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(54) Title: COMPOSITIONS FOR INHIBITING NLRP3 GENE EXPRESSION AND USES THEREOF

(57) Abstract:



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COMPOSITIONS FOR INHIBITING NLRP3 GENE EXPRESSION AND USES
THEREOF

BACKGROUND OF THE INVENTION

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Related Applications

[0001] This application claims the benefit of U.S. provisional patent application serial number 62/250,796, filed on November 4, 2015, the contents of which are incorporated herein by reference in its entirety.

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Field of the invention

[0002] The present invention relates to compounds, compositions, and methods of use for the inhibition of NLR family, pyrin domain containing 3 (NLRP3; also known as CIAS1) gene expression or for diagnosing, treating and/or preventing diseases and/or conditions that respond to the inhibition of NLRP3 gene expression.

15

Summary of the Related Art

[0003] The *NLRP3* gene belongs to a family of genes called NLR (nucleotide-binding domain and leucine rich repeat containing family). NLR proteins are involved in the immune system, helping to start and regulate the immune system's response to injury, toxins, or invasion by microorganisms. These proteins recognize specific molecules, become activated, and respond by helping to engage components of the immune system.

20

[0004] The *NLRP3* gene provides instructions for making a protein called cryopyrin, which is found mainly in white blood cells and in cartilage-forming cells (chondrocytes).

25

Cryopyrin recognizes bacterial particles; chemicals such as asbestos, silica, and uric acid crystals; and compounds released by injured cells.

[0005] Once activated, groups of cryopyrin molecules assemble themselves along with other proteins into structures called inflammasomes, which are involved in the process of inflammation. Inflammation occurs when the immune system sends signaling molecules as well as white blood cells to a site of injury or disease to fight microbial invaders and facilitate tissue repair.

30

[0006] Mutations in the *NLRP3* gene are associated with familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), chronic infantile neurological cutaneous and articular (CINCA) syndrome, and neonatal-onset multisystem

inflammatory disease (NOMID). NLRP3 has also been implicated in the pathogenesis of interstitial cystitis/bladder pain syndrome (IC/BPS). Thus, there exists a need for treatments for diseases or disorders that would benefit from the reduced expression of NLRP3, or from modulation of NLRP3 activity.

5

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention is directed to compounds, compositions, and methods useful for modulating NLRP3 mRNA or protein expression using gene silencing compounds (“GSOs”) comprising two or more single stranded antisense oligonucleotides that are linked through their 5’-ends to allow the presence of two or more accessible 3’-ends. The gene silencing compounds according to the invention effectively inhibit or decrease NLRP3 mRNA or protein expression.

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[0008] Provided herein are methods, compounds, and compositions for modulating expression of NLRP3 mRNA and protein. In certain embodiments, compounds useful for modulating expression of NLRP3 mRNA and protein are gene silencing compounds.

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[0009] In certain embodiments, modulation can occur in a cell or tissue. In certain embodiments, the cell or tissue is in an animal. In certain embodiments, the animal is a human. In certain embodiments, NLRP3 mRNA levels are reduced. In certain embodiments, NLRP3 protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

20

[0010] Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating diseases, disorders, and conditions.

[0011] In certain embodiments, methods of treatment include administering a NLRP3 mRNA or protein expression gene silencing compound or composition to an individual in need thereof.

25

BRIEF DESCRIPTION OF THE FIGURES

[0012] The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

30

[0013] FIG. 1 depicts screening results of exemplary mNLRP3 GSOs in a cell based assay.

[0014] FIG. 2 depicts the silencing of mNLRP3 by exemplary GSOs in mouse J774 cell line.

5 [0015] FIG. 3A and FIG. 3B demonstrate that exemplary mNLRP3 GSO dose dependently silences NLRP3 mRNA expression and protein levels in mouse J774 cells.

[0016] FIG. 4 demonstrates that exemplary mNLRP3 GSO dose dependently reduces LPS plus ATP induced IL-1b secretion in J774 cells.

[0017] FIG. 5 demonstrates that exemplary mNLRP3 GSO specifically silences
10 mouse NLRP3 gene expression in cell based assay.

[0018] FIG. 6 depicts dose response curves of exemplary hNLRP3 GSOs in cell-based assay.

[0019] FIG. 7A and FIG. 7B depict dose response curves of exemplary hNLRP3 GSOs in THP-1 cells.

15 [0020] FIG. 8A and FIG. 8B demonstrate that exemplary hNLRP3 GSOs inhibit LPS/ATP-induced cytokine secretion in THP-1 cells.

[0021] FIG. 9 depicts dose response curves of exemplary hNLRP3 GSOs in human PBMCs.

[0022] FIG. 10A and FIG. 10B demonstrate that exemplary hNLRP3 GSOs inhibit
20 LPS/ATP-induced cytokine secretion in human PBMCs.

[0023] FIG. 11A through FIG. 11C demonstrate the effects of exemplary mNLRP3 GSO in an animal model of interstitial cystitis on bladder weight, urine IL-1 β and urine IL-18.

[0024] FIG. 12A through FIG. 12D demonstrate the effects of exemplary mNLRP3
25 GSO in an animal model of interstitial cystitis on bladder inflammasome gene expression.

[0025] FIG. 13 demonstrates the effects of exemplary mNLRP3 GSO in an animal model of interstitial cystitis on bladder inflammasome gene expression.

[0026] FIG. 14 demonstrates the effects of exemplary mNLRP3 GSO in an animal model of interstitial cystitis on bladder weight.

30 [0027] FIG. 15A and FIG. 15B demonstrate the effects of exemplary mNLRP3 GSO in an animal model of interstitial cystitis on bladder weight, urine IL-1 β .

[0028] FIG. 16A through FIG. 16D demonstrate the effects of exemplary mNLRP3 GSO in an animal model of experimental autoimmune uveitis.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0029] The invention relates to the therapeutic and prophylactic use of gene silencing compounds to down-regulate NLRP3 mRNA or protein expression. Such molecules are useful, for example, in providing compositions for modulation of NLRP3 gene expression or
5 for treating and/or preventing diseases and/or conditions that are capable of responding to modulation of NLRP3 gene expression in patients, subjects, animals or organisms.

[0030] The objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which the following terms have the ascribed
10 meaning. Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Where permitted, all patents, applications, published applications and
15 other publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

[0031] The term “2’-O-substituted” means substitution of the 2’ position of the
20 pentose moiety with an –O- lower alkyl group containing 1-6 saturated or unsaturated carbon atoms (for example, but not limited to, 2’-O-methyl), or with an –O-aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl or allyl group may be unsubstituted or may be substituted, (for example, with 2’-O-methoxyethyl, ethoxy, methoxy, halo, hydroxyl, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups);
25 or with a hydroxyl, an amino or a halo group, but not with a 2’-H group. In some embodiments the oligonucleotides of the invention include four or five 2’-O-alky nucleotides at their 5’ terminus, and/or four or five 2’-O-alky nucleotides at their 3’ terminus.

[0032] The term “3’”, when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 3’ (toward the 3’ end of the nucleotide) from another
30 region or position in the same polynucleotide or oligonucleotide.

[0033] The term “3’ end” generally refers to the 3’ terminal nucleotide of the component oligonucleotides. “Two or more oligonucleotides linked at their 3’ ends” generally refers to a linkage between the 3’ terminal nucleotides of the oligonucleotides

which may be directly via 5', 3' or 2' hydroxyl groups, or indirectly, via a non-nucleotide linker. Such linkages may also be via a nucleoside, utilizing both 2' and 3' hydroxyl positions of the nucleoside. Such linkages may also utilize a functionalized sugar or nucleobase of a 3' terminal nucleotide.

5 **[0034]** The term "5'", when used directionally, generally refers to a region or position in a polynucleotide or oligonucleotide 5' (toward the 5' end of the nucleotide) from another region or position in the same polynucleotide or oligonucleotide.

10 **[0035]** The term "5' end" generally refers to the 5' terminal nucleotide of the component oligonucleotides. "Two or more single-stranded antisense oligonucleotides linked at their 5' ends" generally refers to a linkage between the 5' terminal nucleotides of the oligonucleotides which may be directly via 5', 3' or 2' hydroxyl groups, or indirectly, via a non-nucleotide linker. Such linkages may also be via a nucleoside, utilizing both 2' and 3' hydroxyl positions of the nucleoside. Such linkages may also utilize a functionalized sugar or nucleobase of a 5' terminal nucleotide.

15 **[0036]** The term "about" generally means that the exact number is not critical. Thus, oligonucleotides having one or two fewer nucleoside residues, or from one to several additional nucleoside residues are contemplated as equivalents of each of the embodiments described above.

20 **[0037]** The term "accessible" generally means when related to a compound according to the invention, that the relevant portion of the molecule is able to be recognized by the cellular components necessary to elicit an intended response to the compound.

[0038] The term "agonist" generally refers to a substance that binds to a receptor of a cell and induces a response. An agonist often mimics the action of a naturally occurring substance such as a ligand.

25 **[0039]** The term "antigen" generally refers to a substance that is recognized and selectively bound by an antibody or by a T cell antigen receptor. Antigens may include but are not limited to peptides, proteins, lipids, carbohydrates, nucleosides, nucleotides, nucleic acids, and combinations thereof. Antigens may be natural or synthetic and generally induce an immune response that is specific for that antigen.

30 **[0040]** "Antisense activity" means any detectable or measurable activity attributable to the hybridization of a gene silencing compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

[0041] "Gene silencing compound" (also referred to herein as "GSO" or "GSOs") means an oligomeric compound comprising two or more single stranded antisense oligonucleotides that are linked through their 5'-ends to allow the presence of two or more accessible 3'-ends. Gene silencing compounds are capable of undergoing hybridization to a target nucleic acid through hydrogen bonding. Gene silencing compounds according to the invention include, but are not limited to, antisense oligonucleotides comprising naturally occurring nucleotides, modified nucleotides, modified oligonucleotides and/or backbone modified oligonucleotides.

[0042] "Antisense inhibition" means reduction of target nucleic acid levels or target protein levels in the presence of a gene silencing compound complementary to a target nucleic acid as compared to target nucleic acid levels or target protein levels in the absence of the gene silencing compound.

[0043] "Antisense oligonucleotide" means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid.

[0044] The term "biologic instability" generally refers to a molecule's ability to be degraded and subsequently inactivated *in vivo*. For oligonucleotides, such degradation results from exonuclease activity and/or endonuclease activity, wherein exonuclease activity refers to cleaving nucleotides from the 3' or 5' end of an oligonucleotide, and endonuclease activity refers to cleaving phosphodiester bonds at positions other than at the ends of the oligonucleotide.

[0045] The term "carrier" generally encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microspheres, liposomal encapsulation, or other material for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. The preparation of pharmaceutically acceptable formulations containing these materials is described in, for example, *Remington's Pharmaceutical Sciences*, 18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA, 1990.

[0046] The term "co-administration" or "co-administered" generally refers to the administration of at least two different substances. Co-administration refers to simultaneous administration, as well as temporally spaced order of up to several days apart, of at least two different substances in any order, either in a single dose or separate doses.

[0047] The term “in combination with” generally means administering an oligonucleotide-based compound according to the invention and another agent useful for treating the disease or condition that does not abolish the activity of the compound in the course of treating a patient. Such administration may be done in any order, including
5 simultaneous administration, as well as temporally spaced order from a few seconds up to several days apart. Such combination treatment may also include more than a single administration of the compound according to the invention and/or independently the other agent. The administration of the compound according to the invention and the other agent may be by the same or different routes.

10 [0048] The term “complementary” is intended to mean the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

[0049] “Contiguous nucleobases” means nucleobases immediately adjacent to each other.

[0050] The term “individual” or “subject” or “patient” generally refers to a mammal,
15 such as a human.

[0051] “NLRP3 nucleic acid” means any nucleic acid encoding NLRP3. For example, in certain embodiments, a NLRP3 nucleic acid includes a DNA sequence encoding NLRP3, an RNA sequence transcribed from DNA encoding NLRP3 (including genomic DNA comprising introns and exons), and an mRNA sequence encoding NLRP3. “NLRP3 mRNA”
20 means an mRNA encoding a NLRP3 protein.

[0052] “Fully complementary” or “100% complementary” means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

25 [0053] “Hybridization” means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include an antisense compound and a target nucleic acid.

[0054] “Inhibiting NLRP3 mRNA or protein expression” means reducing expression of NLRP3 mRNA and/or protein levels in the presence of a gene silencing compound
30 according to the invention as compared to expression of NLRP3 and/or protein levels in the absence of a gene silencing compound according to the invention.

[0055] The term “linear synthesis” generally refers to a synthesis that starts at one end of an oligonucleotide and progresses linearly to the other end. Linear synthesis permits

incorporation of either identical or non-identical (in terms of length, base composition and/or chemical modifications incorporated) monomeric units into an oligonucleotide.

[0056] The term “mammal” is expressly intended to include warm blooded, vertebrate animals, including, without limitation, humans, non-human primates, rats, mice, cats, dogs, horses, cattle, cows, pigs, sheep and rabbits.

[0057] The term “nucleoside” generally refers to compounds consisting of a sugar, usually ribose, deoxyribose, pentose, arabinose or hexose, and a purine or pyrimidine base.

[0058] The term “nucleotide” generally refers to a nucleoside comprising a phosphorous-containing group attached to the sugar.

10 [0059] The term “modified nucleoside” or “nucleotide derivative” generally is a nucleoside that includes a modified heterocyclic base, a modified sugar moiety, or any combination thereof. In some embodiments, the modified nucleoside or nucleotide derivative is a non-natural pyrimidine or purine nucleoside, as herein described. For purposes of the invention, a modified nucleoside or nucleotide derivative, a pyrimidine or purine analog or
15 non-naturally occurring pyrimidine or purine can be used interchangeably and refers to a nucleoside that includes a non-naturally occurring base and/or non-naturally occurring sugar moiety. For purposes of the invention, a base is considered to be non-natural if it is not guanine, cytosine, adenine, thymine or uracil and a sugar is considered to be non-natural if it is not β -ribo-furanoside or 2'-deoxyribo-furanoside.

20 [0060] The term “modified oligonucleotide” as used herein describes an oligonucleotide in which at least two of its nucleotides are covalently linked via a synthetic linkage, i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide in which the 5' nucleotide phosphate has been replaced with any number of chemical groups. The term “modified oligonucleotide” also
25 encompasses 2'-O,4'-C-methylene-b-D-ribofuranosyl nucleic acids, arabinose nucleic acids, substituted arabinose nucleic acids, hexose nucleic acids, peptide nucleic acids, morpholino, and oligonucleotides having at least one nucleotide with a modified base and/or sugar, such as a 2'-O-substituted, a 5-methylcytosine and/or a 3'-O-substituted ribonucleotide.

[0061] The term “nucleic acid” encompasses a genomic region or an RNA molecule
30 transcribed therefrom. In some embodiments, the nucleic acid is mRNA.

[0062] The term “linker” generally refers to any moiety that can be attached to an oligonucleotide by way of covalent or non-covalent bonding through a sugar, a base, or the backbone. The non-covalent linkage may be, without limitation, electrostatic interactions,

hydrophobic interactions, π -stacking interactions, hydrogen bonding and combinations thereof. Non-limiting examples of such non-covalent linkage includes Watson-Crick base pairing, Hoogsteen base pairing, and base stacking. The linker can be used to attach two or more nucleosides or can be attached to the 5' and/or 3' terminal nucleotide in the oligonucleotide. Such linker can be either a non-nucleotide linker or a nucleoside linker.

[0063] The term "non-nucleotide linker" generally refers to a chemical moiety, other than a linkage directly between two nucleotides that can be attached to an oligonucleotide by way of covalent or non-covalent bonding. Preferably such non-nucleotide linker is from about 2 angstroms to about 200 angstroms in length, and may be either in a cis or trans orientation.

[0064] The term "internucleotide linkage" generally refer to a chemical linkage to join two nucleosides through their sugars (e.g. 3'-3', 2'-3', 2'-5', 3'-5', 5'-5') consisting of a phosphorous atom and a charged, or neutral group (e.g., phosphodiester, phosphorothioate, phosphorodithioate or methylphosphonate) between adjacent nucleosides.

[0065] The term "oligonucleotide" refers to a polynucleoside formed from a plurality of linked nucleoside units, which may include, for example, deoxyribonucleotides or ribonucleotides, synthetic or natural nucleotides, phosphodiester or modified linkages, natural bases or modified bases natural sugars or modified sugars, or combinations of these components. The nucleoside units may be part of viruses, bacteria, cell debris or oligonucleotide-based compositions (for example, siRNA and microRNA). Such oligonucleotides can also be obtained from existing nucleic acid sources, including genomic or cDNA, but are preferably produced by synthetic methods. In certain embodiments each nucleoside unit includes a heterocyclic base and a pentofuranosyl, trehalose, arabinose, 2'-deoxy-2'-substituted nucleoside, 2'-deoxy-2'-substituted arabinose, 2'-O-substituted arabinose or hexose sugar group. The nucleoside residues can be coupled to each other by any of the numerous known internucleoside linkages. Such internucleoside linkages include, without limitation, phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, alkylphosphonate, alkylphosphonothioate, phosphotriester, phosphoramidate, siloxane, carbonate, carboalkoxy, acetamidate, carbamate, morpholino, borano, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate, and sulfone internucleoside linkages. The term "oligonucleotide" also encompasses polynucleosides having one or more stereospecific internucleoside linkage (e.g., (*RP*)- or (*SP*)-phosphorothioate, alkylphosphonate, or phosphotriester linkages). As used

herein, the terms “oligonucleotide” and “dinucleotide” are expressly intended to include polynucleosides and dinucleosides having any such internucleoside linkage, whether or not the linkage comprises a phosphate group. In certain exemplary embodiments, these internucleoside linkages may be phosphodiester, phosphorothioate or phosphorodithioate linkages, or combinations thereof. In exemplary embodiments, the nucleotides of the synthetic oligonucleotides are linked by at least one phosphorothioate internucleotide linkage. The phosphorothioate linkages may be mixed Rp and Sp enantiomers, or they may be stereoregular or substantially stereoregular in either Rp or Sp form (see Iyer et al. (1995) Tetrahedron Asymmetry 6:1051-1054). In certain embodiments, one or more of the oligonucleotides within the antisense compositions of the invention contain one or more 2'-O,4'-C-methylene-b-D-ribofuranosyl nucleic acids, wherein the ribose is modified with a bond between the 2' and 4' carbons, which fixes the ribose in the 3'-endo structural conformation.

