAACGGAAAGTTTCTAATATGCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCCGTTCTG GCGCAGGCTAACCGAGTTCGCAAAACGTCCTCTCTTACTGGTCCCGGGGTTCTCATCA TCATCATCATCATGTTAAGTCGAC
0117	Mutant MF227N DNA sequence of mutant MF227N	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCGGCAGGCCAGCGGATTGCTAACC GCTTCACTTCTAATATCAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTCTTA TTGCGCAGACCACTGAAGGTGCGCTGAATGAAATCAACAACACCTGCAGCGTGTGCGTGAG
0118	Mutant MF227N mutant MF227N	DNA	Artificial Sequence	

0119	Mutant MF227N mutant MF227N	PRT	Artificial Sequence	TTGTCGTTCAGGCCACTAACGGGACTAACTCTGATTCGGATCTGAAATCTATCCAGGATGAA ATTCAGCAACGCTCGAAGAAATCGATCGCGTTCTAATCAGACTCAATTTAACGGTGTAA GTCTGTCTCAGGACAACCCAGATGAAATCCAGGTTGGTGCTAACGATGGTCCCGGGGAA TACCGTAACCCACTGGCTTCAATTGATTCGATTGTCAAAGTGGACGCGAGTTCGTTCTTC TCTGGGGCAATTCAAAACCGCTTTGATTCAGCCATTACCAACCTTGGCAATACGGTAACCAA TCTGAACTCCGCGGTAGCGGTATCGAAGATGCTGACTATGCAACGGGAAGTTTCTAATATGTC TAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCGTTCTGGCGCAGGCTAACCCAGGTTCCGC AAAACGTCCTCTCTTACTGGTTCGCGGGGTTCTCATCATCATCATCATCATGTTAA MSGLRINSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNNLQRVRELSVQ ATNGTNSDSLKSIQDEIQRLEEIDRVSNQTFNGVKVLSQDNQMKIQVANGDSPGSTANPLA SIDSALSKVDVRRSSLGAIQNRFDSAITNLGNVTNLSARSRIEDADYATEVSNMSKAQILQQAGT SVLAQANQPQNVLSSLVPRGSHHHHHHG AGATCTCCGCGGAACCCAGCAGGTTATCTGGGTCAACACGGCAGCGCTGTTGTATTAATGA CTTGTCATAGTCAGCATCTTCGATACG TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCGCG TAACGCTAACGACGGCATTTCTATTGCGCAGACCCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTTTCAGGCCACTAACGGGACTAACTCTGATTCGCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGCTCAGGACAACCCAGATGAAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTGATGTGAAAAAGCCTTGCGCTTG ATGGGTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGAT TGTCAAAAGTGGACCGAGTTCGTTCTCTCTGGGGCAATTCAAAACCCGCTTGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCGCGCGTAGCCGTATCGAAGATGCT GACTATGCACAAGTCATTAACAACAGCCCTGTCGCTGTGACCCAGAAATAACCTGCTGGTT CCGCGGGGTCTCATCATCATCATCATCATGTTAAGTCGAC ATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATGCGGCAGGCCAGCGGATTGCTAACCC GCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTCCCGTAACGCTAACGACGGCATTTCTA TTGGCGAGACCCACTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGTGCGGTGAG TTGTCGTTCAGGCCACTAACGGGACTAACTCTGATTCGGATCTGAAATCTATCCAGGATGAA ATTACGCAACGCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAA GTCCTGTCTCAGGACAACCCAGATGAAAATCCAGGTTGGTGCTAACGATGGTGAACCCATTAC CATCGATCTGCAAAAAAATTGATGTGAAAAAGCCTTGCGCTTGATGGGTCAATGTTAATTCGCC
0120	Mutant MF233 Reverse primer RMF233	DNA	Artificial Sequence	AGATCTCCGCGGAACCCAGCAGGTTATCTGGGTCAACACGGCAGCGCTGTTGTATTAATGA CTTGTCATAGTCAGCATCTTCGATACG
0121	Mutant MF233 DNA Sequence of construct MF233	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCGCG TAACGCTAACGACGGCATTTCTATTGCGCAGACCCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTTTCAGGCCACTAACGGGACTAACTCTGATTCGCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGCTCAGGACAACCCAGATGAAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTGATGTGAAAAAGCCTTGCGCTTG ATGGGTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGAT TGTCAAAAGTGGACCGAGTTCGTTCTCTCTGGGGCAATTCAAAACCCGCTTGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCGCGCGTAGCCGTATCGAAGATGCT GACTATGCACAAGTCATTAACAACAGCCCTGTCGCTGTGACCCAGAAATAACCTGCTGGTT CCGCGGGGTCTCATCATCATCATCATCATGTTAAGTCGAC
0122	Mutant MF233 MF233	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATGCGGCAGGCCAGCGGATTGCTAACCC GCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTCCCGTAACGCTAACGACGGCATTTCTA TTGGCGAGACCCACTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGTGCGGTGAG TTGTCGTTCAGGCCACTAACGGGACTAACTCTGATTCGGATCTGAAATCTATCCAGGATGAA ATTACGCAACGCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAA GTCCTGTCTCAGGACAACCCAGATGAAAATCCAGGTTGGTGCTAACGATGGTGAACCCATTAC CATCGATCTGCAAAAAAATTGATGTGAAAAAGCCTTGCGCTTGATGGGTCAATGTTAATTCGCC

0123	Mutant MF233 MF233	PRT	Artificial Sequence	GGGAAGTACCGCTAACCACCTGGCTTCAATTGATTCTGCATTGTCAAAAGTGGACGCAGTTC GTTCTTCTGGGGCAATTCAAAACCGCTTTGATTACGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCACAAGTCATTAATA CAACAGCCTGTGCTGTTGACCCAGAATAACCTGCTGGTCCGCGGGTCTCATCATCAT CATCATCATGGTTAA
0124	Mutant MF471 Forward primer F471-77	DNA	Artificial Sequence	MSGLRNSAKDDAAGQAIA NRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRL EIEDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDVVRSSLGAIQNRFD SAINLGNVTVNLNSARSRIED ADYAQVINTNSLSLLTQNNLLVPRGSHHHHHHG
0125	Mutant MF471 Reverse Primer R471-77	DNA	Artificial Sequence	GCTGACTATGCAACGGCAGTTTCTGCTATGCTGCAGCGCAGATTCTGC GCAGAACTGCGCTGCAGACATAGCAGAAACTGCCGTTGCATAGTCAGC
0126	Mutant MF471 DNA Sequence of construct MF471	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGGCCAGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCCG TAACGCTAACGACGCGCATTTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTCTCAGGACAACCATGATAATGATGTGAAAAGCCTTGGCCTTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTG ATGGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAAGTGGACGCGAGTTCGTTCTTCTCTGGGGCAATTCAAAACCGCTTTGATTACGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGCAGTTTCTGCTATGCTGCAGCGCAGATTCTGCAGCAGGCTGGTCTGGT TCCGCGGGTCTCATCATCATCATCATCATGTTAAGTCGAC
0127	Mutant MF471 MF471	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATGCGGACGGCCAGGCGATTGCTAACC GCTTCACCTCTAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGTAACGACGGCATTCTA TTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGCCTGAG TTGTCTGTTACGGCCACTAACGGGACTAACTCTGATCCGATCTGAAATCTATCCAGGATGAA ATTACAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAA GTCCTGTCTCAGGACAACCCAGATGAAATCCAGGTGGTGTGCTAACGATGGTGAACCATTAAC CATCGATCTGCAAAAAAATTGATGTGAAAAGCCTTGGCCTTGATGGGTTCAATGTAAATCCCCC GGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCATTGTCAAAAGTGGACGCAGTTC

				GTTCTTCTGGGGCAATTCAAAACCGCTTTGATTACGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAACCTCGCGCTAGCCGTATCGAAGATGCTGACTATGCAACGGCAGTTTCT GCTATGCTGCAGCGCAGATTCTGCAGCAGGCTGGTCTGGTCCGCGGGTCTCATCATCA TCATCATCATGGTTAA
0128	Mutant MF471 MF471	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTQFNGVKVLSQDNQMKI/VGANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDVRRSLGAIQNRFDSAITNLGNTVTVNLSARSRIED ADYATAVSAMSAQAQILQQAGLVPRGSHHHHHG
0129	Mutant MF479 Forward primer F479-83	DNA	Artificial Sequence	GTTTCTAATATGCTAAAGCGGCGATTCTGGAGCGGCTGGTCTGGTCCGCGG
0130	Mutant MF479 Reverse Primer R479-83	DNA	Artificial Sequence	CCGCGGAACCCAGACCAGCCGCTCCCAGAAATCGCCGCTTTAGACATATTAGAAAC
0131	Mutant MF479 DNA Sequence of construct MF479	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCCG TAACGCTAACGACGCGCATTTCTATTGGCAGACCCACTGAAGTGGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTCAAGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAAACAGATGAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCGATCTGCAAAAAATGATGTGAAAAGCCTTGGCCTTG ATGGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGCA TGTCAAAAGTGGACGCGAGTTCTTCTCTCTGGGGCAATTCAAAACCGCTTGTGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGGAAGTTTCTAATATGCTTAAAGCGGCGATTCTGGAGCGGCTGGTCTGGT TCCGCGGGGTTCTCATCATCATCATCATGTTAAGTCGAC
0132	Mutant MF479 Mutant MF479	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATCGGCGAGGCCAGGCGATTGCTAACC GCTTCACCTCTAATATCAAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTCTA TTGCGCAGACCACTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGCCTGAG TTGCTGTTTCAAGCCACTAACGGGACTAACTCTGATTCGGATCTGAAATCTATCCAGGATGAA ATTACAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAA GTCCTGTCTCAGGACAAACAGATGAAATCCAGGTGGTGGTACGATGGTGAACCATTAAC CATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTGATGGGTTCAATGTTAATCCCC GGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGATTCGAAAGTGGACGCGAGTTC GTTCTTCTCTGGGGCAATTCAAAAACCGCTTTGATTCAGCCATTACCAACCTTGGCAATACGG

					TAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTA ATATGTTCTAAAGCGCGGATTCTGGGAGCGGCTGGTCTGGTCCGCGGGTTCTCATCATCAT CATCATCATGGTTAA
0133	Mutant MF479 Mutant MF479	PRT	Artificial Sequence		MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTQFNQVKVLSQDNQMKIQVANGDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSVDVVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIED ADYATEVSNMSKAAILGAAGLVPRGSHHHHHHG TCTAGAGGATCCGGCAGGCCAGGCGC
0134	Mutant N45 Forward primer N45_F	DNA	Artificial Sequence		
0135	Mutant N45 Reverse R502D0	DNA	Artificial Sequence		CGCAAGCTTGTCGACTTAAGCG
0136	Mutant N45 DNA sequence of Mutant N45	DNA	Artificial Sequence		TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGATAAATTTGTTT AACTTTAAGAAGGAGATATACATATCGGGGTTCTCATCATCATCATCATGATGGCTA GCATGACTGGTGACAGCAAAATGGTCGGGATCTGTACGACCTGGTTCGCGCGGTAGCGC GAAGGATCCGCGCAGCCAGCGATTGCTAACCGTTTCACTTCTAATATCAAAGTCTGACTC AGGCTTCCCGTAACGCTAACGACGGCATTCTATTGCGCAGACCACCTGAAGTGCGCTGAAT GAAATCAACAACAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAAGCCACTAACGGGACTAA CTCTGATTCGGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATCG CGTTTCTAATCAGACTCAATTTAACGGTGTTAAAGTCTGTCTCAGGACAACCCAGATGAAATC CAGTTGGTGTAAACGATGGTGAACCATACCATCGATCTGCAAAAAATTTGATGTGAAAAGC CTTGGCCTTGATGGGTTCATGTTAATTCCTCCGGGAATTCCTGGTGGTGGTGAATTTCTA GACTCCATGGGTACATTAATCAATGAAGACGCTGCCGAGCCCAAGAAAAGTACCGCTAACCC ACTGGCTTCAATTGATTCTGCATTGTCAAAAAGTGACGCAGTTCTTCTCTGGGGCAAT TCAAAACCGTTTTGATTCAGCCATTACCAACCTTGCGCAATACGGTAACCAATCTGAACCTCCGC GCGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTAATATGCTCTAAAGCGCAGAT TCTGCAGCAGGCTGGTACTTCCTGCTGCGCAGGCTAACCCAGTTCCGCAAAAACGTCCTCT CTTTACTGCGTTAAGTCGACAAGCTTGCGG
0137	Mutant N45 Mutant N45	PRT	Artificial Sequence		MRGSHHHHHHGMASMTGQQQMGRLDYLVRPSAKDPAGQAIANRFTSNIKGLTQASRNAND GISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTQFNQVKV LSQDNQMKIQVANGDGETITIDLQKIDVKSLGLDGFNVNSPGISGGGGILDMSGTLINEDAAAAK KSTANPLASIDSALSVDVVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIEDADYATEVSNMSKA QILQQAGTSVLAQANQVPQNVLSLLR
0138	Mutant NGD1 DNA Sequence of NGD1	DNA	Artificial Sequence		TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGATAAATTTGTTT AACTTTAAGAAGGAGATAACATATGAGTAAAGGAGAGAAGCACTTTTCACTGGAGTTGTCCCAA

	construct			TTCTTGTTGATGGTGTATGTTAATGGGCACAAATTTCTGTCACTGGAGAGGGTGAAG GTGATGCAACATACGAAACCTTACCCTTAAATTTATTTGCACACTACGAAACCTACCTGTTCC ATGGCCAAACACTTGTCACTACTCTGACGTATGGTGTCAATGCTTTTCCCGTTATCCGGATCA CATGAAACGGCATGACTTTTCAAGAGTGCCTGCCATGCCGAAAGTTATGACAGGAACGCACATAT ATCTTTCAAAGATGACGGAACTACAAGACGCGTCTGAAAGTCAAGTTGAAGGTGATACCCCT TGTTAATCGTATCGAGTTAAAGGTATGATTTTAAAGAAGATGAAACATTTCTCGGACACAAA CTCGAGTACAACATAACTCACACAATGTATACATCAGGCGACACAACAAGCCCTATCAGGC CGCATTATGAGCGGTTACGGATCAACAGCGCAAGACGATGCGGACGCCAGCGGATTG CTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGCA TTTCTATTGCGCAGACCACTGAAGTGGCTGAATGAAATCAACAACCTGCGAGCGTGTG CGTGAGTTGCTGTTCAAGCCACTAACGGGACTAACTCTGATTCCGATCTGAAATCTATCCAG GATGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACCGGT GTTAAAGTCTGCTCAGGACAACCCAGATGAAATCCAGGTGGTGCTAACGATGGTGAAC CATTACCATCGATCTGCAAAAAATGATGTGAAAGCCCTGGCCCTGATGGGTCAATGTTAAT TCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCATTGTCAAAAAGTGGACGC AGTTCGTTCTCTGCGGGCAATTCAAAACCGCTTGTGATTCAGCCATTACCAACCTTGGCAA TACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCACTGGTTC CGCGGGTCTCATCATCATCATCATGTTAAGTCGAC
0139	Mutant NGD1 Expressed Mutant SY3- GFP	DNA	Artificial Sequence	ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGCCAAATCTTGTGAATTAGATGGTGAT GTTAATGGGCACAAATTTCTGTCACTGGAGGGTGAAGGTGATGCAACATACGGAACACTT ACCTTAAATTTATTTGCACACTAGGAAACCTACCTGTCCATGGCCAAACACTTGTCACTACTC TGACGTATGGTGTCAATGCTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTCAA GAGTGCCATGCCCGAAGTTATGTACAGGAACGCATATCTTTCAAAGATGACGGGAACT ACAAGACCGTGTCTGAAGTCAAGTTGAAGGTGATACCTTGTAAATCGTATCGAGTTAAAG GTATTGATTTTAAAGAAGATGGAACATTTCTCGACACAACTCGAGTACAACATAACTCACA CAATGTATACATCAGGCAGACAAACAAGCCCTATCAGGCCGCAATTATGAGCGGGTTACGGA TCAACAGCGCGAAAGACGATGCGGCAGGCCAGCGGATTGCTAACCGCTTCACTTCTAATATC AAAGGCTGACTCAGGCTTCCCGTAAACGCTAACGACGGCATTTCTATTGCGCAGACCACTGA AGGTGCGCTGAATGAAATCAACAACACCTGACGCGTGTGCGTGAGTTGCTGTTCAGGCCA CTAACGGGACTAACTCTGATTCCGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGG AAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACA ACCAGATGAAATCCAGGTGGTGTACGATGGTGAACCATTACCATCGATCTGCAAAAAA TTGATGTGAAAGCCCTTGGCCTTGATGGGTCAATGTTAATTCGCCGGGAAGTACCGCTAACCC CACTGGCTTCAATTGATTCTGCATTGTCAAAAGTGACGCGAGTTCGTTCTTCTCTGGGGCAA

0140	Mutant NGD1 Expressed Mutant SY3- GFP/Mutant NGD1	PRT	Artificial Sequence	TTCAAAACCGCTTTGATTAGCCATTACCAACCTTGGCAATACGGTAACCAATCTGAACTCCG CGGTAGCCGTATCGAAGATGCTGACTATGCACTGGTTCGCGGGGTTCTCATCATCATCAT CATCATGGTTAA
0141	Mutant S33 Forward F502_S33	DNA	Artificial Sequence	MSKGEELFTGVWPILVELDGVNGHKFSVGEGEDATYGKLTCLKFICTTGKLPVPWPVLVTLLTY GVQCFSRYPDHMKRHHDFKFSAMPEGYVQERTISFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK EDGNILGHKLEYNYNSHNVYITADKQGLSGRIMSGLRINSKDDAAGQAANRFTSNIKGLTQASR NANDGISIAQTTEGALNEINNLRVREL SVQATNGTNSDSLKSIQDEIQRLLEEDRVSNQTQF NGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKSGLDGFNVNSPGSTANPLASIDSALSKVDAV RSSLGAIQNRFDSEITNLGNTVNLNSARSRIEDADYALVPRGSHHHHHHG TCTAGAGGATCCGTCTGGTCTGCGTATCAACAGCGC
0142	Mutant S33 DNA sequence of Mutant S33	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAATCCCTCTAGATAAATTTTGTTT AACTTTAAGAAGGAGATATACATATGCGGGGTTCTCATCATCATCATCATCATGGTATGGCTA GCATGACTGGTGACAGCAAAATGGTGGGATCTGTACGACCTGGTTCGCGCGGTAGCGC GAAGGATCCGTCTGGTCTGCGTATCAACAGCGCGAAGACGATGCGGAGCCAGCGCGATT GCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCGTAACGCTAACGACGGC ATTTCTATTGCGCAGACCCTGAAGTGGCTGAATGAAATCAACAACCTGCAGCGTGT GCGTGAGTTGTCTGTTACAGGCCACTAACGGGACTAACTCTGATCCGATCTGAAATCTATCCA GGATGAAATTCAGCAACGCTGGAAGAAATCGATCGGTTTCTAATCAGACTCAATTTAAACGG TGTTAAAGTCTGCTCAGGACAACAGATGAAATCCAGGTGGTGTCTAACCGATGGTGAAC CATTACCATCGATCTGCAAAAATTTGATGTAAAAGCCTTGGCCTTGATGGGTCAATGTAAAT TCCCCGGGAATTTCCGGTGGTGGTGGGAAATCTAGACTCCATGGGTACATTAATCAATGAA GACGCTGCCGACGCAAGAAAGTACCGCTAACCCACTGGCTTCAATGATTCTGCATTGTC AAAAGTGGACGCAAGTTCTTCTCTGCGGCAATCTGAAACCGTTTGTGATTACGCCATTAC CAACCTTGGCAATACGTTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACT ATGCAACGGAAGTTTCTAATATGCTAAAAGCGCAGATTCTGCAGCAGCGTGGTACTTCCGTTT TGGCGCAGGCTAACCGGTTCCGCAAAACGTCCTCTCTTACTGCGTTAAGTCGAC
0143	Mutant S33 Mutant S33	DNA	Artificial Sequence	ATCGGGGTTCTCATCATCATCATCATGGTATGGTATGGTATGGTATGGTATGGTATGGTATG GGTCGGGATCTGTACGACCTGGTTCGCGCGGTAGCGGAAGGATCCGTCTGGTCTGCGGTA TCAACAGCGCAAGACGATGCGGAGCCAGCGGATTTGCTAACCGCTTCACTTCTAATATC AAAGGTCTGACTCAGGCTTCCGTAACGCTAACGACGGCATTTCTATTGCGCAGACCACTGA AGGTGCGCTGAATGAAATCAACAACCACTGCAGCGTGTGCGTGAGTTGTCTGTTCAAGGCCA CTAACGGGACTAACTCTGATTCCGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGG AAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGGTGTTAAAGTCTCTGCTCAGGACA

				ACCAGATGAAAAATCCAGGTGGTGCTAACGATGGTGAAACCATTACCATCGATCTGCAAAAAA TTGATGTGAAAAGCCTTGGCCTTGATGGTTCAATGTTAATCCCCGGGAATTTCCGGTGGTG GTGGTGAATTTAGACTCCATGGGTACATTAATCAATGAAGACGCTGCCGACGCCAAGAAA AGTACCGCTAACCCACCTGGCTTCAATTGATCTGCTGCTGCAAAAGTGGACGCGAGTTCTGTTCT TCTCTGGGGCAATTCAAAAACCGTTTGGTTCAGCCATTACCAACCTTGGCAATACGGTAACC AATCTGAATCCGCGGTAGCCGTATCGAAGATGCTGACTACTGAACGGAAGTTTCTAATATG TCTAAAGCGCAGATTTGCAGCAGGCTGGTACTCCGTTCTGGCGCAGGCTAACCCAGGTTCC GCAAAACGTCCTCTCTTACTGCGTTAA
0144	Mutant S33 Mutant S33	PRT	Artificial Sequence	MRGSHHHHHGMSMTGGQQMGRDLYDLVPRGSAKPSGLRINSKDDAAGQAANRFTSNIK GLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQRLIEIDRV SNQTQFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDVKSLGLDGFVNNSPGISGGGGILDSM GTLINEDAAAAKKSTANPLASIDSALSVDVAVRSSLGAIQNRFDSAITNLGNTVNLNSARSRIEDA DYATEVSNMSKAQILQQAGTSVLQANQV/PQNVLSSLR
0145	Mutant SY3CT Reverse primer RSY3CT	DNA	Artificial Sequence	AGATCTCCGCGGAACCCAGTGCAATAGTCAGCATCTTCGATACGGC
0146	Mutant SY3CT DNA Sequence of SY3CT construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGGCCAGGCGATTGTAACCGCTTCACTTCTAATATCAAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGCTCAGGACAAACAGATGAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAAATTGATGTGAAAAGCCTTGGCCTTG ATGGGTTCAATGTTAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAAGTGGACGCGAGTTCTTCTCTGGGGCAATTCAAAACCGCTTTCGATTTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGTATCGAAGATGCT GACTATGCATGTTCCGCGGGTTCTCATCATCATCATCATGTTAAGTCGAC
0147	Mutant SY3CT Expressed Mutant SY3CT	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGGAAAGACGATGCGGACAGCCAGGCGATTGCTAACC GCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTA TTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGCAGTGAG TTGCTCTGTTCAAGCCACTAACGGGACTAACTCTGATTCGATCTGAAATCTATCCAGGATGAA ATTACAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATCAGACTCAATTTAACGGTGTAA GTCCTGTCTCAGGACAACCCAGATGAAATCCAGGTTGGTGTGCTAACGATGGTGAACCATTA CATCGATCTGCAAAAAAATTGATGTGAAAAGCCTTGGCCTTGAATGGTTCAATGTTAATCCCC

