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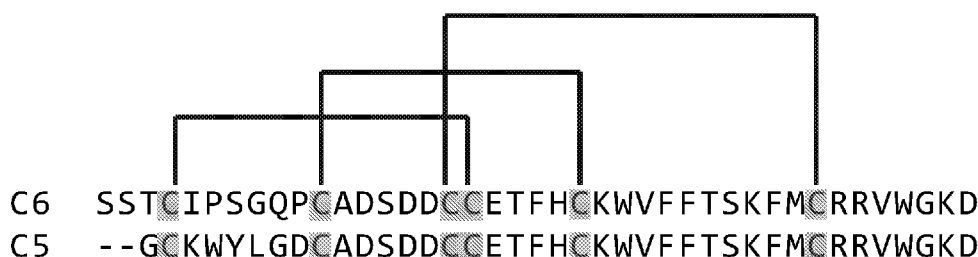
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(54) Title: HV1 MODULATORS AND USES

FIG. 1



(57) Abstract: The present invention provides novel agents for modulation of Hv1 channels. The present invention provides agents for activating and/or inhibiting Hv1 channel function and/or activity, and reagents and methods relating thereto.



Hv1 MODULATORS AND USES

Cross Reference to Related Applications

[1] This application claims benefit to U.S. Provisional Application No. 62/441,097, filed December 30, 2016 and U.S. Provisional Application No. 62/447,433, filed January 17, 2017, the contents of which are incorporated herein by reference.

Sequence Listing

[2] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety.

Background

[3] Voltage-gated ion channels facilitate the transfer of ions across cell membranes and function as key components of essential cellular processes. One particular type of voltage-gated ion channel is the voltage-gated proton channel (Hv1). Hv1 is a transmembrane protein that regulates the transfer of protons across cell membranes. When the Hv1 channel is open, protons permeate the channel and cross the cellular membrane.

[4] The Hv1 channel is expressed in many different tissues and is associated with a wide variety of physiological and pathological processes. For example, Hv1 channels may play a role in immune defense, sperm activity, and cancer progression. For these and other reasons, Hv1 may be an attractive drug target (Seredenina, T., et al. "Voltage-gated proton channels as novel drug targets: from NADPH oxidase regulation to sperm biology." *Antioxid Redox Signal.* 10;23(5): 490-513 (2015)). However, clinically compatible Hv1 activators or inhibitors are not known. For these reasons, there is a need for the development of activators and inhibitors of Hv1 channels.

Summary

[5] The present disclosure provides technologies relating to modulation of Hv1 channels. Among other things, the present disclosure provides Hv1 modulating agents, and various compositions and methods relating thereto.

[6] In some embodiments, an Hv1 modulating agent is or comprises an engineered polypeptide component having an inhibitor cysteine knot (ICK)-like structural motif.

[7] In some embodiments, an Hv1 modulating agent is or comprises an engineered polypeptide component that includes one or more toxin sequence elements, each of which has an amino acid sequence that is substantially identical to, but differs from, that of a corresponding element found in a wild-type toxin.

[8] In some embodiments, an Hv1 modulating agent shares one or more cysteines with a wild-type toxin sequence. In some embodiments, an Hv1 modulating agent shares the same approximate relative position of cysteines with a wild-type toxin.

[9] In some embodiments, an Hv1 modulating agent is or comprises a polypeptide sequence set forth in Tables 2A, 3A, and 4.

[10] In some embodiments, an Hv1 modulating agent is encoded by a nucleotide sequence that is or comprises a sequence set forth in Tables 2C and 3B.

[11] In some embodiments, an Hv1 modulating agent can be expressed from a vector including a nucleic acid sequence encoding the Hv1 modulating agent.

[12] In some embodiments, an Hv1 modulating agent binds to the external surface of human Hv1. In some embodiments, an Hv1 modulating agent binds to the S3-S4 external loop region of human Hv1.

[13] In some embodiments, an Hv1 modulating agent inhibits human Hv1 function. For example, in some embodiments, an Hv1 modulating agent may decrease or block proton current. In some embodiments, an Hv1 modulating agent may reduce the number or likelihood of Hv1 channel opening. In some embodiments, an Hv1 modulating agent may increase the rate of Hv1 channel closing.

[14] In some embodiments, an Hv1 modulating agent activates human Hv1 function. For example, in some embodiments, an Hv1 modulating agent increases proton current. In some embodiments, an Hv1 modulating agent increase the rate of Hv1 channel opening. In some embodiments, an Hv1 modulating agent slows the rate of Hv1 channel closing.

[15] In some embodiments, an Hv1 modulating agent inhibits sperm capacitation.

[16] In some embodiments, an Hv1 modulating agent decreases reactive oxygen species (ROS) production in white blood cells.

[17] The present invention further provides various reagents and methods associated with Hv1 modulating agents including, for example, systems for identifying and characterizing them, strategies for preparing them, and various therapeutic compositions and methods relating to them. Further description of certain embodiments of these aspects, and others, of the present invention, is presented below.

Brief Description of the Drawing

[18] *FIG. 1* Presents an amino acid sequence alignment of exemplary Hv1 modulating agents C5 and C6. Six conserved cysteine residues and three disulfide bridges of an inhibitor cysteine knot (ICK)-like structural motif are indicated.

[19] *FIG. 2* Presents the amino acid sequences of exemplary Hv1 modulating agents C5 and C6 and the amino acid sequence of hanatoxin (HaTx1). Sequence elements corresponding to sequences in Table 3A are labeled. Conserved cysteine residues are highlighted. NT, N-terminus. CT, C-terminus.

[20] *FIG. 3* Presents exemplary T-toxin amino acid sequences and effects of T-toxins on hHv1 function. (FIG. 3A) depicts an exemplary T-toxin comprising the amino acid sequence of Hv1 modulating agent C6 linked to a trypsin secretory signal sequence at the N-terminus, a 16 amino acid linker with embedded C-Myc epitope tag at the C-terminus, and a hydrophobic sequence for GPI attachment from the mammalian Lynx1 peptide. (FIG. 3B) depicts an exemplary T-toxin comprising an N-terminal trypsin secretory signal sequence, the amino acid sequence of HaTx1 linked by a flexible 7 amino acid linker to C6, a C-Myc epitope embedded into a 6 amino acid linker at the C-terminus, and a hydrophobic sequence

for GPI attachment. (FIG. 3C) depicts an exemplary T-toxin comprising an N-terminal trypsin secretory signal sequence, C6 dimers linked by a rigid 10 amino acid linker, a C-Myc epitope embedded into a 6 amino acid linker at the C-terminus, and a hydrophobic sequence for GPI attachment. (FIG. 3D) depicts an exemplary T-toxin comprising an N-terminal trypsin secretory signal sequence, C6 dimers linked by a flexible 10 amino acid linker, a C-Myc epitope embedded into a 6 amino acid linker at the C-terminus, and a hydrophobic sequence for GPI attachment. (FIG. 3E) depicts an exemplary T-toxin comprising an N-terminal trypsin secretory signal sequence, C6 dimers linked by long flexible 38 amino acid linker, a C-Myc epitope embedded into a 6 amino acid linker at the C-terminus, and a hydrophobic sequence for GPI attachment. (FIG. 3F) shows inhibition of wild-type hHv1 measured as unblocked fractional current in oocytes expressing only hHv1 or both hHv1 and T-toxin.