[0066] The term “peptide” generally refers to oligomers or polymers of amino acids that are of sufficient length and composition to affect a biological response, for example, antibody production or cytokine activity whether or not the peptide is a hapten. The term “peptide” may include modified amino acids (whether or not naturally or non-naturally occurring), where such modifications include, but are not limited to, phosphorylation, glycosylation, pegylation, lipidization, and methylation.

[0067] The term “pharmaceutically acceptable” means a non-toxic material that does not interfere with the effectiveness of a compound according to the invention or the biological activity of a compound according to the invention.

[0068] The term “physiologically acceptable” refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism.

Preferably, the biological system is a living organism, such as a mammal, particularly a human.

[0069] The term “prophylactically effective amount” generally refers to an amount sufficient to prevent or reduce the development of an undesired biological effect.

[0070] “Portion” means a defined number of contiguous (i.e., linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

[0071] “Single-stranded oligonucleotide” means an oligonucleotide which is not hybridized to a complementary strand.

[0072] "Specifically hybridizable" refers to a gene silencing compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays and therapeutic treatments.

[0073] "Targeting" or "targeted" means the process of design and selection of a gene silencing compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

[0074] "Target nucleic acid," "target RNA," "target mRNA," and "target RNA transcript" all refer to a nucleic acid capable of being targeted by gene silencing compounds.

[0075] "Target segment" means the sequence of nucleotides of a target nucleic acid to which a gene silencing compound is targeted. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

[0076] The term "therapeutically effective amount" or "pharmaceutically effective amount" generally refers to an amount sufficient to affect a desired biological effect, such as a beneficial result, including, without limitation, prevention, diminution, amelioration or elimination of signs or symptoms of a disease or disorder. Thus, the total amount of each active component of the pharmaceutical composition or method is sufficient to show a meaningful patient benefit, for example, but not limited to, healing of chronic conditions characterized by immune stimulation. Thus, a "pharmaceutically effective amount" will depend upon the context in which it is being administered. A pharmaceutically effective amount may be administered in one or more prophylactic or therapeutic administrations. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

[0077] The term "treatment" generally refers to an approach intended to obtain a beneficial or desired result, which may include alleviation of symptoms, or delaying or ameliorating a disease progression.

[0078] The term "gene expression" generally refers to process by which information from a gene is used in the synthesis of a functional gene product, which may be a protein. The process may involve transcription, RNA splicing, translation, and post-translational modification of a protein, and may include mRNA, preRNA, ribosomal RNA, and other templates for protein synthesis.

[0079] In certain embodiments provided are methods, compounds, and compositions for inhibiting NLRP3 mRNA or protein expression. In certain embodiments the compounds are antisense oligonucleotides, double stranded or single-stranded siRNA compounds, or gene silencing compounds.

5 [0080] As used herein, gene silencing compounds according to the invention comprise two or more single-stranded antisense oligonucleotides linked at their 5' ends, wherein the compounds have two or more accessible 3' ends. The general structure of the oligonucleotide-based compounds of the invention may be described by the following formula I:

10 3'-N_n...N₁N₂N₃N₄-5'-X-5'-N₈N₇N₆N₅...N_m-3' (Formula I),

wherein X is a nucleotide linker or non-nucleotide linker; N₁-N₈, at each occurrence, is independently a nucleotide or nucleotide derivative; N_m and N_n, at each occurrence, are independently a nucleotide or nucleotide derivative; and wherein m and n are independently numbers from 0 to about 40.

15 [0081] The linkage at the 5' ends of the component oligonucleotides is independent of the other oligonucleotide linkages and may be directly via 5', 3' or 2' hydroxyl groups, or indirectly, via a non-nucleotide linker or a nucleoside, utilizing either the 2' or 3' hydroxyl positions of the nucleoside. Linkages may also utilize a functionalized sugar or nucleobase of a 5' terminal nucleotide.

20 [0082] In certain embodiments provided are gene silencing compounds targeted to a human NLRP3 nucleic acid. In certain embodiments, the human NLRP3 nucleic acid is the sequence set forth in GENBANK Accession No. NM_004895.4 (incorporated herein as SEQ ID NO: 95).

25 1 gtagatgagg aaactgaagt tgaggaatag tgaagagttt gtccaatgtc atagccccgt
61 aatcaacggg acaaaaatth tcttgctgat gggtaagat ggcatcgtga agtggttggt
121 caccgtaaac tgtaatacaa tcctgtttat ggatttggtt gcatatthttt ccctccatag
181 ggaaaccttt cttccatggc tcaggacaca ctcttgatc gagccaacag gagaactttc
241 tggtaagcat ttggctaact tttttttttt tgagatggag tcttgctgtg tcgcctaggg
301 tggagtgcag ttggcgtgatc ttggctcact gcagcctcca cttcccgggt tcaatcaatt
361 ctctacctc aacttcctga gtactggga ttacaggcgc ccgccaccac acccggtca
421 tttttgtact tttagtagag acacagtttt gccatgttgg ccaggctggg cttgaattcc
481 tcagctcagg tgatctgcct gccttggcct ctcaaagtgc tgggattaca ggcgtgagcc
541 actgtgcccg gccttggcta acttttcaaa attaaagatt ttgacttggt acagtcattg
601 gacattthttt tctttctgtt tgctgagttt ttgataatth atatctctca aagtggagac
35 661 tttaaaaaag actcatccgt gtgcccgtgt cactgcctgg tatcttagtg tggaccgaag
721 cctaaggacc ctgaaaacag ctgcagatga agatggcaag caccgcgtgc aagctggcca
781 ggtacctgga ggacctggag gatgtggact tgaagaaatt taagatgcac ttagaggact
841 atcctcccca gaagggtgc atccccctcc cgaggggtca gacagagaag gcagaccatg
901 tggatctagc cagctaata atcgacttca atggggagga gaaggcgtgg gccatggccg
40 961 tgtggatctt cgctgcgac aacaggagag acctttatga gaaagcaaaa agagatgagc
1021 cgaagtgggg ttcagataat gcacgtgttt cgaatccac tgtgatatgc caggaagaca
1081 gcattgaaga ggagtggatg gtttactgg agtacctttc gagaatctct atttgtaaaa

	1141	tgaagaaaga	ttaccgtaag	aagtacagaa	agtacgtgag	aagcagattc	cagtgcattg
	1201	aagacaggaa	tgcccgtctg	ggtgagagt	tgagcctcaa	caaacgctac	acacgactgc
	1261	gtctcatcaa	ggagcaccgg	agccagcagg	agagggagca	ggagcttctg	gccatcggca
	1321	agaccaagac	gtgtgagagc	cccgtgagtc	ccattaagat	ggagttgctg	tttgaccccg
5	1381	atgatgagca	ttctgagcct	gtgcacaccg	tggtgttcca	gggggcggca	gggattggga
	1441	aaacaatcct	ggccagggaag	atgatgttgg	actgggctgc	ggggacactc	taccaagaca
	1501	ggtttgacta	tctgttctat	atccactgtc	gggaggtgag	ccttgtgaca	cagaggagcc
	1561	tgggggacct	gatcatgagc	tgctgccccg	acccaaaccc	acccatccac	aagatcgtga
10	1621	gaaaaccctc	cagaatcctc	ttcctcatgg	acggcttcga	tgagctgcaa	ggtgcctttg
	1681	acgagcacat	aggaccgctc	tgactgact	ggcagaaggc	cgagcgggga	gacattctcc
	1741	tgagcagcct	catcagaaag	aagtgtcttc	ccgaggcctc	tctgtctatc	accacgagac
	1801	ctgtggccct	ggagaaactg	cagcacttgc	tggaaccatcc	tcggcatgtg	gagatcctgg
	1861	gtttctccga	ggccaaaagg	aaagagtact	tcttcaagta	cttctctgat	gaggcccaag
15	1921	ccagggcagc	cttcagtctg	attcaggaga	acgaggctct	cttcaccatg	tgcttcatcc
	1981	ccctggtctg	ctggatcgtg	tgactggac	tgaacagca	gatggagagt	ggcaagagcc
	2041	ttgccagac	atccaagacc	accaccgcgg	tgtacgtctt	cttcctttcc	agtttgctgc
	2101	agccccgggg	agggagccag	gagcacggcc	tctgcgcca	cctctggggg	ctctgtctct
	2161	tggctgcaga	tggaatctgg	aaccagaaaa	tcctgtttga	ggagtccgac	ctcaggaatc
20	2221	atggactgca	gaaggcggat	gtgtctgctt	tcctgaggat	gaacctgttc	caaaaggaag
	2281	tggactgcga	gaagtctctac	agcttcatcc	acatgacttt	ccaggagttc	tttgccgcca
	2341	tgtactacct	gctggaagag	gaaaaggaag	gaaggacgaa	cgttccaggg	agtcgtttga
	2401	agcttccag	ccgagacgtg	acagtccctc	tggaaaacta	tgacaaattc	gaaaaggggt
	2461	atttgattht	tggtgtacgt	ttcctctttg	gcctggtaaa	ccaggagagg	acctcctact
25	2521	tggagaagaa	attaagtgtc	aagatctctc	agcaaatacag	gctggagctg	ctgaaatgga
	2581	ttgaagtga	agccaaagct	aaaaagctgc	agatccagcc	cagccagctg	gaattgttct
	2641	actgtttgta	cgagatgcag	gaggaggact	tcgtgcaaag	ggccatggac	tatttcccca
	2701	agattgagat	caatctctcc	accagaatgg	accacatggt	ttcttctctt	tgcattgaga
	2761	actgtcatcg	ggtggagtc	ctgtccctgg	ggtttctcca	taacatgccc	aaggaggaag
30	2821	aggaggagga	aaaggaaggc	cgacaccttg	atatggtgca	gtgtgtcctc	ccaagctcct
	2881	ctcatgtctg	ctgttctctc	ggattggtga	acagccacct	cacttccagt	ttttgccggg
	2941	gcctcttttc	agttctgagc	accagccaga	gtctaactga	attggacctc	agtgacaatt
	3001	ctctggggga	cccagggatg	agagtgttgt	gtgaaacgct	ccagcatcct	ggctgtaaca
	3061	ttcggagatt	gtggttgggg	cgctgtggcc	tctcgcatga	gtgctgcttc	gacatctcct
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	3181	acttcggaat	cagacttctg	tgtgtgggac	tgaagcacct	gttgtgcaat	ctgaagaagc
	3241	tctggttggg	cagctgctgc	ctcacatcag	catgttgtca	ggatcttgca	tcagtattga
	3301	gcaccagcca	ttccctgacc	agactctatg	tgggggagaa	tgccctggga	gactcaggag
	3361	tcgcaatttt	atgtgaaaaa	gccaagaatc	cacagtgtaa	cctgcagaaa	ctgggggttg
40	3421	tgaattctgg	ccttacgtca	gtctgttgtt	cagctttgtc	ctcggtactc	agcactaatc
	3481	agaatctcac	gcacctttac	ctgcgaggca	acactctcgg	agacaaaggg	atcaaaactac
	3541	tctgtgaggg	actcttgac	cccactgca	agcttcagg	gttgaatta	gacaactgca
	3601	acctcacgtc	acactgctgc	tgggatcttt	ccacacttct	gacctccagc	cagagcctgc
	3661	gaaagctgag	cctgggcaac	aatgacctgg	gcgacctggg	ggatcatgat	ttctgtgaag
45	3721	tgctgaaaca	gcagagctgc	ctcctgcaga	acctgggggt	gtctgaaatg	tatttcaatt
	3781	atgagacaaa	aagtgcgtta	gaaacacttc	aagaagaaaa	gcctgagctg	accgtcgtct
	3841	ttgagccttc	ttggtaggag	tggaaacggg	gctgccagac	gccagtgttc	tccggctcct
	3901	ccagctgggg	gccctcaggt	ggagagagct	gcgatccatc	caggccaaga	ccacagctct
	3961	gtgatccttc	cgggtgagtg	tcggagaaga	gagcttgccg	acgatgcctt	cctgtgcaga
50	4021	gcttggtcat	ctcctttacg	ccagggtgag	gaagacacca	ggacaatgac	agcatcgggt
	4081	gttggtgtca	tcacagcgcc	tcagttagag	gatgttcttc	ttggtgacct	catgtaatta
	4141	gctcattcaa	taaagcactt	tctttattht	tctcttctct	gtctaaactt	ctttttctta
	4201	tcttttttct	tctttgttct	gtttacttht	gtcatatca	tcattcccgc	tatctttcta
	4261	ttaactgacc	ataacacaga	actagttgac	tatatattat	gttgaaatth	tatggcagct
55	4321	atthatttat	ttaaatttht	tgtaacagtt	ttgttttcta	ataagaaaaa	tccatgctth
	4381	ttgtagctgg	ttgaaaattc	aggaatatgt	aaaacttht	ggtatttht	taaattgatt
	4441	cctthtctta	atthtaaaaa	aaaaaaaaaa	(SEQ ID NO: 95)		

[0083] In certain embodiments provided are gene silencing compounds targeted to a mouse NLRP3 nucleic acid. In certain embodiments, the mouse NLRP3 nucleic acid is the

sequence set forth in GENBANK Accession No. NM_145827.3 (incorporated herein as SEQ ID NO: 96).

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1  ccaggtccta gcctcgtcac catgggttct ggtcagacac gagtcctggt gactttgtat
5  61  atgcgtgttc tctgtatacc acatctgatt gtgttaatgg ctttcttatt tttatctcta
121 cagaggaact tttcttccat ggctcaggac atacgtctgg atcaagctaa gagaactttc
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15 721 gcagctccaa ctggtcaagg agcatccaag caagcaggag cgggagcatg aactcctgac
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961 caaagacaaa tttgactatt tgttctttat ccactgccga gaggtgagcc tcaggacgcc
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1261 gacgaggccg gttagccttg agaaactgca gcatctcctg gaccaccccc gccatgtgga
25 1321 gatcctaggt ttctctgagg ccaaaggaa ggagtatttc tttaagtatt ttccaacga
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1561 cctgtgcaa tcccgggggg gcattgagga gcatctcttc tctgactacc tacaggggct
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35 1921 gtttgaaaaa ggctatctga ttttgttgt ccgattcctc ttggcccttg taaaccagga
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2161 ggaccacttt cccaaaattg agatcaacct ctctaccaga atggaccacg tggtttcctc
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2281 gcccaggag gaagaagaag agaggagagg aggtcgaccc ttggaccagg ttcagtgtgt
2341 tttccagac actcatgttg cctgttcttc cagactgggt aactgctgcc tcacttctag
2401 cttctgccgt ggtctcttct caagtctaag caccaaccgg agcctcactg aactggacct
2461 cagtgacaat actctgggag acccgggcat gaggggtctg tgtgaggcac tccagcacc
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3301 gactatagtc ttcgagattt cctggtaggc gtggaagcag gaccaccagg tgcctcggtc
3361 ctgccccaag tcctgcccc aagccccagt cgcactgctc ttactgcta tcaagccctc

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3421 cttcaccatc aggatcacag ccgaggctct tctggatatag ggtctggagc aaaggcttgt
 3481 gtgggaccaa atatTTTTcc tcacatcgat aacgtgaaac tgccagaggc tgcccttccc
 3541 atcatatcct cagtgggcaa ggtgttccct cttggtgact tcatggaaac agcttcaaga
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 5 3661 tccccctccc cccctcctc ctccgcttct ccccccacct gtctttctct ctctgggcct
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 3781 cataaggagc tgcccggtggg ctagatggaa ggtttgtggc agcctctcag ctacattgtt
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 3901 aaaacccatt tttgtattta ttgtatgttt gtactgcttt cttaatttaa aaatgtatct
 10 3961 agaattcttt taagttattt atccaaacta ctaaaaataa atcagtttac acatttaaaa
 4021 t (SEQ ID NO: 96)

[0084] Certain embodiments provide gene silencing compounds comprising two
 oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase
 15 sequence comprising a portion of at least 12 contiguous nucleobases complementary to an
 equal length portion of SEQ ID NO: 95. Certain embodiments provide compounds
 comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides
 having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases
 complementary to an equal length portion of SEQ ID NO: 95. Certain embodiments provide
 20 compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having
 a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases
 complementary to an equal length portion of SEQ ID NO: 95. In certain embodiments, the
 two oligonucleotide of the gene silencing compound each, independently, comprise at least 9,
 at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at
 25 least 18, or at least 19 contiguous nucleobases complementary to an equal length portion of
 SEQ ID NO: 95.