				GGGAAGTACCGCTAACCACCTGGCTTCAATTGATTCTGCATTGTCAAAAGTGGACGCGATTCTGTTCTCTGGGGCAATTCAAAACCGCTTTGATTACGCCATTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGTAGCCGTATCGAAGATGCTGACTATGCACTGGTTCGCGGGGGTTCTCATCATCATCATCATCATGTTAA
0148	Mutant SY3CT Expressed Mutant SY3CT	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIV/GANDGETITIDLQKIDVKSLGLDGFNVNSPGSTANPLASIDSALS/KVDAVRSSLGAIQNRFDSAITNLGNTVTVNLNSARSRIEDADYALVPRGSHHHHHHG
0149	Mutant 33MX Mutant 33MX	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATCGGCGAGCGCGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGGCTGACTCAGGCTTCCCGTAACGCTGCAGACGGCATTTCTATTGCGCAGACCACTGAAGGTGCGCTGAATGAATCAACAACAACCTGCAGCGTGTGCGGTGAGTTGCTGTTACGGCCACTGCCGGGCTAACGCTGATGCCGCTCTGAAAGCTATCCAGGCTGAATTCAGCAACGCTTGGAAAGAAATCGATCGGCTTCTCAGCAGACTCAAGCTGCCGCTGTAAAGTCTGCTCAGGACAACGCAATGGCAATCCAGGTGGTGTGCTAACGATGGTGCCGCTATTAATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGGCCTTGATGGGTCAATGTTAAATTTCCC CGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCATTGTCAAAAAGTGGACGCGAGTTCTCTCTCTGGGGCAATTCAAAAACCGCTTTGATTACGCCATTACCAACCTTGGCAATACGCTAATCAATCTGAACCTCCGCGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTCAAATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTCCGTTCTGGCGCAGGCTAACCCAG GTCCGCAAAACGCTCCTCTCTTACTGGTTCCGCGGGGTTCTCATCATCATCATCATCATGTTTAA
0150	Mutant 33MX Mutant 33MX	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNAADGISIAQTTEGALNEINNLRVRELSVQATAGANADAALKAIQAEIQRLLEEIDRV/SQQTQAAAVKVLSDNAMAIVQ/GANDGAATITDLQKIDVKSLGLDGFNVNSPGSTANPLASIDSALS/KVDAVRSSLGAIQNRFDSAITNLGNTVTVNLNSARSRIEDADYATEVSQMSKAQILQQAGTSVLAQANQV/PQNVL SLLVPRGSHHHHHHG
0151	Mutant 33MX DNA sequence of 33MX	DNA	Artificial Sequence	TAATACGACTCACATATAGGGGAATTGTGAGCGGATAACAAATCCCTCTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACATATAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCGG CAGGCCAGGCGATTGTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCGTAACGCTGCAGACGGCATTTCTATTGCGCAGACCACTGAAGGTGCGCTGAATGAATCAACAACAACCTGCAGCGTGTGCGTGAGTTGCTGTTACGCCCACTCCGGGGCTAACGCTGATGC CGCTCTGAAAGCTATCCAGGCTGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTCA GCAGACTCAAGCTGCCGCTGTTAAAGTCTCTCTCAGGACAACGCAATGGCAATCCAGGTTG GTGCTAACGATGTTGCCGCTATTACCATCGATCTGCAAAAAATTGATGTGAAAAAGCCTTGCGC TTGATGGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGCGCTTCAATTGATTCTG

0152	Mutant 485MX DNA Sequence of 485MX construct	DNA	Artificial Sequence	CATTGTCAAAGTGGACGCGAGTTCTTCTCTCTGGGGGCAATTCAAACCGCTTTGATTTCAG CCATTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGTAGCCGTATCGAAGAT GCTGACTATGCAACGGAAGTTTCTCAAATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTACT TCCGTTCTGGCGCAGGCTAACCCAGGTTCCGCAAAACGTCCTCTCTTTACTGGTTCGCGCGGG TTCTCATCATCATCATCATCATGGTTAAGTCGAC TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAGTCTGACTCAGGCTTCCCCG TAACGCTGCAGACGGCATTTCTATTGCGCAGACCACCTGAAGTGCCTGAATGAATCAACA ACAACCTGCAGCGTGTGCGTGAGTTGTCTGTTCAAGCCACTGCCGGCTAACGCTGATGC CGCTCTGAAAGCTATCCAGGCTGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTCA GCAGACTCAAGCTGCCGCTGTAAAGTCTCTCTCAGGACACGCAATGGCAATCCAGGTTG GTCTAACGATGTGCCGCTATTACCATCGATCTGCAAAAAATTGATGTGAAAAAGCCTTGCC TTGATGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTG CATTGTCAAAGTGGACGCGAGTTCCTTCTCTGCGGGGCAATCAAACCGCTTTGATTTCAG CCATTACCAACCTTGGCAATACGTAACCAATCTGAACCTCCGCGTAGCCGTATCGAAGAT GCTGACTATGCAACGGAAGTTTCTCAAATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTCT GGTCCGCGGGTCTCATCATCATCATCATCATGGTTAAGTCGAC
0153	Mutant 485MX 485MX construct	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATCGGCGAGCGCGGATTGCTAACCC GCTTCACTTCTAATATCAAAGTCTGACTCAGGCTTCCCGTAACGCTGCAGACGGCATTTCTA TTGCGCAGACCACTGAAGGTGCGCTGAATGAATCAACAACAACCTGCAGCGTGTGCGTGAG TTGCTGTTCAGGCCACTGCCGGGGCTAACGCTGATGCCGCTCTGAAAGCTATCCAGGCTGA AATTCAGCAACGCTGGAAAGAAATCGATCGCGTTTCTCAGCAGACTCAAGCTGCCGCTGTAA AGTCTGTCTCAGGACAAACGCAATGGCAATCCAGTTGGTGTGCTAACGATGGTGGCGCTATTA CCATCGATCTGCAAAAAATTGATGTGAAAAAGCCTTGGCCTTGATGGTTCAATGTTAATCC CGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCATTGTCAAAAAGTGGACGCGATT GTTCTTCTCTGGGGCAATTCAAAAACCGCTTGTGATTCAGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTC AAATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTCTGGTTCGCGGGGTTCTCATCATCAT CATCATCATGGTTAA
0154	Mutant 485MX 485MX construct	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIAINRFTSNIKGLTQASRNAADGISIAQTTEGALNEINNLRVRELSVQA TAGANADAALKAIQAEIQRLLEEIDRVSSQQTQAAVKVLSQDNAMAIQV/GANDGAATIDLQKIDVK SLGLDGFNVNSPGSTANPLASIDSALSK/DVVRSSLGAIGNRFDSAITNLGNTVTNLNSARSRIEDA DYATEVSQMSKAQLQQAGLVPRGSHHHHHHG

0155	Mutant MIM4 CBLB502 variant	DNA	Artificial Sequence	ATGCGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAAATG GGTCGGGATCTGTACGACGATGACGATAAGGATCCGATGGCACAAGTCATTAATACAAACAG CCTGTCGTGTTGACCCAGAAATAACCTGCAGAAATCTCAGTCTCTCAGTCTCAGTTCGGCTATTGA GCGTCTGCTCTGCTCGGTATCAACAGCGCGAAAGACGATCGGCAGGCCAGCGGAT GCTAACCGCTTCACTTCTAATATCAAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGC ATTCTATTGGCGAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGT GCGTGAGTTGCTGTTCAGGCCACTCAAGGGACTAACTCTGATTCGGATCTGAAATCTATCCCA GGATGAAATTCAGCAACGCTGGAAGAAATCGATCGGTTTCTCAGCAGACTCAATTTAACGG TGTTAAAGTCTCTCAGGACAACCAGATGAAAATCCAGGTGGTGCTAACGATGGTGAAC CATTACCATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGGCCTTGATGGGTCAATGTTAAT TCCCGGGAAATTCGGTGGTGGTGGAATTCAGACTCCATGGGTACATTAAATCAATGAA GACGCTGCCGAGCCAAGAAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCATTGTC AAAAGTGGACGCGAGTTCTTCTCTGCGGGCAATTCAAAACCGTTTTGATTACGCCATTAC CAACCTTGGCAATACGGTAACCAATCTGAACCTCGCGCGTAGCCGTATCGAAGATGCTGACT ATGCAACGGGAAGTTTCTCAATGTCTAAAGCGCAGATCTGCAGCAGGCTGGTACTTCCGTTCT TGGCGCAGGCTAACCGGTTCCGCAAAACGTCCTCTTTACTGCGTTAA
0156	Mutant MIM4 Primers design (for deletion aa Gln439; Asn440; Arg441; Phe442):	DNA	Artificial Sequence	AACCCACTGGCTTCAATTGATTCTGCATTGTCAAAAAGTGGACGAGTTCGTTCTTCTCTGGGG GCAATTCAAAACCGTTTTGATTACGCCATTACCGCCCTTGGCGCTACGGTAACCCGCTCTGGC CTCCGCGCGTAGCGCTATCGAAGATGCTGACTATGCAACGGAAGTTTCTCAAATG
0157	Mutant MIM4 Primers design (for deletion aa Gln439; Asn440; Arg441; Phe442):	PRT	Artificial Sequence	NPLASIDSALSKVDVRSSLGAIQNRFDSSAITALGATVTALASARSAIEDADYATEVSNM
0158	Mutant MIM4 Forward Primer MIM4	DNA	Artificial Sequence	GCAGTTCGTTCTTCTCTGGGGGCAATTGATTCAGCCATTACCGCCCTTGG
0159	Mutant MIM4 Reverse Primer MIM4	DNA	Artificial Sequence	CCAAGGGCGGTAATGGCTGAATCAATTGCCCCCCAGAGAAGAACGAACTGC
0160	Mutant MIM4 MIM4	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCGCCCTCTAGAAAATAATTTTGT TAACCTTTAAGAAGGAGATATACATATGCGGGGTCTCATCATCATCATCATATGGTATGGCTA GCATGACTGGTGACAGCAAAATGGGTGCGGATCTGTACGACGATGACGATAAGGATCCGAT GGCACAAGTCAATTAAACAAACAGCCTGTCGCTGTTGACCCAGAAATAACCTGAACAAAATCTCA

				GTCTCACTGAGTCCGCTATTGAGCGTCTGTCTCTGGTCTGGTATCAACAGCGCGAAAG ACGATCGCGCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGCTGACTCAG GCTCCCGTAACGCTAACGACGGCATTCTATTGCGCAGACCACTGAAGGTGCGCTGAATGA AATCAACAACAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAAGCCACTAACGGGACTAACT CTGATCCGATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCGAAGAAATCGATCGCG TTTCTAATCAGACTCAATTTAACGGGTAAAGTCTCTCAGGACAACCCAGATGAAAATCCA GGTGGTGTAAAGATGGTGAACCATTAACATCGATCTGCAAAAAATTGATGAAAAAGCCT TGGCCTTGATGGGTCAATGTTAAATCCCGGGAAATTCGGGTGGTGGTGGAAATTTCTAG ACTCCATGGGTACATTAATCAATGAAGACGCTCCGCGAGCCAAAGAAAGTACCGCTAACCCCA CTGGCTTCAATTGATTCGATTGTCAAAAGTGACGCGATTCTTCTCTGGGGCAATT GATTCAGCCATTACCGCCCTTGGCGCTACGGTAACCGCTCTGGCCTCCGCGGTAGCCGTAT CGAAGATGCTGACTATGCAACGGAAGTTTCTAATATGCTAAAGCGCAGATTCTGCAGCAGGC TGGTACTCCGTTCTGGCGCAGGCTAACCCAGGTTCCGCAAAACGTCCTCTCTTTACTGCGTTA A
0161	Mutant MIM4 MIM4	PRT	Artificial Sequence	MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPMAQVINTNSLLTQNNLNKSSLSAIE RLSSGLRINSKDDAAGQAANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSV QATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITDLQKI DVKSLGLDGFNVNSPGISGGGGILDSMGLINEDAAAAKKSTANPLASIDSALS KVDVRRSLGAI DSAITALGATVTALASAASRIEDADYATEVSNM SKAQILQQAGTSVL AQANVPQNVL SLLR
0162	Mutant MIM5 Primers design (mutations Gln439Ala; Asn440Lys; Arg441Ala):	DNA	Artificial Sequence	AACCCACTGGCTTCAATTGATTCGATTGTCAAAAGTGGACGCGATTGCTTCTCTCTGCGG GCAATTGCAAAGGCTTTTGATTCAGCCATTACCGCCCTTGGCGCTACGGTAACCGCTCTGGC CTCCGCGGTAGCGCTATCGAAGATGCTGACTATGCAACGGAAGTTTCTCTCAAAATG
0163	Mutant MIM5 Primers design (mutations Gln439Ala; Asn440Lys; Arg441Ala):	PRT	Artificial Sequence	NPLASIDSALS KVDVRRSLGAIKAFDSAITALGATVTALASARSAIEDADYATEVSNM
0164	Mutant MIM5 Forward Primer MIM5	DNA	Artificial Sequence	CGTCTCTCTCTGGGGCAATTGCAAAGGCTTTTGATTACGCCATTACCGC
0165	Mutant MIM5 Reverse Primer MIM5	DNA	Artificial Sequence	GCGGTAATGCGTGAATCAAAAGCCTTTGCAATTGCCCCCGCAGAGAAGAACG
0166	Mutant MIM5	DNA	Artificial	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCCTCTAGAAAATAATTTTGT

	MIM5	Sequence
0167	Mutant MIM5 MIM5	PRT
0168	Mutant MIMX Mutant 33MIMX	DNA

[illegible]

				ATGGGTTCAATGTTAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGTCAT TGTCAAAAGTGGACGAGTTCGTTCTTCTCTGGGGCAATTCAAGCTCGTTTTCGCGGGCC ATTGCTAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAGTTTCTAATATGCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCGGTTCCGCAAAACGTCCTCTCTTTACTGGTTCGCGGGGTTT TCATCATCATCATCATCATGTTAAGTCGAC
0173	Mutant MIXC MIXC	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIV/GANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKV/DAVRSSLGAIQARFAAIAIANLGNVTNLSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQV/PQNVLSSLVPRGSHHHHHG AGATCTCATATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATGC
0174	Mutant MIXN Forward primer FMIMxN	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCCG TAACGCTGCAGACGGCATTTCTATTGCGCAGACCACCTGAAGTGCGCTGAATGAAATCAACA ACAACCTGCAGCGTGTGCGTGAGTTGCTGTTTTCAGGCCACTAACGGGACTAACTCTGATTCC GATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAAT CAGACTCAAGCTAACGGTGTAAAGTCTCTCTCAGGACAACGCAATGAAATCCAGGTTGGT GCTAACGATGGTCCGCTATTACCATCGATCTGCAAAAATGATGTGAAAAGCCTTGGCCTT GATGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGCA TTGTCAAAAGTGGACGAGTTCGTTCTTCTCTGGGGGCAATTCAAAACCGCTTGTGATTCAGCC ATTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAGTTTCTAATATGCTTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCGGTTCCGCAAAACGTCCTCTCTTTACTGGTTCGCGGGGTTT TCATCATCATCATCATCATGTTAAGTCGAC
0176	Mutant MIXN Expressed Mutant MIX.N	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNAADGISIAQTTEGALNEINNLRVRELSVQA TNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIV/GANDGAATIDLQKIDVK SLGLDGFNVNSPGSTANPLASIDSALSKV/DAVRSSLGAIQNRFDSAITNLGNVTNLSARSRIEDA DYATEVSNMSKAQILQQAGTSVLAQANQV/PQNVLSSLVPRGSHHHHHG
0177	Mutants MIM1; MIM2 and MIM3 502 Mutants MIM1; MIM2 and MIM3	DNA	Artificial Sequence	AACCCACTGGCTTCAATTGATTCGCAATGTCAAAAAGTGGACGAGTTCGTTCTCTCTGGGG GCAATTCAAAACCGTTTTGATTCAGCCATTACCAACCTTGGCAATACGGTAACCAATCTGAACCT CCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTCTCAAAATGCTCAAAGCG CAGATTCTGCAGCAGGCTGGTACTTCCGTTCTGGCGCAGGCTAACCGAGGTTCCGCAAAACGCT

	C-terminal part of CBLB502			CCTCTCTTTACTGCGTTAA
0178	Mutants MIM1; MIM2 and MIM3 Primers design (mutant MIM1)	DNA	Artificial Sequence	ATTACCAACCTTGGCAATACGGTAACCAATCTGAACTCCGCGGTAGCCGTATCGAAGAT
0179	Mutants MIM1; MIM2 and MIM3 Primers design (mutant MIM1)	PRT	Artificial Sequence	ITNLGNTVTLNSARSRIED
0180	Mutants MIM1; MIM2 and MIM3 Forward Primer 455-57	DNA	Artificial Sequence	CCTTGGCAATACGGTAACCGCTCTGGCCTCCGCGGTAGCCGTATC
0181	Mutants MIM1; MIM2 and MIM3 Reverse Primer 455-57	DNA	Artificial Sequence	GATACGGCTACGCGCGGAGGCCAGAGCGGTACCGTATTGCCAAGG
0182	Mutants MIM1; MIM2 and MIM3 Primers design (mutant MIM2_MIM1 plus R460A)	DNA	Artificial Sequence	ACGGTAACCGCTCTGGCCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACGGAA
0183	Mutants MIM1; MIM2 and MIM3 Primers design (mutant MIM2_MIM1 plus R460A)	PRT	Artificial Sequence	TVTALASARSRIEDADYATE
0184	Mutants MIM1; MIM2 and MIM3 Forward Primer 460	DNA	Artificial Sequence	GCTCTGGCCTCCGCGGTAGCCGTATCGAAGATG
0185	Mutants MIM1; MIM2 and MIM3 Reverse Primer 460	DNA	Artificial Sequence	CATCTTCGATACGGCTAGCCGCGGAGGCCAGAGC
0186	Mutants MIM1; MIM2	DNA	Artificial	CAAAACCGTTTGTATTAGCCATTACCAACCTTGGCAATACGGTAACCGCTCTGGCCTCC

	and MIM3 Primers design (mutant MIM3_MIM2 plus N448A; N451A)		Sequence	
0187	Mutants MIM1; MIM2 and MIM3 Primers design (mutant MIM3_MIM2 plus N448A; N451A)	PRT	Artificial Sequence	QNRFDSAITNLGNTVTALAS
0188	Mutants MIM1; MIM2 and MIM3 Forward Primer 448-51	DNA	Artificial Sequence	GTTTTGATTACGCCATTACCGCCCTTGGCGCTACGGTAACCGCTCTGG
0189	Mutants MIM1; MIM2 and MIM3 Reverse Primer 448-51	DNA	Artificial Sequence	CCAGAGCGGTACCGTAGCGCCCAAGGGCGGTAATGGCTGAATCAAAAC
0190	Mutant ME42 Forward Primer ME42	DNA	Artificial Sequence	CAACAGCGGAAAGCCGATGCGGAGGCCAGGCGATTGC
0191	Mutant ME42 Reverse Primer ME42	DNA	Artificial Sequence	GCAATCGCCTGGCCTCCCGCATCGGCTTTTCGCGCTGTTG
0192	Mutant ME42 Sequence of ME42 construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCCCTCTAGAAATTTTGT AACTTTAAGAAGGAGATATACATATAGCGGGTTACGGATCAACAGCGCGAAAGCCGATGCG GGAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGGCAGACCACCTGAAGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTACGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAACCCAGATGAAAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTG ATGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAAGTGGACGCGATTCTTCTCTCTGGGGCAATTCAAAACCGCTTTGATTTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAAAGTTTCTAATATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCGGTTCCGCAAAACGTCCTCTCTTTACTGTTCCGCGGGGTTT TCATCATCATCATCATCATGTTAAGTCGAC

0193	Mutant ME42 Mutant ME42	PRT	Artificial Sequence	MSGLRINSADAGGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDVAVRSSLGAIQNRFDISAITNLGNTVTNLNSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVLSSLVPRGSHHHHHHG
0194	Mutant ME110 Forward Primer ME100	DNA	Artificial Sequence	GTCTGTTCAGGCCACTGCCGGGGCTAACTCTGATTCCGATCTG
0195	Mutant ME110 Reverse Primer ME100	DNA	Artificial Sequence	CAGATCGGAATCAGAGTTAGCCCCCGGCAGTGGCCTGAACAGAC
0196	Mutant ME110 Sequence of ME100 construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGGCATTTCTATTGCGCAGACCACCTGAAGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTGCCGGGGCTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACCAACAGATGAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTGATGTGAAAGCCTTGGCCTTG ATGGGTTCAATGTTAATTCCTCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGCAAAAGTGACGCGAGTTCTCTCTCTGGGGCAATTCAAAACCGCTTTGATTACGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGATCGAAGATGCT GACTATGCAACGGAAAGTTTCTAATATGCTAAAGCGCAGATTCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCAAGTTCGGCAAAACGTCCTCTCTTACTGGTTCGCGGGGGTTC TCATCATCATCATCATGTTAAGTCGAC
0197	Mutant ME110 Mutant ME110	PRT	Artificial Sequence	MSGLRINSKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATAGANSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDVAVRSSLGAIQNRFDISAITNLGNTVTNLNSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVLSSLVPRGSHHHHHHG
0198	Mutant ME100/110 Forward Primer ME110	DNA	Artificial Sequence	CTGATTCCGATCTGAAAGCTATCCAGGGCTGAAATTCAGCAACGTC
0199	Mutant ME100/110 Reverse Primer ME110	DNA	Artificial Sequence	GACGTTGCTGAATTTACGCCCTGGATAGCTTTCAGATCGGAATCAG
0200	Mutant ME100/110 Sequence of ME100/110 construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGGTCTGACTCAGGCTTCCCG

0201	Mutant ME104N Intermediate Mutant ME100/110	DNA	Artificial Sequence	TACGCTAACGACGGCATTCTATTGGCAGACCACCTGAAGGTGGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTTCAGGCCACTCCGGGGCTAACTCTGATTCGG ATCTGAAAGCTATCCAGGCTGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTCTGCTCAGGACAACCCAGATGAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATGATGTGAAAAGCCTTGGCCTTG ATGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATCTGCAT TGTCAAAAGTGGACGCGAGTTCCTTCTCTCTGGGGCAATTCAAAACCGCTTTCGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAAAGTTTCTAATATGCTAAAGCGCAGATTCCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCGGTTCCGCAAAACGCTCCTCTCTTTACTGGTTCCGCGGGGTTT TCATCATCATCATCATCATGGTTAAGTCGAC ATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATCGGCGAGGCCAGGCGATTGCTAACC GCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTTCTA TTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAACAACCTGCAGCGTGTGCGTGAG TTGCTGTTTACGGCCACTGCCGGGGCTAACTCTGATTCGGATCTGAAAGCTATCCAGGCTGA AATTCAGCAACGCTCTGGAAGAAATCGATCGGCTTCTAATCAGACTCAATTTAACGGTGTAAA GTCCTGTCTCAGGACAACCCAGATGAAATCCAGGTGGTGTGCTAACGATGGTGAACCATTAAC CATCGATCTGCAAAAAATGATGTGAAAAGCCTTGGCCTTGATGGTTCAATGTTAATTCCTCC GGGAAGTACCGCTAACCCACTGGCTTCAATGATTCGATTCGCAAAAGTGGACGCAGTTT GTTCTTCTCTGGGGCAATTCAAAACCGCTTTCGATTCAGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAATCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTA ATATGCTAAAGCGCAGATTCGCAGCAGGCTGGTACTCCGTTCTGGCGCAGGCTAACCCAG GTTCCGCAAAACGCTCCTCTTTACTGGTTCCGCGGGGTTCTCATCATCATCATCATGTTGTT TAA MSGLRINSKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNENNNLQRVRELSVQ ATAGANSDDLKAIQAEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDVAVRSSLGAIQNRFDISAITNLGNTVTNLNSARSRIED ADYATEVSNMSKAQILQQAGTSVLQANQVPQNVLSLLVPRGSHHHHHHG GCCACTAACGGGACTAACGCTGATGCCGCTCTGAAATCTATCCAG CTGGATAGATTCAGAGCGGGCATCAGCGTTAGTCCCGTTAGTGGC
0202	Mutant ME100/110 Mutant ME110/110	PRT	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCCTCTAGAATAATTTGTTT AACITTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGGAAAGACGATGCG
0203	Mutant ME104 Forward Primer ME104	DNA	Artificial Sequence	
0204	Mutant ME104 Reverse Primer ME104	DNA	Artificial Sequence	
0205	Mutant ME104 Sequence of ME104	DNA	Artificial Sequence	

	construct			GCAGGCCAGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGGCATTCTATTGGCGAGACCACCTGAAGTGGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTCAGGCCACTAACGGGACTAACGCTGATGCCG CTCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAACCCAGATGAAATCCAGGTTGGTG CTAACGATGGTGAACCATTAACATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGGCCCTTG ATGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATCTGCAT TGTCAAAAGTGGACGCGAGTTCTTCTCTGGGGCAATTCAAACCCGCTTTGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAGTTTCTAATATGCTTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCGGTTCCGCAAAAACGTCCTCTCTTTACTGGTCCCGCGGGTTC TCATCATCATCATCATCATGTTAAGTCGAC
0206	Mutant ME104 Mutant ME104	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIAINRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNADAALKSIQDEIQRLLEEIDRVSNQTQFNGVKVLSQDNQMKIQVGANDGETITDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDVRSLSGAIQNRFDSAITNLGNTVTNLSARSRIED ADYATEVSNMSKAQILQQAGTSVL AQANVPQNVL SLLVPRGSHHHHHHG
0207	Mutant ME104N Primer FME104New	DNA	Artificial Sequence	GCCACTGCCGGGGCTAACGCTGATGCCGCTCTGAAAGCTATCCAG
0208	Mutant ME104N Primer RME104New	DNA	Artificial Sequence	CTGGATAGCTTTCAGAGCGGGCATCAGCGTTAGCCCCGGCAGTGCG
0209	Mutant ME104N Sequence of construct ME104New	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGGCCAGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGGCGAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTACGGCCACTGCCGGGGCTAACGCTGATGCC GCTCTGAAAGCTATCCAGGCTGAAATTCAGCAACGCTTGGAAAGAAATCGATCGCGTTTCTAAT CAGACTCAATTTAACGGTGTAAAGTCCGTCTCAGGACAACCCAGATGAAATCCAGGTTGGT GCTAACGATGGTGAACCATTAACCATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGGCCCTT GATGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATCTGCA TTGTCAAAAGTGGACGCGAGTTCTTCTCTGGGGGCAATTCAAACCGCTTTGATTCAGCC ATTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAGTTTCTAATATGCTTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCGGTTCCGCAAAAACGTCCTCTCTTTACTGGTCCCGCGGGTTC TCATCATCATCATCATCATGTTAAGTCGAC

