CLAIMS

1. A method of identifying a compound capable of binding to a Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing 3 (NLRP3) polypeptide, the method comprising:

1) contacting a compound suspected of being capable of binding an NLRP3 polypeptide with an NLRP3 variant, wherein the NLRP3 variant is immobilized on a surface;

2) washing the compound and immobilized NLRP3 variant with a buffer; and

3) detecting the compound,

wherein if the compound is detected, the compound is capable of binding an NLRP3 polypeptide,

wherein NLRP3 variant comprises at least a partial deletion of an amino terminal pyrin domain (PYD).

2. The method of claim 1, wherein the compound is linked to a polynucleotide sequence, optionally wherein:

detection of the compound comprises detecting the polynucleotide sequence;

detecting the polynucleotide sequence comprises sequencing the polynucleotide sequence;

the polynucleotide sequence comprises at least 5 nucleotides, optionally between 5 and 50 nucleotides in length;

the polynucleotide sequence is single stranded DNA (ssDNA);

the NLRP3 variant is at a concentration of between 1 nM and 300 nM, optionally at a concentration of between 25 nM and 250 nM.

3. The method of any one of claims 1-2, wherein the NLRP3 variant is contained in a buffer comprising:

1) 5mM to 500 mM Tris-HCl, optionally 50 mM Tris-HCl;

2) 15 to 1500 mM NaCl, optionally 150 mM NaCl;

3) 1 to 100 mM $MgCl_2$, optionally 10 mM $MgCl_2$;

4) 1% to 20% Glycerol, optionally 10% Glycerol; and

5) 0.0005% to 0.05% Tween-20, optionally 0.005% Tween-20,

optionally wherein the buffer further comprises:

1) 1 to 500 mM imidazole, optionally 10 mM Imidazole; and

2) 0.03 to 3 mg/mL single-stranded DNA (ssDNA), optionally 0.3 mg/mL ssDNA.

4. The method of any one of claims 1-3, wherein the contacting step 1) comprises incubating the compound with the NLRP3 variant and between 0.01 mM to 50 mM ATP, optionally between 0.1 mM to 1 mM ATP.

5. The method of any one of claims 1-4, wherein the NLRP3 variant is immobilized on a solid support, optionally wherein the solid support comprises a nickel-immobilized resin.

6. The method of any one of claims 1-5, further comprising:

4) incubating the compound capable of binding an NLRP3 polypeptide with a cell; and

5) measuring at least one pro-inflammatory cytokine secreted from the cell,

wherein a decrease in secretion of at least one pro-inflammatory cytokine from the cell indicates that the compound is an inhibitor of NLRP3, optionally wherein:

the at least one pro-inflammatory cytokine is IL-1β; and/or

the cell is a human monocytic THP-1 cell.

7. A method of identifying an inhibitor of Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing 3 (NLRP3), the method comprising:

1) contacting a compound suspected of being capable of binding an NLRP3 polypeptide with a NLRP3 variant, wherein the NLRP3 variant is immobilized on a surface;

2) washing the compound and immobilized NLRP3 variant with a buffer;

3) detecting the compound, wherein if the compound is detected, the compound is capable of binding an NLRP3 polypeptide;

4) incubating the compound capable of binding an NLRP3 polypeptide from step 3) with a cell; and

5) measuring at least one pro-inflammatory cytokine secreted from the cell,

wherein a decrease in secretion of at least one pro-inflammatory cytokine from the cell indicates that the compound is an inhibitor of NLRP3,

wherein NLRP3 variant comprises at least a partial deletion of an amino terminal pyrin domain (PYD).

8. The method of claim 7, wherein the at least one pro-inflammatory cytokine is IL-1β, and/or the cell is a human monocytic THP-1 cell.

9. The method of any one of claims 1-8, wherein:

the NLRP3 variant comprises a deletion of amino acids 1-142 of the amino acid sequence of SEQ ID NO: 1;

the NLRP3 variant comprises an amino acid sequence with at least 80% identity to SEQ ID NO: 2;

the NLRP3 variant comprises an amino acid sequence with at least 80% identity to SEQ ID NO: 3; and/or

the NLRP3 variant is linked to one or more polypeptide domains, optionally wherein:

the one or more polypeptide domains comprise a solubility enhancing domain, optionally wherein the one or more polypeptide domains are selected from the group consisting of maltose binding protein (MBP), glutathione S-transferase (GST), N-utilization substance A (NusA), and small ubiquitin-related modifier (SUMO); and/or

the one or more polypeptide domains comprise an affinity purification domain, optionally wherein the affinity purification domain comprises a His domain, 6xHis domain, biotin, streptavidin, glutathione S-transferase (GST), FLAG (DYKDDDDK; SEQ ID NO: 4), and an antibody Fc domain.