[0085] In certain embodiments, the two oligonucleotide of the gene silencing
 compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least
 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least
 30 21, at least 22, or at least 23, contiguous nucleobases complementary to an equal length
 portion of SEQ ID NO: 95.

[0086] Certain embodiments provide gene silencing compounds comprising two
 oligonucleotides each, independently, comprising a portion which consists of least 12
 contiguous nucleobases of SEQ ID NOs: 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55,
 35 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80,
 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94. In certain embodiments, the gene
 silencing compounds comprise two oligonucleotides each, independently, comprising a
 portion which consists of least 12 contiguous nucleobases of SEQ ID NOs: 42, 43, 44, 45, 46,
 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71,

72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, and is at least 80% complimentary to SEQ ID NO: 95. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion which consists of least 12 contiguous nucleobases of SEQ ID NOs: 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, and is at least 85% complimentary to SEQ ID NO: 95. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion which consists of least 12 contiguous nucleobases of SEQ ID NOs: 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, and is at least 90% complimentary to SEQ ID NO: 95. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion which consists of least 12 contiguous nucleobases of SEQ ID NOs: 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, and is at least 95% complimentary to SEQ ID NO: 95.

[0087] In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are, independently, at least 90% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 95. In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are, independently, at least 95% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 95. In certain embodiments, the oligonucleotides of the gene silencing compound are at least 99% complementary over its entire length to SEQ ID NO: 95. In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are 100% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 95.

[0088] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, consisting of 12 to 30 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 96. Certain embodiments provide compounds comprising two oligonucleotides each, independently, consisting of 15 to 25 nucleotides having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 96. Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 18 to 21 nucleotides having

a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 96. In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 96.

[0089] In certain embodiments, the two oligonucleotide of the gene silencing compound each, independently, comprise at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, or at least 23, contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 96.

[0090] Certain embodiments provide gene silencing compounds comprising two oligonucleotides each, independently, comprising a portion which consists of least 12 contiguous nucleobases of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion which consists of least 12 contiguous nucleobases of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41, and is at least 80% complimentary to SEQ ID NO: 96. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion which consists of least 12 contiguous nucleobases of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41, and is at least 85% complimentary to SEQ ID NO: 96. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion which consists of least 12 contiguous nucleobases of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41, and is at least 90% complimentary to SEQ ID NO: 96. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising a portion which consists of least 12 contiguous nucleobases of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41, and is at least 95% complimentary to SEQ ID NO: 96.

[0091] In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are, independently, at least 90% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 96. In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are, independently, at least 95% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 96. In certain embodiments, the oligonucleotides of the gene silencing compound are at least 99% complementary over its entire length to SEQ ID NO: 96. In certain embodiments, the nucleobase sequence of the oligonucleotides of the gene silencing compound are 100% complementary over its entire length to a nucleobase sequence of SEQ ID NO: 96.

[0092] In certain embodiments, the oligonucleotides of the gene silencing compound are, independently, 4 to 44 nucleotides in length. In certain embodiments, the oligonucleotides of the gene silencing compound are, independently, 12 to 30 nucleotides in length. In other words, the oligonucleotides are from 12 to 30 linked nucleobases. In other embodiments, the oligonucleotides, independently, consist of 15 to 28, 18 to 24, 19 to 22, or 20 linked nucleobases. In certain such embodiments, the oligonucleotides, independently, consist of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 linked nucleobases in length, or a range defined by any two of the above values.

[0093] In certain such embodiments, the oligonucleotides are 19 linked nucleobases in length.

[0094] In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for NLRP3 can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

[0095] Certain embodiments provide a composition comprising a gene silencing compound as described herein, or a salt thereof, and a pharmaceutically acceptable carrier or diluent. Certain embodiments provide a composition comprising two or more gene silencing compounds as described herein, or a salt thereof, and a pharmaceutically acceptable carrier or diluent. The two or more gene silencing compounds can inhibit the mRNA or protein expression of the same target or can inhibit the mRNA or protein expression of different targets.

[0096] In certain embodiments, gene silencing compounds according to the invention comprise two identical or different sequences linked at their 5'-5' ends via a phosphodiester, phosphorothioate or non-nucleoside linker. Gene silencing compounds according to the invention that comprise identical sequences are able to bind to a specific mRNA via Watson-Crick hydrogen bonding interactions and inhibit mRNA and protein expression. Gene silencing compounds according to the invention that comprise different sequences are able to bind to two or more different regions of one or more mRNA targets and inhibit mRNA and protein expression. Such compounds are comprised of heteronucleotide sequences complementary to target mRNA and form stable duplex structures through Watson-Crick hydrogen bonding.

[0097] The oligonucleotides of the gene silencing compounds are linked through their 5'-ends to allow the presence of two or more accessible 3'-ends. In certain embodiments, the oligonucleotides are linked through one or more of the non-nucleotide linkers listed in Table 1. In certain embodiments, a single linker listed in Table 1 is used to link the oligonucleotides of the gene silencing compounds. In certain embodiments, the linker is small molecule linker such as glycerol or a glycerol homolog of the formula $\text{HO}-(\text{CH}_2)_o\text{-CH(OH)-}(\text{CH}_2)_p\text{-OH}$, wherein o and p independently are integers from 1 to about 6, from 1 to about 4 or from 1 to about 3. In some other embodiments, the small molecule linker is a derivative of 1,3-diamino-2-hydroxypropane. Some such derivatives have the formula $\text{HO}-(\text{CH}_2)_m\text{-C(O)NH-CH}_2\text{-CH(OH)-CH}_2\text{-NHC(O)-}(\text{CH}_2)_m\text{-OH}$, wherein m is an integer from 0 to about 10, from 0 to about 6, from 2 to about 6 or from 2 to about 4. Representative non-nucleotide linkers are set forth in Table 1.

Table 1: Representative Non-Nucleotide Linkers

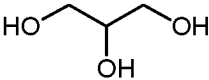
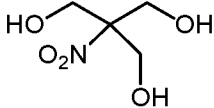
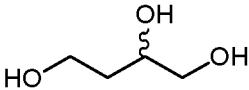
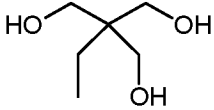
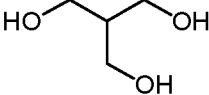
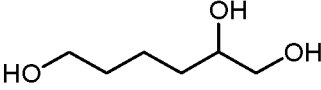
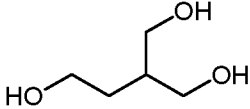
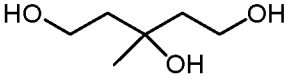
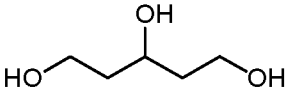
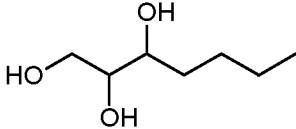
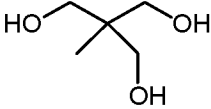
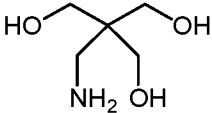
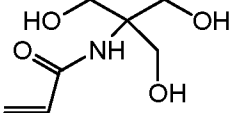
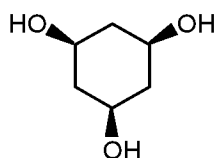
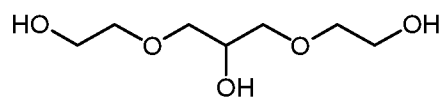
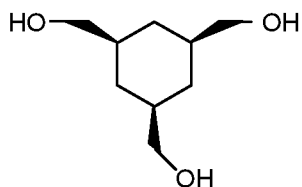
 Glycerol (1,2,3-Propanetriol)	 1,1,1-Tris(hydroxy methyl)nitromethane
 1,2,4-Butanetriol	 1,1,1-Tris(hydroxymethyl)propane
 2-(hydroxymethyl)-1,3-propanediol	 1,2,6-Hexanetriol
 2-(hydroxymethyl)1,4-butanediol	 3-Methyl-1,3,5-pentanetriol
 1,3,5-Pentanetriol	 1,2,3-Heptanetriol
 1,1,1-Tris(hydroxymethyl)ethane	 2-Amino-2-(hydroxymethyl)-1,3-propanediol
	 N-[Tris(hydroxymethyl)methyl]acrylamide

Table 1: Continued

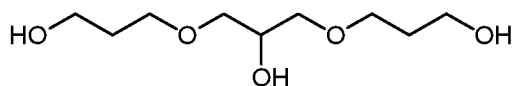
cis-1,3,5-Cyclohexanetriol



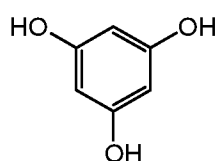
1,3-Di(hydroxyethoxy)-2-hydroxyl-propane



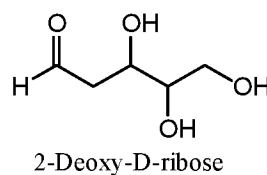
cis-1,3,5-Tri(hydroxymethyl)cyclohexane



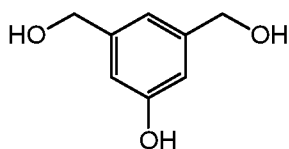
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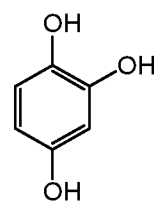
1,3,5-Trihydroxyl-benzene



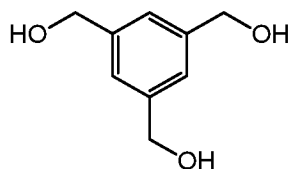
2-Deoxy-D-ribose



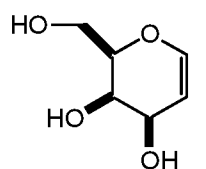
3,5-Di(hydroxymethyl)phenol



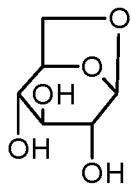
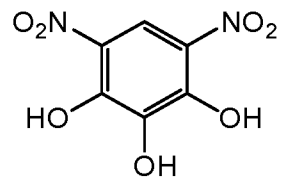
1,2,4-Trihydroxyl-benzene



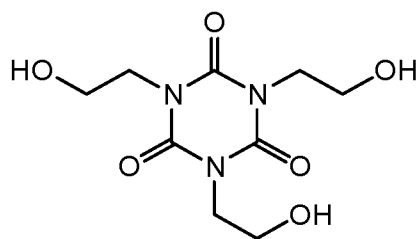
1,3,5-Tri(hydroxymethyl)benzene



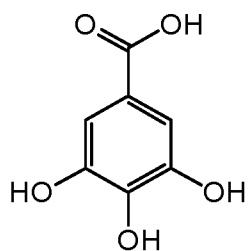
D-Galactool

Table 1: Continued1,6-anhydro- β -D-Glucose

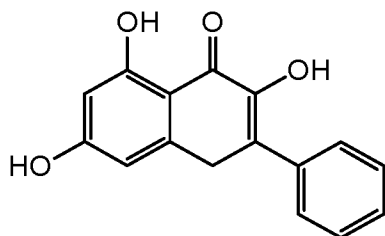
4,6-Nitropyrogallol



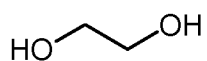
1,3,5-Tris(2-hydroxyethyl)-Cyanuric acid



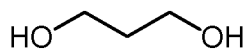
Gallic acid



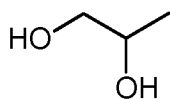
3,5,7-Trihydroxyflavone

Table 1: Continued

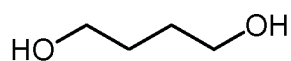
Ethylene glycol



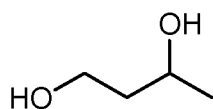
1,3-Propanediol



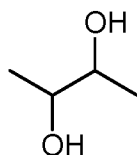
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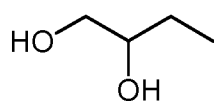
1,4-Butanediol



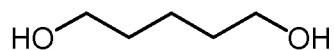
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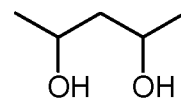
2,3-Butanediol



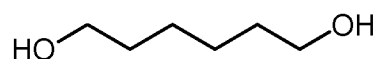
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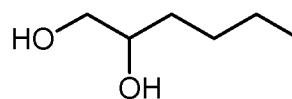
1,5-Pentanediol



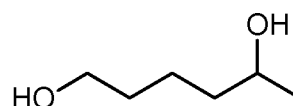
2,4-Pentanediol



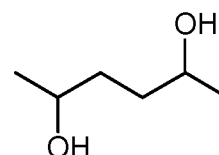
1,6-Hexanediol



1,2-Hexanediol

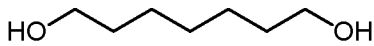
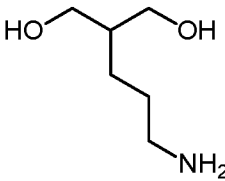
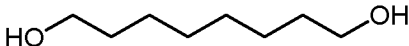
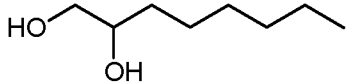
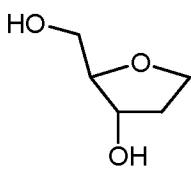
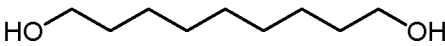
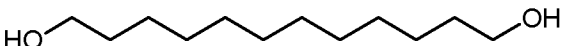
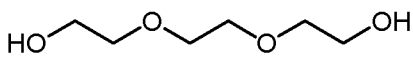
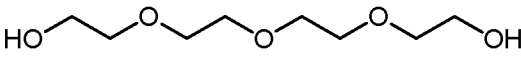
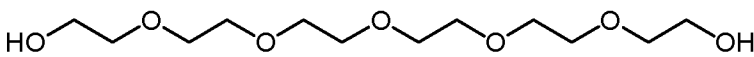


1,5-Hexanediol



2,5-Hexanediol

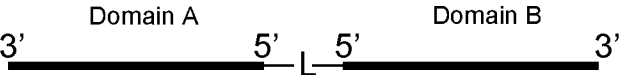
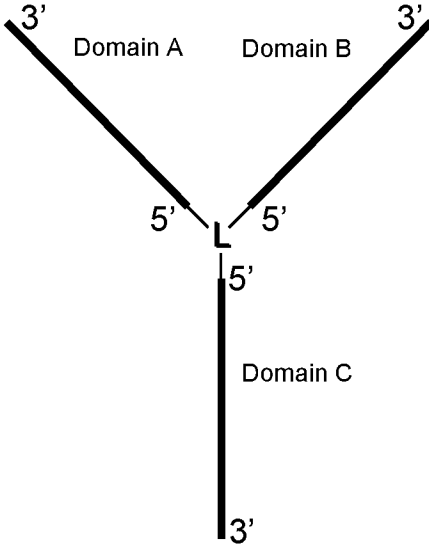
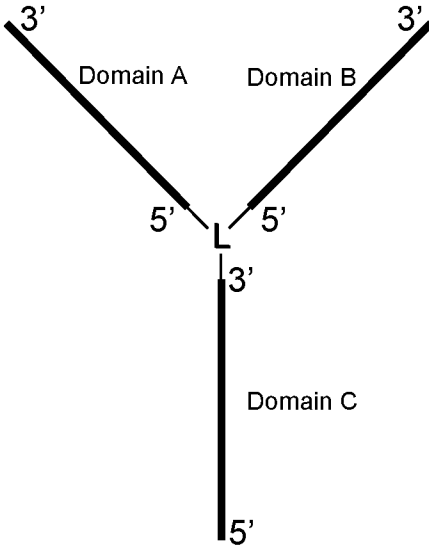
Table 1: Continued

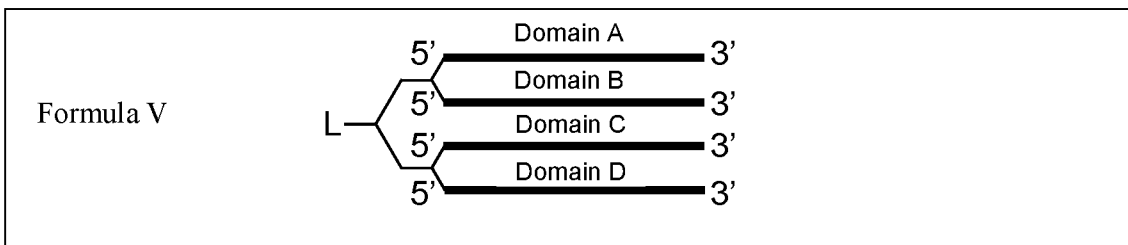
	
1,7-Heptanediol	2-(1-Aminopropyl)-1,3-propanediol
	
1,8-Octanediol	
	
1,2-Octanediol	1,2-Dideoxyribose
	
1,9-Nonanediol	
	
1,12-Dodecanediol	
	
Triethylene glycol	
	
Tetraethylene glycol	
	
Hexaethylene glycol	

[0098] In some embodiments, the small molecule linker is glycerol or a glycerol
5 homolog of the formula $\text{HO}-(\text{CH}_2)_o-\text{CH}(\text{OH})-(\text{CH}_2)_p-\text{OH}$, wherein o and p independently are
integers from 1 to about 6, from 1 to about 4 or from 1 to about 3. In some other
embodiments, the small molecule linker is a derivative of 1,3-diamino-2-hydroxypropane.
Some such derivatives have the formula

$\text{HO}-(\text{CH}_2)_m-\text{C}(\text{O})\text{NH}-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-\text{NHC}(\text{O})-(\text{CH}_2)_m-\text{OH}$, wherein m is an integer from 0 to about 10, from 0 to about 6, from 2 to about 6 or from 2 to about 4.