0210	Mutant ME104N Mutant ME104N	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATAGANADAALKAIQAEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDARSSLGAIQNRFDSAITNLGNTVTNLSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVL SLLVPRGSHHHHHHG
0211	Mutant ME110 Sequence of ME110 construct	DNA	Artificial Sequence	TAATACGACTCACATATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGATAATTTTGT AACTTTAAGAAGGAGATATACATATAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGCGCAGACCACCTGAAGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAAGCTATCCAGGCTGAAATTCAGCAACGCTCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTCTGTCTCAGGACAACCCAGATGAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTG ATGGTTCAATGTAAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAAGTGGACGCGATTCTTCTCTCTGGGGCAATTCAAAACCGCTTGTGATTGACGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGTAGCCGATCGAAGATGCT GACTATGCAACGGAAGTTCTAAATATGTCTAAAGCGCAGATCTCGACGAGGCTGGTACTCC GTTCTGGCGCAGGCTAACGAGTTCGCAAAACGCTCTCTCTTACTGGTTCGCGCGGGTTC TCATCATCATCATCATGTTAAGTCGAC
0212	Mutant ME110 Mutant ME110	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKAIQAEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKVDARSSLGAIQNRFDSAITNLGNTVTNLSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVL SLLVPRGSHHHHHHG
0213	Mutant ME117 Forward Primer ME117	DNA	Artificial Sequence	CTATCCAGGATGAAATTCAGGCACGCTCTGGCAGAAATCGATCGCG
0214	Mutant ME117 Reverse Primer ME117	DNA	Artificial Sequence	CGCGATCGATTCTGCCAGACGTCCTGAAATTCATCTCGGATAG
0215	Mutant ME117 Sequence of 33ML construct (should this say ME117?)	DNA	Artificial Sequence	TAATACGACTCACATATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGATAATTTTGT AACTTTAAGAAGGAGATATACATATAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGCGCAGACCACCTGAAGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGGCACGCTCTGGCAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTCTGTCTCAGGACAACCCAGATGAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTG

				ATGGGTTCAATGTTAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGCAT TGTCAAAAGTGGACGCGAGTTCGTTCTTCTCTGGGGCAATTCAAAACCGCTTGTATCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAAGTTTCTAAATATGCTAAAGCGCAGATCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCCAGTTCCGCAAAACGTCCTCTCTTTACTGTTCCGCGGGGTTCTC TCATCATCATCATCATGTTAAGTCGAC
0216	Mutant ME117 Mutant ME117	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLAEIDRVSNQTFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDV KSLGLDGFNVNPGSTANPLASIDSALSKVDVVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVL SLLVPRGSHHHHHHGG
0217	Mutant ME124 Forward Primer ME104	DNA	Artificial Sequence	GGAAAGAAATCGATGCCGTTTCTGCTGCGACTCAATTTAACGGTGTTAAAGTCCTGTCTCTC
0218	Mutant ME124 Reverse Primer ME104	DNA	Artificial Sequence	GAGACAGGACTTTAACACCCGTTAAATTGAGTCGCAGCAGAAACGGCATCGATTTCTTCTCC
0219	Mutant ME124 Sequence of ME124 construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGAATTGTGAGCGGATAACAATTCCTCTAGATAAATTTGTTTT AACTTTAAGAAGGAGATATACATATAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGCGCAGACCCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGGTGTCGTGAGTTGCTGTTACGGCCACTAACGGGACTAACTCTGATTCGG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCGAAGAAATCGATGCCGTTTCTGCTG CGACTCAATTTAACGGTGTAAAGTCTGCTCAGGACAACCCAGATGAAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTGATGTAAAAAGCCTTGCCCTTG ATGGGTTCAATGTTAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCGCAT TGTCAAAAGTGGACGCGAGTTCGTTCTTCTCTGGGGCAATTCAAAACCGCTTTGATTCAGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCGCGCGTAGCCGTATCGAAGATGCT GACTATGCAACGGAAGTTTCTAAATATGCTAAAGCGCAGATCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCCAGTTCCGCAAAACGTCCTCTCTTTACTGTTCCGCGGGGTTCTC TCATCATCATCATCATGTTAAGTCGAC
0220	Mutant ME124 Mutant ME124	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLAEIDAVSAATQFNGVKVLSQDNQMKIQVGANDGETITIDLQKIDV KSLGLDGFNVNPGSTANPLASIDSALSKVDVVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQVPQNVL SLLVPRGSHHHHHHGG
0221	Mutant ME124P Forward Primer ME124P	DNA	Artificial Sequence	CAGCAACGCTCTGGAAGAAATCGATGCCGTTTCTAATCAGACTCAATTTAAGCG

0222	Mutant ME124P Reverse Primer ME124P	DNA	Artificial Sequence	CCGTTAAATTGAGTCTGATTAGAAAACGGCATCGATTTCTTCCAGACGTTGCTG
0223	Mutant ME124 Expressed Mutant ME124P	DNA	Artificial Sequence	ATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATCGGCAGGCCAGCGGATTGCTAACC GCTTCACTTCTAATATCAAAGGCTGACTCAGGCTTCCCGTAACGCTAACGACGGCATTCTTA TTGCGCAGACCACTGAAGGTGCGTGAATGAATCAACAACAACCTGCAGCGTGTGCGTGAG TTGCTGTTCAGGCCACTAACGGGACTAACTCTGATCCGATCTGAATCTATCCAGGATGAA ATTACGCAACGCTCTGGAAGAAATCGATGCCGTTTCTAATCAGACTCAATTTAACGGTGTAAA GTCCTGCTCAGGACAACCCAGATGAAAATCCAGGTGGTGCTAACGATGGTGAACCATTAAC CATCGATCTGCAAAAAATTGATGTGAAAAGCCTTGGCCTTGATGGTCAATGTTAATTCCTCC GGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAATTGTCAAAAAGTGGACGCAGTTC GTTCTTCTGGGGCAATTCAAAAACCGCTTTGATTACGCCATTACCAACCTTGGCAATACGG TAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCTGACTATGCAACGGAAGTTTCTA ATATGCTAAAGCGCAGATCTGCAGCAGGCTGGTACTTCCGTTCTGGCGCAGGCTAACCCAG GTTCCGCAAAACGCTCCTCTCTTACTGGTTCCGCGGGGTTCTCATCATCATCATCATGTTGGT TAA
0224	Mutant ME124P ME124P	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTAGCGGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGATTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTACGGCCACTAACGGGACTAACTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCTGGAAGAAATCGATGCCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTCTGCTCAGGACAACCCAGATGAAAATCCAGGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTG ATGGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAAGTGGACGCGAGTTCGTTCTTCTCTGGGGCAATTCAAAACCGCTTTGATTACGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGGTAGCCGTATCGAAGATGCT GACTATGCAACGGAGTTTCTAATATGCTTAAAGCGCAGATCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCCAGGTTCCGCAAAACGTCCTCTCTTACTGGTTCGCGGGGTTTC TCATCATCATCATCATCATGTTAAGTCGAC
0225	Mutant ME124P ME124P	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDAVSNQTFNGVKVLSQDNQMKIQV/GANDGETITDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSKV/DAVRSSLGAIQNRFDSAITNLGNTVNLNSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQV/PQNVLSLLVPRGSHHHHHHG
0226	Mutant ME132	DNA	Artificial	CGTTTCTAATCAGACTCAATTTGCCGCTGTTAAAGTCCCTGCTCAGGACAACC

0227	Forward Primer ME132	DNA	Sequence	GGTTGTCTGAGACAGGACTTTAACAGCGGCAAAATTGAGTCTGATTAGAAACG
0228	Mutant ME132 Reverse Primer ME132 Sequence of ME132 construct	DNA	Artificial Sequence	TAATACGACTCACATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGAATAATTTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGGCATTCTATTGGCAGACCACTGAAGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGTCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTGCCGCTGTTAAAGTCCGTCTCAGGACAACCAAGATGAAATCCAGTTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTTG ATGGTTCAATGTTAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAAGTGGACGCGAGTTCCTTCTCTGCGGGCAATTCAAAACCGCTTGTGATTGAGCCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCCGCGTAGCCGTATCGAAGATGCT GACTATGCAACGGAAATTTCTAATATGCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCAAGTTCCGCAAAACGTCTCTCTTACTGGTTCCCGCGGGTTC TCATCATCATCATCATGTTAAGTCGAC
0229	Mutant ME132 Mutant ME117 (ME132?)	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLIEIDRVSNQTQFAAVKVLSDNQMKIQV/GANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSK/DVAVRSSLGAIQNRFDSAITNLGNTVTNLNSARSRIED ADYATEVSNMSKAQILQQAGTSVLQANQV/PQNVLSLLVPRGSHHHHHG
0230	Mutant ME142 Forward Primer ME142	DNA	Artificial Sequence	GTTAAAGTCCTGTCTCAGGACAACGCGATGGCAATCCAGGTTGGTGCTAACG
0231	Mutant ME142 Reverse Primer ME142	DNA	Artificial Sequence	CGTTAGCACCAACCTGGATTGCCATCGCGTTGTCCTGAGACAGGACTTTAAC
0232	Mutant ME142 Sequence of ME142 construct	DNA	Artificial Sequence	TAATACGACTCACATAGGGGAATTGTAGCGGATAACAAATCCCTCTAGAATAATTTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGGCATTCTATTGGCAGACCACTGAAGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGTCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGTCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAACGCGATGGCAATCCAGGTTGGT GCTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCTT GATGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCA

0233	Mutant ME142 Mutant ME142	PRT	Artificial Sequence	TTGTCAAAGTGGACGCGAGTTCTTCTCTCTGGGGGCAATTCAAACCGCTTTGATTACGCC ATTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCGCGCGTAGCCGTATCGAAGATGCT GACTATGCAACGGAGTTTCTAATATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCGAGTTCCGCAAAACGTCCTCTCTTTACTGGTTCCGCGGGGTTT TCATCATCATCATCATCATGTTAAGTCGAC
0234	Mutant ME150 Forward Primer ME150	DNA	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNAMAIQV/GANDGETITIDLQKIDV KSLGLDGFVNSPGSTANPLASIDSALSKV/DAVRSSLGAIQNRFDISAITNLGNTVTVNLSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQV/PQNVLSSLVPRGSHHHHHG
0235	Mutant ME150 Reverse Primer ME150	DNA	Artificial Sequence	GATGAAAATCCAGGTTGGTGCTAGCGCTGCTGAAACCATTAACCATCGATCTGCTGC
0236	Mutant ME150 Sequence of ME150 construct	DNA	Artificial Sequence	GCAGATCGATGGTAATGGTTTCAGCAGCGGCTAGCACCAACCTGGATTTTCATC TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCTCTAGAAATAATTTGTTT AACTTTAAGAAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAAGACGATGCG GCAGGCCAGCGATTGCTAACCGCTTCACTTCTAATATCAAAGGTCTGACTCAGGCTTCCCG TAACGCTAACGACGCGCATTTCTATTGCGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTACGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTTAAAGTCTGCTCAGGACACCCAGATGAAATCCAGGTTGGTG CTAGCGCTGCTGAAACCATTAACCATCGATCTGCAAAAAATTTGATGTGAAAAGCCTTGGCCCTG ATGGGTTCAATGTTAATCCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAGTGGACGCGAGTTCTTCTCTCTGGGGCAATTCAAACCGCTTTGATTACGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAACCTCGCGCGTAGCCGTATCGAAGATGCT GACTATGCAACGGAGTTTCTAATATGTCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTCC GTTCTGGCGCAGGCTAACCGAGTTCCGCAAAACGTCCTCTCTTTACTGGTTCCGCGGGGTTT TCATCATCATCATCATCATGTTAAGTCGAC
0237	Mutant ME150 Mutant ME150	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTQASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNMQIKV/GASAAETITIDLQKIDV KSLGLDGFVNSPGSTANPLASIDSALSKV/DAVRSSLGAIQNRFDISAITNLGNTVTVNLSARSRIED ADYATEVSNMSKAQILQQAGTSVLAQANQV/PQNVLSSLVPRGSHHHHHG
0238	Mutant ME468 Forward Primer ME468	DNA	Artificial Sequence	GCCGTATCGAAGATGCTGACGCTGGAGCGGAAGTTGCTAATATGCTTAAAGCGCAG

0239	Mutant ME468 Reverse Primer ME468	DNA	Artificial Sequence	CTGCGCTTTAGACATATTAGCAACTTCGGCTCCAGCGTCAGCATCTTCGATACGGC
0240	Mutant ME468 Sequence of ME468 construct	DNA	Artificial Sequence	TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAAATCCCCCTCTAGAAATAATTTGTTT AACTTTAAGAGGAGATATACATATGAGCGGGTTACGGATCAACAGCGCGAAGACGATGCG GCAGCCAGGCGATTGCTAACCGCTTCACTTCTAATATCAAAAGTCTGACTCAGGCTTCCCG TAACGCTAACGACGGCATTCTATTGGCAGACCACCTGAAGGTGCGCTGAATGAAATCAACAA CAACCTGCAGCGTGTGCGTGAGTTGCTGTTCAGGCCACTAACGGGACTAACTCTGATTCCG ATCTGAAATCTATCCAGGATGAAATTCAGCAACGCTCGGAAGAAATCGATCGCGTTTCTAATC AGACTCAATTTAACGGTGTAAAGTCTGTCTCAGGACAACCCAGATGAAATCCAGGTGGTG CTAACGATGGTGAACCAATTACCATCGATCTGCAAAAAAATGATGTGAAAAGCCTTGGCCTTG ATGGGTCAATGTAAATCCCGGGAAGTACCGCTAACCCACTGGCTTCAATTGATTCTGCAT TGTCAAAAGTGGACGCGAGTTCGTTCTTCTCTGGGGCAATTCAAAACCGCTTTCGATTACGCCA TTACCAACCTTGGCAATACGGTAACCAATCTGAATCCGCGGTAGCCGATCGAAGATGCT GACGCTGGAGCGGAAGTTGCTAATATGCTAAAGCGCAGATTCTGCAGCAGGCTGGTACTTC CGTCTGGCGCAGGCTAACCGGTTCCGCAAAACGCTCTCTCTTTACTGTTCCGCGGGGTT CTCATCATCATCATCATGTTAAGTCGAC
0241	Mutant ME468 Mutant ME468	PRT	Artificial Sequence	MSGLRNSAKDDAAGQAIANRFTSNIKGLTOASRNANDGISIAQTTEGALNEINNLRVRELSVQ ATNGTNSDSLKSIQDEIQRLLEEIDRVSNQTFNGVKVLSQDNQMKIVGANDGETITIDLQKIDV KSLGLDGFNVNSPGSTANPLASIDSALSVDVAVRSSLGAQNRFDSAITNLGNTVNLNSARSRIED ADAGAEVANMSKAQILQQAGTSVLAQANQVPQNVL SLLVPRGSHHHHHG
0242	Linker	PRT	Artificial Sequence	SPG
0243	Lru283	PRT	Artificial Sequence	mgthhhhhsgMEEFNMRINTNVAAMNTYSRLTAANTAKSNSLAKLSSGLRINKAGDDAAGLAISEKM KSQIGGLTQAKRNAQDGLSLVQTAEAGALNETHSILERMRLAVQGSNGTLTSSDRGSINKELKALH QELTRISNTTEFNTQKLFQTKQKSVTFTFQIGANAGQTLVAITAMSGEALLVSTDAKFSLNAAGT NAGAMIKSIDAAIAKVSDQRADLGAVQNRLEHTINNLTATNENLSDANSRIRDVDMAEEMMTFTKS NILSQAATSM LAQANAMPNSVLNLLQG
0244	Tpe270	PRT	Artificial Sequence	mgthhhhhsgMRINHNISALNAWRNIDQTYQSM SKTLERLSSGLRINRAGDDAAGLAISEKMRGQIK GLNMAIKNAQDAISLIQTAEAGALTEVHSILQRMRELAVQAASDTNTNVDRQIQKEIDQLREEIDRIA RTTEFNTKKLLDGKLEGRFSQVDAKVVTGGNINVLQGTVSSKAVEGTYVIEVGaAERAIMVDDAI HRVSTARAALGAQNRLHTISNLGVAENLTAESRIRDADMAKEMMEFTKQILLQSSMAMLAQ SNTLPQNVLQLMR
0245	Tpe159w	PRT	Artificial Sequence	mgthhhhhhsGLNMAIKNAQDAISLIQTAEAGALTEVHSILQRMRELAVQAASDTNTNVDRQIQKEIDQ LREEIDRIARTTEFNTKKLLDGKLEGRFSQVDAKVVTGGNINVLQGTVSSKAVEGTYVIEVGaAERA

0246	Chy275	PRT	Artificial Sequence	IMVVDAAIHRVSTARAALGAIGNRLEHTISNLG mg h h h h h s g M S L R I N N I E A L N A W R A L N S T S N A L Q K S M E K L S S G L R I N R A G D D A A G L A I S E K L R A Q I R G L N Q A I R N A Q D G I S L I Q T A E G G L S E I Q N I L Q R M R E L G V Q A A N G T L N N Q D I S A I T T E L N Q L F N E I D R I A G A T E F N T K N I L A V S T G L V W T L Q V G A N A G Q V I A F T I D N A G T A S L G L S S A D L A I N D N A S A S A F I S K V D S A L Q K V S T Y R A N L G S I Q N R L E H T I A N L G I A S E N L S A S E S R I R D V D M A E M M N F T K N I L Q Q A G V A I L A Q A N Q A P Q A V L Q L L R
0247	Chy162w	PRT	Artificial Sequence	mg h h h h h s G L N Q A I R N A Q D G I S L I Q T A E G G L S E I Q N I L Q R M R E L G V Q A A N G T L N N Q D I S A I T T E L N Q L F N E I D R I A G A T E F N T K N I L A V S T G L V W T L Q V G A N A G Q V I A F T I D N A G T A S L G L S S A D L A I N D N A S A S A F I S K V D S A L Q K V S T Y R A N L G S I Q N R L E H T I A N L G
0248	ChyU137	PRT	Artificial Sequence	mg h h h h h s G L N Q A I R N A Q D G I S L I Q T A E G G L S E I Q N I L Q R M R E L G V Q A A N G T L N N Q D I S A I T T E L N Q L F N E I D R I A G A T E F N T K N I L A A G T A S L G L S S A D L A I N D N A S A S A F I S K V D S A L Q K V S T Y R A N L G S I Q N R L E H T I A N L G
0249	ChyN108	PRT	Artificial Sequence	mg h h h h h s S A S A F I S K V D S A L Q K V S T Y R A N L G S I Q N R L E H T I A N L G p d G L N Q A I R N A Q D G I S L I Q T A E G G L S E I Q N I L Q R M R E L G V Q A A N G T L N N Q D I S A I T T E L N Q L F N E I D R I A
0250	ChyZ94	PRT	Artificial Sequence	mg h h h h h s N N Q D I S A I T T E L N Q L F N E I D R I A G A T g s G G L S E I Q N I L Q R M R E L G V Q A A N G T L N g g S A S A F I S K V D S A L Q K V S T Y R A N L G S I Q N R L E H T I A N L G
0251	Fir161B	PRT	Artificial Sequence	mg h h h h h s G L A Q A S R N A Q D A I S I A Q T A E G A L D E T Q S I L Q R V R E L G V Q G A N G T L T A D D I N A L Q A E V D Q L I A E I D R I A G A T E F N T Q N L L D G S F T T K A F Q V G A N S G Q N M T L T I G K M D T T T L G L S S A D L A I N D N A F A N G A I S T V D S A L Q K V S A E R A K L G A I Q N R L E H T I A N L G
0252	Fir161MNB	PRT	Artificial Sequence	mg h h h h h s G L A Q A S R Q A Q D A I S I A Q T A E G A L D E T Q S I L Q R V R E L G V Q G A D G T L T A D D I D A L Q A E V D Q L I A E I D R I A G A T E F A T Q K L L D G S F T T K A F Q V G A A S G Q D V T L T I G K V D T T T L G L S S A D L A I D S A A F A D G A I S T V D S A L Q K V S A E R A K L G A I Q N R L E H T I A Q L G

[0052] In some embodiments, the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and potassium aluminum sulfate, AS04 (which is composed of aluminum salt and MPL), and ALHYDROGEL. In some embodiments, the aluminum gel or salt is a formulation or mixture with any of the additional adjuvants described herein.

5 **[0053]** In some embodiments, adjuvants in addition to the described flagellin-based agent and an aluminum gel or salt find use in the present invention. In some embodiments, the additional adjuvant is selected from, oil-in-water emulsion formulations, saponin adjuvants, ovalbumin, Freund's Adjuvant, cytokines, and chitosans. Illustrative additional adjuvants include, but are not limited to: (1) ovalbumin (e.g. ENDOFIT), which is often used for biochemical studies; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer such as, for example, Model HOy microfluidizer (Microfluidics, Newton, Mass.), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, (c) RIBI adjuvant system (RAS), (RIBI IMMUNOCHEM, Hamilton, MO.) containing 2% Squalene, 0.2% Tween 80, and, optionally, one or more bacterial cell wall components from the group of monophosphoryl lipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), including MPL+CWS (DETOX™); and (d) ADDAVAX (Invitrogen); (3) saponin adjuvants, such as STIMULON (Cambridge Bioscience, Worcester, Mass.) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (by way of non-limiting example, IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; (6) chitosans and other derivatives of chitin or poly-N-acetyl-D-glucosamine in which the greater proportion of the N-acetyl groups have been removed through hydrolysis (see, e.g., European Patent Application 460 020, which is hereby incorporated by reference in its entirety, disclosing pharmaceutical formulations including chitosans as mucosal absorption enhancers; and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition, e.g., monophosphoryl lipid A. In other embodiments, the additional adjuvant is one or more of a flagellin-based agent (e.g. CBLB502 or any of the agents of Table 1), an aluminium salt or gel, a pattern recognition receptors (PRR) agonist, CpG ODNs and imidazoquinolines. In some embodiments, the additional adjuvant is one or more of cyclic [G(3',5')pA(3',5')p] (e.g. 3'3'-cGAMP VACCIGRADE); cyclic [G(2',5')pA(3',5')p]2'3' (e.g. 2'3' cGAMP VACCIGRADE); cyclic [G(2',5')pA(2',5')p] (e.g. 2'2'-cGAMP VACCIGRADE), cyclic diadenylate monophosphate (e.g. c-di-AMP VACCIGRADE); cyclic diguanylate monophosphate (e.g. c-di-GMP VACCIGRADE); TLR7 agonist-imidazoquinolines compound (e.g. TLR7 agonists, such as, for example, Gardiquimod VACCIGRADE, Imiquimod VACCIGRADE, R848 VACCIGRADE); lipopolysaccharides (e.g. TLR4 agonists), such as that from E. coli 0111:B4 strain (e.g. LPS-EB VACCIGRADE); monophosphoryl lipid A (e.g. MPLA-SM VACCIGRADE and MPLA Synthetic VACCIGRADE); N-glycolylated

muramyl dipeptide (e.g. N-Glycolyl-MDP VACCIGRADE); CpG ODN, class A and/or CpG ODN, class B and/or CpG ODN, class C (e.g. ODN 1585 VACCIGRADE, ODN 1826 VACCIGRADE, ODN 2006 VACCIGRADE, ODN 2395 VACCIGRADE), a triacylated lipoprotein (e.g. Pam3CSK4 VACCIGRADE); Polyinosine-polycytidylic acid (e.g. Poly(I:C) (HMW) VACCIGRADE); and cord factor (i.e. mycobacterial cell wall component trehalose 6,6'-dimycolate (TDM),) or an analog thereof (e.g. TDB VACCIGRADE, TDB-HS15 VACCIGRADE). In some embodiments, the additional adjuvant is a TLR agonist (e.g. TLR1, and/or TLR2, and/or TLR3, and/or TLR4, and/or TLR5, and/or TLR6, and/or TLR7, and/or TLR8, and/or TLR9, and/or TLR10, and/or TLR11, and/or TLR12, and/or TLR13), a nucleotide-binding oligomerization domain (NOD) agonist, a stimulator of interferon genes (STING) ligand, or related agent.