[21] *FIG. 4* Illustrates activating and inhibiting effects of exemplary Hv1 modulators C5 and C6 on hHv1. Whole-cell patch clamp recordings were performed on HEK-293T cells over-expressing hHv1. Proton currents are shown with C5 (FIG. 4A) and C6 (FIG. 4B) versus current without any peptide or modifications (black traces).

[22] *FIG. 5* Illustrates that Hv1 modulating agent C6 affects response of sperm to progesterone, but not other changes related to sperm capacitation. C6 did not affect the vitality (FIG. 5A), the protein tyrosine phosphorylation (FIG. 5F and FIG. 5G), or the cholesterol content of the membranes (FIG. 5H). C6 did not significantly alter the mobility of sperm (FIGS. 5B-5E). C6 did affect the response of sperm to progesterone. The increase of cytosolic calcium triggered by progesterone is diminished in the presence of the Hv1 modulating agent and the acrosome reaction induced by the hormone is inhibited (FIGS. 5I-5K). All responses with C6 were compared to control peptide. VSL, velocity straight line; PROG, progression; VAP, velocity average path; Capac, capacitating medium (Human tubal fluid (HTF) media supplemented with 5 mg/mL BSA; as described, for example, in Pocognoni, C.A., et al., "PerfringolysinO as a useful tool to study human sperm physiology," *Fertility & Sterility* 99(1): 99-106(2013)).

[23] *FIG. 6* Demonstrates that Hv1 modulating agent C6 blocks production of ROS in human blood cells in a dose-dependent manner. Phorbol myristate acetate (PMA) was used to stimulate ROS production as shown by the increase of fluorescence over baseline (blood). The known inhibitor of Hv1, zinc (Zn), blocks to background levels.

Various concentrations of C6 also blocked fluorescence intensity in a dose-dependent manner. Two other toxins that block potassium channels with nM affinity (Moka and KTX) had no effect. FIG. 6A shows relative fluorescence intensity, measured at 590 nM (excited at 530 nM) using Amplex Red, which reacts with ROS to give a fluorescent product, for whole blood alone, PMA-stimulated whole blood, and PMA-stimulated whole blood with various inhibitors. 10 μ M of MOKA toxin was used as a control. FIG. 6B shows dose response curve plotted as the percentage of fluorescence from PMA-stimulated whole blood blocked versus concentration of C6 present. MOKA toxin and KTX were used as controls and showed no effect at 10 μ M.

[24] FIG. 7 Presents an exemplary T-toxin nucleotide (FIG. 7A; SEQ ID NO: 807) and amino acid (FIG. 7B; SEQ ID NO: 808) sequence comprising a PDGFR transmembrane helix which links an internal mVenus fluorescent protein to an external C6.

[25] FIG. 8 Illustrates effects of Hv1 modulating agent C6 tethered to HEK-293T cell surfaces via a PDGFR transmembrane link. (FIG. 8A) shows current recordings for WT Hv1 without any tether or peptide (top), expressed with the transmembrane mVenus without a peptide sequence (middle), or expressed with tethered mVenus-C6 (bottom). (FIG. 8B) shows WT Hv1 current with various amounts of tethered toxin plasmid normalized to current with the transmembrane tether without peptide ($I(C6)/I(notox)$) or to WT Hv1 with no tether expressed ($I(C6)/I(Hv1)$). (FIG. 8C) demonstrates small shifts observed in g-V normalized to the maximum seen for each condition. Black line is WT Hv1, Green line is WT Hv1 with transmembrane without tether, Red line is WT-Hv1 with tethered C6. (FIG. 8D) demonstrates current-voltage (I-V) showing the decrease in current in WT (black), WT with tether without toxin (green) or WT with tethered C6 (red). (FIG. 8E) shows amount of current blocked by 1 μ g of expressed tethered C6 in peak (end of pulse) or tail current. Current is normalized to the maximum of either WT alone ($I(C6)/I(Hv1)$) or the WT co-expressed with a tether without toxin ($I(C6)/I(notox)$). (FIG. 8F) shows FRET measurements between Hv1-TFP and mVenus transmembrane without toxin or with C6. Normalized fluorescence versus time is fit with a single exponential decay to determine the time constants for fluorescence decay with or without toxin. (FIG. 8G) shows average τ measured with the tether without toxin and with the tether with C6 from the fit in FIG. 8F. Increase in decay rate indicates FRET and indicates an interaction between C6 and Hv1.

[26] *FIG. 9* Demonstrates that Hv1 modulating agent C6 targets an S3-S4 external loop region of hHv1. (FIG. 9A) illustrates sequence alignments of *Ciona intestinalis* Hv1 (CiHv1, yellow), human Hv1 (hHv1, cyan), a chimeric Hv1 where an S3-S4 external loop region from hHv1 replaces a corresponding region from CiHv1 (hS3S4CiHv1), a chimeric Hv1 where an S3-S4 external loop region from CiHv1 replaces an hHv1 loop region, as well as other examples of chimeric sequences. If C6 blocks (YES) or does not (NO) block the channel is indicated. X indicates that proton currents were not measurable. (FIG. 9B) demonstrates a representative trace (left) for CiHv1 with 1 μ M C6 (red trace) or without (black trace), a representative trace (middle) for hS3S4CiHv1 which is sensitive to C6 (red trace, 1 μ M C6), and a representative trace (right) for CiS3S4hHv1 which is insensitive to C6 (red trace, 1 μ M C6). Black traces are current without any applied peptide. (FIG. 9C) demonstrates results from a cysteine scan of part of the transferred epitope. Bars are the amount of current with 1 μ M C6 normalized to current without toxin (Itox/Ictr). Many residues show decreased affinity but only G199 and E192 show dramatically different effects to the WT (first bar).

Definitions

[27] **Component:** The term “component” as used herein refers to a relevant part, portion, or moiety of an entity of interest. For example, in some embodiments, an entity of interest may be a polypeptide component.

[28] **Comprising:** A composition or method described herein as "comprising" one or more named elements or steps is open-ended, meaning that the named elements or steps are essential, but other elements or steps may be added within the scope of the composition or method. To avoid prolixity, it is also understood that any composition or method described as "comprising" (or which "comprises") one or more named elements or steps also describes the corresponding, more limited composition or method "consisting essentially of" (or which "consists essentially of") the same named elements or steps, meaning that the composition or method includes the named essential elements or steps and may also include additional elements or steps that do not materially affect the basic and novel characteristic(s) of the composition or method. It is also understood that any composition or method described herein as "comprising" or "consisting essentially of" one or more named elements

or steps also describes the corresponding, more limited, and closed-ended composition or method "consisting of" (or "consists of") the named elements or steps to the exclusion of any other unnamed element or step. In any composition or method disclosed herein, known or disclosed equivalents of any named essential element or step may be substituted for that element or step.