[0099] In certain embodiments, the two or more oligonucleotides of the gene silencing compounds of the invention can be linked as shown in Table 2.

Table 2: Oligoribonucleotide Formulas II – V	
Formula II	
Formula III	
Formula IV	



[00100] In certain embodiments of Formulas II and/or V, L is a linker or a nucleotide linkage and Domain A and/or Domain B are antisense oligonucleotides that are designed to selectively hybridize to the same target RNA sequence or different target RNA sequences.

5 **[00101]** In certain embodiments of Formulas II, III, IV or V, L is a linker and Domain A and/or Domain B and/or Domain C and/or Domain D are antisense oligonucleotides that are designed to selectively hybridize to the same target RNA sequence or different target RNA sequences. For example, in one embodiment, Domain A and/or Domain B and/or Domain C of Formulas II and/or III are antisense oligonucleotides that are designed to selectively hybridize to the same target RNA sequence. In this embodiment, Domain A and/or Domain B and/or Domain C can be designed to hybridize to the same region on the target RNA sequence or to different regions of the same target RNA sequence.

[00102] In a further embodiment of this aspect of the invention, Domain A, Domain B, Domain C, and Domain D are independently RNA or DNA-based oligonucleotides. In certain aspects of this embodiment, the oligonucleotides comprise mixed backbone oligonucleotides.

[00103] In another embodiment, one or more of Domain A and/or Domain B and/or Domain C and/or Domain D is an antisense oligonucleotide that is designed to selectively hybridize to one target RNA sequence and one or more of the remaining Domain A and/or Domain B and/or Domain C and/or Domain D is an antisense oligonucleotide that is designed to selectively hybridize to a different target RNA sequence.

[00104] In another embodiment, one or more of Domain A and/or Domain B and/or Domain C and/or Domain D is an RNA-based oligonucleotide hybridized to a complementary RNA-based oligonucleotide such that the domain comprises an siRNA molecule.

25 **[00105]** These gene silencing compounds of the invention can be prepared by the art recognized methods such as phosphoramidate or H-phosphonate chemistry which can be carried out manually or by an automated synthesizer. The synthetic antisense oligonucleotides of the invention may also be modified in a number of ways without compromising their ability to hybridize to mRNA. Such modifications may include at least

one internucleotide linkage of the oligonucleotide being an alkylphosphonate, phosphorothioate, phosphorodithioate, methylphosphonate, phosphate ester, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate hydroxyl, acetamidate or carboxymethyl ester or a combination of these and other internucleotide linkages between the 5' end of one nucleotide and the 3' end of another nucleotide in which the 5' nucleotide phosphodiester linkage has been replaced with any number of chemical groups.

[00106] The synthetic antisense oligonucleotides of the invention may comprise combinations of internucleotide linkages. For example, U.S. Pat. No. 5,149,797 describes traditional chimeric oligonucleotides having a phosphorothioate core region interposed between methylphosphonate or phosphoramidate flanking regions. Additionally, U.S. Pat. No. 5,652,356 discloses "inverted" chimeric oligonucleotides comprising one or more nonionic oligonucleotide region (e.g. alkylphosphonate and/or phosphoramidate and/or phosphotriester internucleoside linkage) flanked by one or more region of oligonucleotide phosphorothioate. Various synthetic antisense oligonucleotides with modified internucleotide linkages can be prepared according to standard methods. In certain embodiments, the phosphorothioate linkages may be mixed Rp and Sp enantiomers, or they may be made stereoregular or substantially stereoregular in either Rp or Sp form.

[00107] Other modifications of gene silencing compounds of the invention include those that are internal or at the end(s) of the oligonucleotide molecule and include additions to the molecule of the internucleoside phosphate linkages, such as cholesterol, cholesteryl, or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose, deoxyribose and phosphate modifications which cleave, or crosslink to the opposite chains or to associated enzymes or other proteins which bind to the genome. Examples of such modified oligonucleotides include oligonucleotides with a modified base and/or sugar such as 2'-O,4'-C-methylene-b-D-ribofuranosyl, or arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide having a sugar which, at both its 3' and 5' positions, is attached to a chemical group other than a hydroxyl group (at its 3' position) and other than a phosphate group (at its 5' position).

[00108] Other examples of modifications to sugars of the oligonucleotide-based compounds of the invention include modifications to the 2' position of the ribose moiety which include but are not limited to 2'-O-substituted with an -O-alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an -O-aryl, or -O-allyl group having 2-6 carbon atoms wherein such -O-alkyl, -O-aryl or -O-allyl group may be unsubstituted or may

be substituted, for example with halo, hydroxyl, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxy, carbalkoxyl or amino groups. None of these substitutions are intended to exclude the presence of other residues having native 2'-hydroxyl group in the case of ribose or 2' H- in the case of deoxyribose.

5 [00109] The gene silencing compounds according to the invention can comprise one or more ribonucleotides. For example, US Pat No. 5,652,355 discloses traditional hybrid oligonucleotides having regions of 2'-O-substituted ribonucleotides flanking a DNA core region. U.S. Pat. No. 5,652,356 discloses an "inverted" hybrid oligonucleotide that includes an oligonucleotide comprising a 2'-O-substituted (or 2' OH, unsubstituted) RNA region
10 which is in between two oligodeoxyribonucleotide regions, a structure that "inverted relative to the "traditional" hybrid oligonucleotides. Non-limiting examples of particularly useful oligonucleotides of the invention have 2'-O-alkylated ribonucleotides at their 3', 5', or 3' and 5' termini, with at least four, and in some exemplary embodiments five, contiguous nucleotides being so modified. Non-limiting examples of 2'-O-alkylated groups include 2'-
15 O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-butyls and 2'-O-methoxy-ethyl.

[00110] The oligonucleotide-based compounds of the invention may conveniently be synthesized using an automated synthesizer and phosphoramidite approach further described in Example 1. In some embodiments, the oligonucleotide-based compounds of the invention are synthesized by a linear synthesis approach.

20 [00111] An alternative mode of synthesis is "parallel synthesis", in which synthesis proceeds outward from a central linker moiety. A solid support attached linker can be used for parallel synthesis, as is described in U.S. Patent No. 5,912,332. Alternatively, a universal solid support (such as phosphate attached controlled pore glass) support can be used.

[00112] Parallel synthesis of the oligonucleotide-based compounds of the invention
25 has several advantages over linear synthesis: (1) parallel synthesis permits the incorporation of identical monomeric units; (2) unlike in linear synthesis, both (or all) the monomeric units are synthesized at the same time, thereby the number of synthetic steps and the time required for the synthesis is the same as that of a monomeric unit; and (3) the reduction in synthetic steps improves purity and yield of the final immune modulatory oligoribonucleotide product.

30 [00113] At the end of the synthesis by either linear synthesis or parallel synthesis protocols, the oligonucleotide-based compounds of the invention may conveniently be deprotected with concentrated ammonia solution or as recommended by the phosphoramidite supplier, if a modified nucleoside is incorporated. The product oligonucleotide-based

compounds is preferably purified by reversed phase HPLC, detritylated, desalted and dialyzed.

[00114] In certain embodiments, the oligonucleotides of the gene silencing compound according to the invention are selected from the non-limiting list of the oligonucleotides shown in Table 3 below. The oligonucleotides shown in Table 3 have phosphorothioate (PS) linkages, but may also include phosphodiester linkages. Those skilled in the art will recognize, however, that other linkages, based on phosphodiester or non-phosphodiester moieties may be included.

Table 3

Oligo # / SEQ ID NO:	Target	Target Site	Sequence 5' → 3'
1	mNLRP3	21	TGTCTGACCAGAACCCATG
2	mNLRP3	155	CTCTTAGCTTGATCCAGAC
3	mNLRP3	211	CATCTTCAGCAGCAGCCCT
4	mNLRP3	242	GATACTGAGCCAGCTTGCA
5	mNLRP3	349	TGCCTTCTCCATCTGGCCC
6	mNLRP3	359	CCAAGTGATCTGCCTTCTC
7	mNLRP3	456	TCCCAGAGGTCTCGCCTGT
8	mNLRP3	485	TCCACTCTGGCTGGTCCTT
9	mNLRP3	531	CTGTCCTCCTGGCATAACCA
10	mNLRP3	532	GCTGTCCTCCTGGCATAACC
11	mNLRP3	728	GATGCTCCTTGACCAGTTG
12	mNLRP3	888	CCGATGCCTGCTGCTCCCT
13	mNLRP3	934	TCCCAGTGCCCAGTCCAAC
14	mNLRP3	936	TTTCCCAGTGCCCAGTCCA
15	mNLRP3	1007	TCCTTGCGTCCTGAGGCT
16	mNLRP3	1050	GGGTTTGGGTCAGGCCAGC

17	mNLRP3	1116	AGCTCATCAAAGCCATCCA
18	mNLRP3	1153	GCAGACCTCCCCAATGTGC
19	mNLRP3	1178	CCCGCACAGCCTTTTGCCA
20	mNLRP3	1291	GGGGTGGTCCAGGAGATGC
21	mNLRP3	1399	CTCTTGGATCAGCCTGAAG
22	mNLRP3	1614	GCCAGTGAACAGAGCCCCT
23	mNLRP3	1664	GCAGATCACACTCCTCAAA
24	mNLRP3	1738	GCAGTCCACTTCCTTCTGG
25	mNLRP3	1847	CTCCTGGTCCTTTCCTCAC
26	mNLRP3	1848	CCTCCTGGTCCTTTCCTCA
27	mNLRP3	1965	CTCTCCTGGTTTACAAGGC
28	mNLRP3	2050	GGCTTTCACCTTCAATCCAC
29	mNLRP3	2190	TGGTCCATTCTGGTAGAGA
30	mNLRP3	2320	AACTGAACCTGGTCCAAG
31	mNLRP3	2439	TCCAGTTCAGTGAGGCTCC
32	mNLRP3	2505	CCTGGGTGCTGGAGTGCCT
33	mNLRP3	2632	CCCCAGGGCATTGTCACTG
34	mNLRP3	2810	CTGAGTCTCCCAAGGCATT
35	mNLRP3	2813	CTCCTGAGTCTCCCAAGGC
36	mNLRP3	2951	AGAGGTGTGTGAAGTTCTG
37	mNLRP3	3014	GGTGCAGAAGCCCCTCACA
38	mNLRP3	3131	TGCCCAGGTTCAGCTTCCG
39	mNLRP3	3218	CACCCAAGTGTAGGCTCTG
40	mNLRP3	3332	CCTGGTGGTCCTGCTTCCA
41	mNLRP3	3459	AGCCTTTGCTCCAGACCCT

42	hNLRP3	715	CAGGGTCCTTAGGCTTCGG
43	hNLRP3	743	GCTTGCCATCTTCATCTGC
44	hNLRP3	796	TTCAAGTCCACATCCTCCA
45	hNLRP3	827	AGGATAGTCCTCTAAGTGC
46	hNLRP3	892	GCTAGATCCACATGGTCTG
47	hNLRP3	985	TTCTCATAAAGGTCTCTCC
48	hNLRP3	1057	TCCTGGCATATCACAGTGG
49	hNLRP3	1079	CCACTCCTCTTCAATGCTG
50	hNLRP3	1094	CTCCAGTAAACCCATCCAC
51	hNLRP3	1191	TCCTGTCTTCAATGCACTG
52	hNLRP3	1359	GGTCAAACAGCAACTCCAT
53	hNLRP3	1427	TGTTTTCCCAATCCCTGCC
54	hNLRP3	1434	CCAGGATTGTTTTCCCAAT
55	hNLRP3	1435	GCCAGGATTGTTTTCCCAA
56	hNLRP3	1537	CTCTGTGTCACAAGGCTCA
57	hNLRP3	1546	CCCAGGCTCCTCTGTGTCA
58	hNLRP3	1590	GGATGGGTGGGTTTGGGTC
59	hNLRP3	1664	GTCAAAGGCACCTTGCAGC
60	hNLRP3	1701	CCTTCTGCCAGTCAGTGCA
61	hNLRP3	1702	GCCTTCTGCCAGTCAGTGC
62	hNLRP3	2017	TTGCCACTCTCCATCTGCT
63	hNLRP3	2018	CTTGCCACTCTCCATCTGC
64	hNLRP3	2034	ATGTCTGGGCAAGGCTCTT
65	hNLRP3	2046	TGGTGGTCTTGGATGTCTG
66	hNLRP3	2150	TGCAGCCAAAGAGCAGAGC

67	hNLRP3	2342	CTCTTCCAGCAGGTAGTAC
68	hNLRP3	2429	TTTGCCATAGTTTTCCAGA
69	hNLRP3	2552	CAGCTCCAGCCTGATTTGC
70	hNLRP3	2773	CCCAGGGACAGTGACTCCA
71	hNLRP3	2849	GACACACTGCACCATATCA
72	hNLRP3	2959	GTTAGACTCTGGCTGGTGC
73	hNLRP3	3012	ACAACACTCTCATCCCTGG
74	hNLRP3	3121	TGGTTGCTGCTGAGGACCA
75	hNLRP3	3192	GTCCCACACACAGAAGTCT
76	hNLRP3	3296	GGAATGGCTGGTGCTCAAT
77	hNLRP3	3315	CCACATAGAGTCTGGTCAG
78	hNLRP3	3316	CCCACATAGAGTCTGGTCA
79	hNLRP3	3464	CTGATTAGTGCTGAGTACC
80	hNLRP3	3540	GCAAGAGTCCCTCACAGAG
81	hNLRP3	3610	AGATCCCAGCAGCAGTGTG
82	hNLRP3	3673	CCCAGGTCATTGTTGCCCA
83	hNLRP3	3814	GTCAGCTCAGGCTTTTCTT
84	hNLRP3	3815	GGTCAGCTCAGGCTTTTCT
85	hNLRP3	3844	CACTCCTACCAAGAAGGCT
86	hNLRP3	3849	GTTTCCACTCCTACCAAGA
87	hNLRP3	3929	TGGCCTGGATGGATCGCAG
88	hNLRP3	3981	GGCAAGCTCTCTTCTCCGA
89	hNLRP3	4013	GATGCCCAAGCTCTGCACA
90	hNLRP3	4014	AGATGCCCAAGCTCTGCAC
91	hNLRP3	4015	GAGATGCCCAAGCTCTGCA

92	hNLRP3	4018	AAGGAGATGCCCAAGCTCT
93	hNLRP3	4101	AGGAACATCCTCTAACTGA
94	hNLRP3	4265	AGTTCTGTGTTATGGTCAG
95	mNLRP3	3285	GTCAGCTCAGGCTTTTCTT

[00115] Compound names for GSOs directed to human NLRP3 are based on the oligonucleotide target sites as depicted in SEQ ID NO: 95. Compound names for GSOs directed to mouse NLRP3 are based on their target sites of SEQ ID NO: 96. For example, a GSO comprising two copies of Oligo # 13 (e.g., 3'-CAACCTGACCCGTGACCCT-5'-X-5'-TCCCAGTGCCCAGTCCAAC-3', wherein X represents a non-nucleotidic linker) will be referred to herein, for example, as "943", or "m943", or "GSO 934", "mGSO-934", or "GSO-m934", or "NLRP-934" or "GSO NLRP-934". Additionally, a GSO comprising two different oligonucleotides such as Oligo # 93 and Oligo # 94 (e.g., 3'-AGTCAATCTCCTACAAGGA-5'-X-5'-AGTTCTGTGTTATGGTCAG-3', wherein X represents a non-nucleotidic linker) will be referred to herein, for example, as "4101/4265", "GSO 4101/4265", or "NLRP-4101/4265" or "GSO NLRP-4101/4265".

[00116] Certain embodiments provide gene silencing compounds comprising two oligonucleotides independently selected from the oligonucleotides listed in Table 3. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising the sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, or combinations thereof. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising the sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41, or combinations thereof. In certain embodiments, the gene silencing compounds comprise two oligonucleotides each, independently, comprising the sequence of SEQ ID NO: 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, or combinations thereof. In certain embodiments, the oligonucleotides of the gene silencing compound are the

same. In certain embodiments, the oligonucleotides of the gene silencing compounds are different.

[00117] In certain embodiments, the invention provides a composition comprising a gene silencing compound according to the invention and one or more vaccines, antigens, antibodies, cytotoxic agents, chemotherapeutic agents (both traditional chemotherapy and modern targeted therapies), kinase inhibitors, allergens, antibiotics, agonist, antagonist, antisense oligonucleotides, ribozymes, RNAi molecules, siRNA molecules, miRNA molecules, aptamers, proteins, gene therapy vectors, DNA vaccines, adjuvants, co-stimulatory molecules or combinations thereof.

[00118] In certain embodiments, the invention provides a method for inhibiting NLRP3 mRNA or protein expression, the method comprising contacting a cell with a gene silencing compound according to the invention. In certain embodiments, the cell can be contacted with two or more gene silencing compounds targeting different regions of NLRP3.

[00119] In certain embodiments, gene silencing compounds according to the invention are useful in treating and/or preventing diseases wherein inhibiting NLRP3 expression would be beneficial.