[0054] In some embodiments, the additional adjuvants is one or more of a mineral adjuvant, gel-based adjuvant, tensoactive agent, bacterial product, oil emulsion, particulated adjuvant, fusion protein, and lipopeptide. Other mineral salt adjuvants, besides the aluminum adjuvants described elsewhere, include salts of calcium (e.g. calcium phosphate), iron and zirconium. Other gel-based adjuvants, besides the aluminum gel-based adjuvants described elsewhere, include Acemannan. Tensoactive agents include Quil A, saponin derived from an aqueous extract from the bark of *Quillaja saponaria*; saponins, tensoactive glycosides containing a hydrophobic nucleus of triterpenoid structure with carbohydrate chains linked to the nucleus, and QS-21. Bacterial products include cell wall peptidoglycan or lipopolysaccharide of Gram-negative bacteria (e.g. from *Mycobacterium* spp., *Corynebacterium parvum*, *C. granulosum*, *Bordetella pertussis* and *Neisseria meningitidis*), N-acetyl muramyl-L-alanyl-D-isoglutamine (MDP), different compounds derived from MDP (e.g. threonyl-MDP), lipopolysaccharides (LPS) (e.g. from the cell wall of Gram-negative bacteria), trehalose dimycolate (TDM), and DNA containing CpG motifs. Oil emulsions include FIA, Montanide, Adjuvant 65, Lipovant, the montanide family of oil-based adjuvants, and various liposomes. Among particulated and polymeric systems, poly (DL-lactide-coglycolide) microspheres have been extensively studied and find use herein.

[0055] Further, in some embodiments, cytokines are an adjuvant of the present invention (e.g. IFN- γ and granulocyte-macrophage colony stimulating factor (GM-CSF)). Also carbohydrate adjuvants (e.g. inulin-derived adjuvants, such as, gamma inulin, algammulin (a combination of γ -inulin and aluminum hydroxide), and polysaccharides based on glucose and mannose, such as glucans, dextrans, lentinans, glucomannans and galactomannans) find use in the present invention. In some embodiments, adjuvant formulations are useful in the present invention and include alum salts in combination with other adjuvants such as Lipid A, algammulin, immunostimulatory complexes (ISCOMS), which are virus like particles of 30–40 nm and dodecahedral structure, composed of Quil A, lipids, and cholesterol.

[0056] In some embodiments, the additional adjuvants are described in Jennings *et al.* Adjuvants and Delivery Systems for Viral Vaccines-Mechanisms and Potential. In: Brown F, Haaheim LR, (eds). *Modulation of the Immune Response to Vaccine Antigens. Dev. Biol. Stand.*, Vol. 92. Basel: Karger 1998; 19–28 and/or Sayers

et al. J Biomed Biotechnol. 2012; 2012: 831486, and/or Petrovsky and Aguilar, *Immunology and Cell Biology* (2004) 82, 488–496 the contents of which are hereby incorporated by reference in their entireties.

[0057] In various embodiments, the present adjuvants (e.g. flagellin-based agent and an aluminum gel or salt) may be part of live and attenuated, or killed or inactivated, or toxoid, or subunit or conjugate vaccines.

5 **[0058]** In various embodiments, the present adjuvants (e.g. flagellin-based agent and an aluminum gel or salt) may be part of one or more approved vaccines and/or the antigens of one or more approved vaccines may be the antigens of the present invention. In some embodiments, the approved vaccines include: Adenovirus; Anthrax (Biothrax); BCG (Tice); DT (Sanofi); DTaP (Daptacel); DTaP (Infanrix); DTaP-HepB-IPV (Pediarix); DTaP-IPV (Kinrix); DTaP-IPV/Hib (Pentacel); Hib (ActHIB); Hib (Hiberix); Hib (PevaxHIB); Hib/Hep B (Comvax);
 10 Hib/Mening. CY (MenHibrix); Hep A (Havrix); Hep A (Havrix); Hep B (Engerix-B); Hep B (Recombivax); Hep A/Hep B (Twinrix); Human Papillomavirus (HPV) (Cerverix); Human Papillomavirus (HPV) (Gardasil); Influenza (Afluria); Influenza (Agrimflu); Influenza (Fluarix); Influenza (Flublok); Influenza (Flucelvax); Influenza (Fluvirin); Influenza (Flulaval); Influenza (Fluzone: Standard, High-Dose, & Intradermal); Influenza (FluMist); Japanese Encephalitis (Ixiaro); Meningococcal (MCV4-Menactra); Meningococcal (MCV4-Menveo); Meningococcal
 15 (MPSV4-Menomune); MMR (MMR-II); MMRV (ProQuad); Pneumococcal (PCV13 – Prevnar 13); Pneumococcal (PPSV-23 – Pneumovax); Polio (IPV – Ipol); Rabies (Imovax); Rabies (RabAvert); Rotavirus (RotaTeq); Rotavirus (Rotarix); Smallpox (Vaccinia – ACAM2000); Td (Decavac); Td (Tenivac); Td (Mass Biologics); Tdap (Adacel); Tdap (Boostrix); Typhoid (inactivated – Typhim Vi); Typhoid (oral – Ty21a); Varicella (Varivax); Yellow Fever (YF-Vax); and Zoster (Shingles – Zostavax).

20 **[0059]** In various embodiments, the present adjuvants (e.g. flagellin-based agent and an aluminum gel or salt) may be part of one or more illustrative vaccines and/or the antigens of one or more illustrative vaccines may be the antigens of the present invention. Illustrative vaccines include, by way of example, subunit vaccine and inactivated or “killed” vaccine (e.g. Infanrix-IPV/Hib (Bordetella pertussis), Infanrix-IPV/Hib (Haemophilus influenzae), Infanrix-IPV/Hib (Poliovirus), Infanrix-IPV/Hib (Clostridium tetani), Infanrix-IPV/Hib (Corynebacterium diphtheriae),
 25 Infanrix-hexa (Bordetella pertussis), Infanrix-hexa (Haemophilus influenzae), Infanrix-hexa (Poliovirus), Infanrix-hexa (Hepatitis B virus), Infanrix-hexa (Clostridium tetani), Infanrix-hexa (Corynebacterium diphtheriae), Infanrix-IPV (Bordetella pertussis), Infanrix-IPV (Poliovirus), Infanrix-IPV (Clostridium tetani), Infanrix-IPV (Corynebacterium diphtheriae), Infanrix/Hib (Corynebacterium diphtheriae), Pediarix (Clostridium tetani), Pediarix (Poliovirus), Pediarix (Hepatitis B virus), ViVaxim (Salmonella spp.), ViVaxim (Hepatitis A virus);
 30 subunit vaccines (e.g. 5CVMB (Neisseria meningitidis), B. pertussis CyaA protein vaccine (Bordetella pertussis), B. pertussis PTx protein vaccine (Bordetella pertussis), Cancer VEGFA protein vaccine (Cancer), E. coli vaccine using intimin polypeptide (Escherichia coli), Engerix-B (Hepatitis B virus), H. pylori VacA protein vaccine (Helicobacter pylori), HC of type C and D (Clostridium botulinum), Infanrix/Hib (Bordetella pertussis), Infanrix/Hib (Haemophilus influenzae), Infanrix/Hib (Clostridium tetani), M. gallisepticum TM-1 Protein Subunit Vaccine
 35 (Mycoplasma gallisepticum), MDA-modified human apo B-100 peptide Vaccine (Atherosclerosis), MSP3-LSP

with aluminium hydroxide (*Plasmodium* spp.), Mumps HN Protein Subunit Vaccine (Mumps virus), N. meningitidis TBP2 Protein Vaccine (*Neisseria meningitidis*), *P. aeruginosa* OprI Protein Vaccine (*Pseudomonas aeruginosa*), *P. falciparum* Subunit SE36 Protein Vaccine (*Plasmodium* spp.), Phleum pratense Allergy Phl p 12 Subunit Vaccine (Allergy), Recombivax HB (Hepatitis B virus), *S. pneumoniae* ClpP protein Vaccine (*Streptococcus pneumoniae*); toxoid vaccine (e.g. BoNT/F(Hc) (*Clostridium botulinum*), DAPTACEL (*Corynebacterium diphtheriae*), Infanrix (*Bordetella pertussis*), Infanrix (*Clostridium tetani*), KINRIX (*Clostridium tetani*), PBT (*Clostridium botulinum*), Pediarix (*Bordetella pertussis*), inactivated or "killed" vaccines (e.g. Avaxim (Hepatitis A virus), Avaxim-Pediatric (Hepatitis A virus), FSME-IMMUN (Tick-borne Encephalitis Virus (TBEV)), Infanrix (*Corynebacterium diphtheriae*), Ixiaro (Japanese encephalitis virus), KINRIX (*Corynebacterium diphtheriae*), and Pediarix (*Corynebacterium diphtheriae*)); and conjugate vaccines (e.g., Arabinomannan-tetanus toxoid conjugate (*Mycobacterium tuberculosis*)), CCPS-P64kR (*Neisseria meningitidis*), COMVAX (*Haemophilus influenzae*), Menjugate (*Neisseria meningitidis*), Neisvac-C (*Neisseria meningitidis*), and PedvaxHIB (*Haemophilus influenzae*)).

[0060] In some embodiments, the present adjuvants (e.g. flagellin-based agent and an aluminum gel or salt) are combined in a vaccine targeting a substance abuse. For example, in one embodiment, the present adjuvants are used in vaccines against addition to fentanyl, heroin, morphine, opium, oxycodone, hydrocodone, ketamine, PCP, barbiturates, benzodiazepines, flunitrazepam, GHB, methaqualone, hashish, marijuana, LSD, mescaline, psilocybin, amphetamine, cocaine, MDMA, methamphetamine, methylphenidate, and nicotine (e.g. TA-CD (Celtic Pharma), those described in US Patent Publication No. 2013/0011432, the contents of which are hereby incorporated by reference (e.g. using 6-(2R,3S)-3-(benzoyloxy)-8-methyl-8-azabicyclo [3.2.1]octane-2-carboxyloxy-hexanoic acid (GNC) or 6-((2R,3S)-3-(benzoyloxy)-8-methyl-8-azabicyclo [3.2.1] octane-2-carboxamido)hexanoic acid) (GNE) as the antigen, and TA-NIC (Celtic Pharma)).

[0061] In some embodiments, the present adjuvants (e.g. flagellin-based agent and an aluminum gel or salt) and/or the present vaccines may comprise any one of the adjuvants or antigens annotated in the VIOLIN or Vaxjo databases (as described in He *et al. Nucleic Acids Research*. 2014. 42 (D1): D1124-D1132 and Xiang *et al. Nucleic Acids Res.* 2008 Jan; 36: D923-8, the contents of which are hereby incorporated by reference in their entirety).

[0062] In various embodiments, the present adjuvants (e.g. flagellin-based agent and an aluminum gel or salt) may be part of one or more cancer vaccines and/or the antigens of one or more cancer vaccines may be the antigens of the present invention. Illustrative cancer vaccines include therapeutic and preventative vaccines. For instance, cancer vaccines include ONCOPHAGE (ANTIGENICS INC., approved in Russia in 2008 for kidney cancer), APC8015/Sipuleucel-T/PROVENGE (DENDREON, for, e.g. metastatic hormone-refractory prostate cancer), CANCERVAX (CANVAXIN), GENITOPE CORP (MYVAX personalized immunotherapy), and FAVRILLE INC (FAVID), preventive vaccines which attack the cancer-causing viruses human papillomavirus (e.g. CERVARIX (GSK) and GARDASIL (MERCK)), hepatitis A virus (e.g. CERVARIX (GSK) and GARDASIL

(MERCK)), and hepatitis B virus (e.g. RECOMBIVAX HB (MERCK), ENGERIX-B (GSK), ELOVAC B (HUMAN BIOLOGICALS INSTITUTE), GENEVAC B (SERUM INSTITUTE), SHANVAC B, *etc.*

[0063] In various embodiments, the present adjuvants (e.g. flagellin-based agent and an aluminum gel or salt) may be part of one or more allergen vaccines and/or the antigens of one or more allergen vaccines may be the antigens of the present invention. For instance, subcutaneous immunotherapy (SCIT) allergen compositions and methods are applicable to the present invention (e.g. "allergy shots"). For example, vaccinations for allergic rhinitis and conjunctivitis (e.g. pollen (including ragweed), dust mites, animal dander and airborne mold spores); allergic or extrinsic bronchial asthma (e.g. house dust mites, pollen, animal dander, mold (*Cladosporium*), latex); and insect venom hypersensitivity. Allergens include pollen (e.g. tree, grass, weed), pet dander (e.g. cat pelt), dust mites, airborne molds, occupational aeroallergens, honey bee venom, yellow jacket venom, hornet venom, wasp venom, and fire ant venom.

[0064] In various embodiments, the antigens of the present vaccines may be the antigens of live and attenuated or killed or inactivated or toxoid or a subunit or conjugate vaccines. In various embodiments, the antigen of the present vaccines is an antigen of any of the vaccines described herein. For example, in some embodiments, the present antigen is that of one or more of the following vaccines: DTP (diphtheria-tetanus-pertussis vaccine), DTaP (diphtheria-tetanus-acellular pertussis vaccine), Hib (*Haemophilus influenzae* type b) conjugate vaccines, Pneumococcal conjugate vaccine, Hepatitis A vaccines, Poliomyelitis vaccines, Yellow fever vaccines, Hepatitis B vaccines, combination DTaP, Tdap, Hib, Human Papillomavirus (HPV) vaccine, Anthrax vaccine, *Bacillus Calmette–Guérin* (Tb), and Rabies vaccine.

[0065] In various embodiments, the flagellin-based agent and antigen are adsorbed to the aluminum gel or salt. In some embodiments, the flagellin-based agent and aluminum gel or salt are mixed to form a stable complex. In some embodiments, the flagellin-based agent and aluminum gel or salt are mixed in a ratio that is substantially below a loading capacity of the aluminum salt. In some embodiments, the flagellin-based agent and aluminum gel or salt are present in a ratio that is substantially below a loading capacity of the aluminum salt. In various embodiments, the flagellin-based agent and aluminum gel or salt are mixed or present in a ratio (w/w) of about 1:500, or about 1:600, or about 1:700, or about 1:800, or about 1:900, or about 1:1000, or about 1:2000, or about 1:5000, or about 1:6000, or about 1:7000, or about 1:8000, or about 1:9000, or about 1:10000. In some embodiments, the flagellin-based agent and aluminum gel or salt are mixed in a ratio (w/w) of about 1:500 or less. In some embodiments, the flagellin-based agent and aluminum gel or salt are present in a ratio that is substantially below a loading capacity of the aluminum salt even in the presence of antigen.

[0066] The loading (or adsorption) capacity of the adjuvant (e.g. the aluminum gel or salt) can be measured using a variety of analytical methods. In general, it is done by comparing the protein content in the aqueous phase of the agent being loaded or adsorbed (e.g. flagellin-based agent and/or antigen solution) before and after adsorption onto the adjuvant. For instance, the Ramon flocculation test may be used (as is used to determine the adsorption of diphtheria and tetanus toxoid). Further, loading can be measured using immunoprecipitation

techniques (e.g. quantitative immunoelectrophoresis or single radial immunodiffusion) or spectrophotometric techniques (e.g. the BCA method). ELISA methods may also be used as can immunoelectrophoresis or HPLC. The aluminum content in the final vaccine can be monitored using a number of known techniques, including spectrometric methods, such as for example atomic adsorption spectrometry.

5 [0067] In some embodiments, the amount of aluminum gel or salt in the vaccines and/or adjuvants described herein is about 0.05 to about 1.0 mg/dose, or about 0.125 to about 0.625 mg/dose. In some
embodiments, the amount of aluminum gel or salt in the vaccines and/or adjuvants described herein is about 0.05, or about 0.10, or about 0.15, or about 0.20, or about 0.25, or about 0.30, or about 0.35, or about 0.40, or
about 0.45, or about 0.50, or about 0.55, or about 0.60, or about 0.65, or about 0.70, or about 0.75, or about
10 0.80, or about 0.85, or about 0.90, or about 0.95, or about 1.0 mg/dose.

[0068] In some embodiments, the amount of flagellin-based agent is about 0.03 to about 5 µg/dose (e.g. about 0.03 µg/dose, about 0.1 µg/dose, about 0.3 µg/dose, about 0.5 µg/dose about 1.0 µg/dose, about 1.5
µg/dose, about 2.0 µg/dose, about 2.5 µg/dose, about 3.0 µg/dose, about 4.0 µg/dose, about 4.5 µg/dose, about 5.0 µg/dose). In various embodiments, the present compositions and methods comprise doses of flagellin-
15 based agent that are less than about 5 µg/dose, or less than 4 µg/dose, or less than 3 µg/dose, or less than 2 µg/dose, or less than 1 µg/dose, or less than 0.5 µg/dose. In some embodiments, the present compositions and
methods comprise low doses of flagellin-based agent.

[0069] In various embodiments, the present compositions and methods do not involve covalently attaching an antigen to the flagellin-based agent either as a fusion protein or via chemical conjugation. In various
20 embodiments, the present compositions do not have either equimolar ratio of antigen to flagellin-based agent (as in a fusion) or several molecules of hapten per one molecule of flagellin-based agent (as in chemical conjugates).
In various embodiments, the amount of flagellin-based agent in any of the present vaccines is less than the amount of antigen. In various embodiments, the amount of flagellin-based agent in any of the present vaccines is
less than the amount of antigen. In various embodiments, the amount of flagellin-based agent in any of the
25 present vaccines is substantially less than the amount of antigen. In various embodiments, the amount of
flagellin-based agent in any of the present vaccines is about 500-fold, or about 450-fold, or about 400-fold, or
about 350-fold, or about 325-fold, or about 300-fold, or about 250-fold, or about 200-fold, or about 150-fold, or
about 100-fold, or about 50-fold less than the amount of antigen.

[0070] In various embodiments, the combination of flagellin-based agent and alum do not substantially effect
30 TLR5 interaction by the flagellin-based agent.

[0071] In various embodiments, the present compositions and methods do not induce production of TNFα.

[0072] In various embodiments the present combination of flagellin-based agent and alum is substantially
stable at low temperatures for about one week (e.g. at about 4°C for about 3 days, or about 5 days, or about 6
days, or about 7 days, or about 10 days).

[0073] In another aspect, the present invention relates to a method of vaccinating a subject against a disorder, comprising administering an effective amount of a vaccine comprising an adjuvant comprising a flagellin-based agent and an aluminum gel or salt and an antigen associated with the disorder. In another aspect, the invention relates to a use of vaccine comprising an adjuvant comprising a flagellin-based agent and an aluminum gel or salt and an antigen associated with a disorder for vaccinating a subject against the disorder. In another aspect, the invention relates to a use of an effective amount of vaccine comprising an adjuvant comprising a flagellin-based agent and an aluminum gel or salt and an antigen associated with a disorder in the manufacture of a medicament for vaccinating a subject against the disorder.

[0074] In another aspect, the present invention relates to a method of immunostimulating a subject in advance of or concurrent with vaccination, comprising administering an effective amount of an adjuvant comprising a flagellin-based agent and an aluminum gel or salt, wherein both T_{H1} and T_{H2} -mediated immune responses are immunostimulated. In another aspect, the invention relates to a use of an effective amount of an adjuvant comprising a flagellin-based agent and an aluminum gel or salt for immunostimulating a subject in advance of or concurrent with vaccination. In another aspect, the invention relates to a use of an effective amount of an adjuvant comprising a flagellin-based agent and an aluminum gel or salt in the manufacture of a medicament for immunostimulating a subject in advance of or concurrent with vaccination.

[0075] In various embodiments, the vaccine described herein causes an improvement in adjuvant properties relative to a vaccine comprising the antigen and the aluminum gel or salt alone (or flagellin-based agent and antigen alone). In various embodiments, the vaccine and/or adjuvant described herein causes a broader, more diverse, more robust and longer lasting immunostimulatory effect than the vaccine comprising the antigen and the aluminum gel or salt alone (or flagellin-based agent and antigen alone) and/or the adjuvant comprising the aluminum gel or salt alone (or the adjuvant comprising the flagellin-based agent alone).

[0076] In some embodiments, the described vaccine and/or described adjuvant causes an increase in titer of 1 or more of, or 2 or more of, or 3 or more of, or all of IgG1, IgG2a, IgG2b, and IgG3 antibodies (e.g. relative to the adjuvant comprising the aluminum gel or salt or flagellin-based agent alone, or relative to the vaccine comprising the antigen and the aluminum gel or salt alone or flagellin-based agent alone)). In some embodiments, the described vaccine and/or described adjuvant causes a relative increase in the titer of all of IgG1, IgG2a, IgG2b, and IgG3 antibodies. In some embodiments, the described vaccine and/or described adjuvant causes a relative increase in the titer of more IgG3 antibodies than the described vaccine and/or described adjuvant in the absence of a flagellin-based agent (or the described vaccine and/or described adjuvant in the absence of an aluminum gel or salt alone).

[0077] In some embodiments, the antigen is administered simultaneously with or sequentially to the adjuvant.

[0078] In some embodiments, the disorder is selected from infectious diseases, cancer, allergy, and autoimmune diseases.

[0079] In some embodiments, the disorder is selected from diphtheria, tetanus, pertussis, influenza, pneumonia, hepatitis A, hepatitis B, polio, yellow fever, Human Papillomavirus (HPV) infection, anthrax, rabies, Japanese Encephalitis, meningitis, measles, mumps, rubella, gastroenteritis, smallpox, typhoid fever, varicella (chickenpox), rotavirus, and shingles.

[0080] In some embodiments, the disorder is a cancer is selected from, but not limited to, a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (e.g., small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

[0081] In some embodiments, the disorder is an allergy, selected from, by way of non-limiting example, allergic rhinitis and conjunctivitis, allergic or extrinsic bronchial asthma, and insect venom hypersensitivity.

[0082] In some embodiments, the disorder is a substance abuse disorder (e.g. of fentanyl, heroin, morphine, opium, oxycodone, hydrocodone, ketamine, PCP, barbiturates, benzodiazepines, flunitrazepam, GHB, methaqualone, hashish, marijuana, LSD, mescaline, psilocybin, amphetamine, cocaine, MDMA, methamphetamine, methylphenidate, and nicotine).

[0083] In some embodiments, the compositions of the present invention (e.g. the described adjuvants and vaccines) can possess a sufficiently basic functional group, which can react with an inorganic or organic acid, or a carboxyl group, which can react with an inorganic or organic base, to form a pharmaceutically acceptable salt.

A pharmaceutically acceptable acid addition salt is formed from a pharmaceutically acceptable acid, as is well known in the art. Such salts include the pharmaceutically acceptable salts listed in, for example, *Journal of Pharmaceutical Science*, 66, 2-19 (1977) and *The Handbook of Pharmaceutical Salts; Properties, Selection, and Use*. P. H. Stahl and C. G. Wermuth (eds.), Verlag, Zurich (Switzerland) 2002, which are hereby incorporated by reference in their entirety.

[0084] Pharmaceutically acceptable salts include, by way of non-limiting example, sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, camphorsulfonate, pamoate, phenylacetate, trifluoroacetate, acrylate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, isobutyrate, phenylbutyrate, α -hydroxybutyrate, butyne-1,4-dicarboxylate, hexyne-1,4-dicarboxylate, caprate, caprylate, cinnamate, glycollate, heptanoate, hippurate, malate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, phthalate, teraphthalate, propiolate, propionate, phenylpropionate, sebacate, suberate, p-bromobenzenesulfonate, chlorobenzenesulfonate, ethylsulfonate, 2-hydroxyethylsulfonate, methylsulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, naphthalene-1,5-sulfonate, xylenesulfonate, and tartarate salts.

[0085] The term "pharmaceutically acceptable salt" also refers to a salt of the compositions of the present invention having an acidic functional group, such as a carboxylic acid functional group, and a base. Suitable bases include, but are not limited to, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or tri-alkylamines, dicyclohexylamine; tributyl amine; pyridine; N-methyl, N-ethylamine; diethylamine; triethylamine; mono-, bis-, or tris-(2-OH-lower alkylamines), such as mono-, bis-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N,N-di-lower alkyl-N-(hydroxyl-lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)amine or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like.

[0086] In some embodiments, the compositions of the present invention (e.g. the described adjuvants and vaccines) described herein are in the form of a pharmaceutically acceptable salt.

[0087] In some embodiments, the compositions of the present invention (e.g. the described adjuvants and vaccines) may comprise a pharmaceutically acceptable carrier or vehicle. Such compositions can optionally comprise a suitable amount of a pharmaceutically acceptable excipient so as to provide the form for proper administration.

[0088] Pharmaceutical excipients can be liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical excipients can be, for example, saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating, and coloring agents can be used.

5 In one embodiment, the pharmaceutically acceptable excipients are sterile when administered to a subject. Water is a useful excipient when any agent described herein is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, specifically for injectable solutions. Suitable pharmaceutical excipients also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, 10 propylene, glycol, water, ethanol and the like. Any composition described herein, if desired, can also comprise minor amounts of wetting or emulsifying agents, or pH buffering agents.

[0089] The present invention includes the compositions of the present invention (e.g. the described adjuvants and vaccines) in various formulations. Any composition of the present invention can take the form of solutions, suspensions, emulsion, drops, tablets, pills, pellets, capsules, capsules containing liquids, powders, 15 sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment, the composition is in the form of a capsule (see, e.g., U.S. Patent No. 5,698,155). Other examples of suitable pharmaceutical excipients are described in *Remington's Pharmaceutical Sciences* 1447-1676 (Alfonso R. Gennaro eds., 19th ed. 1995), incorporated herein by reference.