[29] ***Corresponding to:*** As used herein, the term “corresponding to” designates the position/identity of a structural element in a compound or composition through comparison with an appropriate reference compound or composition. For example, in some embodiments, a monomeric residue in a polymer (e.g., an amino acid residue in a polypeptide or a nucleic acid residue in a polynucleotide) may be identified as “corresponding to” a residue in an appropriate reference polymer. For example, those of ordinary skill will appreciate that, for purposes of simplicity, residues in a polypeptide are often designated using a canonical numbering system based on a reference related polypeptide, so that an amino acid "*corresponding to*" a residue at position 190, for example, need not actually be the 190th amino acid in a particular amino acid chain but rather corresponds to the residue found at 190 in the reference polypeptide; those of ordinary skill in the art readily appreciate how to identify "*corresponding*" amino acids.

[30] ***Engineered:*** In general, the term “engineered” refers to the aspect of having been manipulated by the hand of man. For example, a polynucleotide is considered to be “engineered” when two or more sequences, that are not linked together in that order in nature, are manipulated by the hand of man to be directly linked to one another in the engineered polynucleotide. For example, in some embodiments of the present invention, an engineered polynucleotide comprises a regulatory sequence that is found in nature in operative association with a first coding sequence but not in operative association with a second coding sequence, is linked by the hand of man so that it is operatively associated with the second coding sequence. Comparably, a cell or organism is considered to be “engineered” if it has been manipulated so that its genetic information is altered (*e.g.*, new genetic material not previously present has been introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by mating protocols). As is common practice and is understood by those in the

art, progeny of an engineered polynucleotide or cell are typically still referred to as “engineered” even though the actual manipulation was performed on a prior entity.

[31] ***Inhibitor Cysteine Knot (ICK)-like structural motif:*** As used herein, the term “inhibitor cysteine knot (ICK)-like structural motif” designates a peptide structure that has substantial structural similarity to an ICK structural motif. In some embodiments, an ICK-like structural motif has three disulfide bridges. In some embodiments, an ICK-like structural motif has two, one, or zero disulfide bridges. In some embodiments, an ICK-like structural motif has three beta strands. In some embodiments, an ICK-like structural motif has two, one, or zero beta strands. In some embodiments, an ICK-like structural motif has an amino acid sequence with six conserved cysteine residues of an ICK structural motif. In some embodiments, an ICK-like structural motif has an amino acid sequence with 5, 4, 3, 2, 1, or 0 conserved cysteine residues of an ICK structural motif.

[32] ***Hv1 associated disease or condition:*** As used herein, the phrase “Hv1 associated disease or condition” refers to a disease, disorder, or condition in which cells exhibit relatively abnormal, uncontrolled, or undesired Hv1 channel function. Abnormal or uncontrolled Hv1 function may arise from, among other mechanisms, dysregulation of phosphorylation, differential isoform expression, or single nucleotide polymorphisms (SNPs) that alter Hv1 properties. In some embodiments, abnormal or uncontrolled Hv1 function includes abnormal activation or opening of Hv1 channels. In some embodiments, abnormal or uncontrolled Hv1 function includes abnormal closing of Hv1 channels. In some embodiments, cells that exhibit abnormal or uncontrolled Hv1 function display an abnormal level or regulation of transmembrane proton flux, transmembrane voltage and/or transmembrane pH gradient (ΔpH , defined as $\text{pH}_o - \text{pH}_i$). In some embodiments, such cells display an abnormal level or regulation of NOX enzyme activity and/or reactive oxygen species (ROS) production. A variety of types of Hv1 associated diseases or conditions may exist, for example, inflammation, autoimmunity, cancer, asthma, brain damage in ischemic stroke, Alzheimer’s disease, infertility, and numerous other conditions. In some embodiments, an Hv1 associated disease or condition refers to a condition in which Hv1 channel function is within normal, but undesired range. For example, an Hv1 associated disease or condition may refer to a condition in which changing Hv1 function would achieve a more preferred physiological outcome than not changing Hv1 function. For example,

suppression of Hv1 function in human sperm may be used as a form of birth control to block fertilization.

[33] **Library:** As used herein, the term "library" refers to a collection of members. A library may be comprised of any type of members. For example, in some embodiments, a library comprises a collection of phage particles. In some embodiments, a library comprises a collection of peptides. In some embodiments, a library comprises a collection of cells. A library typically includes diverse members (i.e., members of a library differ from each other by virtue of variability in an element, such as a peptide sequence, between members). For example, a library of phage particles can include phage particles that express unique peptides. A library of peptides can include peptides having diverse sequences. A library can include, for example, at least 10^1 , 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , or more unique members.

[34] **Modulate:** The term "modulate" is used to refer to the characteristic of changing the state and/or nature of an entity of interest. For example, a particular agent is considered to modulate an entity of interest if the presence, level, and/or form of the agent correlates with a change in the presence, level, and/or form of the entity of interest. In some embodiments, to modulate means to increase activity. In some embodiments, to modulate means to antagonize, inhibit, or reduce activity. In some embodiments, modulation involves binding or direct interaction between a modulator and the entity of interest. In some embodiments, to modulate means to affect level of a target entity of interest; alternatively or additionally, in some embodiments, to modulate means to affect activity of a target entity without affecting level of the target entity. In some embodiments, to modulate means to affect both level and activity of a target entity of interest. In some embodiments, effects of a modulator are apparent at the level of the whole-cell, tissue, system (e.g. immune system), or whole organism.

[35] **Pharmaceutical Composition:** As used herein, the term "pharmaceutical composition" refers to a composition that is suitable for administration to a human or animal subject. In some embodiments, a pharmaceutical composition comprises an active agent formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, the active agent is present in a unit dose amount appropriate for administration in a therapeutic regimen. In some embodiments, a therapeutic regimen comprises one or more doses administered according to a schedule that has been determined

to show a statistically significant probability of achieving a desired therapeutic effect when administered to a subject or population in need thereof. In some embodiments, a pharmaceutical composition may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces. In some embodiments, a pharmaceutical composition is intended and suitable for administration to a human subject. In some embodiments, a pharmaceutical composition is sterile and substantially pyrogen-free.

[36] ***Polypeptide:*** As used herein, the term “polypeptide” refers to any polymeric chain of amino acids. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. In some embodiments, a polypeptide may comprise or consist of natural amino acids, non-natural amino acids, or both. In some embodiments, a polypeptide may comprise or consist of only natural amino acids or only non-natural amino acids. In some embodiments, a polypeptide may comprise D-amino acids, L-amino acids, or both. In some embodiments, a polypeptide may comprise only D-amino acids. In some embodiments, a polypeptide may comprise only L-amino acids. In some embodiments, a polypeptide is referred to as a “peptide.”

[37] ***Substantial identity:*** As used herein, the term “substantial identity” refers to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be “substantially identical” if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of

algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul et al., Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410, 1990; Altschul et al., Methods in Enzymology; Altschul et al., Nucleic Acids Res. 25:3389-3402, 1997; Baxevanis et al., Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener, et al, (eds.), Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues.

[38] ***Substantial structural similarity:*** As used herein, the term “substantial structural similarity” refers to presence of shared structural features such as presence and/or identity of particular amino acids at particular positions. In some embodiments the term “substantial structural similarity” refers to presence and/or identity of structural elements (for example: loops, sheets, helices, H-bond donors, H-bond acceptors, glycosylation patterns, salt bridges, and disulfide bonds). In some embodiments, the term “substantial structural similarity” refers to three dimensional arrangement and/or orientation of atoms or moieties relative to one another (for example: distance and/or angles between or among them between an agent of interest and a reference agent).