[00120] Certain embodiments further provide a method to reduce NLRP3 mRNA or protein expression in an animal comprising administering to the animal a gene silencing compound or composition as described herein to reduce NLRP3 mRNA or protein expression in the animal. In certain embodiments, the animal is a human. In certain embodiments, reducing NLRP3 mRNA or protein expression prevents, treats, ameliorates, or slows progression of disease. In certain embodiments two or more gene silencing compounds targeting different regions of NLRP3 can be administered.

[00121] In certain embodiments provided are methods for treating diseases or disorders comprising administering to the animal a gene silencing compound or composition as described herein to reduce NLRP3 mRNA or protein expression in the animal. In certain embodiments, the animal is a human. In certain embodiments two or more gene silencing compounds targeting different regions of NLRP3 can be administered.

[00122] In certain embodiments provided are methods, compounds, and compositions for the treatment, prevention, or amelioration of diseases, disorders, and conditions associated with NLRP3 in an individual in need thereof. Also contemplated are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of a disease, disorder, or condition associated with NLRP3. In certain embodiments two or more gene silencing compounds targeting different regions of NLRP3 can be administered.

[00123] NLRP3 associated diseases, disorders, and conditions include, but are not limited to, familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), chronic infantile neurological cutaneous and articular (CINCA) syndrome, neonatal-onset multisystem inflammatory disease (NOMID), interstitial cystitis/bladder pain syndrome (IC/BPS) multiple sclerosis, rheumatoid arthritis, gout, Alzheimer's disease, allergy and asthma, inflammatory bowel disease, atherosclerosis, type II diabetes, uveitis, hypertension, psoriasis, obesity, chronic obstructive pulmonary disease, nonalcoholic steatohepatitis, mucositis, Parkinson's disease, asbestosis, hepatomas, mesothelioma, chronic kidney disease, Schnitzler syndrome, cellulitis, conjunctivitis, dry eye syndrome, pyoderma gangrenosum, PAPA syndrome (pyogenic arthritis, pyoderma gangrenosum and acne) and any other disease, disorder or condition that would benefit from the modulation of NLRP3 mRNA or protein expression.

[00124] In certain embodiments provided are NLRP3 gene silencing compounds for use in treating, preventing, or ameliorating a NLRP3 associated disease. In certain embodiments, NLRP3 gene silencing compounds are capable of inhibiting the expression of NLRP3 mRNA and/or NLRP3 protein in a cell, tissue, or animal.

[00125] Certain embodiments provide methods comprising administering to an animal a gene silencing compounds as described herein. In certain embodiments two or more gene silencing compounds targeting different regions of NLRP3 can be administered.

[00126] Also provided are methods and gene silencing compounds for the preparation of a medicament for the treatment, prevention, or amelioration of disease.

[00127] Certain embodiments provide the use of gene silencing compounds as described herein in the manufacture of a medicament for treating, ameliorating, or preventing disease.

[00128] Certain embodiments provide gene silencing compounds as described herein for use in treating, preventing, or ameliorating disease as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

[00129] Certain embodiments provide the use of a gene silencing compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating disease as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

[00130] Certain embodiments provide the use of a gene silencing compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating

disease as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

[00131] In any of the methods according to the invention, the gene silencing compound according to the invention can variously act by producing direct gene expression modulation effects alone and/or in combination with any other agent useful for treating or preventing the disease or condition that does not diminish the gene expression modulation effect of the gene silencing compound according to the invention. In any of the methods according to the invention, the agent(s) useful for treating or preventing the disease or condition includes, but is not limited to, vaccines, antigens, antibodies, preferably monoclonal antibodies, cytotoxic agents, kinase inhibitors, allergens, antibiotics, siRNA molecules, antisense oligonucleotides, TLR antagonist (e.g. antagonists of TLR3 and/or TLR7 and/or antagonists of TLR8 and/or antagonists of TLR9), chemotherapeutic agents (both traditional chemotherapy and modern targeted therapies), targeted therapeutic agents, activated cells, peptides, proteins, gene therapy vectors, peptide vaccines, protein vaccines, DNA vaccines, adjuvants, and co-stimulatory molecules (e.g. cytokines, chemokines, protein ligands, trans-activating factors, peptides or peptides comprising modified amino acids), or combinations thereof. Alternatively, the gene silencing compound according to the invention can be administered in combination with other compounds (for example lipids or liposomes) to enhance the specificity or magnitude of the gene expression modulation of the oligonucleotide-based compound according to the invention.

[00132] In any of the methods according to the invention, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, can be by any suitable route, including, without limitation, intramuscular, parenteral, mucosal, oral, sublingual, intratumoral, transdermal, topical, inhalation, intrathecal, intranasal, aerosol, intraocular, intratracheal, intrarectal, vaginal, by gene gun, dermal patch or in eye drop or mouthwash form. In any of the methods according to the invention, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, can be directly to a tissue or organ such as, but not limited to, the eye, bladder, liver, lung, kidney or lung. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by intramuscular administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by mucosal administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other

agent, is by intraocular administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by oral administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by
5 intrarectal administration. In certain embodiments, administration of gene silencing compounds according to the invention, alone or in combination with any other agent, is by intrathecal administration.

[00133] Administration of the therapeutic compositions of gene silencing compounds according to the invention can be carried out using known procedures using an effective
10 amount and for periods of time effective to reduce symptoms or surrogate markers of the disease. For example, an effective amount of a gene silencing compound according to the invention for treating a disease and/or disorder could be that amount necessary to alleviate or reduce the symptoms, or delay or ameliorate the disease and/or disorder. In the context of administering a composition that modulates gene expression, an effective amount of a gene
15 silencing compound according to the invention is an amount sufficient to achieve the desired modulation as compared to the gene expression in the absence of the gene silencing compound according to the invention. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular compound being administered, the size of the subject, or the severity of the disease or
20 condition. One of ordinary skill in the art can empirically determine the effective amount of a particular compound without necessitating undue experimentation.

[00134] When administered systemically, the therapeutic composition is preferably administered at a sufficient dosage to attain a blood level of gene silencing compound according to the invention from about 0.0001 micromolar to about 10 micromolar. For
25 localized administration, much lower concentrations than this may be effective, and much higher concentrations may be tolerated. Preferably, a total dosage of gene silencing compound according to the invention ranges from about 0.001 mg per patient per day to about 200 mg per kg body weight per day. In certain embodiments, the total dosage may be 0.08, 0.16, 0.32, 0.48, 0.32, 0.64, 1, 10 or 30 mg/kg body weight administered daily, twice
30 weekly or weekly. It may be desirable to administer simultaneously, or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention to an individual as a single treatment episode.

[00135] The methods according to this aspect of the invention are useful for model studies of gene expression. The methods are also useful for the prophylactic or therapeutic

treatment of human or animal disease. For example, the methods are useful for pediatric and veterinary inhibition of gene expression applications.

[00136] The examples below are intended to further illustrate certain preferred embodiments of the invention, and are not intended to limit the scope of the invention.

5 Examples

Cell Culture Conditions and Reagents:

[00137] Cell lysis buffer, NLRP3 and α/β -tubulin antibodies were from Cell Signaling Technology (Danvers, MA). Anti-rabbit IgG-horse radish peroxidase (HRP) conjugate was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Bio-Rad protein reagent, Ready Gels, 10 Laemmli sample buffer and PVDF membranes were from BioRad Laboratories (Hercules, CA), whereas Western Lightning Plus Chemiluminescence kit was from Perkin Elmer Life Sciences (Waltham, MA). RNeasy kits and Taqman gene expression assays and PCR reagents were purchased from Qiagen and ThermoFisher Scientific, respectively. HyBlot CL autoradiography film was purchased from Denville Scientific (Metuchen, NJ). Human and 15 mouse IL-18 and IL-1 β ELISA kits were purchased from R&D Systems (Minneapolis, MN). ATP was purchased from Invivogen (San Diego, CA). All other chemicals and reagents were purchased either from Sigma (St. Louis, MO).

[00138] Murine macrophage-like cells, J774A.1 (American Type Culture Collection, Rockville, MD) were cultured in Dulbecco's modified Eagle's medium supplemented with 20 10% heat-inactivated defined FBS (Hyclone) and antibiotics (100 IU/mL of penicillin G/streptomycin). All other culture reagents were purchased from Mediatech (Gaithersburg, MD).

Preparation of GSO/lipid complexes

25 [00139] For all gene silencing experiments, culture medium without antibiotics was used. For primary screens, exemplary GSOs shown in Table 4 were transfected at 5 and 25 nM final concentration while for dose-response curve experiments, the GSOs were serially diluted starting at 50 or 100 nM. For transfection, appropriate GSO concentrations were prepared either in culture medium (no serum) or Opti-MEM just before use, mixed with 30 lipofectamine RNAiMax (final concentration, 3 μ l/ml) and incubated at room temperature for 20 minutes.

[00140] Table 4

GSO	GSO sequence
242	3'-ACGTTGACCGAGTCATAG-5'-X-5'-GATACTGAGCCAGCTTGCA-3'
934	3'-CAACCTGACCCGTGACCCT-5'-X-5'-TCCCAGTGCCCAGTCCAAC-3'
1664	3'-AAACTCCTCACACTAGACG-5'-X-5'-GCAGATCACACTCCTCAAA-3'
531	3'-ACCATACGGTCCTCCTGTC-5'-X-5'-CTGTCCTCCTGGCATACCA-3'
155	3'-CAGACCTAGTTTCGATTCTC-5'-X-5'-CTCTTAGCTTGATCCAGAC-3'
21	3'-GTACCCAAGACCAGTCTGT-5'-X-5'-TGTCTGACCAGAACCCATG-3'
2050	3'-CACCTAACTTCACTTTCGG-5'-X-5'-GGCTTTCACCTTCAATCCAC-3'
1738	3'-GGTCTTCCTTCACCTGACG-5'-X-5'-GCAGTCCACTTCCTTCTGG-3'
1399	3'-GAAGTCCGACTAGGTTCTC-5'-X-5'-CTCTTGGATCAGCCTGAAG-3'
1965	3'-CGGAACATTTGGTCCTCTC-5'-X-5'-CTCTCCTGGTTTACAAGGC-3'
532	3'-CCATACGGTCCTCCTGTCG-5'-X-5'-GCTGTCCTCCTGGCATAACC-3'
936	3'-ACCTGACCCGTGACCCTTT-5'-X-5'-TTTCCCAGTGCCCAGTCCA-3'
3459	3'-TCCCAGACCTCGTTTCCGA-5'-X-5'-AGCCTTTGCTCCAGACCCT-3'
2320	3'-GAACCTGGTCCAAGTCACA-5'-X-5'-ACACTGAACCTGGTCCAAG-3'
1094	3'-CACCTACCCAAATGACCTC-5'-X-5'-CTCCAGTAAACCCATCCAC-3'
1359	3'-TACCTCAACGACAACTGG-5'-X-5'-GGTCAAACAGCAACTCCAT-3'
1537	3'-ACTCGGAACACTGTGTCTC-5'-X-5'-CTCTGTGTCACAAGGCTCA-3'
1546	3'-ACTGTGTCTCCTCGGACCC-5'-X-5'-CCCAGGCTCCTCTGTGTCA-3'

where X is glycerol.

Monitoring gene expression in J774, THP-1 or PBMCs treated with GSOs

5 [00141] For gene silencing, J774 cells were plated overnight at a concentration of 0.3×10^6 cells/ml in 12-well culture plates. Media was changed the next morning and the GSO /lipid complexes were added and cells were incubated at 37°C for 24 hours.

[00142] THP-1 (1 million cells/ ml in 12-well plate) cells were differentiated with phorbol myristate acetate (PMA, 500 ng/ml) for 3hr, washed and resuspended in RPMI
10 complete medium and incubated overnight. GSO/lipid complexes were added the next day and incubation continued for 24 hours.

[00143] Freshly isolated human PBMCs (10×10^6 cells/ml) in 6-well culture plates were incubated with the GSO/lipid for 24 hours.

[00144] At the end of the experiment, media was removed and the pelleted cells were
15 lysed and homogenized using a QIAshredder Kit.

RNA analysis

[00145] RNA was isolated using the Qiagen RNeasy Mini Kit, following the manufacturer's protocol and reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit. Real Time PCR was performed on the cDNAs generated, using TaqMan Fast Universal PCR Master Mix and probes (Applied Biosystems, Carlsbad, CA) specific for mouse (Mm00840904_m1) or human (Hs00918082_m1) NLRP3 on StepOnePlus TaqMan Real-Time PCR System. Target mRNA levels in the samples were normalized using peptidylprolyl isomerase B, PPIB (Mm00478295_m1 and Hs00168719_m1 for mouse and human, respectively) as an endogenous control. The expression data are shown either as relative quantities or log2FC (fold control) of NLRP3 in the samples treated with GSOs compared with a PBS control.

Western blotting and quantitation of protein expression

[00146] For monitoring protein expression, cells were harvested at the end of the experiment, washed with chilled PBS containing protease inhibitors and then suspended in cell lysis buffer with protease inhibitors. Cells were lysed on ice for 15 minutes, sonicated briefly and centrifuged at 14000g for 20 minutes. The supernatants were transferred to fresh vials and stored at -70° C till further use. Protein concentration in the samples was measured by the method of Bradford using the Bio-Rad protein assay. Samples (20-30 µg/lane) were subjected to gel electrophoresis using 10% or 4-15% gradient Tris-HCl Ready gels, and western blotted onto PVDF membranes. After blocking in 5% non-fat milk in PBS-Tween 20 for 1 h, the membranes were incubated overnight with the appropriate primary antibody. Labeled proteins were visualized by the enhanced chemiluminescence method using HRP-coupled secondary antibodies and quantitated using a Scion Image Analysis Program (Scion Corp., Fredrick, MD). For re-probing of Western blots, the blots were washed in PBS, incubated for 30 min in the stripping buffer (Thermo Scientific). They were then washed thoroughly in PBST and probed with another primary antibody using the protocol described above.

Functional inhibition studies

[00147] Following incubation of cells with GSOs for 24 hours, media was changed and cells were primed with lipopolysaccharide (100 ng ml) for 4 hours and stimulated with ATP (5mM) for 1 hour. Culture supernatants were analyzed for IL-1 β and IL-18 secretion by

ELISA. Cell lysates were processed for RNA isolation and quantitative real-time PCR analysis for NLRP3.

mNLRP3 GSO in an animal model of interstitial cystitis

5 1.) Acute CYP-IC model treated with GSO s.c.

[00148] Interstitial cystitis was induced in four groups (n=10) of female CD1 mice, 7 to 9 weeks of age (Charles River Laboratories, Wilmington, MA) by intraperitoneal injection with 200 mg/kg of cyclophosphamide (Sigma, St. Louis, MO) diluted in PBS. The mice were treated by subcutaneous injection of different doses of NLRP GSO or PBS as vehicle 1 h post-cyclophosphamide administration. Five naïve CD1 mice of the same sex and age, without any treatment, were used as controls.

[00149] All mice were sacrificed at 24 h post disease induction. Urine samples were collected and stored at -20°C for cytokine assay later. Bladders were collected, weighed, and stored in 10% neutral buffered formalin for histology process, or stored in RNALater for gene expression analysis. Results are shown in Figures 11-13.

15 2.) Chronic CYP-IC model treated with GSO s.c.

[00150] Interstitial cystitis was induced in two groups (n=10) of female CD1 mice, 7 to 9 weeks of age by intraperitoneal injection with 150 mg/kg of cyclophosphamide at day 0, 1 and 3. The mice were treated by subcutaneous injection of 25 mg/kg of NLRP GSO or PBS as vehicle 1, 2 and 3 days post-cyclophosphamide administration. Five naïve CD1 mice of the same sex and age, without treatment, were used as controls. Results are shown in Figure 14.

 3.) Acute CYP-IC model treated with intrabladder instillation of GSO

[00151] Interstitial cystitis was induced in four groups (n=5) of female CD1 mice, 7 to 9 weeks of age by intraperitoneal injection with 200 mg/kg of cyclophosphamide diluted in PBS. The mice were treated by intra-bladder (i.b.) instillation of different doses of NLRP GSO or PBS as vehicle 1 h post-cyclophosphamide administration. Five naïve CD1 mice of the same sex and age, without any treatment, were used as controls.

[00152] All mice were sacrificed at 24 h post disease induction. Urine samples were collected and stored at -20°C for cytokine assay later. Bladders were collected, weighed, and stored in 10% neutral buffered formalin for histology. Results are shown in Figure 15A and B.

mNLRP3 GSO in an animal model of experimental autoimmune uveitis

[00153] To induce experimental autoimmune uveitis, 6 to 7 week old male B10-RIII mice (Jackson Laboratories, Bar Harbor, ME, USA) were injected at base of the tail and two thighs with 100 µg of IRBP161-180 peptide (AnaSpec, San Jose, CA) and 1 mg of bovine eye homogenate (InVision BioResources, Seattle, WA) emulsified 1:1 vol/vol in complete Freund's adjuvant (Sigma, St Louis, MO) supplemented with Mycobacterium tuberculosis (Voigt Global Distribution Inc., Lawrence, KS) to 10 mg/ml concentration. Four hours after the first immunization, mice with injected i.p. with 0.5 µg of pertussis Toxin (List Biological Laboratories, Campbell, California).

[00154] All mice received a boost immunization of 100 µg of IRBP/1 mg bovine eye homogenate emulsified 1:1 vol/vol in incomplete Freund's adjuvant (Sigma) on day 7.

[00155] Immunized mice were treated by subcutaneous injection of 15 mg/kg of NLRP GSO or PBS as vehicle at day 8, 10 and 13. Five naïve B10.RIII mice of the same sex and age, without any treatment, were used as controls.