[0090] Where necessary, the compositions of the present invention can also include a solubilizing agent. 20 Also, the agents can be delivered with a suitable vehicle or delivery device as known in the art. Combination therapies outlined herein can be co-delivered in a single delivery vehicle or delivery device. Compositions for administration can optionally include a local anesthetic such as, for example, lignocaine to lessen pain at the site of the injection.

[0091] The formulations comprising the compositions of the present invention may conveniently be 25 presented in unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods generally include the step of bringing the therapeutic agents into association with a carrier, which constitutes one or more accessory ingredients. Typically, the formulations are prepared by uniformly and intimately bringing the therapeutic agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into dosage forms of the desired formulation (e.g., wet or dry 30 granulation, powder blends, etc., followed by tableting using conventional methods known in the art).

[0092] In one embodiment, any composition of the present invention is formulated in accordance with routine procedures as a composition adapted for a mode of administration described herein.

[0093] Routes of administration include intramuscular, e.g. by injection or infusion. In some embodiments, the described adjuvant of a flagellin-based agent (e.g. CBLB502) and aluminum gel or salt may prevent systemic

delivery of the flagellin-based agent and induce localized delivery. In other embodiments, routes of administration include nasal, oral, and sublingual delivery.

[0094] Routes of administration may also be intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, oral, sublingual, intranasal, transdermal, or by inhalation. In some embodiments, the administering is effected orally or by parenteral injection. The mode of administration can be left to the discretion of the practitioner, and depends in-part upon the site of the medical condition. In most instances, administration results in the release of any agent described herein into the bloodstream.

[0095] Any composition of the present invention (e.g. the described adjuvants and vaccines) can be administered orally. Such compositions can also be administered by any other convenient route, for example, by intravenous infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and can be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer.

[0096] Dosage forms suitable for parenteral administration (e.g. intravenous, intramuscular, intraperitoneal, subcutaneous and intra-articular injection and infusion) include, for example, solutions, suspensions, dispersions, emulsions, and the like. They may also be manufactured in the form of sterile solid compositions (e.g. lyophilized composition), which can be dissolved or suspended in sterile injectable medium immediately before use. They may contain, for example, suspending or dispersing agents known in the art.

[0097] The dosage of any composition of the present invention (e.g. the described adjuvants and vaccines) as well as the dosing schedule can depend on various parameters, including, but not limited to, the disorder being treated, the subject's general health, and the administering physician's discretion.

[0098] *In vitro* or *in vivo* assays can be employed to help identify optimal dosage ranges. For example, doses may be determined with reference *Physicians' Desk Reference*, 66th Edition, PDR Network; 2012 Edition (December 27, 2011), the contents of which are incorporated by reference in its entirety.

[0099] For administration of a composition of the present invention (e.g. the described adjuvants and vaccines) by parenteral injection, the dosage is normally about 0.1 mg to about 250 mg per day, about 1 mg to about 20 mg per day, or about 3 mg to about 5 mg per day. Injections may be given up to four times daily. Generally, when orally or parenterally administered, the dosage of any agent described herein is normally about 0.1 mg to about 1500 mg per day, or about 0.5 mg to about 10 mg per day, or about 0.5 mg to about 5 mg per day. A dosage of up to about 3000 mg per day can be administered.

[00100] In another embodiment, delivery can be in a vesicle, in particular a liposome (see Langer, 1990, *Science* 249:1527-1533; Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989).

[00101] Any composition of the present invention (e.g. the described adjuvants and vaccines) can be administered by controlled-release or sustained-release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,556, each of which is incorporated herein by reference in its entirety. Such dosage forms can be useful for providing controlled- or sustained-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled- or sustained-release formulations known to those skilled in the art, including those described herein, can be readily selected for use with the active ingredients of the agents described herein. The invention thus provides single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled- or sustained-release.

[00102] Controlled- or sustained-release of an active ingredient can be stimulated by various conditions, including but not limited to, changes in pH, changes in temperature, stimulation by an appropriate wavelength of light, concentration or availability of enzymes, concentration or availability of water, or other physiological conditions or compounds.

[00103] In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, *Ann. Neurol.* 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 71:105).

[00104] In another embodiment, a controlled-release system can be placed in proximity of the target area to be treated, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, *Science* 249:1527-1533) may be used.

[00105] Administration of a composition of the present invention (e.g. the described adjuvants and vaccines) can, independently, be once per patient or may be used in a booster strategy. Administration may be about one to about four times daily or about one to about four times per month or about one to about six times per year or once every two, three, four or five years. Administration can be for the duration of about one day or about one month, about two months, about three months, about six months, about one year, about two years, about three years, and may even be for the life of the subject. The dosage may be administered as a single dose or divided into multiple doses.

[00106] The dosage regimen utilizing any flagellin related composition (and/or additional agents) described herein can be selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the subject; the severity of the condition to be treated; the route of administration; the renal or hepatic function of the subject; the pharmacogenomic makeup of the individual; and the specific compound of the invention employed. Any flagellin related composition (and/or additional agents) described herein can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, any flagellin related composition (and/or additional agents) described herein can be administered continuously rather than intermittently throughout the dosage regimen.

[00107] In some embodiments, the composition of the present invention (e.g. the described adjuvants and vaccines) may be used in conjunction with one or more additional agents. In some embodiments, the invention pertains to co-administration and/or co-formulation. Any of the compositions described herein may be co-formulated and/or co-administered.

[00108] In some embodiments, any composition described herein acts synergistically when co-administered with another agent and is administered at doses that are lower than the doses commonly employed when such agents are used as monotherapy. In various embodiments, any agent referenced herein may be used in combination with any of the composition described herein.

[00109] In some embodiments, the present invention pertains to additional agents described elsewhere herein. In one embodiment, any flagellin-related agent or composition comprising the same may be used with agents that stimulate NOD receptors (e.g. NOD1 and NOD2 agonists, such as peptidoglycan, C12-iE-DAP and L18-MDP) and as described in Infect Immun. Oct 2013; 81(10): 3855–3864, the contents of which are hereby incorporated by reference in their entirety.

[00110] In some embodiments, the present invention pertains to chemotherapeutic agents as additional agents.

[00111] Examples of chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and CYTOXAN cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (e.g., bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; cally statin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (e.g., cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB 1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine,

and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., Agnew, Chem. Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, 5 authramycin, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN doxorubicin (including morpholino- doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxy doxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, 10 streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as 15 minoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; def of amine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; 20 procarbazine; PSK polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (e.g., T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE Cremophor-free, albumin-engineered 25 nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, 111.), and TAXOTERE doxetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; GEMZAR gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE. vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) 30 (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); lapatinib (Tykerb); inhibitors of PKC- α , Raf, H-Ras, EGFR (e.g., erlotinib (Tarceva)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above. In addition, the methods of treatment can further include the use of 35 radiation. In addition, the methods of treatment can further include the use of photodynamic therapy.

[00112] In some embodiments, the flagellin-based agents (and/or additional agents) described herein, include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the composition such that covalent attachment does not prevent the activity of the composition. For example, but not by way of limitation, derivatives include composition that have been modified by, *inter alia*, glycosylation, lipidation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, *etc.* Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of turicamycin, *etc.* Additionally, the derivative can contain one or more non-classical amino acids.

[00113] In still other embodiments, the flagellin-based agents (and/or additional agents) described herein further comprise a cytotoxic agent, comprising, in illustrative embodiments, a toxin, a chemotherapeutic agent, a radioisotope, and an agent that causes apoptosis or cell death. Such agents may be conjugated to a composition described herein.

[00114] The flagellin-based agents (and/or additional agents) described herein may thus be modified post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent moieties, or functional moieties such as for example streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, and radioactive materials.

[00115] Illustrative cytotoxic agents include, but are not limited to, methotrexate, aminopterin, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine; alkylating agents such as mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU), mitomycin C, lomustine (CCNU), 1-methylnitrosourea, cyclophosphamide, mechlorethamine, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin and carboplatin (paraplatin); anthracyclines include daunorubicin (formerly daunomycin), doxorubicin (adriamycin), detorubicin, carminomycin, idarubicin, epirubicin, mitoxantrone and bisantrene; antibiotics include dactinomycin (actinomycin D), bleomycin, calicheamicin, mithramycin, and anthramycin (AMC); and antimetabolic agents such as the vinca alkaloids, vincristine and vinblastine. Other cytotoxic agents include paclitaxel (taxol), ricin, pseudomonas exotoxin, gemcitabine, cytochalasin B, gramicidin D, ethidium bromide, emetine, etoposide, tenoposide, colchicin, dihydroxy anthracin dione, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons, and mixtures of these cytotoxic agents.

[00116] Further cytotoxic agents include, but are not limited to, chemotherapeutic agents such as carboplatin, cisplatin, paclitaxel, gemcitabine, calicheamicin, doxorubicin, 5-fluorouracil, mitomycin C, actinomycin D, cyclophosphamide, vincristine, bleomycin, VEGF antagonists, EGFR antagonists, platins, taxols, irinotecan, 5-fluorouracil, gemcytabine, leucovorine, steroids, cyclophosphamide, melphalan, vinca alkaloids (*e.g.*, vinblastine,

vincristine, vindesine and vinorelbine), mustines, tyrosine kinase inhibitors, radiotherapy, sex hormone antagonists, selective androgen receptor modulators, selective estrogen receptor modulators, PDGF antagonists, TNF antagonists, IL-1 antagonists, interleukins (e.g. IL-12 or IL-2), IL-12R antagonists, Toxin conjugated monoclonal antibodies, tumor antigen specific monoclonal antibodies, Erbitux, Avastin, Pertuzumab, anti-CD20 antibodies, Rituxan, ocrelizumab, ofatumumab, DXL625, HERCEPTIN®, or any combination thereof. Toxic enzymes from plants and bacteria such as ricin, diphtheria toxin and Pseudomonas toxin may be conjugated to the therapeutic agents (e.g. antibodies) to generate cell-type-specific-killing reagents (Youle, *et al.*, Proc. Nat'l Acad. Sci. USA 77:5483 (1980); Gilliland, *et al.*, Proc. Nat'l Acad. Sci. USA 77:4539 (1980); Krolick, *et al.*, Proc. Nat'l Acad. Sci. USA 77:5419 (1980)).

[00117] Other cytotoxic agents include cytotoxic ribonucleases as described by Goldenberg in U.S. Pat. No. 6,653,104. Embodiments of the invention also relate to radioimmunoconjugates where a radionuclide that emits alpha or beta particles is stably coupled to the antibody, or binding fragments thereof, with or without the use of a complex-forming agent. Such radionuclides include beta-emitters such as Phosphorus-32, Scandium-47, Copper-67, Gallium-67, Yttrium-88, Yttrium-90, Iodine-125, Iodine-131, Samarium-153, Lutetium-177, Rhenium-186 or Rhenium-188, and alpha-emitters such as Astatine-211, Lead-212, Bismuth-212, Bismuth-213 or Actinium-225.

[00118] Illustrative detectable moieties further include, but are not limited to, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, beta-galactosidase and luciferase. Further illustrative fluorescent materials include, but are not limited to, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin and dansyl chloride. Further illustrative chemiluminescent moieties include, but are not limited to, luminol. Further illustrative bioluminescent materials include, but are not limited to, luciferin and aequorin. Further illustrative radioactive materials include, but are not limited to, Iodine-125, Carbon-14, Sulfur-35, Tritium and Phosphorus-32.

[00119] In some embodiments, the subject and/or animal is a mammal, e.g., a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, rabbit, sheep, or non-human primate, such as a monkey, chimpanzee, or baboon. In other embodiments, the subject and/or animal is a non-mammal, such, for example, a zebrafish. In some embodiments, the subject and/or animal may comprise fluorescently-tagged cells (with e.g. GFP). In some embodiments, the subject and/or animal is a transgenic animal comprising a fluorescent cell.

[00120] In some embodiments, the subject and/or animal is a human. In some embodiments, the human is a pediatric human. In other embodiments, the human is an adult human. In other embodiments, the human is a geriatric human. In other embodiments, the human may be referred to as a patient.

[00121] In certain embodiments, the human has an age in a range of from about 0 months to about 6 months old, from about 6 to about 12 months old, from about 6 to about 18 months old, from about 18 to about 36 months old, from about 1 to about 5 years old, from about 5 to about 10 years old, from about 10 to about 15

years old, from about 15 to about 20 years old, from about 20 to about 25 years old, from about 25 to about 30 years old, from about 30 to about 35 years old, from about 35 to about 40 years old, from about 40 to about 45 years old, from about 45 to about 50 years old, from about 50 to about 55 years old, from about 55 to about 60 years old, from about 60 to about 65 years old, from about 65 to about 70 years old, from about 70 to about 75 years old, from about 75 to about 80 years old, from about 80 to about 85 years old, from about 85 to about 90 years old, from about 90 to about 95 years old or from about 95 to about 100 years old.

[00122] In other embodiments, the subject is a non-human animal, and therefore the invention pertains to veterinary use. In a specific embodiment, the non-human animal is a household pet. In another specific embodiment, the non-human animal is a livestock animal.

[00123] The invention provides kits that can simplify the administration of any agent described herein. An illustrative kit of the invention comprises any composition described herein in unit dosage form. In one embodiment, the unit dosage form is a container, such as a pre-filled syringe, which can be sterile, containing any agent described herein and a pharmaceutically acceptable carrier, diluent, excipient, or vehicle. The kit can further comprise a label or printed instructions instructing the use of any agent described herein. The kit may also include a lid speculum, topical anesthetic, and a cleaning agent for the administration location. The kit can also further comprise one or more additional agent described herein. In one embodiment, the kit comprises a container containing an effective amount of a composition of the invention and an effective amount of another composition, such those described herein.

[00124] The following definitions are used in connection with the invention disclosed herein. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of skill in the art to which this invention belongs.

[00125] As used herein, “a,” “an,” or “the” can mean one or more than one.

[00126] Further, the term “about” when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 10% of that referenced numeric indication. For example, the language “about 50” covers the range of 45 to 55.

[00127] An “effective amount,” when used in connection with medical uses is an amount that is effective for providing a measurable treatment, prevention, or reduction in the rate of pathogenesis of a disease of interest.

[00128] As used herein, something is “decreased” if a read-out of activity and/or effect is reduced by a significant amount, such as by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or more, up to and including at least about 100%, in the presence of an agent or stimulus relative to the absence of such modulation. As will be understood by one of ordinary skill in the art, in some embodiments, activity is decreased and some downstream read-outs will decrease but others can increase.

[00129] Conversely, activity is “increased” if a read-out of activity and/or effect is increased by a significant amount, for example by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or more, up to and including at least about 100% or more, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 50-fold, at least about 100-fold, in the presence of an agent or stimulus, relative to the absence of such agent or stimulus.

[00130] As referred to herein, all compositional percentages are by weight of the total composition, unless otherwise specified. As used herein, the word “include,” and its variants, is intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that may also be useful in the compositions and methods of this technology. Similarly, the terms “can” and “may” and their variants are intended to be non-limiting, such that recitation that an embodiment can or may comprise certain elements or features does not exclude other embodiments of the present technology that do not contain those elements or features.

[00131] Although the open-ended term “comprising,” as a synonym of terms such as including, containing, or having, is used herein to describe and claim the invention, the present invention, or embodiments thereof, may alternatively be described using alternative terms such as “consisting of” or “consisting essentially of.”

[00132] As used herein, the words “preferred” and “preferably” refer to embodiments of the technology that afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the technology.

[00133] The amount of compositions described herein needed for achieving a therapeutic effect may be determined empirically in accordance with conventional procedures for the particular purpose. Generally, for administering therapeutic agents (e.g. flagellin-based agents, flagellin-based agents (and/or additional agents) described herein) for therapeutic purposes, the therapeutic agents are given at a pharmacologically effective dose. A “pharmacologically effective amount,” “pharmacologically effective dose,” “therapeutically effective amount,” or “effective amount” refers to an amount sufficient to produce the desired physiological effect or amount capable of achieving the desired result, particularly for treating the disorder or disease. An effective amount as used herein would include an amount sufficient to, for example, delay the development of a symptom of the disorder or disease, alter the course of a symptom of the disorder or disease (e.g., slow the progression of a symptom of the disease), reduce or eliminate one or more symptoms or manifestations of the disorder or disease, and reverse a symptom of a disorder or disease. For example, administration of therapeutic agents to a patient suffering from cancer provides a therapeutic benefit not only when the underlying condition is eradicated or ameliorated, but also when the patient reports a decrease in the severity or duration of the symptoms associated with the disease, e.g., a decrease in tumor burden, a decrease in circulating tumor cells, an increase

in progression free survival. Therapeutic benefit also includes halting or slowing the progression of the underlying disease or disorder, regardless of whether improvement is realized.

[00134] Effective amounts, toxicity, and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to about 50% of the population) and the ED50 (the dose therapeutically effective in about 50% of the population). The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. In some embodiments, compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from in vitro assays, including, for example, cell culture assays. Also, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 as determined in cell culture, or in an appropriate animal model. Levels of the described compositions in plasma can be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

[00135] In certain embodiments, the effect will result in a quantifiable change of at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 70%, or at least about 90%. In some embodiments, the effect will result in a quantifiable change of about 10%, about 20%, about 30%, about 50%, about 70%, or even about 90% or more. Therapeutic benefit also includes halting or slowing the progression of the underlying disease or disorder, regardless of whether improvement is realized.

[00136] In certain embodiments, a pharmacologically effective amount that will treat cancer will modulate the symptoms typically by at least about 10%, at least about 20%, at least about 30%, at least about 40%, or at least about 50%. In illustrative embodiments, such modulations will result in, for example, statistically significant and quantifiable changes in the numbers of cancerous cells.

[00137] This invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1: Preparation of the Model Vaccine

[00138] Alum adjuvant (IMJECT ALUM, THERMO) was used in combination with CBLB502 to challenge the mice with a model antigen, ovalbumin (OVA). Vaccine was comprised of the following components as shown in

TABLE A:

Component	Amount (per mouse)
Ovalbumin	10mg
CBLB502	1ug/10ug
Alum Adjuvant	500ug

[00139] To achieve excellent absorption CBLB502 (either 1 ug or 10 ug) was mixed with 10 mg of OVA and 500 µg of Alum adjuvant for 30 minutes at 300 rpm at room temperature.

Example 2: Mouse Immunization Study

[00140] C57Bl/6 male mice (10 weeks of age, n=6 for each experiment) were used for injection. Mice were divided into five groups with six animals in each group (n=6). Mice were injected with 100 µl of above described vaccine in each of the hind legs (200 µl total per mouse) as shown in **TABLE B**.

	Ovalbumin	CBLB502	Alum Adjuvant
Group 1			
Group 2	10mg		
Group 3	10mg		500ug
Group 4	10mg	1ug	500ug
Group 5	10mg	10ug	500ug

Two weeks after the initial immunization mice were challenged with an additional booster following the same setup as the original injection. Plasma was collected one, two and four weeks after booster via mandibular vein.

Using an ELISA based assay total IgG, IgG1, IgG2a, IgG2b and IgG3 were measured. Results are shown in **FIGs. 2-3**. In the figures, Group 1 is also called "Untreated," Group 2 is also called "OVA," Groups 3 is also called "OVA + Alum," Group 4 is also called "SA 1 µg," and Group 5 is also called "SA 10 µg."

[00141] **FIG. 2** shows ELISA data for the average response of the five aforementioned mouse treatment groups at the time point of 1 week post-boost. **FIG. 3** shows ELISA data for the average response of the five aforementioned mouse treatment groups at the time point of 2 weeks post-boost. **FIG. 4** shows ELISA data for the average response of the five aforementioned mouse treatment groups at the time point of 4 weeks post-boost.

Example 3: Evaluation of CBLB502 Binding to ALHYDROGEL

[00142] The binding capacity of ALHYDROGEL for CBLB502 was evaluated. ALHYDROGEL adjuvant 2%, aluminium hydroxide wet gel (colloidal) suspension (INVIVIGEN Catalog # vac-alu-250), was mixed with CBLB502 at different volume ratios, resulting in 10 to 52% ALHYDROGEL suspension in the reaction. CBLB502 concentration was maintained at 10 µg/ml, corresponding to a 1 µg/100 µl inoculation dose (100 µl inoculation volume is the recommended maximum volume for subcutaneous injection of antigen/adjuvant mixtures per injection site for mice). After overnight incubation at 4°C, the tubes were centrifuged to sediment ALHYDROGEL and unbound CBLB502, remaining in solution was measured by ELISA. The amounts of adsorbed CBLB502 were calculated as difference between total protein added to the mixture and unbound CBLB502 (**TABLE C**). The maximum adsorption of CBLB502, more than 99.9% bound, was observed when the reactions were formulated with 40 to 52 µl ALHYDROGEL/100 µl; at lower ratios, the binding efficiency decreased gradually to about 83% at 10 µl/100 µl ALHYDROGEL suspension.

TABLE C: CBLB502 adsorption at different ALHYDROGEL 2% volume ratios. The binding reactions were composed of CBLB502, PBS and the indicated volumes of ALHYDROGEL adjuvant 2%.

	ALHYDROGEL 2% μl/100μl	CBLB502 dose μg/100μl	Total CBLB502 ng/ml	Unbound ng/ml	Bound ng/ml	Bound (%)
1	52	1	10000	2.1	9997.9	99.98
2	50	1	10000	1.6	9998.4	99.98
3	40	1	10000	2.9	9997.1	99.97
4	30	1	10000	22.7	9977.3	99.77
5	20	1	10000	173.4	9826.6	98.27
6	10	1	10000	1692.5	8307.5	83.07

[00143] CBLB502 binding to ALHYDROGEL was also evaluated at different CBLB502 doses. Adsorption of CBLB502 to ALHYDROGEL (52 μl/100 μl ALHYDROGEL adjuvant 2%) was determined at CBLB502 doses, ranging from approximately 0.02 to 20 μg per 100 μl. The reactions were incubated as described above and unbound CBLB502 was measured by ELISA. The results (**TABLE D**) demonstrated that almost complete binding of the drug to ALHYDROGEL, 99.82 to 99.99% bound, was accomplished at all tested CBLB502 concentrations.

TABLE D: Efficiency of CBLB502 binding to ALHYDROGEL in PBS. The incubations were performed with CBLB502 at the indicated doses in PBS and 52 μl/100 μl ALHYDROGEL adjuvant 2%.

	CBLB502 dose μg/100μl	Total CBLB502 ng/ml	Unbound ng/ml	Bound ng/ml	Bound (%)
1	20	200000	199.3	199800.7	99.90
2	5	50000	90.6	49909.4	99.82
3	1.25	12500	0.79	12499.2	99.99
4	0.31	3125.0	0.029	3124.97	99.99
5	0.078	781.25	0.023	781.23	99.99
6	0.02	195.31	0.011	195.30	99.99

[00144] CBLB502 binding to ALHYDROGEL in the presence of TT-SMA vaccine was evaluated. Binding efficiency of CBLB502 to ALHYDROGEL was tested in the presence of a vaccine candidate TT-SMA, succinyl methamphetamine (SMA) hapten conjugated to tetanus toxoid. In a separate study, shown below, this vaccine was administered to mice at a 32μg/100 μl dose to compare efficacy with and without CBLB502. This TT-SMA dose was co-incubated with CBLB502 (0.02 to 20 μg/100 μl) and ALHYDROGEL (52 μl/100 μl) under standard conditions and unbound CBLB502 was measured by ELISA. The binding efficiency was 99.7 to 99.9% at all tested CBLB502 concentrations, indicating that CBLB502 adsorption to ALHYDROGEL was unaffected with addition of TT-SMA to the reaction mixture

TABLE E. CBLB502 binding to ALHYDROGEL in the presence of TT-SMA. The incubations were performed with CBLB502 at the indicated doses in PBS, 52 µl/100 µl ALHYDROGEL adjuvant 2% and 32µg/100 µl TT-SMA conjugate.

	CBLB502 dose µg/100µl	Total CBLB502 ng/ml	Unbound ng/ml	Bound ng/ml	Bound (%)
1	20	200000	668.88	199331.1	99.67
2	5	50000	50.21	49949.8	99.90
3	1.25	12500	11.44	12488.6	99.91
4	0.31	3125.0	1.65	3123.4	99.95
5	0.078	781.25	0.13	781.1	99.98
6	0.02	195.31	0.06	195.2	99.96

5 Example 4: Analysis of *in vitro* CBLB502 Activity after Adsorption to ALHYDROGEL

[00145] An activity assay of CBLB502/ALHYDROGEL using 293-hTLR5-LacZ reporter cells was undertaken. Biological activity of CBLB502 after adsorption to ALHYDROGEL was tested using an *in vitro* assay which measures activation of a NF-κB-controlled reporter enzyme, β-galactosidase, in HEK293-hTLR5::NF-κB-lacZ (293-hTLR5-LacZ) cells. Since 293-hTLR5-LacZ cells express only one cell membrane-bound toll-like receptor, human TLR5, CBLB502 activity could be determined without interference from other toll-like receptor ligands such as LPS (endotoxin).