[39] ***Toxin:*** As used herein, the term “toxin” refers to all peptides and/or proteins, of any amino acid length and sequence, in either monomeric or multimeric forms, naturally present in animal venoms or poisons and their non-venom homologues. Non-venom homologues include any molecule present outside of a venom gland or not used as a venom component but similar in sequence, structure and/or function to toxins. Animal toxins include all molecules identified or inferred by any means (e.g., physical, chemical, biochemical, genetic, genomic, proteomic) from animal venoms or poisons, including but

not limited to isolation from crude venoms, isolation from venom gland tissues or extracts, identification based on venom gland proteome/proteomics, venome/venomics, transcriptome, and/or EST analysis. In some embodiments, a toxin is a toxin from a venom or poison of a centipede, lizard, scorpion, sea anemone, snail, snake, spider, or toad. In some embodiments, the amino acid sequence of a toxin can be a sequence that encodes an expressed and/or active toxin, or a sequence showing substantial identity thereto. In some embodiments, the amino acid sequence of a toxin is substantially identical to that of a wild-type toxin. In some embodiments, the amino acid sequence of a toxin is less than 100, 90, 80, 70, 60, 50, 40, 30, 20 or fewer amino acids long. In some embodiments, the amino acid sequence of a toxin is more than 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 or more amino acids long. Representative toxins, and their amino acid sequences and source designations are presented in Table 1.

[40] *Toxin Sequence Element:* The phrase “toxin sequence element” is used herein to refer to a stretch of amino acid sequence, typically at least 5 amino acids in length, that corresponds to an element found in a wild-type toxin. In some embodiments, a toxin sequence element has a length within a range of about 5 to about 100 amino acids. In some embodiments, a toxin sequence has a length of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more amino acids. In some embodiments, a toxin sequence element has a length within a range of about 5 to about 25 amino acids. In some embodiments, a toxin sequence element differs from a corresponding sequence element found in the wild-type toxin; for example, in some embodiments, a toxin sequence element differs from its corresponding wild-type sequence element, by a sequence variation that includes an addition, substitution, or deletion of at least one amino acid residue. In some embodiments, the variation alters (e.g., adds, substitutes or deletes) 1, 2, 3, 4, 5 or more residues. In some embodiments, the variation alters exactly 1 residue. In some embodiments, the variation alters exactly 2 residues. In some embodiments, the variation alters exactly 3 residues. In some embodiments, the variation alters not more than 5, 4, 3, 2, or 1 residues. In some embodiments, the variation alters fewer than 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of the relevant residues. In some embodiments, a toxin sequence element corresponds to a full-length toxin. In some embodiments, a toxin sequence element corresponds to a full-length reference wild-type toxin. In some embodiments, a toxin sequence element corresponds to a portion of a reference wild-type toxin. In some embodiments, a toxin sequence element corresponds to a

portion of a wild-type reference toxin, which portion is bounded on at least one end by a cysteine residue (e.g., a cysteine residue that, in the wild-type toxin, may participate in a disulfide bond).

[41] **Wild-type:** As used herein, the term “wild-type” refers to a form of an entity (e.g., a polypeptide or nucleic acid) that has a structure and/or activity as found in nature in a “normal” (as contrasted with mutant, diseased, altered) state or context. In some embodiments, more than one “wild type” form of a particular polypeptide or nucleic acid may exist in nature, for example as “alleles” of a particular gene or normal variants of a particular polypeptide. In some embodiments, that form (or those forms) of a particular polypeptide or nucleic acid that is most commonly observed in a population (e.g., in a human population) is the “wild-type” form.

Detailed Description of Certain Embodiments

Hv1 Channel

[42] The voltage-gated proton channel (Hv1), also known as the hydrogen voltage-gated channel 1 (HVCN1), is a protein that in humans is encoded by the *HVCN1* gene. There are at least ten species with functionally confirmed Hv1 genes, including human (hHv1) and mouse (mHv1), in addition to several species with predicted Hv1 genes that have not yet been confirmed by expression and electrophysiology studies. Among its functions, Hv1 transports protons (H^+) across cell membranes (DeCoursey, T.E. “The voltage-gated proton channel: a riddle, wrapped in a mystery, inside an enigma.” *Biochemistry* 54(21): 3250-68 (2015)). In humans, the Hv1 protein is expressed in a variety of tissues and body systems, including the immune system, the circulatory system, and the reproductive system. In these and other areas, Hv1 plays important physiological functions, such as regulation of cell charge and pH. In the present disclosure the terms Hv1 channel and Hv1 are equivalent.

[43] Hv1 belongs to a superfamily of voltage-gated ion channels. Similar to other voltage-gated ion channels, Hv1 is a transmembrane protein that facilitates the transfer of ions (e.g. H^+) across cell membranes. Also like other voltage-gated ion channels, Hv1 has a voltage sensor domain (VSD). However, Hv1 channels also have several unique features that distinguish them from other voltage-gated ion channels. For example, Hv1 channels are

exquisitely selective for protons, whereas other ion channels such as potassium channels have some permeability to other ions besides K^+ .

[44] According to the National Center for Biotechnology Information (NCBI) Gene database, there are three transcript variants for human *HVCN1*. Variant 1 (NM_001040107.1) represents the longest transcript. Variant 2 (NM_032369.3) differs in the 5' untranslated region (UTR) compared to variant 1. Variants 1 and 2 encode the same protein (isoform 1), which is 273 amino acids. Variant 3 (NM_001256413.1) differs in the 5' UTR, lacks a portion of the 5' coding region, and initiates translation at a downstream start codon compared with variant 1. The resulting protein (isoform 2) is shorter (253 amino acids) and has a distinct N-terminus compared to isoform 1. The longer isoform (isoform 1) is considered to be most widely expressed, while the shorter isoform (isoform 2) has been found only in B-lymphocytes and exhibits functionally important differences compared to the full-length protein. Recently, another Hv1 isoform (Hv1Sper, post-translationally cleaved) was reported in human sperm. At least seven validated, nonsynonymous single-nucleotide polymorphisms (SNPs) for human *HVCN1* have been identified. Only two of these seven have a frequencies above 1%.

Hv1 Channel Structure

[45] The structure of the Hv1 channel differs from other voltage-gated ion channels. Other voltage-gated ion channels consist of six transmembrane segments, with segments S1-S4 constituting the VSD that detects changes in membrane potential and S5-S6 forming the pore domain responsible for selective ion permeation. In contrast, Hv1 channels lack the pore domain (S5-S6). Instead, Hv1 channels have the first four transmembrane segments (S1-S4) and assemble as a dimer with each subunit containing its own permeation pathway. The N-terminus and C-terminus of Hv1 are on the cytoplasmic side. The main region of attachment of the Hv1 channel dimer is in the intracellular C-terminus.