[00156] All mice were sacrificed at day 14 after blood samples were collected. The left eyes from each mouse were collected and stored in 10% neutral buffered formalin for histology process, and right eyes are stored in RNA Later for gene expression analysis. Results are shown in Figure 16A-16D.

EQUIVALENTS

[00157] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. For example, antisense oligonucleotides that overlap with the oligonucleotides may be used. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

[00158] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A synthetic oligonucleotide compound comprising two single stranded antisense
5 oligonucleotides that are linked through their 5'-ends to allow the presence of two or more
accessible 3'-ends, each oligonucleotide, independently, comprises 12 to 30 nucleotides
having a nucleobase sequence comprising a portion of at least 12 contiguous nucleobases
complementary to an equal length portion of SEQ ID NO: 95 or SEQ ID NO: 96.
2. The compound according to claim 1, wherein the oligonucleotides comprise the same
10 sequence.
3. The compound according to claim 1, wherein the oligonucleotides are each,
independently, between 15 to 25 nucleotides in length.
4. The compound according to claim 1, wherein the nucleobase sequence of each
oligonucleotide is, independently, at least 90% complementary over its entire length to a
15 nucleobase sequence of SEQ ID NO: 95.
5. The compound according to claim 1, wherein the nucleobase sequence of each
oligonucleotide is, independently, at least 90% complementary over its entire length to a
nucleobase sequence of SEQ ID NO: 96.
6. The compound according to claim 1, wherein each oligonucleotide, independently,
20 comprises a portion of at least 12 contiguous nucleobases of SEQ ID NO: 42, 43, 44, 45, 46,
47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71,
72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94.
7. The compound according to claim 1, wherein each oligonucleotide, independently,
comprises a portion of at least 12 contiguous nucleobases of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8,
25 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33,
34, 35, 36, 37, 38, 39, 40, or 41.
8. The compound according to claim 1, wherein each oligonucleotide, independently,
comprises a portion of at least 12 contiguous nucleobases of SEQ ID NO: 42, 43, 44, 45, 46,
47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71,

72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, and is at least 80% complimentary to its target site with SEQ ID NO: 95.

9. The compound according to claim 1, wherein each oligonucleotide, independently, comprises a portion of at least 12 contiguous nucleobases of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8,
5 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41, and is at least 80% complimentary to its target site with SEQ ID NO: 96.

10. A composition comprising a compound according to claim 1 and a pharmaceutically acceptable carrier.

10 11. A synthetic oligonucleotide compound comprising two single stranded antisense oligonucleotides that are linked through their 5'-ends to allow the presence of two or more accessible 3'-ends, wherein the oligonucleotides, independently, comprise a sequence selected from SEQ ID NOs: 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58,
15 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94.

12. The compound according to claim 11, wherein the oligonucleotides comprise the same sequence.

13. A composition comprising a compound according to claim 11 and a pharmaceutically acceptable carrier.

20 14. A synthetic oligonucleotide compound comprising two single stranded antisense oligonucleotides that are linked through their 5'-ends to allow the presence of two or more accessible 3'-ends, wherein the oligonucleotides, independently, comprise a sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41.

25 15. The compound according to claim 14, wherein the oligonucleotides comprise the same sequence.

16. A composition comprising a compound according to claim 14 and a pharmaceutically acceptable carrier.

17. The composition according to claim 10, further one or more vaccines, antigens, antibodies, cytotoxic agents, chemotherapeutic agents, kinase inhibitors, allergens, antibiotics, agonist, antagonist, antisense oligonucleotides, ribozymes, RNAi molecules, siRNA molecules, miRNA molecules, aptamers, proteins, gene therapy vectors, DNA
5 vaccines, adjuvants, co-stimulatory molecules or combinations thereof.
18. The composition according to claim 13, further one or more vaccines, antigens, antibodies, cytotoxic agents, chemotherapeutic agents, kinase inhibitors, allergens, antibiotics, agonist, antagonist, antisense oligonucleotides, ribozymes, RNAi molecules, siRNA molecules, miRNA molecules, aptamers, proteins, gene therapy vectors, DNA
10 vaccines, adjuvants, co-stimulatory molecules or combinations thereof.
19. The composition according to claim 16, further one or more vaccines, antigens, antibodies, cytotoxic agents, chemotherapeutic agents, kinase inhibitors, allergens, antibiotics, agonist, antagonist, antisense oligonucleotides, ribozymes, RNAi molecules, siRNA molecules, miRNA molecules, aptamers, proteins, gene therapy vectors, DNA
15 vaccines, adjuvants, co-stimulatory molecules or combinations thereof.
20. A method for inhibiting NLRP3 mRNA or protein expression, the method comprising contacting a cell with at least one compound according to claim 1.
21. The method according to claim 20, wherein the cell is contacted with two or more compounds targeting different regions of NLRP3.
- 20 22. A method for inhibiting NLRP3 mRNA or protein expression, the method comprising contacting a cell with at least one compound according to claim 11.
23. The method according to claim 22, wherein the cell is contacted with two or more compounds targeting different regions of NLRP3.
24. A method for inhibiting NLRP3 mRNA or protein expression, the method comprising
25 contacting a cell with at least one compound according to claim 14.
25. The method according to claim 24, wherein the cell is contacted with two or more compounds targeting different regions of NLRP3.

26. A method for the treatment of a disease, disorder, or condition associated with NLRP3 in an individual in need thereof, the method comprising administering a compound according to claim 1.

27. A method for the treatment of a disease, disorder, or condition associated with
5 NLRP3 in an individual in need thereof, the method comprising administering a compound according to claim 11.

28. A method for the treatment of a disease, disorder, or condition associated with NLRP3 in an individual in need thereof, the method comprising administering a compound according to claim 14.

Screening of mNLRP3 in cell based assay

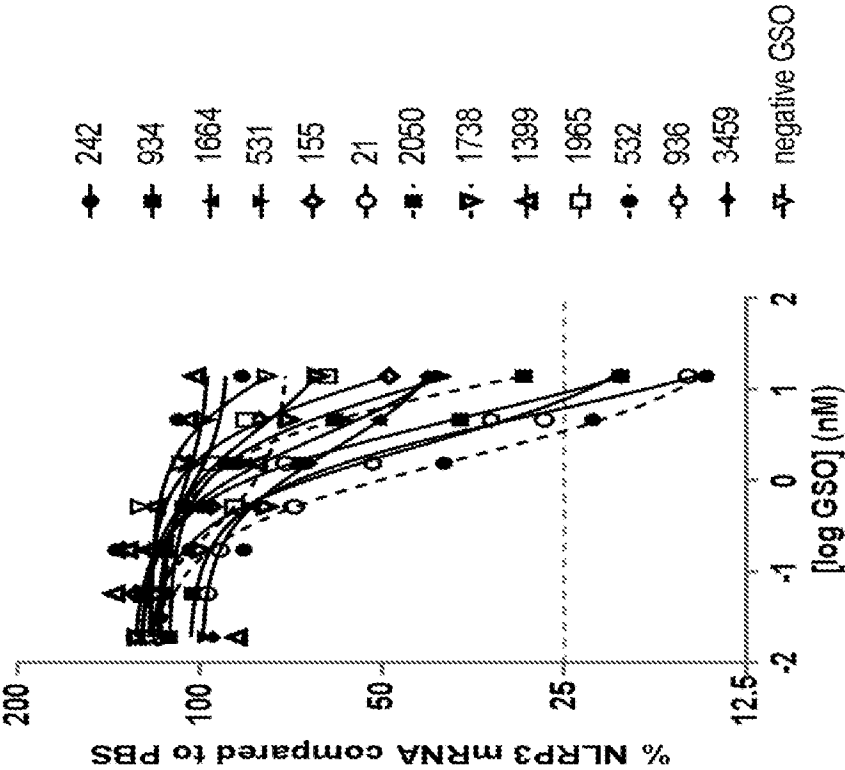


FIG. 1

Silencing of mNLRP3 by selected GSOs in mouse J774 cell line

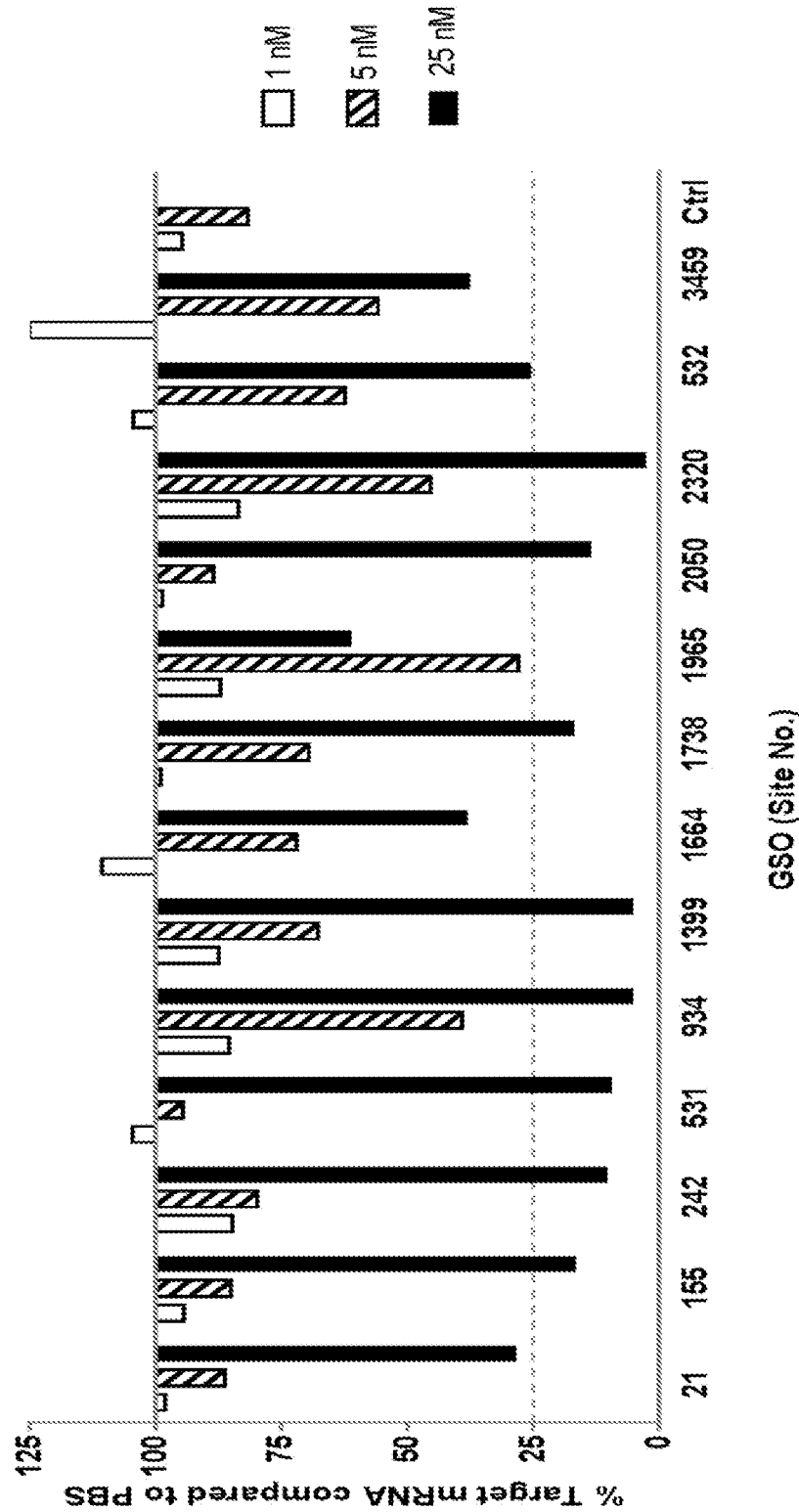


FIG. 2

mNLRP3 GSO dose dependently silences NLRP3 mRNA expression and protein levels in mouse J774 cells

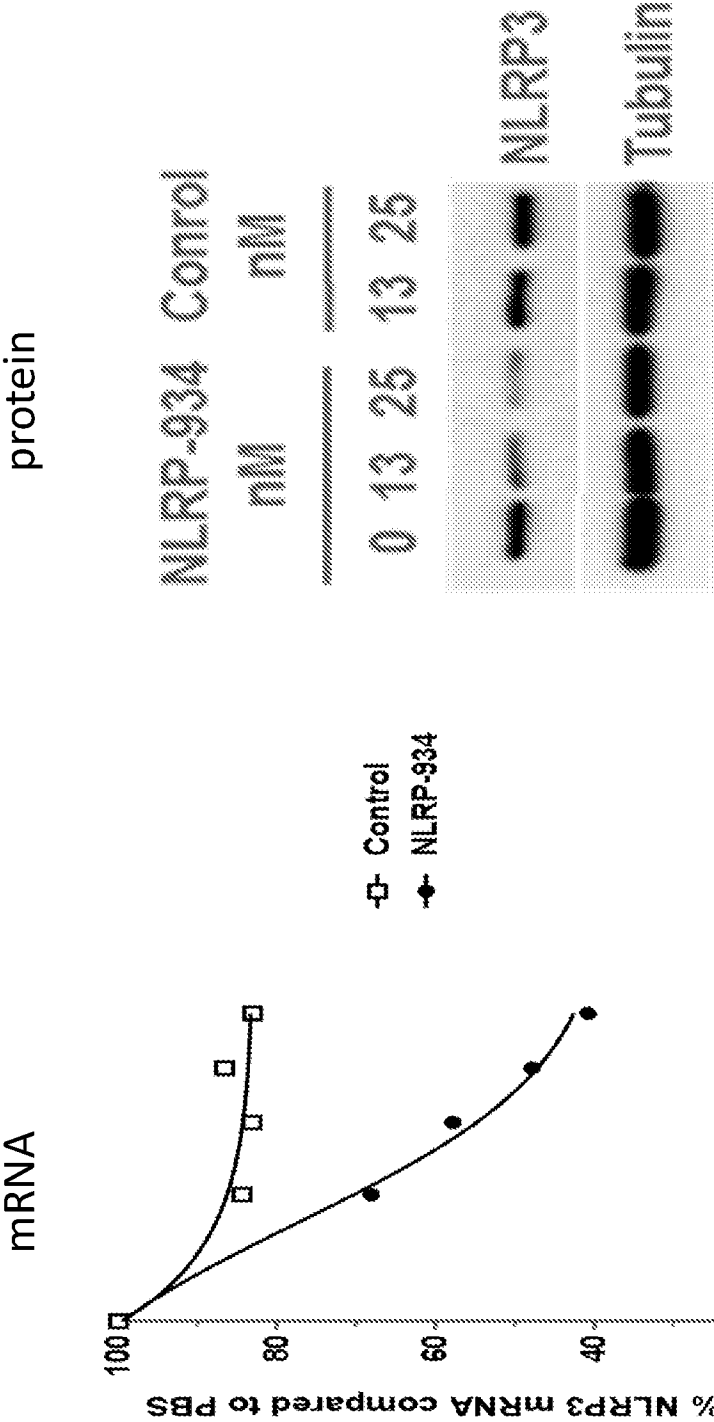


FIG. 3B

mNLRP3 GSO dose dependently reduces LPS plus ATP induced IL-1 β secretion in J774 cells