[00146] Specific induction of the β-galactosidase reporter by CBLB502/ALHYDROGEL was demonstrated after incubating the cells with a serially diluted CBLB502/ALHYDROGEL formulation (5 µg/100 µl), starting with a 2000-fold dilution in cell growth media and resulting in the concentration range of ALHYDROGEL-adsorbed CBLB502 from 25 to 0.004 ng/ml (**FIG. 5**). The maximum β-galactosidase activity was observed at 0.93 ng/ml ALHYDROGEL-adsorbed CBLB502; this value was consistent with typical results of the assay using soluble CBLB502 where the reporter enzyme activity would normally peak around 1 ng/ml. ALHYDROGEL alone tested at same dilutions did not induce the reporter. Therefore, it could be concluded, *inter alia*, that CBLB502 retains its biological activity after adsorption to ALHYDROGEL and becomes readily available for interaction with its target receptor (TLR5) under regular cell culture conditions.

[00147] Next, CBLB502/ALHYDROGEL suspensions were prepared at CBLB502 inoculation doses ranging from 2.5 to 0.00015 µg/100 µl and were added to 293-hTLR5-LacZ cells at 200-fold dilution (**FIG. 6**). NF-κB-inducing activity followed a typical dose-response pattern and was measured at sub-nanogram doses of CBLB502/ALHYDROGEL (less than 0.001 µg/100 µl), indicating that at such low doses, CBLB502 was not irreversibly bound to the alum adjuvant and remained active.

[00148] An activity titration of CBLB502/ALHYDROGEL at different CBLB502 doses was undertaken. CBLB502/ALHYDROGEL suspensions were titrated in cell culture media and incubated with 293-hTLR5-LacZ cells. CBLB502 standards were included in the assay to compare the recovered activity. The resulting reporter

enzyme activity was plotted against theoretical concentrations of ALHYDROGEL-bound CBLB502, assuming that the protein was completely available (**FIG. 7**).

[00149] The results of titration assay demonstrated that at a formulation dose of 20 µg/100 µl, the activity of CBLB502 followed the expected concentrations, essentially matching the activity of soluble standards. However, reduction of the dose 64- and 256-fold to respectively, about 0.31 and 0.078 µg/100 µl, resulted in lower relative recovery of NF-κB-inducing activity to approximately 15-30%. This apparent loss of CBLB502 activity could be due to stronger binding of the particular amounts of the protein relative to the amounts of alum in the formulation. These fixed amounts could represent a small fraction of total CBLB502 at the higher doses such as 20 µg/100 µl and would not affect significantly the measured activity. However, at lower doses, this tightly adsorbed fraction may become substantial compared to the total protein, leading to a lower measurable activity of CBLB502

Example 5: Assessment of CBLB502/ALHYDROGEL Short-Term Storage Stability at 4°C

[00150] A formulation of 1 µg/100 µl CBLB502 (52 µl/100 µl ALHYDROGEL 2%) was prepared and tested for activity after 2 and 6 days storage at 4°C. The results of CBLB502 activity assay using 293-hTLR5-LacZ reporter cells are shown in **FIG. 8**. Based on comparison with the soluble CBLB502 standard, it can be concluded that NF-κB-inducing activity of ALHYDROGEL-adsorbed CBLB502 remained unchanged after 6 days under the storage conditions.

Example 6: Evaluation of CBLB502 Dissociation from ALHYDROGEL in PBS and Cell Media

[00151] Aliquots of CBLB502/ALHYDROGEL (1 µg/100 µl dose; 52 µl/100 µl ALHYDROGEL 2%) were re-suspended in PBS or cell media (DMEM supplemented with 10% FBS) and incubated for 3 hr at 22°C and 37°C. After centrifugation to remove ALHYDROGEL, dissociated CBLB502 was measured in supernatants by ELISA. The calculation of CBLB502 amounts that were recovered during incubation demonstrated that the protein remained stably associated with ALHYDROGEL in PBS; however, in cell media, 28 and 53% CBLB502 was released from the complex at 22°C and 37°C, respectively (**TABLE F**). Such rapid dissociation from ALHYDROGEL in media may be facilitated by serum proteins which could displace CBLB502 from the complex with alum, explaining how the protein becomes readily available in the cell-based activity assay.

TABLE F: CBLB502 recovery from ALHYDROGEL after incubation in PBS and cell media

Incubation conditions	CBLB502 dose µg/100µl	Total CBLB502 ng/ml	22°C		37°C	
			Recovered ng/ml	Desorption (%)	Recovered ng/ml	Desorption (%)
PBS	1	10000	2.3	0.02	33.2	0.33
Media	1	10000	2807.6	28.1	5300.9	53.0

Example 7: Quantitation of CBLB502 and Cytokine Levels in Mouse Serum after Subcutaneous Administration of CBLB502/ALHYDROGEL.

[00152] An animal study was conducted to evaluate feasibility of methods to determine the levels of CBLB502 and cytokines in serum after administration of a CBLB502/ALHYDROGEL formulation. The study design is presented in **TABLE G**. In this study, mice were injected subcutaneously with a 1 µg dose CBLB502 adsorbed to 52 µl/100 µl ALHYDROGEL 2% and serum samples were collected between 0.5 and 24 hr post inoculation.

TABLE G. Study design.

Group No.	Group Size	Age Strain Sex	Treatment	Injection volume, µl	Time Point, blood collection	Evaluation
1	3	8 wk old C57BL/6 Female	ALHYDROGEL/PBS, sc	100	1 hr	Serum prepared from collected blood and analyzed for entolimod concentration and cytokine levels by ELISA
2	3		ALHYDROGEL/PBS/CBLB502, (1 µg/100 µl CBLB502; 52 µl/100 µl ALHYDROGEL 2%) sc	100	30 min	
3	3			100	1h	
4	3			100	2h	
5	3			100	4h	
6	3			100	8h	
7	3			100	24h	

[00153] Serum concentrations of CBLB502 were determined by ELISA and were found at measurable levels in all groups of animals inoculated with CBLB502/ALHYDROGEL. The pharmacokinetic profile representing per-group mean serum CBLB502 concentrations is shown in **FIG. 9**. The maximum mean CBLB502 concentration, 0.516 ng/ml, was observed at the first blood collection time point, 0.5 hr post administration; the minimum concentration was 0.012 ng/ml at 24 hr. Half-time elimination of CBLB502 was calculated at 2.4 hr. As discussed above, CBLB502 dissociated rapidly from the complex with ALHYDROGEL after incubation in cell media, containing 10% fetal bovine serum. Therefore, it should be expected to see the protein released from alum into animal bloodstream shortly after inoculation.

[00154] The concentrations of three cytokines, KC, G-CSF and IL-6, were measured in the collected mouse serum samples by ELISA (**FIG. 10**). Rapid induction of KC and IL-6 was detected 1 hr after CBLB502/ALHYDROGEL administration; the maximum per-group mean concentrations, 29624 and 479 pg/ml, respectively, were determined at 2 hr time point. After reaching maximum, the blood levels of these cytokines decreased to baseline at 4 to 8 hr post injection. The serum G-CSF concentration increased about an hour later compared to KC and IL-6 and was maximal between 2 and 4 hr after CBLB502/ALHYDROGEL administration (7141 and 7169 pg/ml per-group mean concentrations at 2 and 4 hr, respectively). However, CBLB502-induced G-CSF level was decreasing at a slower rate, remaining above baseline at 24 hr time point. In general, the kinetics of cytokine induction after CBLB502/ALHYDROGEL administration was similar to the kinetics observed after injection of the soluble CBLB502 preparations, suggesting consistent effects of this drug formulation.

Example 8: CBLB502 Adjuvant with Methamphetamine Vaccine

[00155] This study evaluated CBLB502 adjuvant at a 32µg dose of human methamphetamine vaccine (SMA-TT) to achieve high and long lasting levels of anti-MA antibody in mice. A low to high dose of CBLB502 ranging from 0.03 to 20µg with TT-SMA and alum was studied. Ten groups (n=5) of Balb/c female mice were employed to test 0, 0.03, 0.1, 0.3, 1, 3, 10, 20 µg of 502 in combination with 32µg of TT-SMA and 1.5 mg alum compared to unvaccinated controls and TT-SMA with 502 alone. All vaccinated groups were administered two boosters, at 3 and 6 weeks after the initial vaccination. Levels of anti-MA IgG were assessed by ELISA in pooled sera samples collected at 2, 4, and 6 weeks after initial vaccination. The antibody levels were higher at 0.03 to 0.3 µg 502 in comparison to the higher doses (1 to 20 µg) of 502. In this experiment, antibody levels were 2 fold higher in the groups with 0.1 µg of 502 in combination with TT-SMA and alum (group 4) than in the comparison group with TT-SMA and alum alone (group 1) and approx. 4 fold higher when compared to a group with TT-SMA and 502 alone (group 2), as demonstrated in **FIG. 11** (panel A). Anti-502 antibodies in the same sera sample were also evaluated. As shown in **FIG. 11** (panel B), the level of anti-502 antibodies was minimal at the low dose range of 502 and increases at higher doses of 502.

Example 9: Generation of Vaccines with Various Antigens

[00156] In this Example, the composition of an adjuvant component for adjuvant-enhanced vaccine is established by testing a range of doses of CBLB502 and CBLB502 optionally combined with an antigen, such as, by way of non-limitation, NOD1 agonist C12-iE-DAP. Additionally, characterization of the effects of these agents on humoral immune response to a model antigen (ovalbumin) is undertaken.

[00157] As shown above, aluminum hydroxide and CBLB502 form a complex which, without wishing to be bound by theory, is stabilized by electrostatic forces as substances with opposite charges in aqueous medium. To test relative efficacy of different compositions of the adjuvant component of the vaccine, a standard dose of aluminum is used (1.5 µg per injection) loaded with a range of doses of CBLB502 (up to 30 µg and including 10 µg, 3 µg, 1 µg, 0.3 µg and 0.1 µg). CBLB502 in aqueous solution is added to the alum suspension in PBS and stirred for 10 min at room temperature. Aluminum hydroxide is then spun down by centrifugation and traces of CBLB502 in supernatants are determined using two quantitative analytical assays: (i) ELISA-based and (ii) reporter cell based (TLR5-positive 293 cells carrying NF-kB responsive reporter and calibrated for detection of CBLB 502 in the medium). In light of, *inter alia*, the above Examples, it is expected that a substantial fraction of CBLB 502 will be stably bound to alum under these conditions and within the chosen range of doses of TLR5 agonists.

[00158] To determine whether this adjuvant remains localized at the vaccine injection site, adult BALB/c female mice (n=10) carrying in their germ line firefly luciferase cDNA under the control of NF-kB-responsive promoter will be used. These “NF-kB reporter mice” are a sensitive tool for the detection of release of CBLB502 from the injection site by monitoring the luciferase activity in TLR5-positive tissues such as liver or small

intestine. Only one dose of CBLB502-containing adjuvant formulation will be used that corresponds to the highest dose that demonstrated stability as a composition with Alum in vitro (presumably 30 µg). Two doses of free CBLB502 (1 and 3 µg in aqueous solution, s.c. injection) will be used for comparison as positive controls of systemically distributed TLR5 agonist. Luciferase detection will be done using a luminescent imager in vivo following luciferin injection at 2, 4, 6 and 18 hours post injection of the adjuvant. For a more accurate and sensitive detection, 3 animals from each group are sacrificed 6 hours post injection of the adjuvant formulation and tissues will be collected from the injection site, lymph nodes nearest to injection site and the liver. The level of luciferase activity indicative of the presence of CBLB 502 is quantitated in these tissue samples following tissue lysis. In addition to functional detection of CBLB 502 based on its expected NF-κB-inducing activity, an alternative analytical method of an established sensitive ELISA assay for direct detection of CBLB502 in tissue extracts is used.

[00159] Adjuvant formulations containing the above-described range of doses of CBLB502 are mixed with 10 mg of ovalbumin and vaccination is performed, as described herein, followed by the ELISA determination of titers of different classes of anti-albumin IgG (IgG1, IgG2a, IgG2b and IgG3) antibodies. The results are compared with “Alum only” control. Determination of the effects on different IgG types provides definition of the degrees of engagement of different immunization paths (e.g. T_{H1}- versus T_{H2}-mediated routes) by the adjuvant.

[00160] After determination of the composition of the adjuvant (in terms of anti-ovalbumin antibody inducing efficacy), additional enhancement of immunization is tested by adding into the formulation a NOD1 agonist, C12-iE-DAP.

[00161] Also, determination of vaccine efficacy at different CBLB 502 doses to generate antibodies to antigen is undertaken in mice. Female mice are chosen because they have stronger immune responses than male mice and BALB/c mice are a commonly used strain in immunological studies due to their robust immune responses. At the beginning of the study, mice weigh about 25 g and are group-housed (5 per cage) in standard acrylic cages (7" X 11" X 5") with corncob bedding polycarbonate and tops. Mice will have *ad libitum* access to food (Harlan) and water. The vivarium will be maintained at 22 ± 1°C and on a 12:12 light/dark cycle (lights on at 6 AM). All experimental procedures are approved by the Institutional Animal Care and Use Committee (IACUC) and are within the guidelines delineated in the Guide for the Care and Use of Laboratory Animals.

[00162] Mice are intramuscularly injected with the vaccine in the gluteal muscle. Each group is optionally immunized with a booster dose with the same vaccine formulation at 3 and 6 weeks post initial immunization. If persistent antibody levels are not maintained for at least 4 weeks, an additional immunization will be given at week 10.

[00163] Blood is drawn at 14, 28, 42, 56 and 84 days post initial immunization and allowed to clot at room temperature for 2 hours. Serum is collected after centrifugation at 4000 rpm for 15 min. Samples are stored at -80 degrees until ready to run the enzyme linked immunosorbent assays (ELISA). In the context of a vaccine with

antigen X, anti-antigen X specific antibodies are assessed by ELISAs. To measure the antibodies, ELISA plates (Immulon 2HB, Daigger, Vernon Hills, IL) are coated overnight in carbonate buffer (0.05 M; pH 9.6) using fish gelatin, which is a heterologous carrier protein, as the conjugate partner for SMA. Pooled or individual serum samples are added to plates in 2 fold serial dilutions starting at 1K in phosphate buffered saline (PBS)-Tween (0.1%) and incubated for 2 hours. Plates are washed with PBS-Tween prior to adding goat anti-mouse IgG conjugated to horse-radish peroxidase (HRP) (Southern Biotech, Birmingham, AL). Plates are incubated for another 30 min and washed before adding substrate (Tetramethylbenzidine, Sigma, St. Louis, MO). Plates are incubated for 45 min in the dark prior to stopping the reaction with 1M HCl. The optical density (OD) of the resulting dark spots on the plates from the antibody with antigen X linking are used to measure titer levels on a microplate reader (iMark Microplate Absorbance Reader) using Microplate Manager v 6.1 software.

[00164] ELISA data is analyzed using SigmaPlot (Systat Software Inc.) and background antibody binding to the carrier alone will be subtracted from each sample. Comparisons are made in each plate with a standard curve of purified mouse IgG (Sigma) bound directly in the wells in serial dilution.

Example 10: Comparison of CBLB502-based Adjuvants/Vaccines with Flagellin-based Adjuvants/Vaccines

[00165] The CBLB502/alum adjuvant of the above Examples is tested head to head against a flagellin/alum adjuvant using the methods and study design of, for instance, Example 9 (optionally with an antigen). Further, the alum binding and dissociation studies, as well as the mouse evaluation studies, all described in the above Examples, are repeated with a flagellin/alum adjuvant for comparison to the CBLB502/alum adjuvant. The efficacy of the CBLB502 variant, *i.e.*, CBLB502-S33MX is also tested.

In one comparison experiment, a group of mice (n=6) is immunized with TT-SMA, which is a methamphetamine vaccine and is used illustratively, co-adsorbed onto Alhydrogel with either flagellin, CBLB502, or CBLB502-S33MX. Immunization is carried out on days 0, 14, and 28 of the study. Each immunization dose includes 32 µg of TT-SMA and adjuvants as follows: Group 1: 0.03 µg flagellin, Group 2: 0.1 µg flagellin, Group 3: 0.3 µg flagellin, Group 4: 1 µg flagellin, Group 5: 0.03 µg CBLB502, Group 6: 0.1 µg CBLB502, Group 7: 0.3 µg CBLB502, Group 8: 1 µg CBLB502, Group 9: 0.03 µg CBLB502-S33MX, Group 10: 0.1 µg CBLB502-S33MX, Group 11: 0.3 µg CBLB502-S33MX, and Group 12: 1 µg CBLB502-S33MX

Serum samples are collected and analyzed on days -1 (pre-immune serum), 14 (post-prime), 28 (post first boost), and 35 (post second boost). For example, the serum samples are analyzed for the levels of anti-methamphetamine and anti-entolimod antibodies. Analysis indicates that CBLB502/alum and CBLB502-S33MX/alum cause a broader, more diverse, more robust and longer immunostimulatory effect than flagellin/alum. In addition, CBLB502/alum and CBLB502-S33MX/alum activate both T_{H1} and T_{H2} -mediated immune response and a greater T_{H1} -mediated immune response than flagellin/alum.

EQUIVALENTS

[00166] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

[00167] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

INCORPORATION BY REFERENCE

[00168] All patents and publications referenced herein are hereby incorporated by reference in their entireties.

[00169] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[00170] As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

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CLAIMS

What is claimed is:

1. A vaccine composition, comprising
 - (a) an adjuvant comprising a flagellin-based agent, or variants thereof having TLR5 agonist activity and an aluminum gel or salt and
 - (b) an antigen.
2. The vaccine of claim 1, wherein the vaccine causes a relative increase in a titer of at least 1, at least 2, at least 3, or all of IgG1, IgG2a, IgG2b, and IgG3 antibodies as compared to the vaccine in the absence of the flagellin-based agent.
3. The vaccine of any one of claims 1-2, wherein the flagellin-based agent comprises an amino acid sequence of one or more of the sequences in Table 1, or variants thereof having TLR5 agonist activity.
4. The vaccine of any one of claims 1-3, wherein the flagellin-based agent comprises an amino acid sequence having at least 80% identity, or at least 85% identity, or at least 90% identity, or at least 95% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity with one or more of SEQ ID Nos.: 1-252.
5. The vaccine of claim 1, wherein the flagellin-based agent is a variant of SEQ ID NO: 1.
6. The vaccine of claim 1, wherein the flagellin-based agent comprises the amino acid sequence of SEQ ID NO: 2.
7. The vaccine of any one of claims 1-6, wherein the flagellin-based agent is a variant of CBLB 502 (*i.e.* an amino acid sequence of SEQ ID NO: 2).
8. The vaccine of any one of claims 1-7, wherein the flagellin-based agent comprises an amino acid sequence having at least 80% identity, or at least 85% identity, or at least 90% identity, or at least 95% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity with one or more sequences in Table 1.
9. The vaccine of any one of claims 1-8, wherein the flagellin-based agent comprises an amino acid sequence having at least 80% identity, or at least 85% identity, or at least 90% identity, or at least 95% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity with one or more of CBLB502-S33ML (SEQ ID NO: 35), CBLB502-485CT (CBLB533, SEQ ID NO: 71), and CBLB502-S33MX (SEQ ID NO: 150).
10. The vaccine of any one of claims 1-9, wherein the flagellin-based agent is a flagellin derivative from a thermophilic microorganism, optionally selected from SEQ ID NOs: 243-252.
11. The vaccine of any one of claims 1-10, wherein the flagellin-based agent is a flagellin derivative from a microorganism well-tolerated by human, optionally selected from SEQ ID NOs: 243-252.

12. The vaccine of any one of claims 1-11, wherein the flagellin-based agent comprises an amino acid sequence having at least 80% identity, or at least 85% identity, or at least 90% identity, or at least 95% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity with one or more of SEQ ID NOs: 243-252.
- 5 13. The vaccine of any one of claims 1-12, wherein the aluminum gel or salt is selected from aluminum hydroxide, aluminum phosphate, and potassium aluminum sulfate, AS04, and ALHYDROGEL.
14. The vaccine of any one of claims 1-13, wherein the vaccine comprises any one of the adjuvants or antigens annotated in the VIOLIN database.
- 10 15. The vaccine of any one of claims 1-14, wherein the antigen is that of one or more of the following vaccines: DTP (diphtheria-tetanus-pertussis vaccine), DTaP (diphtheria-tetanus-acellular pertussis vaccine), Hib (Haemophilus influenzae type b) conjugate vaccines, Pneumococcal conjugate vaccine, Hepatitis A vaccines, Poliomyelitis vaccines, Yellow fever vaccines, Hepatitis B vaccines, combination DTaP, Tdap, Hib, Human Papillomavirus (HPV) vaccine, Anthrax vaccine, and Rabies vaccine.
- 15 16. The vaccine of any one of claims 1-15, wherein the vaccine further comprises an additional adjuvant selected from oil-in-water emulsion formulations, saponin adjuvants, ovalbumin, Freund's Adjuvants, cytokines, and chitosans.
17. The vaccine of any one of claims 1-16, wherein one or more of the flagellin-based agent and antigen are adsorbed to the aluminum gel or salt.
18. The vaccine of any one of claims 1-17, wherein the flagellin-based agent and aluminum gel or salt are mixed to form a stable complex.
- 20 19. The vaccine of any one of claims 1-18, wherein the flagellin-based agent and aluminum gel or salt are mixed in a ratio (w/w) of about 1:500 or less.
20. The vaccine of any one of claims 1-19, wherein the flagellin-based agent and aluminum gel or salt are mixed in a ratio (w/w) of about 1:500, or about 1:600, or about 1:700, or about 1:800, or about 1:900, or about 1:1000, or about 1:2000, or about 1:5000, or about 1:10000.
- 25 21. The vaccine of any one of claims 1-20, wherein the flagellin-based agent and aluminum gel or salt are mixed in a ratio that is substantially below a loading capacity of the aluminum salt.
22. The vaccine of any one of claims 1-21, wherein the vaccine causes an improvement in adjuvant properties relative to a vaccine comprising the antigen and the aluminum gel or salt alone.
- 30 23. The vaccine of any one of claims 1-22, wherein the vaccine and/or adjuvant causes immunostimulation of one or more of T_{H1} and T_{H2} -mediated immune response.

24. The vaccine of any one of claims 1-23, wherein the vaccine and/or adjuvant causes immunostimulation of T_{H1} -mediated immune response at levels greater than a vaccine comprising the antigen and the aluminum gel or salt alone.
25. A method of vaccinating a subject against a disorder, comprising administering an effective amount of a vaccine comprising:
- 5 (a) an adjuvant comprising a flagellin-based agent, or variants thereof having TLR5 agonist activity and an aluminum gel or salt and
- (b) an antigen associated with the disorder.
26. The method of claim 25, wherein the disorder is selected from infectious diseases, cancers, and allergies.
- 10 27. The method of any one of claims 25-26, wherein the disorder is selected from diphtheria, tetanus, pertussis, influenza, pneumonia, hepatitis A, hepatitis B, polio, yellow fever, Human Papillomavirus (HPV) infection, anthrax, rabies, Japanese Encephalitis, meningitis, measles, mumps, rubella, gastroenteritis, smallpox, typhoid fever, varicella (chickenpox), rotavirus, and shingles.
- 15 28. The method of any one of claims 25-27, wherein the antigen is administered simultaneously with or sequentially to the adjuvant.
29. The method of any one of claims 25-28, wherein the flagellin-based agent comprises the amino sequence of SEQ ID No: 2.
30. A method of immunostimulating a subject in advance of or concurrent with vaccination, comprising administering an effective amount of an adjuvant comprising a flagellin-based agent, or variants thereof having TLR5 agonist activity and an aluminum gel or salt, wherein both T_{H1} and T_{H2} -mediated immune responses are immunostimulated.
- 20 31. The method of claim 29, wherein the disorder is selected from infectious diseases, cancers, and allergies.
- 25 32. The method of claim 29 or 30, wherein the vaccination is for a disorder selected from diphtheria, tetanus, pertussis, influenza, pneumonia, hepatitis A, hepatitis B, polio, yellow fever, Human Papillomavirus (HPV) infection, anthrax, rabies, Japanese Encephalitis, meningitis, measles, mumps, rubella, gastroenteritis, smallpox, typhoid fever, varicella (chickenpox), rotavirus, and shingles.
- 30 33. The method of any one of claims 30-32, wherein the flagellin-based agent comprises the amino sequence of SEQ ID No: 2.