[46] Hv1 from several multicellular species (human, mouse, and the sea squirt *Ciona intestinalis*) exist as dimers, whereas Hv1 in several unicellular species are predicted to exist as monomers. When Hv1 from species with dimeric channels are forced to express as monomers, the channels open (activate) several-fold faster than dimeric forms and their g_H - V relationship is somewhat (10-15 mV) more positive. The promoters of each monomer in an Hv1 channel dimer gate cooperatively, such that both promoters must undergo voltage-

induced conformational change before either conduction pathway is open. Consequently, the probability that dimeric Hv1 will open is more dependent on voltage compared to the monomeric form.

[47] Certain amino acids may be important for Hv1 selectivity for protons. The acidic amino acid aspartate at position 112 (Asp112), which is located in the middle of the S1 transmembrane helix, is one such amino acid important for Hv1 proton selectivity. Mutating Asp112 to any other amino acid except Glu (another acidic amino acid) converts hHv1 channel into an anion channel. Asp112 interacts with arginine (Arg208) in the S4 segment via two hydrogen bonds, with Asp or Arg protonated. Introducing a hydronium ion, H_3O^+ , into either configuration results in protonation of Asp, breaking of the hydrogen bonds, and resulting in a neutral water molecule that mediates interactions in $\text{AspH}^0\text{-H}_2\text{O}^0\text{-Arg}^+$. From this protonated selectivity filter configuration, reprotonation of the water molecule results in net H^+ permeation. Therefore, the unique abilities of protons to travel with a water molecule and to transfer readily and reversibly with other groups is exploited by Hv1 in achieving proton selectivity. Proton selectivity can also be preserved when Asp is replaced with Glu, or when Arg is replaced with Lys.

[48] Besides the selectivity filter, there is another hydrophobic region in hHv1 predicted by molecular dynamic simulations. This second region is a highly conserved Phe150. The proton may inject its own water wire through this hydrophobic region. Thus, protons are uniquely able to open the selectivity filter and to hydrate dry regions of the pore.

Hv1 Channel Mechanism

[49] Hv1 channels are uniquely selective for protons, with detectably no other ion permeation. The requirement of such selectivity is crucial because the concentration of protons in mammalian cells or bodily fluids is orders of magnitude lower than that of other major cations like Na^+ and K^+ . As already discussed above, Hv1 channel selectivity is dependent on specific amino acids, including Asp112 and Arg208.

[50] The primary determinants of Hv1 channel activation are transmembrane voltage and transmembrane pH gradient (ΔpH , defined as $\text{pH}_o\text{-pH}_i$). Hv1 opens at relatively positive transmembrane voltages (i.e. depolarization), but voltage-dependence is strongly modulated by pH. When the cytosol becomes more acidic relative to the extracellular or

intraluminal space, the entire conductance-voltage relationship of the channel shifts by 40 mV to more negative voltages for each unit increase in ΔpH . Conversely, when the extracellular or intraluminal side becomes more acidic than the cytosol, the conductance-voltage relationship shifts by 40 mV to more positive voltages for each unitary change in ΔpH . Parameters that are useful in determining Hv1 channel activation include: (1) the membrane voltage (measured on the cytosolic side, relative to the extracellular or luminal side); (2) the cytosolic pH (whereby acidification favors activation at any given voltage); and (3) the extracellular or intra-luminal pH (whereby acidification opposes activation at any given voltage). Three charged Arg residues in the S4 transmembrane segment of Hv1 confer voltage dependency, while the structural basis for pH sensing is not fully understood.

[51] Besides voltage and ΔpH , other parameters can also influence Hv1 channel activation. For example, phosphorylation of the channel by PKC can produce an enhanced responsiveness mode, allowing for more channels to open more quickly. PKC phosphorylates Hv1 at Thr29 located in the intracellular N-terminus. A situational example of enhanced gating of Hv1 is phagocyte exposure to pathogenic stimuli, such as bacteria. A diversity of stimuli can induce enhanced gating, including chemotactic peptides such as fMLF in neutrophils, lipopolysaccharide (LPS) in dendritic cells, IgE in basophils, IL-5 in eosinophils, and arachidonic acid in neutrophils and eosinophils. Such enhanced Hv1 gating is only functional in certain cells. The intensity of enhanced gating response may be associated with the presence of an active NADPH oxidase complex. Additionally, slower tail current decay (channel closing), is temporally correlated with NADPH oxidase activity. Enhanced gating makes Hv1 channels more likely to open or remain open, thereby requiring a smaller stimulus to activate H^+ flux. Hv1 proton currents are also sensitive to temperature, and have a small (15 fF) unitary conductance.

Hv1 Channel Expression

[52] Hv1 channels have been identified directly by voltage-clamp recordings in many primary tissue cell types, including neutrophils, basophils, eosinophils, cardiac fibroblasts, cultured myotubes, tracheal epithelium, and monocytes. Neutrophil and eosinophil granulocytes express the highest levels of Hv1.

[53] In most cells, Hv1 is expressed in plasma membranes, though there is evidence that Hv1 can also be expressed on intracellular membranes such as Golgi

membranes in some cells. Full-length Hv1 can be detected in human granulocytes by western blot as a 30 kDa monomer or 70 kDa dimer. Based on immunocytochemistry, Hv1 partially colocalizes with NOX2 in the membrane of intracellular granules and in the plasma membrane.

Hv1 Channel Functions

[54] Functions of Hv1 channels differ depending on the cells in which they are expressed. Cells in which high activity and a physiological function for Hv1 channels have been documented include immune cells, central nervous system cells, airway epithelia, spermatozoa, and cardiac fibroblasts. Under normal circumstances, when Hv1 channels open, H^+ efflux occurs and thereby increases pH_i , decreases pH_o and hyperpolarizes the membrane potential. These changes can have different consequences in different cells.

[55] In some cells, Hv1 channel expression and function is closely linked to expression and function of the enzyme NADPH oxidase (NOX), of which there are four isoforms: NOX1, NOX2, NOX3 and NOX4. NOX is a membrane-bound enzyme that transfers electrons from NADPH across cell membranes and couples these electrons to molecular oxygen to produce superoxide anion. In some locations, superoxide can undergo further reactions to generate reactive oxygen species (ROS). One of the functions of Hv1 linked to NOX activity is the extrusion of protons to compensate for the loss of electrons, as discussed in more detail below.

[56] Hv1 expression and/or function has been detected in both innate and adaptive immune cells. A major role of Hv1 channels is in the phagosome, an intracellular organelle in white blood cells where pathogens such as bacteria are engulfed and destroyed. The primary role for Hv1 channels in the phagosome is to allow NOX2 (the NADPH oxidase enzyme complex) to produce large quantities of reactive oxygen species (ROS) to kill pathogens, in a process called “respiratory burst”. During the respiratory burst, NOX enzymes catalyze the transfer of electrons from NADPH across the plasma membrane to reduce molecular oxygen to O^{2-} , generating two protons in the cytoplasm. The resulting depolarization and cytoplasmic acidification inhibits NOX2 activity. Depolarization opens Hv1 channels to sustain NOX2 activity by extruding protons from the cytoplasm, thereby maintaining physiological membrane potential and re-establishing normal pH. Such contributions of Hv1 channels to NOX2-dependent ROS release are characterized in

granulocytes and in particular neutrophils. H^+ current has also been detected in basophils. In these cells, IgE stimulates Hv1 channels, which facilitate release of histamine. Hv1 may also participate in ROS production and/or histamine release by mast cells.