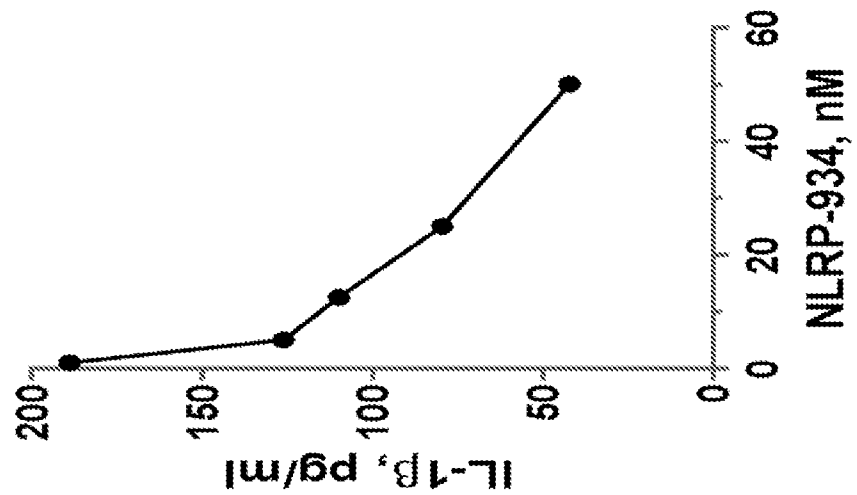


FIG. 4

mNLRP3 GSO specifically silences mouse NLRP3 gene expression in cell based assay



FIG. 5

hNLRP3 GSO dose response curves in cell-based assay

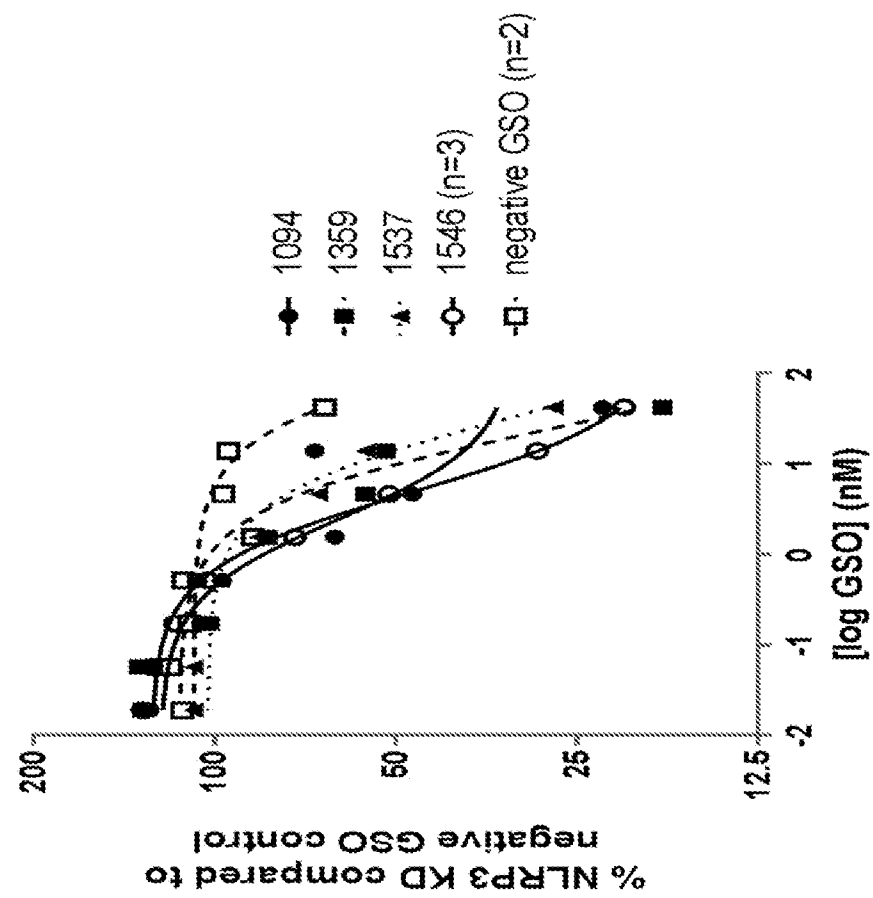


FIG. 6

hNLRP3 GSO dose response curves in THP-1 cells

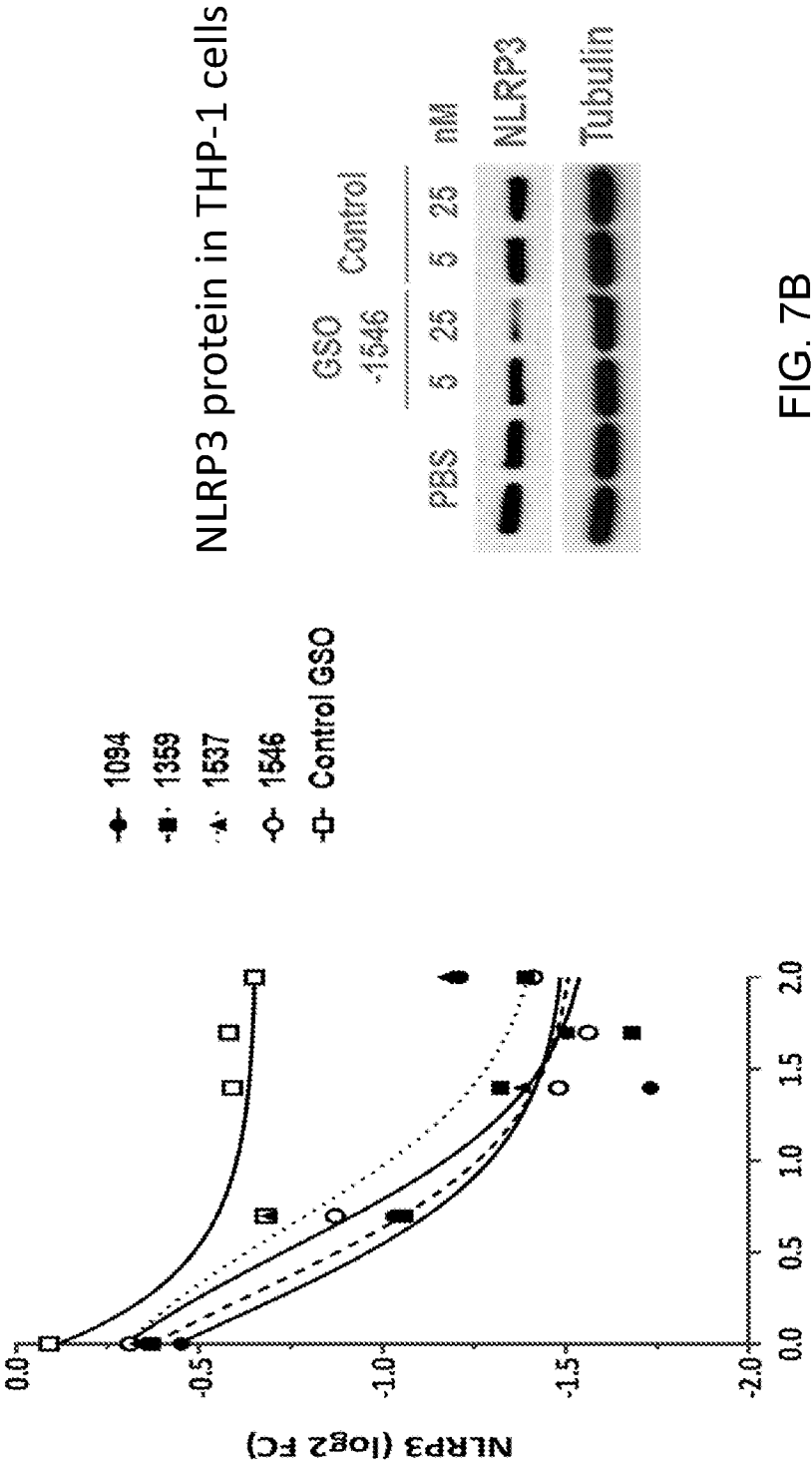


FIG. 7A

FIG. 7B

hNLRP3 GSOs inhibit LPS/ATP-induced cytokine secretion in THP-1 cells

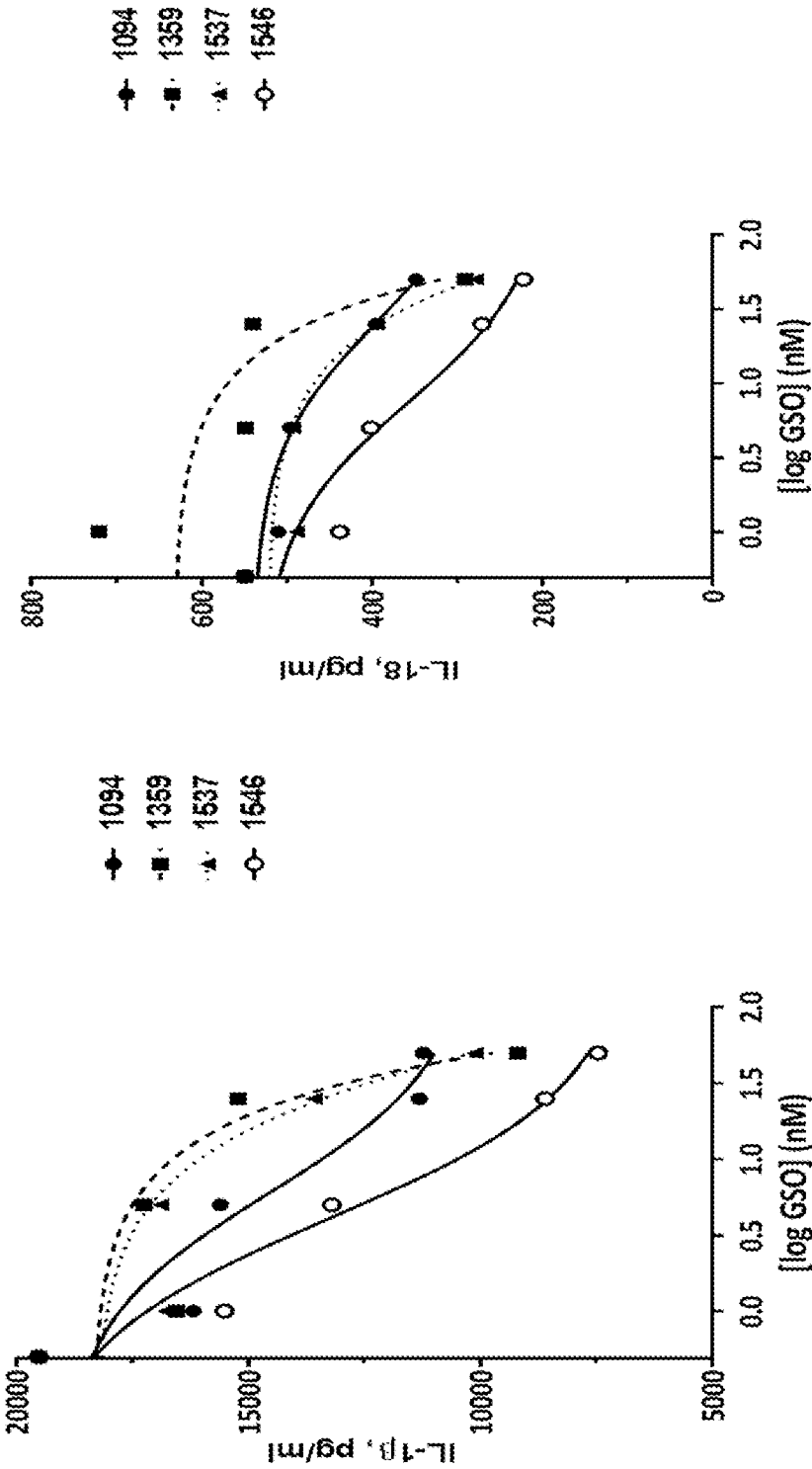
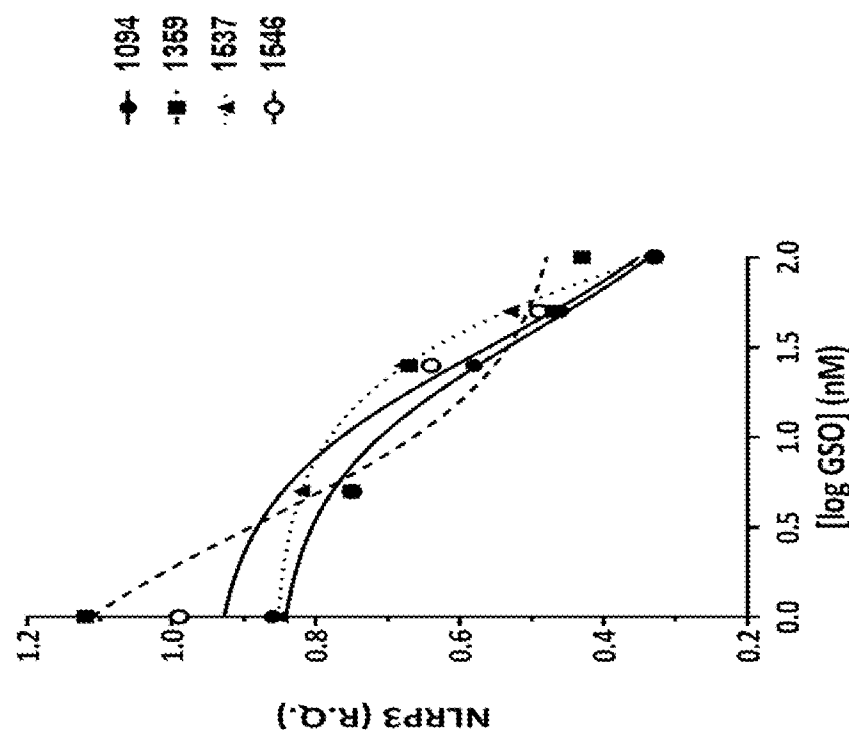


FIG. 8A

FIG. 8B

NLRP3 GSOs plus RNAiMax for 24 hr and LPS (100 ng/ml) for 4hr, ATP (5 mM), 1hr. In PMA-differentiated THP-1 cells

hNLRP3 GSO dose response curves in human PBMCs



Freshly isolated human PBMCs (10×10^6 cells/ml) in 6-well culture plates were incubated with GSO/RNAiMax for 24

FIG. 9

hNLRP3 GSOs inhibit LPS/ATP-induced cytokine secretion in human PBMCs

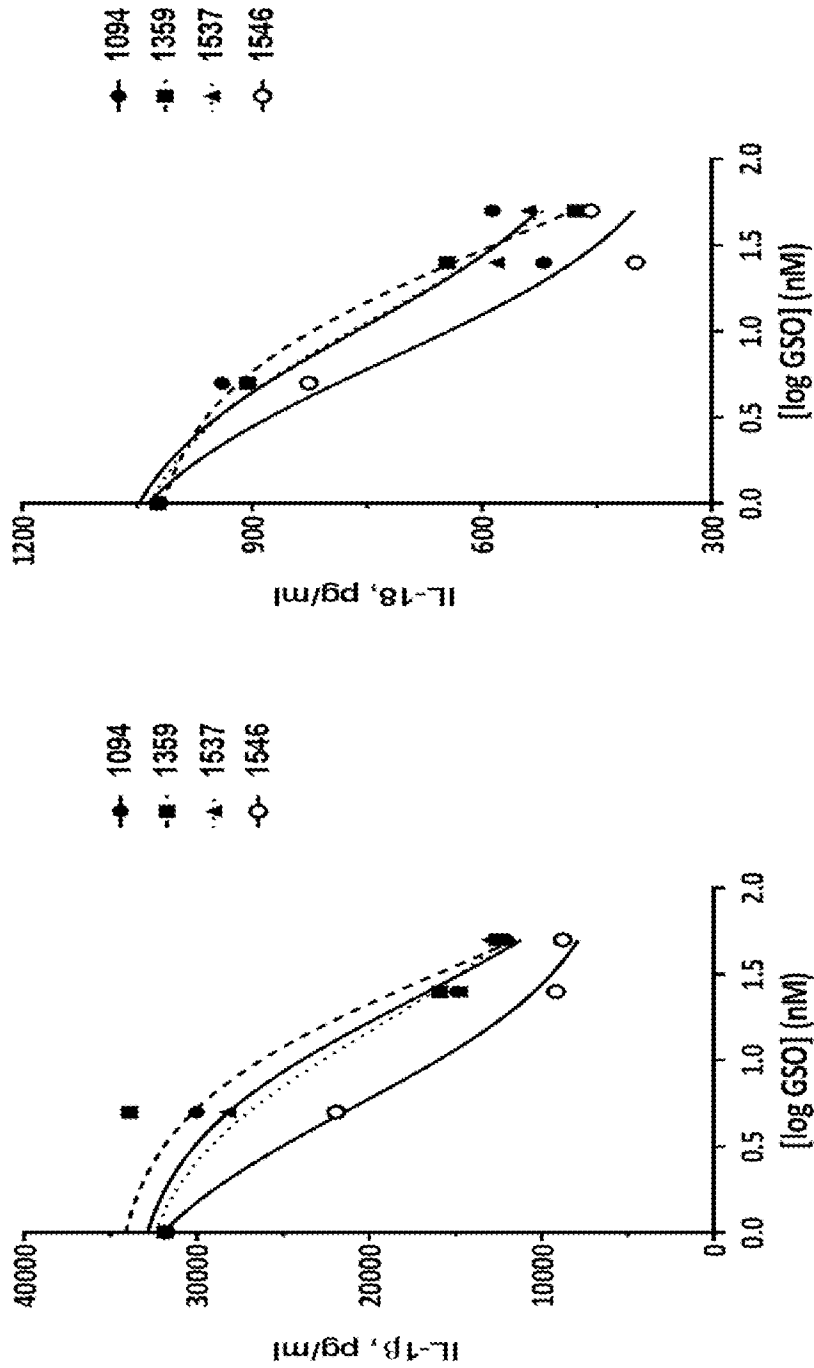


FIG. 10A

FIG. 10B

Freshly isolated human PBMCs (10×10^6 cells/ml) in 6-well culture plates were incubated with GSO/RNAiMax for 24 hours followed by stimulation with LPS (100 ng/ml) for 4hr and ATP (5 mM), 1hr.