FIG. 1A

Q93279	71	GISIAQTTGEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
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Q6XEM6	71	GISIAQVAGEANQHTYVLOKRAHESTLAKSTKMSHDAKIQSIEQLSELRLIACMTEFSGQILLO
Q6VMV6	71	GISIAQTTGEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
F13713	71	GISIAQTTGEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
Q93RKS	69	GISLIQTAGEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
Q62551	71	GIELLIQVADSLGEMSESLORHGHQAVSGSGFQGHKQKQTPGSLTIKELSDVASTIYNNHILINQ
Q63012	69	GIEVAQTIEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
Q8QNT8	71	GISIAQTTGEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
Q9FAE7	71	GIERAQVAGEALKEITDLCRTKRLKQASATISGDSKATQAPGGLLSHCORACKEFTFPGQILLQ
Q82F76	70	GISIAQTTGEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
Q7R6J4	71	GISIAQTTEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
Q53578	70	GKELVDTTTCAGAVESVSMLOREKRAALSSQDTVTADRGSLAAGQGLIAIEIVASSPTFNGKTVLQ
Q55838	71	GISIAQTTEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
F42273	71	GISEVQITTEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
Q31059	69	GIEAVTAGEALTYVHNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
Q7V5C2	71	GISIAQTTGEGALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
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Q82E43	71	GISIAQVAGEALNEINNNLQVRESEVYATGYNHDSOLRSTQDSIQCLEXIDRVNQTFQNGEVVLQ
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```

Q53970 141 DQK-MK---IQVGNAGD-----ETITIELQ-----KID-VESLG---LDSPN
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Q5X5M6 141 SFEGAS--FQVGNASR-----QTINFEIG-----SLK-ASSGSLATATGTG
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Q825E1 139 TATGASQVQSILASDPAK-----DALNIDLPNARGLSAGTILGSSSTVAYYSALSVD
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Q8NTE7 141 DQK-LT---IQVGNAGS-----ETIDILK-----KIN-AQSLG---LKNFN
Q9PAK7 141 SFQBAT--FQVGNANR-----QVITATGNETNRY--GQILT-SEASG--AAATGAS
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Q32578 140 SPIDNG--LQIGAGDS-----QTMAKNVDSAAATISIGANKISGASTVVDAAALHTT
Q568Z6 143 IYTKMK--IQVGNAGN-----ETIEIKILK-----KIN-SKSLG---LKEFS
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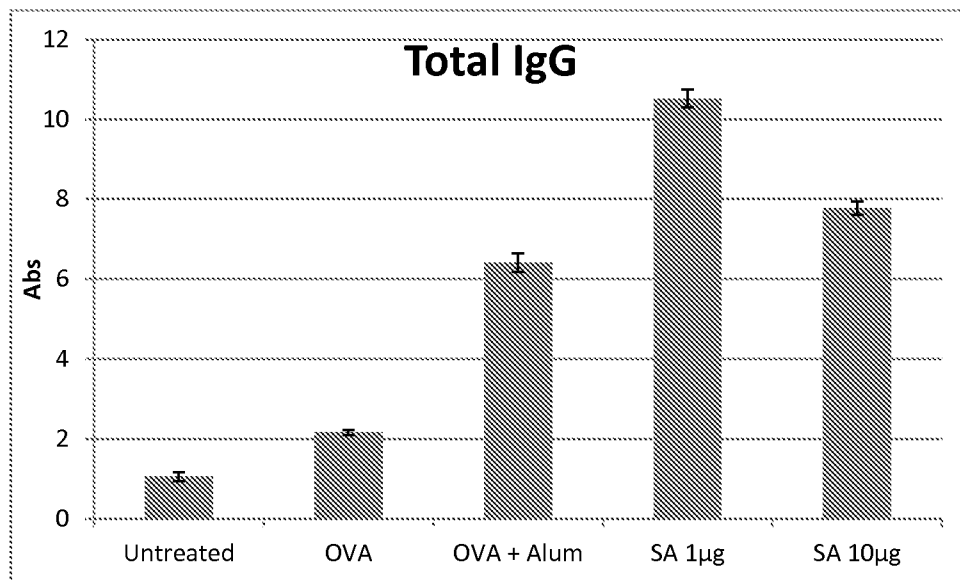
FIG. 1B

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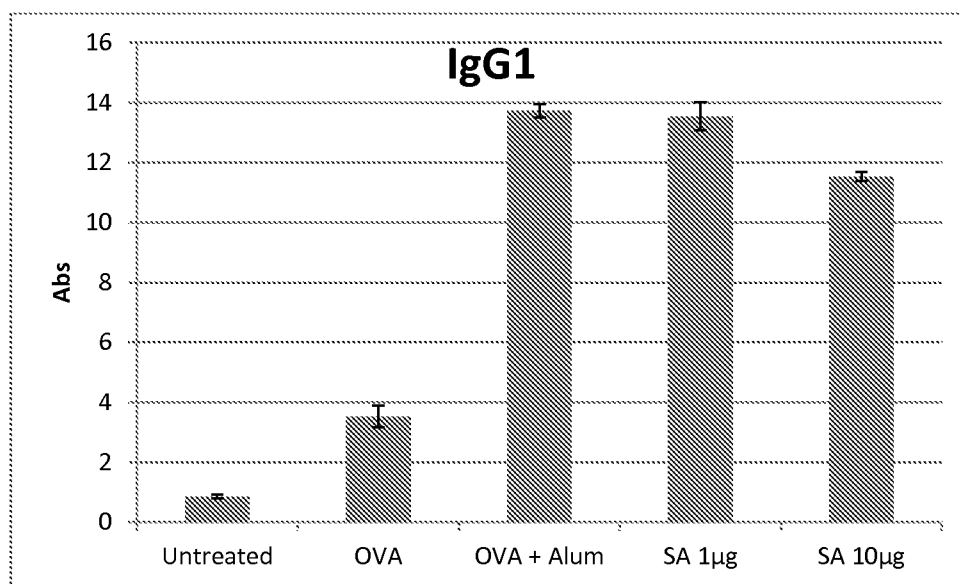
3/16

FIG. 2

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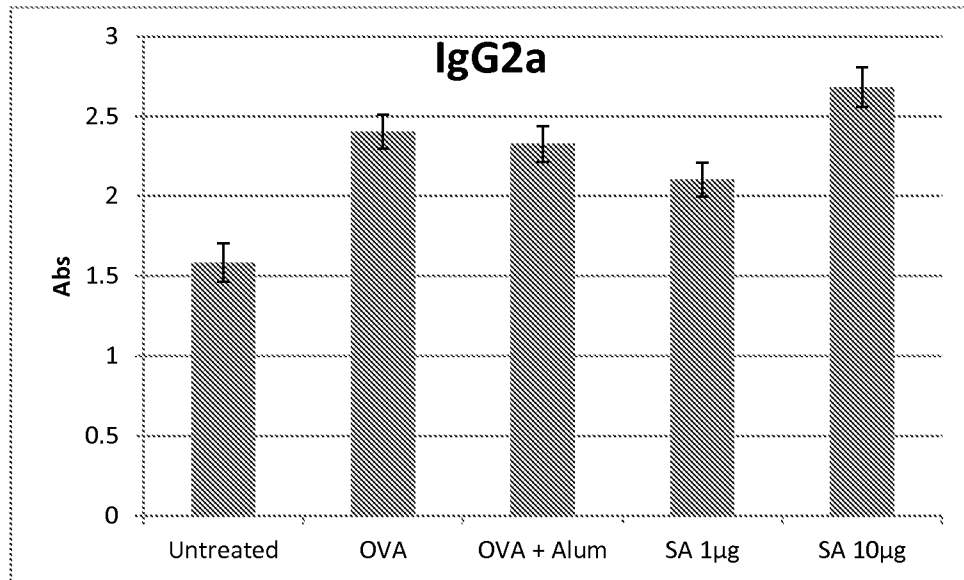


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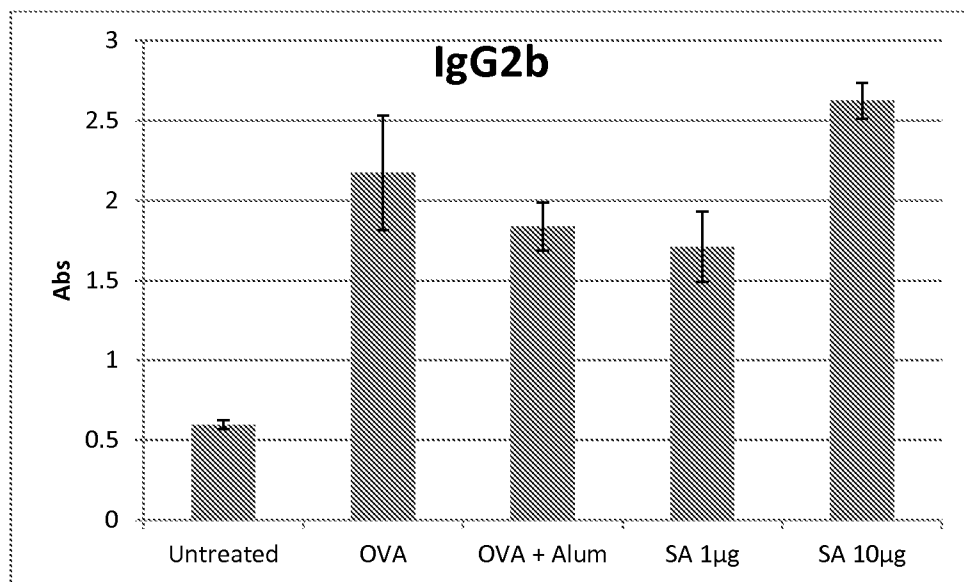


4/16
FIG. 2 (CONT.)

C.

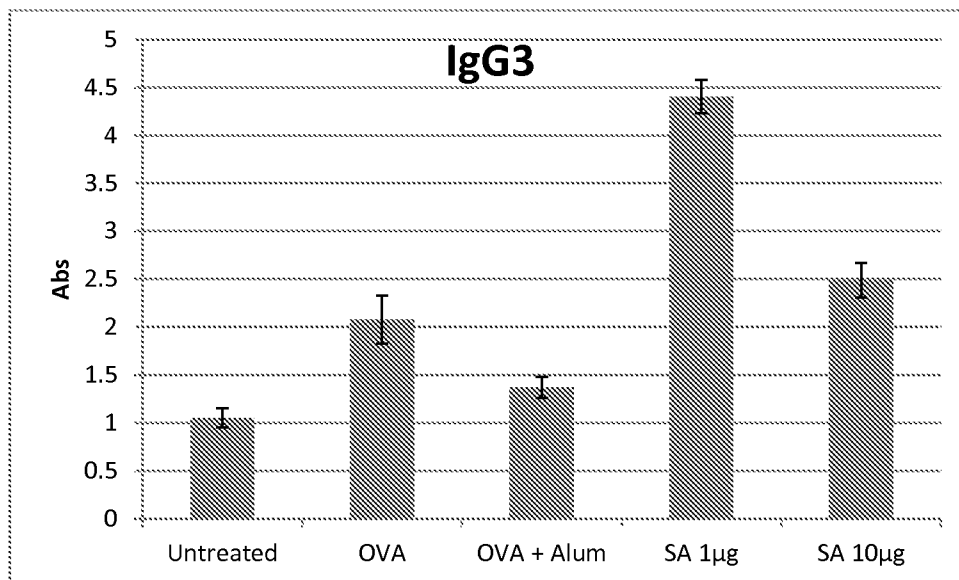


D.



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FIG. 2 (CONT.)

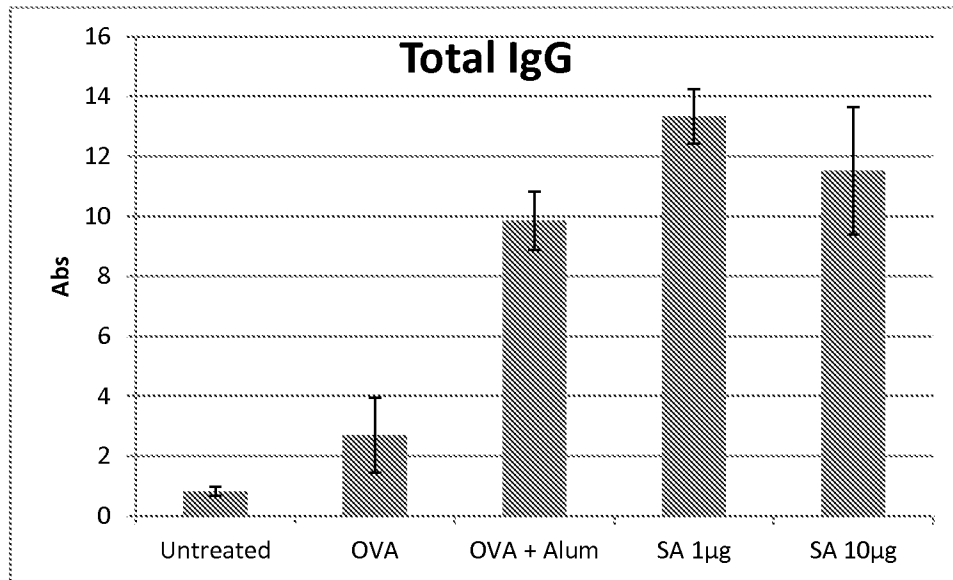
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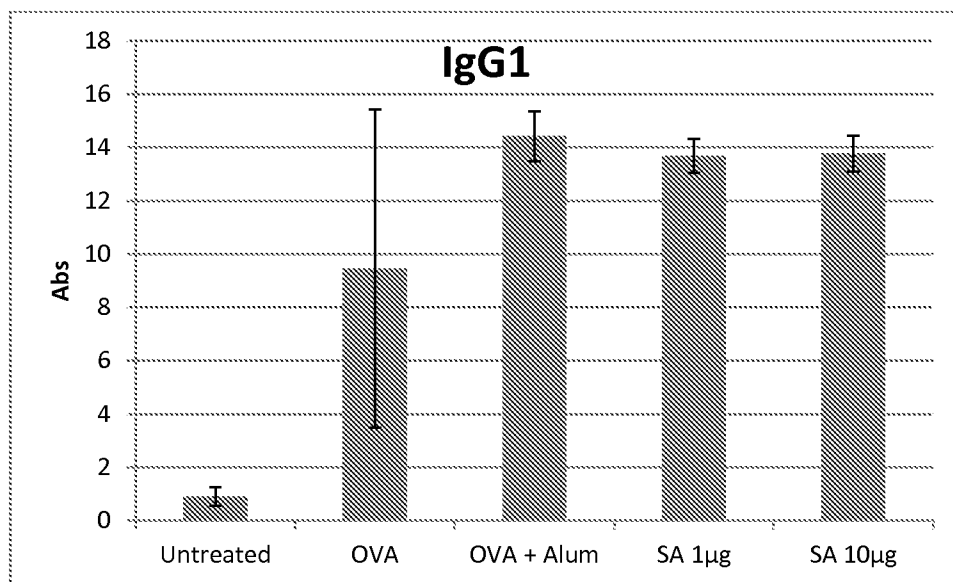
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FIG. 3

A.

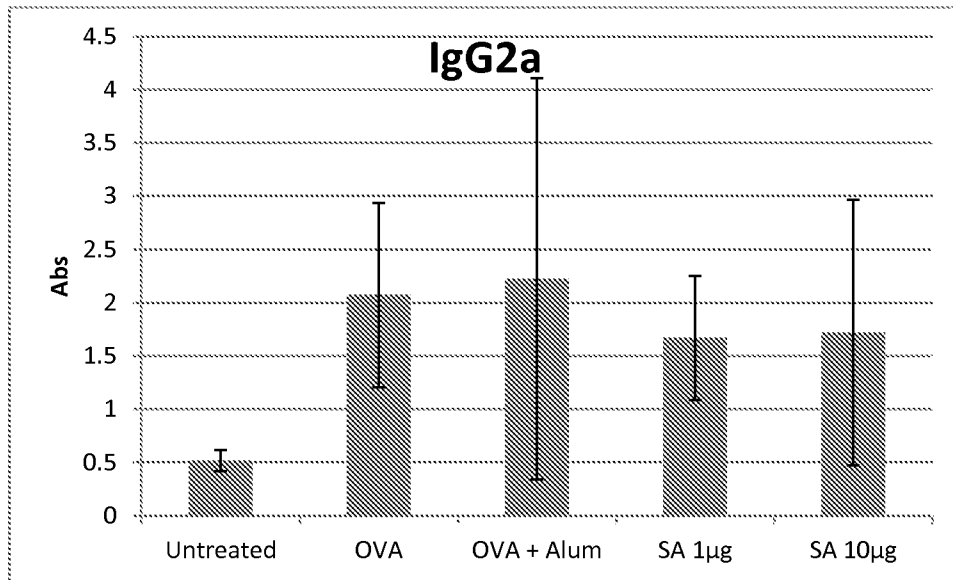


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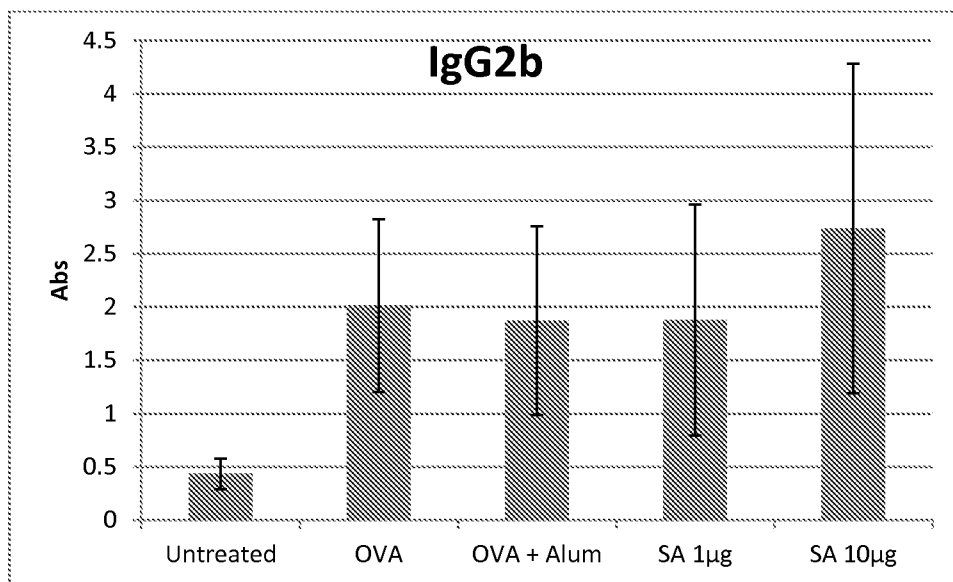


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FIG. 3 (CONT.)

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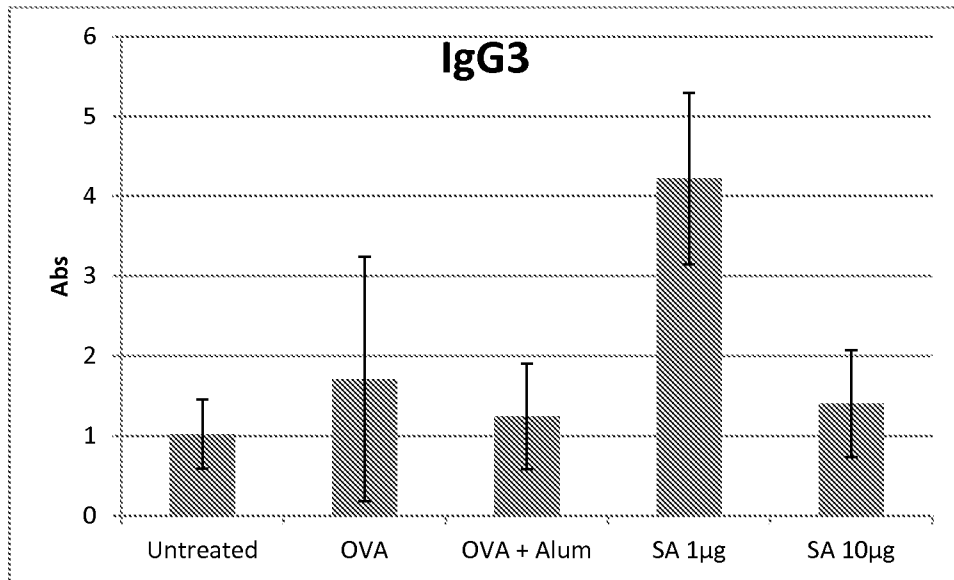


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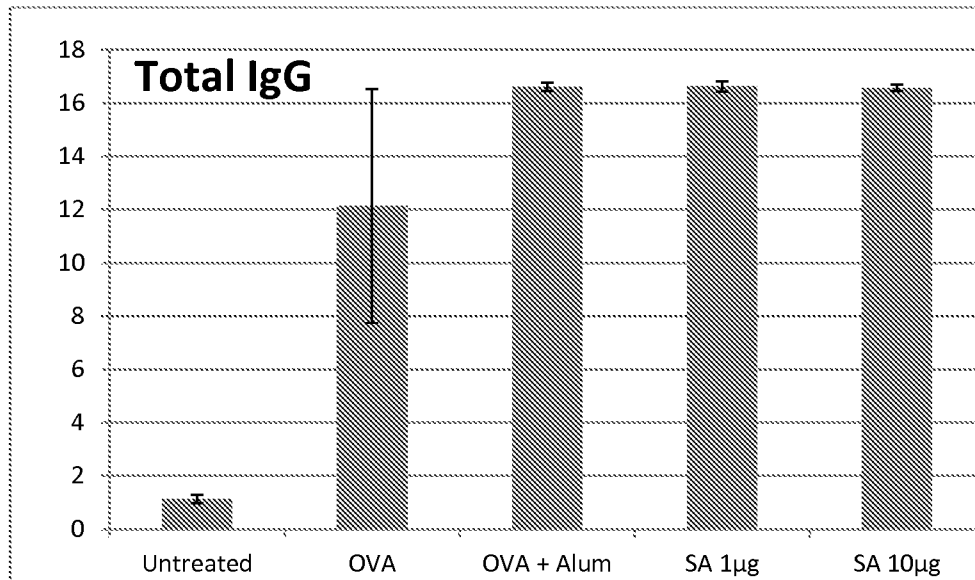
8/16
FIG. 3 (CONT.)

E.

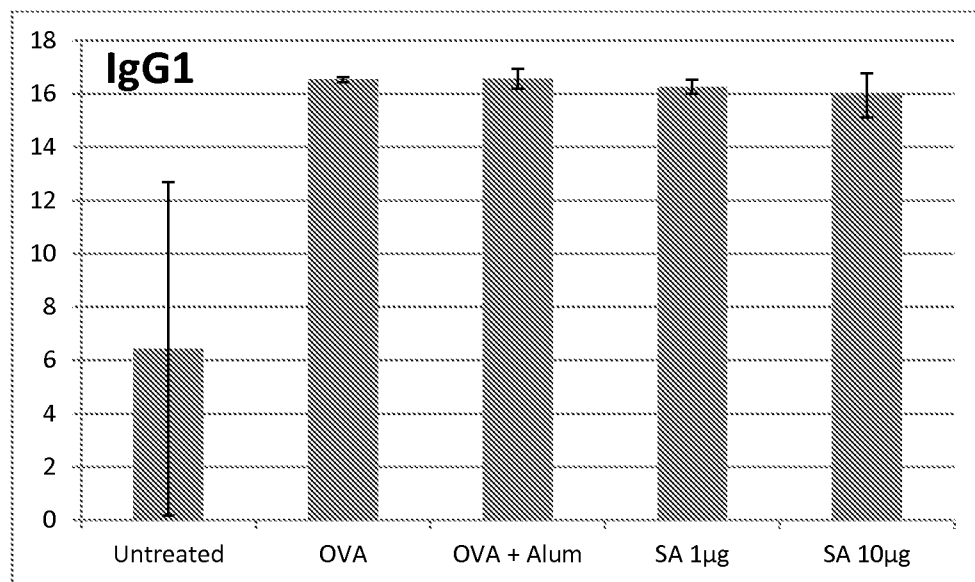


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FIG. 4

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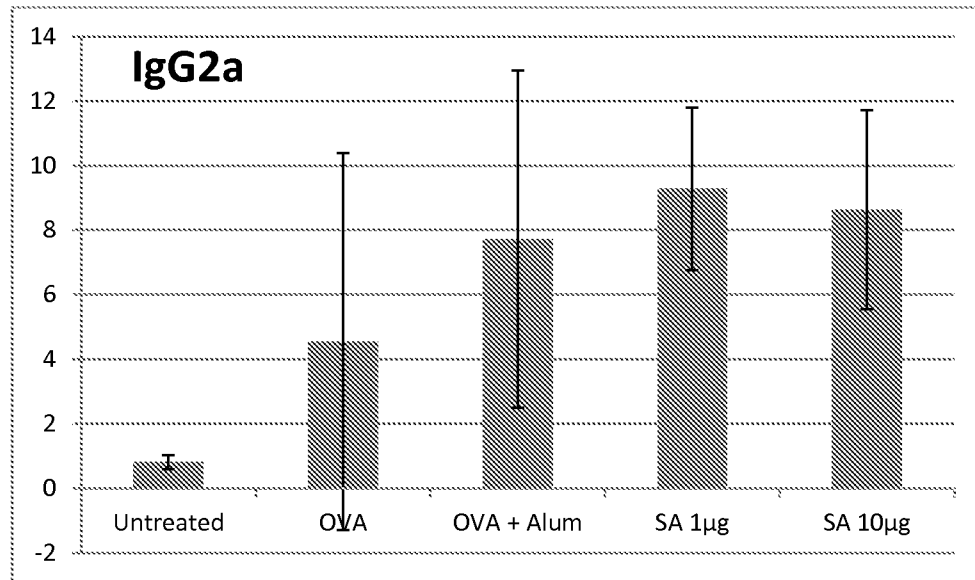


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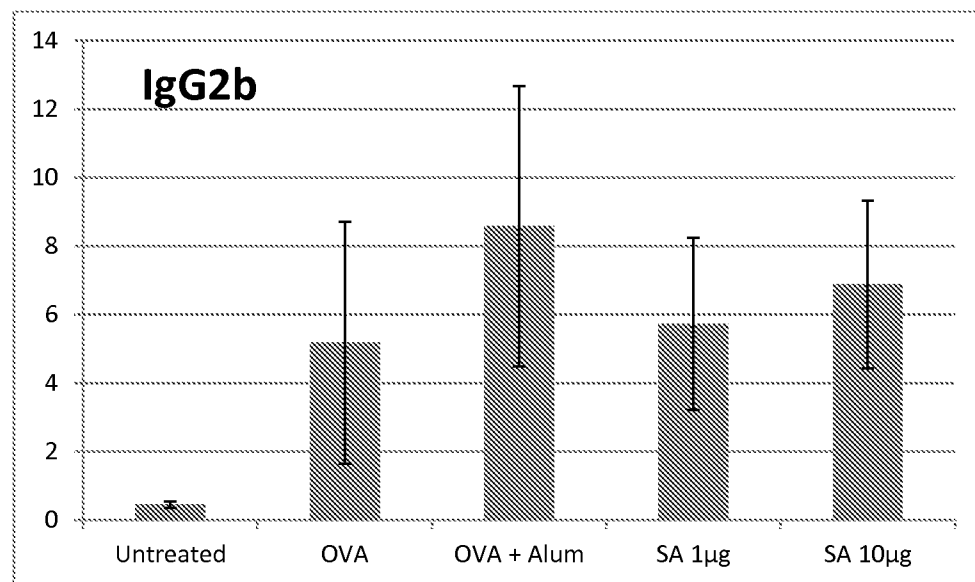


10/16
FIG. 4 (CONT.)