[57] B lymphocytes of the adaptive immune system, which are responsible for antibody production, express Hv1 protein. It has been suggested that Hv1 mediates signaling in the antibody maturation process upon B-cell receptor activation by antigen binding. For example, Hv1 channels may be required for ROS production by NOX2 in mature B lymphocytes upon antigen stimulation. Given the involvement of Hv1 in B cell receptor signaling, inhibitors of Hv1 may be useful for treating autoimmune diseases and B cell malignancies.

[58] Hv1 channels are also expressed in T lymphocytes, which are cells of the adaptive immune system that recognize antigens presented by major histocompatibility complex I or II. Here, Hv1 may function to facilitate ROS production and help regulate the number of activated T lymphocytes, thereby opposing an autoimmune phenotype.

[59] Expression and activity of Hv1 has been confirmed in human sperm. Functional data indicate that Hv1 activity may be necessary for sperm activation and mobility of human sperm to achieve fertilization. The process that prepares sperm to fertilize an oocyte is called capacitation, a kind of maturation process that is triggered by an increase in intracellular pH and ROS. Changes related to capacitation include: changes in sperm motility, decrease of cholesterol in the membranes, increase of tyrosine phosphorylation in several proteins, and maturation of the sperm response to progesterone. Interestingly, seminal fluid has an unusually high concentration of Zn^{2+} (which is known to inhibit Hv1), whereas the female reproductive tract has low Zn^{2+} concentrations. It is hypothesized that on arrival of sperm in the female reproductive tract, Hv1 becomes activated and cooperates with the sperm-specific Ca^{2+} channel CatSper and NOX5 to activate sperm effector functions such as sperm movement, capacitation, sperm-zona pellucida interaction, acrosome reaction, and sperm-oocyte fusion.

[60] There is evidence to suggest the presence of Hv1 channels in mammalian brain tissue. Without wishing to be bound by any particular theory, the present disclosure proposes that Hv1 channels may play a neuroprotective role by extruding protons from metabolically active neurons and regulating neuronal pH homeostasis. Hv1 expression has

been detected in human microglia, the macrophage-like cells of the central nervous system. Hv1 in microglia may contribute to CNS disease by supporting NOX function. Microglia can become activated in acute and chronic brain disorders including brain injury, ischemia, and neurodegeneration. The expression and function of Hv1 is correlated with the expression and function of NOX2. Hv1 channels may support the activity of NOX2 in microglia by extruding excess protons from the cytoplasm. Oxidative stress, at least in part due to ROS generation by NOX, can contribute to the development of CNS disease. Hv1 inhibition could be beneficial for the treatment of neurodegenerative processes accompanied by excessive production of ROS by microglia, such as stroke, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, among others.

[61] The present disclosure proposes that NOX-independent Hv1 channel functions may also exist, given that certain cell types (e.g., basophil granulocytes) that exhibit Hv1 channel activity are not known to express NOX.

[62] Evidence indicates that Hv1 also functions in airway epithelia (e.g. tracheal epithelia) in functions such as providing a protective mechanism through acidification of the airway surface liquid. Hv1 may also function in cardiac fibroblasts, though its function in these cells is not fully known. Some experimental data also suggests Hv1 function in monocytes and macrophages. Hv1 channels may promote osteoclast cell function, for instance by promoting bone resorption by osteoclast cells. Hv1 channels may also mediate antigen presentation by dendritic cells.

[63] Many other tissues and cells not mentioned here also express Hv1 channels, some at relatively low levels. For most of the cell types and tissues that have been reported to express low levels of Hv1 channels, a specific function has not been assigned.

[64] Given the widespread expression and function of Hv1 channels, it may not be surprising that channel dysfunction can cause or enhance pathologic states. Moreover, genomic studies have identified the *HVCN1* gene as being relevant to multiple diseases. For example, *HVCN1* has been associated with Crohn's disease activity and cystic fibrosis. A study in *HVCN1* knockout rats indicated that Hv1 may contribute to the development of hypertension and renal disease with a high-salt diet. The link between Hv1 function and ROS production provides some insight on the mechanism of some Hv1-associated disorders. Excessive ROS production is thought to cause local tissue damage and contribute to several

pathological conditions, including atherosclerosis, ischemic stroke, Parkinson's disease, ischemic liver disease, Alzheimer's disease, and aging. A study on ischemic stroke has confirmed that Hv1 can exacerbate brain damage by facilitating production of ROS by NOX in microglia. Moreover, it was recently shown that Hv1-deficient mice are protected in models of stroke, suggesting that pharmacological inhibition of Hv1 channels may have neuroprotective benefits (see Seredenina, T., et al. "Voltage-gated proton channels as novel drug targets: from NADPH oxidase regulation to sperm biology." *Antioxid Redox Signal.* 10;23(5): 490-513 (2015) and references cited therein).

[65] Hv1 channels may contribute to the malignancy of several cancers, including breast cancer, colorectal cancer and leukemia. For example, higher levels of Hv1 expression occur in breast cancer cell lines with greater metastatic likelihood and knockdown of Hv1 in breast cancer cell lines reduced proliferation and invasiveness. In human patients, a high level of Hv1 expression was correlated with poor prognosis. One mechanism by which Hv1 contributes to cancer cell malignancy is related to the abnormal metabolism of cancer cells, which use glycolysis in preference to oxidative phosphorylation even in the presence of adequate oxygen. This altered metabolism creates a buildup of lactic acid that acidifies the cells, thus requiring enhanced activity of H^+ extrusion to prevent cell death.

[66] The particular isoform of Hv1 that is expressed may contribute to certain malignancies. The levels of the short isoform of Hv1 are higher in malignant B cell lines as compared to normal B lymphocytes. Moreover, the short isoform comprises approximately one-third of the Hv1 protein in malignant B cells from patients with chronic lymphocytic leukemia. The enhanced gating response is substantially more pronounced in the short compared to the long isoform of Hv1. Hv1 channel activity, proliferation and cell migration are all promoted by the expression of the short isoform.

Hv1 Channel Modulators

[67] Modulation of Hv1 channel activity is an attractive strategy for treating Hv1-related pathologies, including the ones described above. For example, agents that modulate Hv1 may be expected to ameliorate inflammation, allergies, autoimmunity, cancer, asthma, brain damage from ischemic stroke, Alzheimer's disease, infertility, and numerous other conditions. Depending on the disease and affected tissue, either Hv1 activation or inhibition

could prove useful. However, clinically compatible Hv1 modulators are not known. Therefore, there is an unmet need in identifying potent and selective modulators of Hv1.

[68] One of the challenges in creating modulators of Hv1 channels stems from the channel structure. The extracellular loops of hHv1 are fewer than a dozen amino acids, resulting in a relatively small extracellular portion of the Hv1 molecule to which drugs can bind. For example, this limits the epitope possibilities for antibodies to bind externally. Additionally, inhibition of Hv1 by physical occlusion is also a challenge, since the channel is structured to be closely packed and prevent other ions from permeating.