mNLRP3 GSO in an animal model of interstitial cystitis

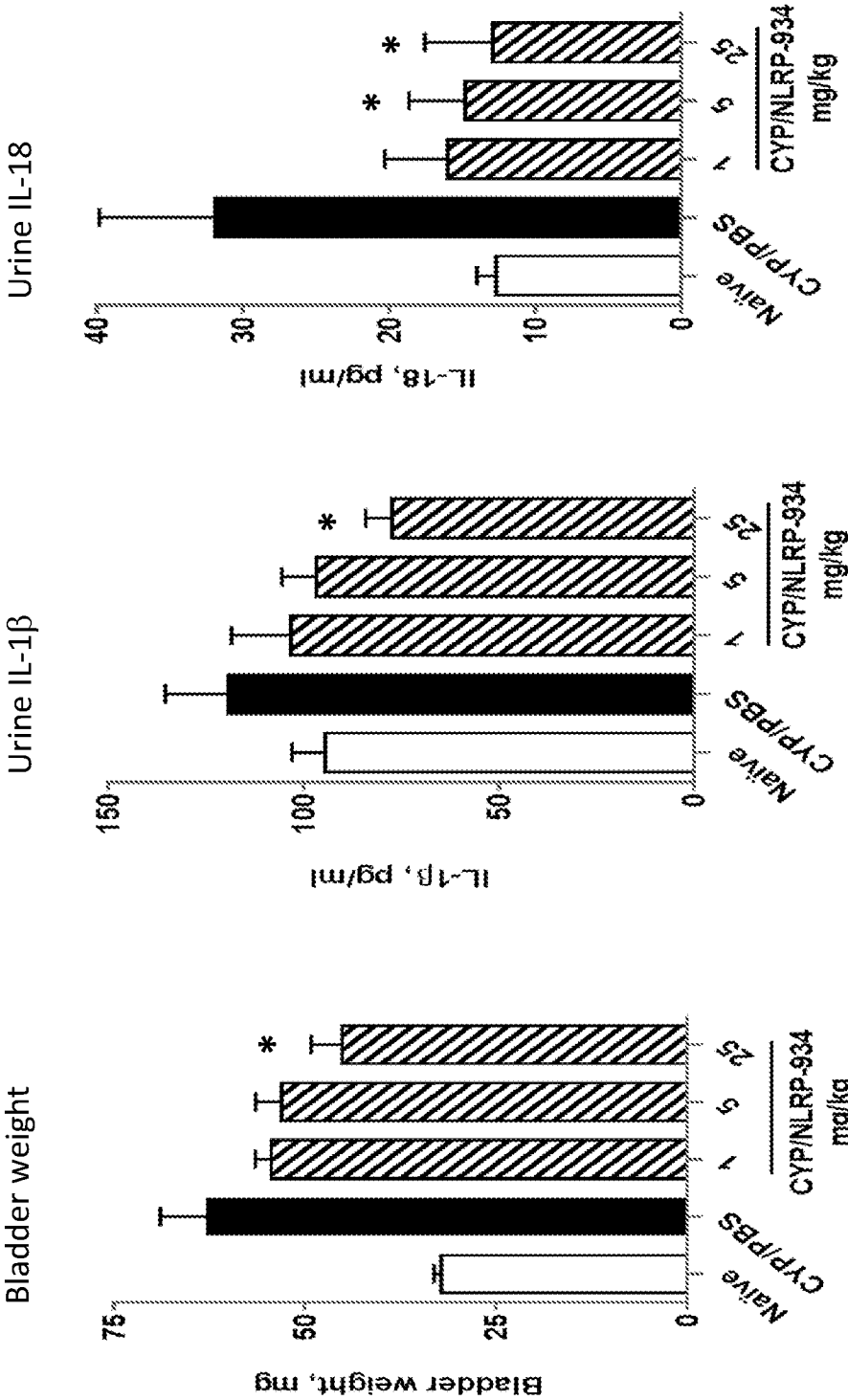


FIG. 11A

FIG. 11B

FIG. 11C

*, $p < 0.05$ when compared to PBS treated group. Statistical analysis was done by unpaired two-tailed Student's t test.

mNLRP3 GSO in an animal model of interstitial cystitis

Bladder inflammasome gene expression

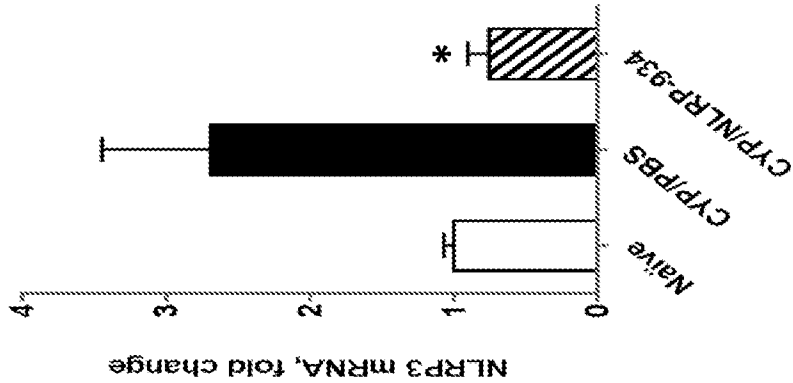


FIG. 12A

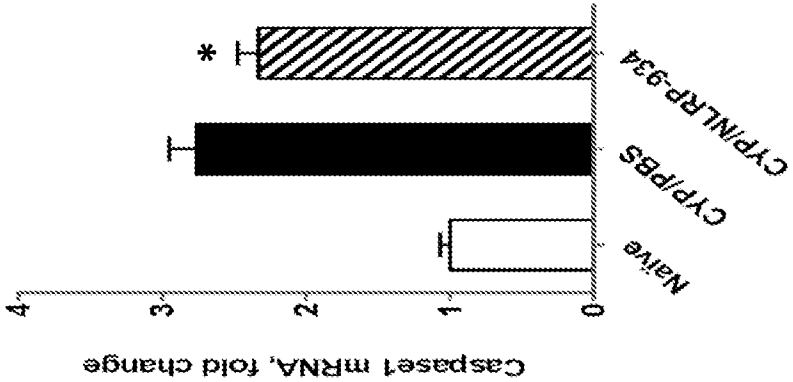


FIG. 12B

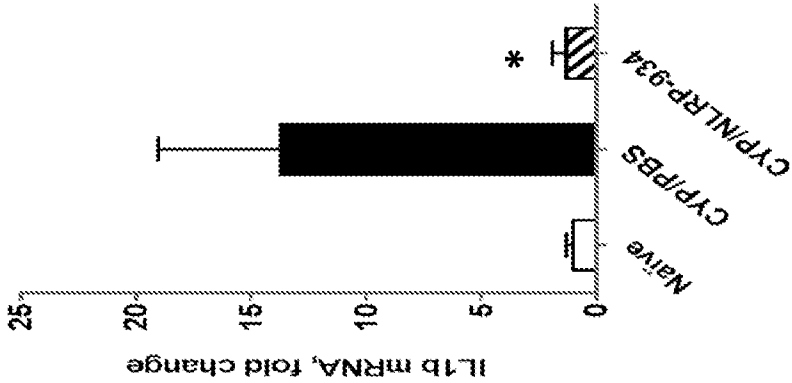


FIG. 12C

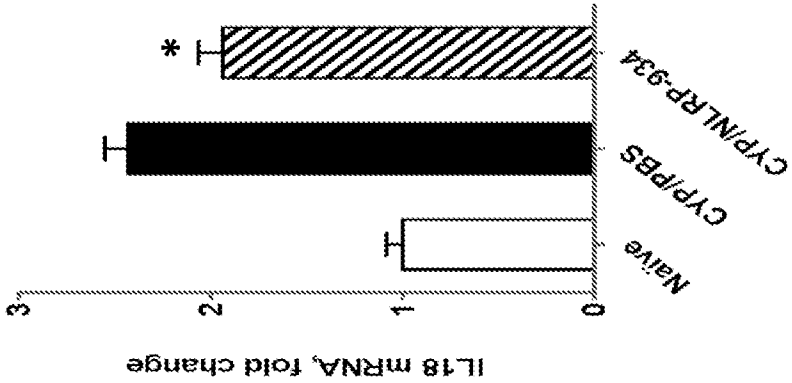


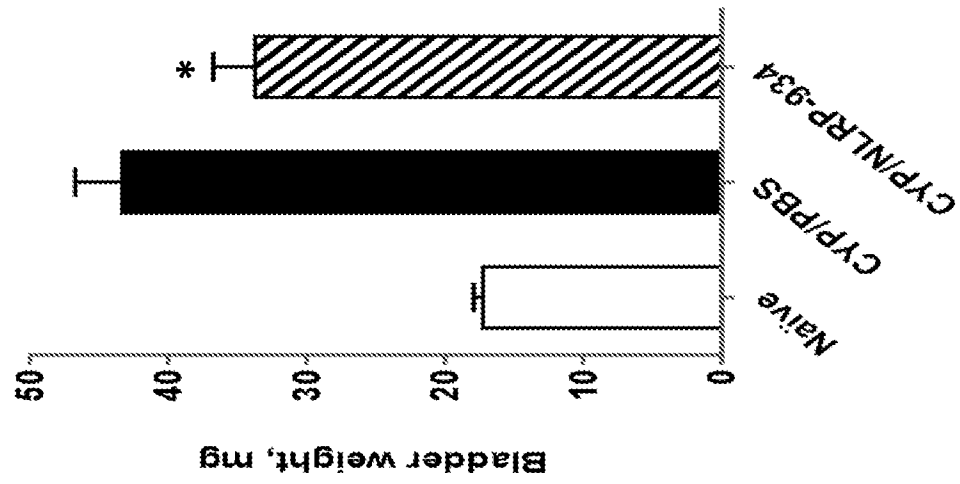
FIG. 12D

*, $p < 0.05$ when compared to PBS treated group. Statistical analysis was done by unpaired two-tailed Student's t test.

mNLRP3 GSO in an animal model of interstitial cystitis

Fold change over naïve			
	CYP/PBS	CYP/NLRP-934	p-value
Casp1	2.2	1.7	0.09
Ccl7	59.1	35.8	0.04
Cflar	1.7	1.3	0.01
Chuk	1.7	-1.4	0.00
Ctsb	2.1	1.6	0.02
Hsp90b1	2.0	1.6	0.02
Il1b	16.9	10.7	0.05
Il6	48.1	32.3	0.05
Il18	1.6	1.0	0.00
Ilr1	1.8	1.3	0.00
Mefv	7.4	4.4	0.03
Myd88	2.9	1.3	0.00
Nlrp4	1.6	1.2	0.03
Nlrp3	5.0	2.7	0.01
Nod2	3.0	1.8	0.00
P2rx7	2.4	2.2	0.55
Ptgs2	6.4	6.2	0.60
Il33	9.4	7.3	0.2

FIG. 13



*, $p < 0.05$ when compared to PBS treated group. Statistical analysis was done by unpaired two-tailed Student's t test.

FIG. 14

mNLRP3 GSO in an animal model of interstitial cystitis

Urine cytokine IL-1 β concentration was determined with mouse cytokine kits from R&D Systems, following manufacturer's instructions.

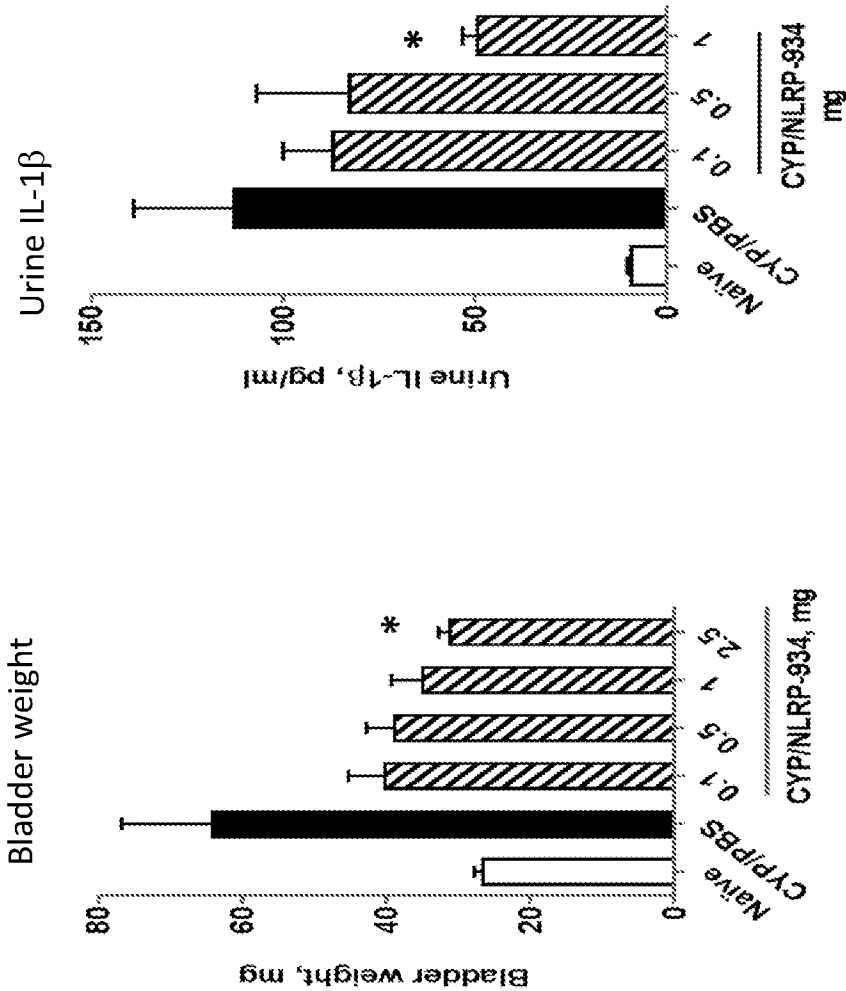


FIG. 15A

FIG. 15B

* , $p < 0.05$ when compared to PBS treated group. Statistical analysis was done by unpaired two-tailed Student's t test.

mNLRP3 GSO in an animal model of experimental autoimmune uveitis

Total RNA from eyes (PBS and 15 mg/kg group, n=6) is isolated using phenol/chloroform. 500 ng of total RNA was reverse transcribed to cDNA and quantitative PCR was performed using gene specific primers/probes (NLRP3, IL1b, IL18 and Caspase1, Lifetechnologies). Gene expression was calculated using 2^{-ΔΔCT} method and HPRT was used as an endogenous control.

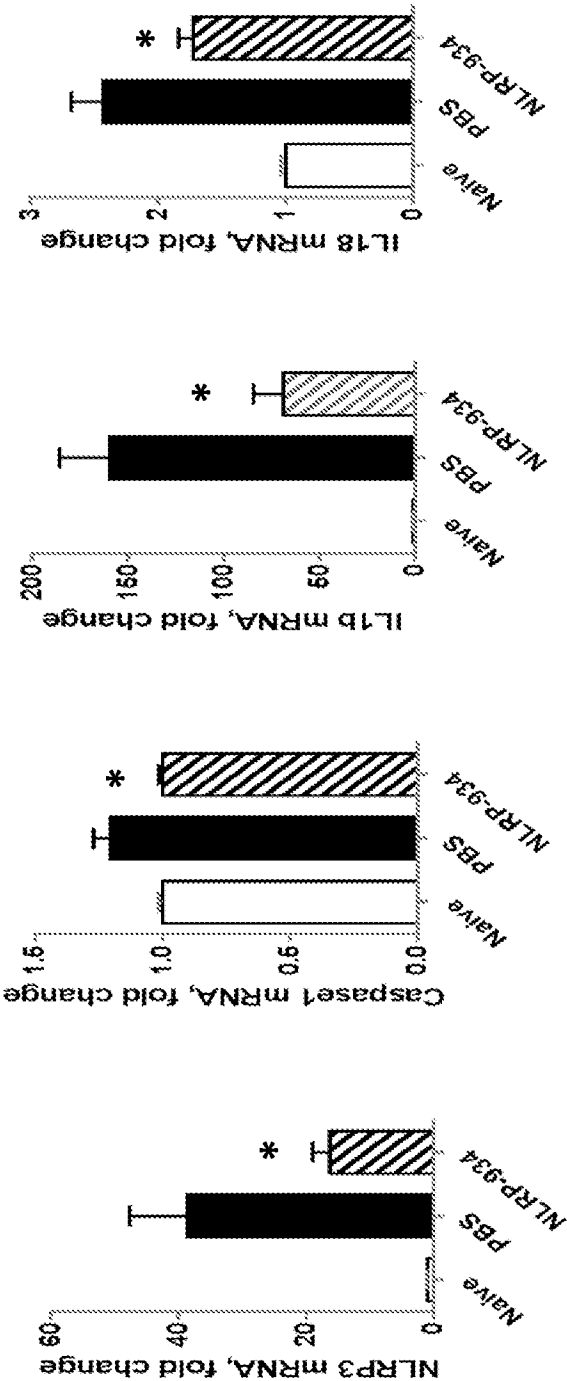


FIG. 16A

FIG. 16B

FIG. 16C

FIG. 16D

*, $p < 0.05$ when compared to PBS treated group. Statistical analysis was done by unpaired two-tailed Student's t test.

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13^{ter}.1(c) and (d) and 39)

Applicant's or agent's file reference 4204.3004 WO	IMPORTANT DECLARATION	Date of mailing (day/month/year) 31 JAN 2017
International application No. PCT/US16/60186	International filing date (day/month/year) 02 November 2016	(Earliest) Priority Date (day/month/year) 04 November 2015
International Patent Classification (IPC) or both national classification and IPC IPC: C12Q 1/68; CPC: C12Q 2600/158		
Applicant IDERA PHARMACEUTICALS, INC.		

This International Searching Authority hereby declares, according to Article 17(2)(a), that no international search report will be established on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:

- a. ☐ scientific theories
- b. ☐ mathematical theories
- c. ☐ plant varieties
- d. ☐ animal varieties
- e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes
- f. ☐ schemes, rules or methods of doing business
- g. ☐ schemes, rules or methods of performing purely mental acts
- h. ☐ schemes, rules or methods of playing games
- i. ☐ methods for treatment of the human body by surgery or therapy
- j. ☐ methods for treatment of the animal body by surgery or therapy
- k. ☐ diagnostic methods practised on the human or animal body
- l. ☐ mere presentations of information
- m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art

2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

- ☐ the description ☐ the claims ☐ the drawings

3. ☒ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:

- ☒ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13^{ter}.1(a) or (b).

4. Further comments:

Applicant failed to submit a valid electronic seq. listing in response to the ISA/225.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Authorized officer Blaine Copenheaver
Facsimile No. 571-273-8300	PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774