10. The method of any one of claims 1-9, wherein:

the NLRP3 variant retains at least about 25% of ATPase activity relative to the ATPase activity of a wild type NLRP3 of SEQ ID NO: 1;

the NLRP3 variant retains the ability to form an NLRP3 inflammasome, relative to the ability of a wild type NLRP3 of SEQ ID NO: 1; and/or

the NLRP3 variant retains the ability to initiate release of IL-1β in a cell, relative to the ability of a wild type NLRP3 of SEQ ID NO: 1.

11. Use of the NLRP3 inhibitor identified in any one of claims 1-10 in the manufacture of a medicament for treating an inflammatory disorder.

12. A composition comprising:

1) an NLRP3 variant;

2) 5mM to 500 mM Tris-HCl, optionally 50 mM Tris-HCl;

3) 15 to 1500 mM NaCl, optionally 150 mM NaCl;

4) 1 to 100 mM $MgCl_2$, optionally 10 mM $MgCl_2$;

5) 1% to 20% Glycerol, optionally 10% Glycerol; and

6) 0.0005% to 0.05% Tween-20, optionally 0.005% Tween-20,

wherein NLRP3 variant comprises at least a partial deletion of an amino terminal pyrin domain (PYD), optionally wherein the composition further comprises:

1) 1 to 500 mM imidazole, optionally 10 mM Imidazole; and

2) 0.03 to 3 mg/mL single-stranded DNA (ssDNA), optionally 0.3 mg/mL ssDNA.

13. The composition of claim 12, wherein the composition further comprises between 0.01 mM to 50 mM ATP.

14. A Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing 3 (NLRP3) variant, comprising at least a partial deletion of an amino terminal pyrin domain (PYD).

15. The NLRP3 variant of claim 14, comprising:

a deletion of amino acids 1-142 of the amino acid sequence of SEQ ID NO: 1;

an amino acid sequence with at least 80% identity to SEQ ID NO: 2;

an amino acid sequence with at least 80% identity to SEQ ID NO: 3; and/or

the NLRP3 variant is linked to one or more polypeptide domains, optionally wherein:

the one or more polypeptide domains comprise a solubility enhancing domain, optionally wherein the one or more polypeptide domains are selected from the group consisting of maltose binding protein (MBP), glutathione S-transferase (GST), N-utilization substance A (NusA), and small ubiquitin-related modifier (SUMO); and/or

the one or more polypeptide domains comprise an affinity purification domain, optionally the affinity purification domain comprises a His domain, 6xHis domain, biotin, streptavidin, glutathione S-transferase (GST), FLAG (DYKDDDDK; SEQ ID NO: 4), and an antibody Fc domain.

16. The NLRP3 variant of any one of claims 14-15, wherein:

the NLRP3 variant retains at least about 25% of ATPase activity relative to the ATPase activity of a wild type NLRP3 of SEQ ID NO: 1;

the NLRP3 variant retains the ability to form an NLRP3 inflammasome, relative to the ability of a wild type NLRP3 of SEQ ID NO: 1; and/or

the NLRP3 variant retains the ability to initiate release of IL-1β in a cell, relative to the ability of a wild type NLRP3 of SEQ ID NO: 1.

17. A nucleic acid encoding the NLRP3 variant of any one of claims 14-16, optionally wherein the nucleic acid is in a vector.

18. A host cell comprising the nucleic acid or vector of claim 17, optionally wherein the host cell is an *e. coli* cell, a yeast cell, an insect cell, or a mammalian cell, optionally wherein the insect cell is an Sf21 cell.

19. A method of purifying the NLRP3 variant of any one of claims 14-16, comprising:

1) introducing a vector encoding the NLRP3 variant into a host cell;

2) culturing the host cell under conditions to allow expression of the NLRP3 variant in the host cell; and

3) isolating the NLRP3 variant from the host cell, optionally wherein the isolating step 3) comprises contacting a lysate of the host cell with one or more affinity purification resins, optionally wherein the one or more affinity purification resins are selected from the group consisting of an immobilized metal-affinity chromatography (IMAC) resin, a maltose or amylose affinity chromatography resin, a glutathione affinity chromatography resin, a protein A affinity chromatography resin, and a anti-FLAG affinity chromatography resin.