[69] In some instances, such as autoimmune disease and male infertility, Hv1 activation may be an attractive pharmacological strategy. Unsaturated long-chain fatty acids such as oleic acid and arachidonic acid have been shown to enhance Hv1 proton currents. This appears to be a direct pharmacological activation of Hv1. Arachidonic acid has been observed to activate a proton conductance in phagocytes. However, arachidonic acid can also activate multiple signaling pathways, which in certain cases can lead to activation of NOX enzymes and therefore indirectly activate H^+ currents. Activators of enhanced gating can also enhance Hv1 function. In general, ion channel activators are more difficult to identify than inhibitors since binding to the channel usually produces inhibition.

[70] Zn^{2+} and other polyvalent cations are known to inhibit Hv1 channels. Hv1 channels can be blocked by Zn^{2+} at concentrations ranging from 100 nM to 1 mM depending on the extracellular pH and on the presence of other polyvalent cations. The mechanism by which Zn^{2+} inhibits Hv1 involves Zn^{2+} competing with H^+ for binding to the external surface of Hv1 channels. Two His residues (His140 and His193) located at the interface between the channel monomers coordinate Zn^{2+} in the closed channel and thereby oppose channel opening. This mechanism changes the membrane potential perceived by the channel, and therefore requires stronger voltage to elicit proton current. Zn^{2+} shifts the current-voltage relationship positively and slows the kinetics of Hv1 channel activation. However, Zn^{2+} ions are implicated in many other physiological processes, and therefore the usefulness of Zn^{2+} as a specific H^+ channel blocker is limited.

[71] There are no documented high-affinity blockers of Hv1 channels that originate in venom or toxin. Tarantula toxins, including hanatoxin, can inhibit Hv1 at low micromolar concentrations by interacting with the S3 and S4 helices from the membrane

interior and shifting the g_H - V relationship in the positive direction. However, hanatoxin is not specific for Hv1. Different voltage-sensing proteins, including Hv1 and other ion channels, contain the highly conserved voltage sensor regions composed of S3 and S4 helices, termed the paddle motif. Binding of hanatoxin to the paddle motif inhibits ion fluxes through various voltage-dependent ion channels besides Hv1.

[72] Guanidine derivatives have been shown to inhibit depolarization-induced H^+ current (see Seredenina, T., et al. "Voltage-gated proton channels as novel drug targets: from NADPH oxidase regulation to sperm biology." *Antioxid Redox Signal.* 10;23(5): 490-513 (2015); DeCoursey, T.E. "The voltage-gated proton channel: a riddle, wrapped in a mystery, inside an enigma." *Biochemistry* 54(21): 3250-68 (2015); and references cited therein). The derivative 2GBI [2-guanidinobenzimidazole ($IC_{50} = 38 \mu M$)] was found to have an intracellular site of action and to only bind when the channel was open. Other identified derivatives include 1-(1,3-benzothiasol-2-yl)guanidine and 5-chloro-2-guanidinobenzimidazole. Guanidine derivatives have shown neuroprotective potential in an *in vitro* model of ischemia. The biggest challenge for pharmaceutical application of guanidine derivatives is their intracellular site of action.

[73] Several other compounds have been observed to block H^+ currents. Examples of such other compounds include weak bases (e.g. 4-aminopyridine, amiloride, verapamil or D600), tricyclic antidepressants (imipramine, amitriptyline, and desipramine), the selective serotonin reuptake inhibitor fluoxetine, the morphine-derivative dextromethorphan (DM), and a tea catechin flavonoid EGCG. These other potential inhibitors have several drawbacks, including mechanisms of action that do not directly involve Hv1 channels, multiple other targets, and effective concentrations that are too high to be of pharmaceutical interest.

To-date, there are no selective inhibitors of Hv1 channels. There is an unmet need to develop such inhibitors, especially ones that are compatible with clinical use.

Hv1 Modulating Agents

Hv1 Modulating Agent Activities

[74] The present disclosure provides agents that modulate Hv1. Among other things, the present disclosure provides agents that, for example, modulate one or more Hv1 activities when contacted with an Hv1 channel, for example, *in vitro* and/or *in vivo*. In some embodiments, Hv1 modulating agents modulate Hv1 activities of Hv1 monomers and dimers with similar IC₅₀. In some embodiments, Hv1 modulating agents specifically bind Hv1. In some embodiments, Hv1 modulating agents inhibit Hv1. In some embodiments, Hv1 modulating agents activate Hv1.

[75] In some embodiments, Hv1 modulating agents do not physically occlude Hv1 channels. In some embodiments, Hv1 modulating agents bind to Hv1 but do not bind to other voltage-gated channels or other ion channels.

[76] In some embodiments, Hv1 modulating agents bind to the external surface of Hv1. In some embodiments, Hv1 modulating agents target or bind to the S3-S4 external loop region of hHv1. For example, Hv1 modulating agents may bind to hHv1 at amino acid residues I183 to L204. In some embodiments, Hv1 modulating agents bind to regions of hHv1 comprising an amino acid sequence corresponding to ILDIVLLFQEHQFEALGLLILL (SEQ ID NO: 111).

[77] In some embodiments, Hv1 modulating agent binding to Hv1 is reversible. In some embodiments, Hv1 modulating agent binding to Hv1 may be irreversible. In some embodiments, Hv1 modulating agent binding to Hv1 is strong but not irreversible.

[78] In some embodiments Hv1 modulating agents bind to open Hv1 channels. In some embodiments, Hv1 modulating agents bind to closed channels. In some embodiments, affinity of Hv1 modulating agents for closed states of Hv1 is about 1 nM. In some embodiments, affinity of Hv1 modulating agents is lower for open states of the channel (e.g. about 200 nM) as compared to closed states of the channel. In some embodiments, Hv1 modulating agents slow opening of closed states of Hv1 even as they unbind.

[79] In some embodiments, provided Hv1 modulating agents may change transmembrane voltage of a cell. Hv1 modulating agents may hyperpolarize the membrane potential. Hv1 modulating agents may depolarize the membrane potential. Effects of Hv1 modulating agents may be measured by, for example, direct electrophysiological recordings of voltage-gated proton currents, such as patch-clamp recordings.

[80] In some embodiments, provided Hv1 modulating agents are characterized in that, for example, they decrease or block proton current. In some embodiments, Hv1 modulating agents may reduce the number or likelihood of Hv1 channel opening. In some embodiments, Hv1 modulating agents may speed up the rate of Hv1 channel closing. In some embodiments, Hv1 modulating agents may cause an Hv1 channel to require stronger voltage to elicit proton current.

[81] In some embodiments, provided Hv1 modulating agents are characterized in that, for example, they increase proton currents and/or slow the closing of Hv1 channels. In some embodiments, Hv1 modulating agents may provide an enhanced responsiveness mode, allowing more channels to open more quickly, increasing likelihood that Hv1 channels will open or remain open, and/or reducing the stimulus required to activate H^+ flux.

[82] In some embodiments, Hv1 modulating agents may increase or decrease proton (H^+) current but do not directly alter current of other ions (e.g. Na^+ , K^{2+} , Ca^{2+}).