C.

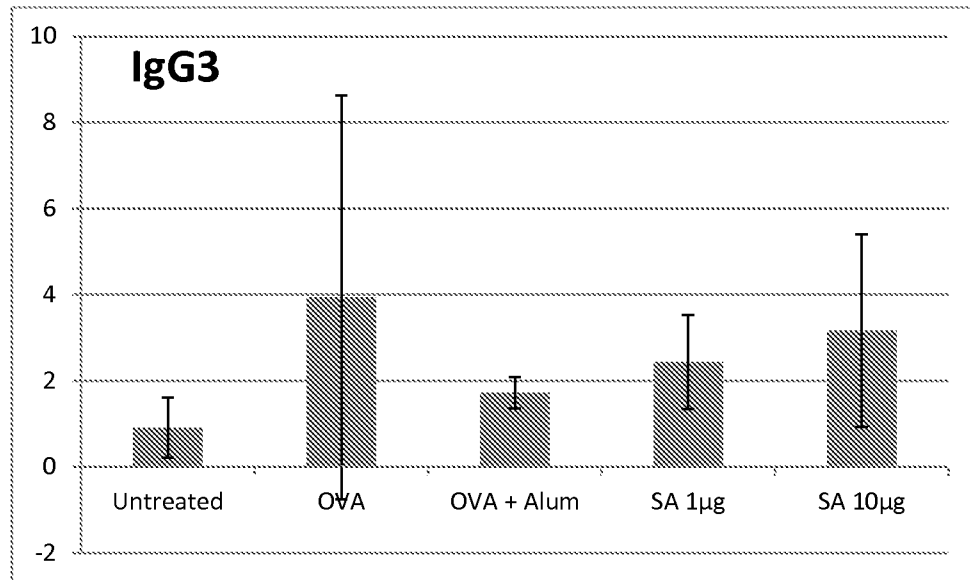


D.



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FIG. 4 (CONT.)

E.



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FIG. 5

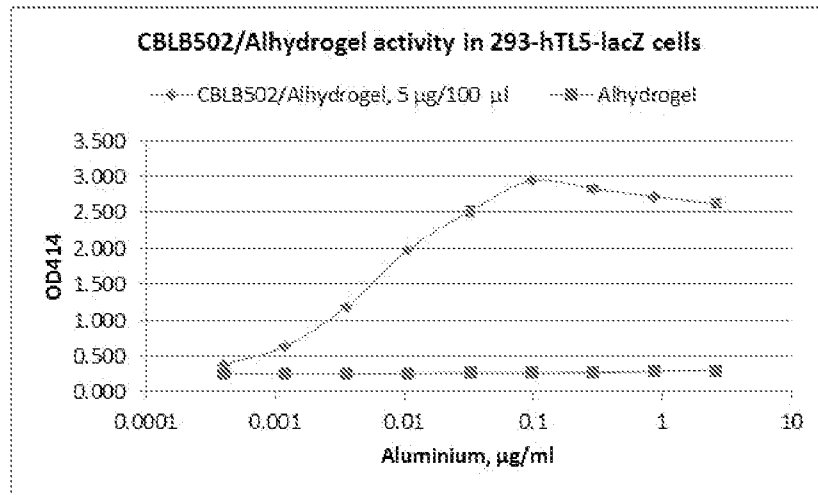
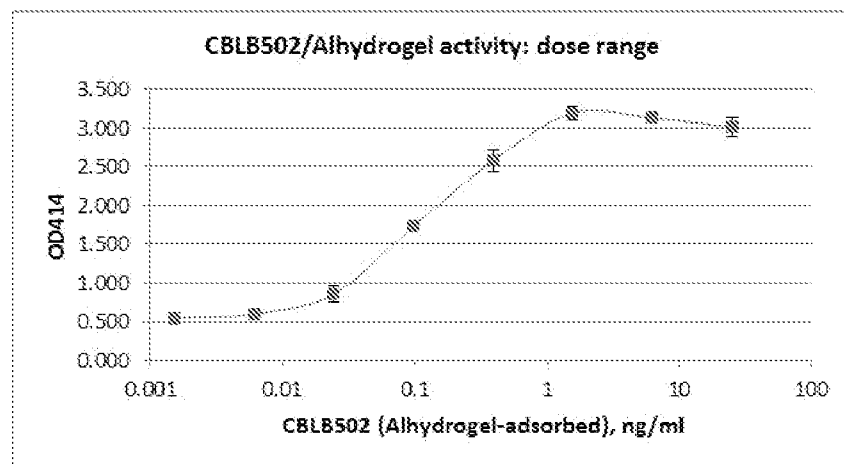
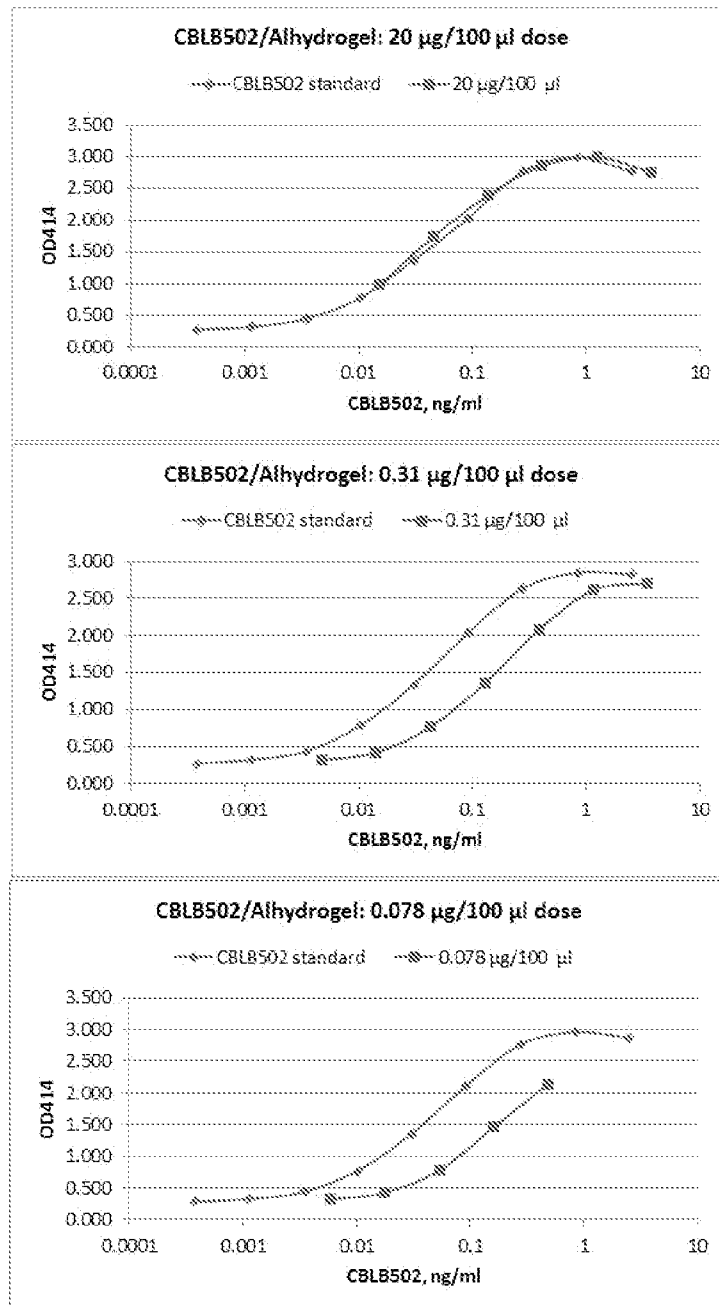


FIG. 6



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FIG. 7



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FIG. 8

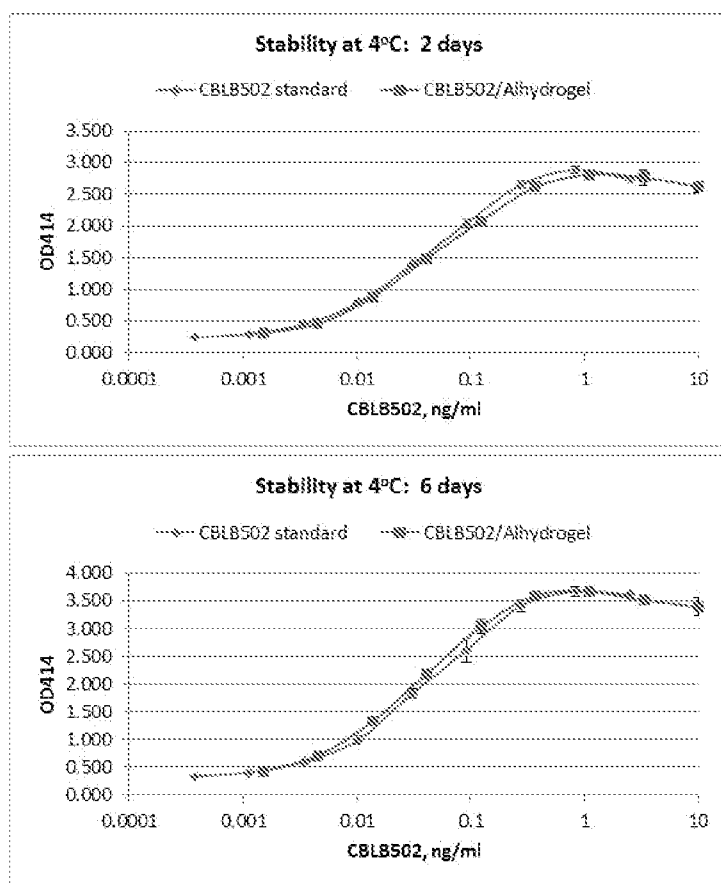
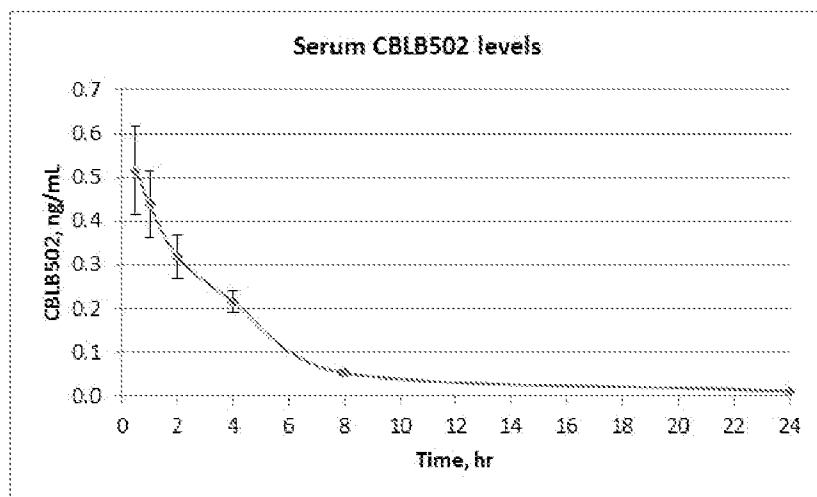
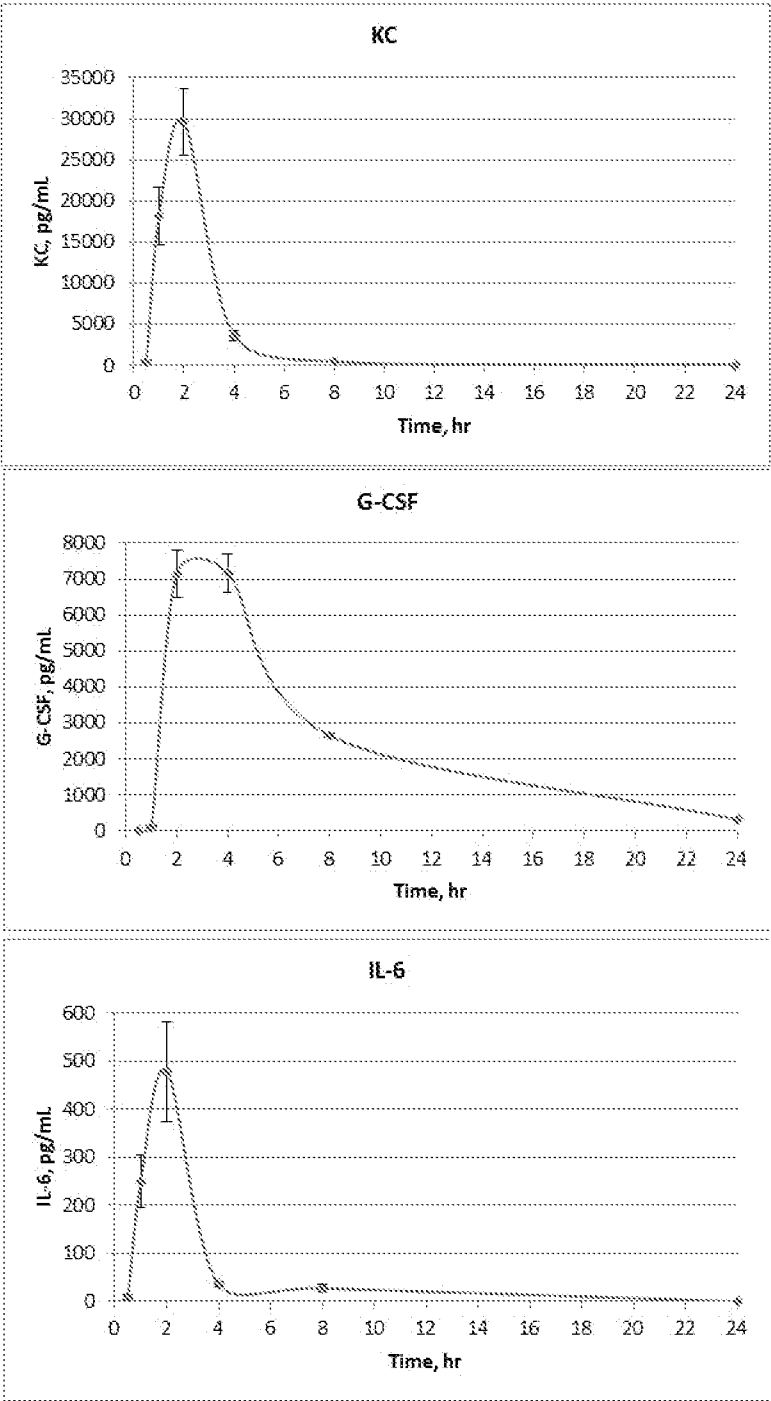


FIG. 9



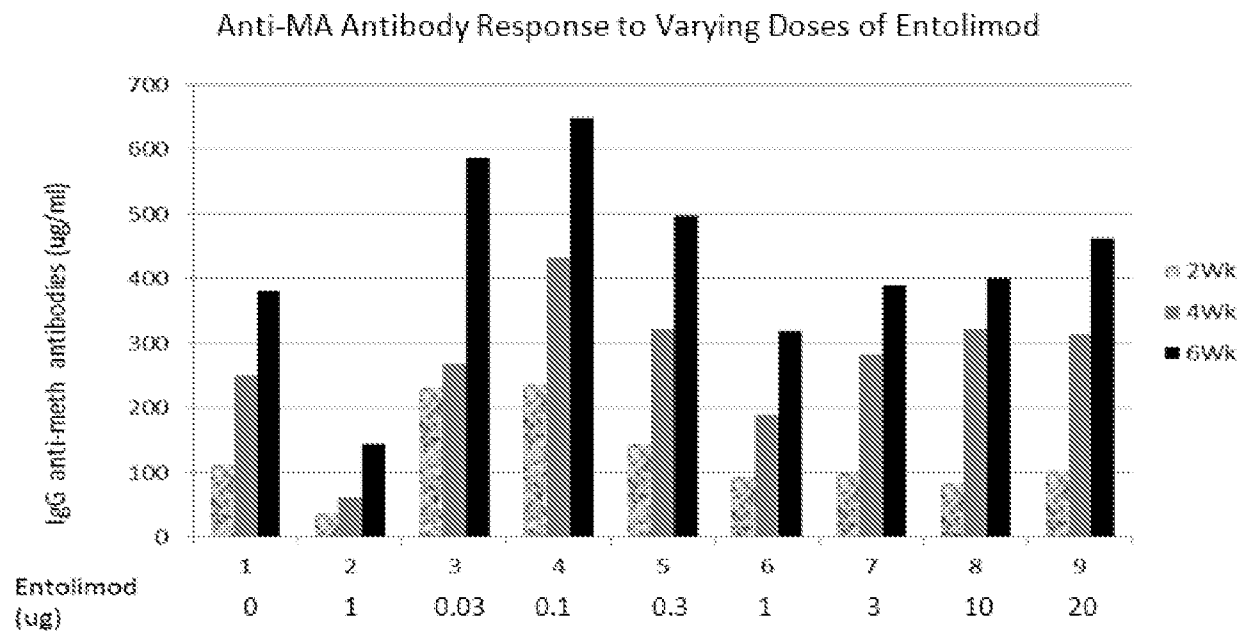
15/16
FIG. 10



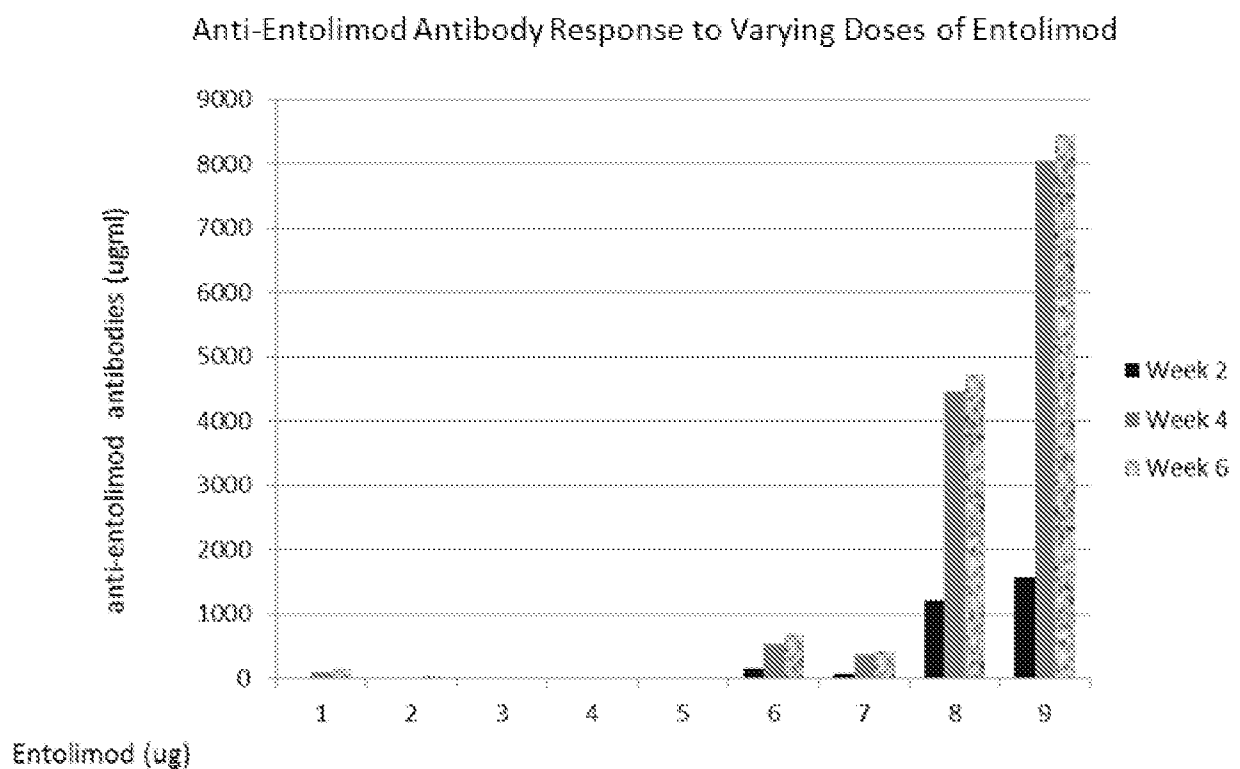
16/16

FIG. 11

A.



B.



INTERNATIONAL SEARCH REPORT	International application No. PCT/US 2015/042887												
A. CLASSIFICATION OF SUBJECT MATTER <i>A61K 47/42 (2006.01)</i> <i>C07K 14/255 (2006.01)</i> <i>A61P 37/02 (2006.01)</i> 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) A61K 47/42, C07K 14/255, A61P 37/02 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Esp@cenet, USPTO, PubMed, RUPAT, PatSearch (RUPTO internal), PAJ													
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Category*</th> <th style="width: 70%;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="width: 20%;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">Y</td> <td>WO 2010/040096 A2 (EMORY UNIVERSITY et al.) 08.04.2010, pp.25-32, 38-53, claims</td> <td style="text-align: center;">1-3, 5, 6, 25-27, 30-32</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>ROBERT L.COFFMAN et al. Vaccine Adjuvants: Putting Innate Immunity to Work, Immunity, 2010 October 29, Vol.33, no.4, pp.492-503.doi:10.1016/j.immuni.2010.10.002, pp.1-21</td> <td style="text-align: center;">1-3, 5, 6, 25-27, 30-32</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>US 2013/0004515 A1 (CLEVELAND CLINIC FOUNDATION et al.) 03.01.2013, SEQ ID NOs:1 и 8, claims</td> <td style="text-align: center;">3, 5, 6</td> </tr> </tbody> </table>		Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	Y	WO 2010/040096 A2 (EMORY UNIVERSITY et al.) 08.04.2010, pp.25-32, 38-53, claims	1-3, 5, 6, 25-27, 30-32	Y	ROBERT L.COFFMAN et al. Vaccine Adjuvants: Putting Innate Immunity to Work, Immunity, 2010 October 29, Vol.33, no.4, pp.492-503.doi:10.1016/j.immuni.2010.10.002, pp.1-21	1-3, 5, 6, 25-27, 30-32	Y	US 2013/0004515 A1 (CLEVELAND CLINIC FOUNDATION et al.) 03.01.2013, SEQ ID NOs:1 и 8, claims	3, 5, 6
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.											
Y	WO 2010/040096 A2 (EMORY UNIVERSITY et al.) 08.04.2010, pp.25-32, 38-53, claims	1-3, 5, 6, 25-27, 30-32											
Y	ROBERT L.COFFMAN et al. Vaccine Adjuvants: Putting Innate Immunity to Work, Immunity, 2010 October 29, Vol.33, no.4, pp.492-503.doi:10.1016/j.immuni.2010.10.002, pp.1-21	1-3, 5, 6, 25-27, 30-32											
Y	US 2013/0004515 A1 (CLEVELAND CLINIC FOUNDATION et al.) 03.01.2013, SEQ ID NOs:1 и 8, claims	3, 5, 6											
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.													
<table style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;"> * 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 document 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 </td> <td style="width: 50%; vertical-align: top;"> “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 </td> </tr> </table>		* 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 document 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										
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Date of the actual completion of the international search 06 November 2015 (06.11.2015)	Date of mailing of the international search report 03 December 2015 (03.12.2015)												
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhevskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	Authorized officer O.Fedonova Telephone No. 495 531 65 15												

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2015/042887

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.: 4, 7-24, 28-29, 33
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.