[83] In some embodiments, Hv1 modulating agents may change the transmembrane pH gradient (ΔpH , defined as $pH_o - pH_i$). In some embodiments, provided Hv1 modulating agents may increase or decrease intracellular or cytosolic pH (pH_i). Thus, Hv1 modulating agents may decrease or increase the cytoplasmic acidity. In some embodiments, provided Hv1 modulating agents may increase or decrease the extracellular, intraluminal, or organelle pH (pH_o). Thus, Hv1 modulating agents may decrease or increase the extracellular, intraluminal, or organelle acidity.

[84] In some embodiments, Hv1 modulating agents may increase or decrease cellular ROS production.

[85] In some embodiments, Hv1 modulating agents may increase or decrease the function and/or activity of NOX enzymes, including NOX1, NOX2, NOX3, and/or NOX4. In some such embodiments, Hv1 modulating agents may enhance or reduce the ability of NOX enzymes to transfer electrons. In some such embodiments, Hv1 modulating agents may increase or decrease the production of superoxide anion by a cell. In some such embodiments, Hv1 modulating agents may increase or decrease the quantity of ROS production mediated by NOX enzymes. In some embodiments, Hv1 modulating agents may sustain NOX activity by extruding protons from the cytoplasm.

[86] In some embodiments, Hv1 modulating agents may alter signaling pathways that can be affected by Hv1 activity. In some embodiments, Hv1 modulating agents may affect cellular, physiological, or pathological processes that can be affected by Hv1 activity. In some such embodiments, Hv1 modulating agents may influence inflammation, allergies, autoimmunity, cancer, asthma, brain damage from ischemic stroke, Alzheimer's disease, and fertility.

[87] In some embodiments, Hv1 modulating agents may alter sperm activity or fertilization. Hv1 modulating agents may affect sperm mobility, capacitation, sperm-zona pellucida interaction, acrosome reaction, and sperm-oocyte fusion. In a particular example, Hv1 modulating agents inhibit properties associated with sperm capacitation.

[88] In another example, Hv1 modulating agents may alter the ability of white blood cells to fight infections. Hv1 modulating agents may alter the activity of phagosomes, NOX enzymes, or ROS production. In a particular example, Hv1 modulating agents may decrease ROS production in white blood cells.

Hv1 Modulating Agent Structure

[89] In some embodiments, an Hv1 modulating agent is or comprises a polypeptide. In some embodiments, a polypeptide component of an Hv1 modulating agent is 10-100 amino acids in length.

[90] In many embodiments in which a provided Hv1 modulating agent includes a polypeptide component, the polypeptide component of the agent has an amino acid tertiary structure that is characterized as an inhibitor cysteine knot (ICK)-like structural motif. In some embodiments, a polypeptide component has a structure that has substantial structural similarity to an ICK structural motif. In some embodiments, a polypeptide component has three disulfide bridges. In some embodiments, a polypeptide component has three beta strands. In some embodiments, a polypeptide component has an amino acid sequence with six conserved cysteine residues of an ICK motif (FIG. 1).

[91] In many embodiments in which a provided Hv1 modulating agent includes a polypeptide component, the polypeptide component of the agent has an amino acid sequence that includes one or more elements that is substantially identical to, but different from, that of wild-type toxin sequences (e.g., of a wild-type voltage sensor toxin). In some

embodiments, such a sequence element has a length of about 5 to about 20 amino acids. In some embodiments, such a sequence element shows at least 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a corresponding element of a wild-type toxin. In some embodiments, a polypeptide component of a provided Hv1 modulating agent may show significant (e.g., at least 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or higher) overall sequence identity with, while differing from, a wild-type toxin. In some embodiments, a polypeptide component has an amino acid sequence that includes a plurality of toxin sequence elements, each of which is substantially identical to a sequence element that is found in the same, or a different, reference wild-type toxin. In some such embodiments, the plurality of toxin sequence elements are assembled in linear order so that the amino acid sequence shows overall correspondence with (e.g., shares one or more certain structural features, such as number and/or [relative] location of one or more cysteine residues) a full-length toxin. In some embodiments, a polypeptide component has an amino acid sequence that includes one or more residues found in a wild-type toxin that participates in binding by that wild-type toxin to a voltage-sensing protein.

[92] Exemplary wild-type toxin sequences are presented in Table 1. In some embodiments, a wild-type toxin is a venom toxin. In some embodiments, a venom toxin is a toxin found in venom of organisms such as scorpion (e.g., *Pandinus imperator*), sea anemone, snails (e.g. *Conus marmoreus*), snakes, and spiders (e.g., *Grammostola rosea*).

Table 1

SEQ ID NO:	Toxin Name	Sequence	NCBI Accession NO:	Animal Species
1	HwTx-IV	ECLEIFKACNPSNDQCCKSSKLVCSRKTRWCKYQI	P83303.2	Haplopelma schmidtii (Chinese bird spider)
2	HnTx-IV	ECLGFGKGCNPSNDQCCKSSNLVCSRKHRWCKYEI	D2Y232.1	Haplopelma hainanum (Chinese bird spider)
3	HnTx-V	ECLGFGKGCNPSNDQCCKSANLVCSRKHRWCKYEI	P60975.1	Haplopelma hainanum (Chinese bird spider)
4	PaurTx3	DCLGFLWKCNPNDKCCRPNLVCSRKDKWCKYQI	P84510.1	Paraphysa scrofa (Chilean copper tarantula)
5	CcoTx1	DCLGWFKSCDPKNDKCKKNYTCRRDRWCKYDL	P84507.1	Ceratogyrus marshalli (Straightthorned baboon tarantula)

6	CcoTx2	DCLGWFKSCDPKNDKCKKNYTCRRDRWCKYYL	P84508.1	Ceratogyrus marshalli (Straighthorned baboon tarantula)
7	VSTX3	DCLGWFKGCDPDNDKCEGYKCNRRDKWCKYKLW	P0C2P5.1	Grammostola rosea (Chilean rose tarantula)
8	TlTx1	AACLGMFESCDPNNDKCCPNRECNRKHKWCKYKLW	P83745.1	Theraphosa blondi (Goliath birdeating spider)
9	JZTX-25	DDCLGMFSSCPNDNDKCEGRKCDRRDQWCKWNPW	B1P1F1.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
10	JZTX-27	DCLGLFWICNYMDDKCCPGYKCERSSPWCKIDI	B1P1H2.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
11	TlTx3	DDCLGMFSSCDPNNDKCCPNRVCRVRDQWCKYKLW	P83747.1	Theraphosa blondi (Goliath birdeating spider)
12	TlTx2	DDCLGMFSSCDPKNDKCCPNRVCSRDRDQWCKYKLW	P83746.1	Theraphosa blondi (Goliath birdeating spider)
13	HwTx-I	ACKGVFDACTPGKNECCPNRVCSDKHKWCKWKL	P56676.2	Haplopelma schmidtii (Chinese bird spider)
14	GsMTx4	GCLEFWWKCNPNDKCCRPKLKCSKLFKLCNFSFGK	Q7YT39.1	Grammostola rosea (Chilean rose tarantula)
15	Omega-AcTx-Hv1b	ADCGWLFHSCESNADCCENWACATTGRFRYLCKYQI	P81595.1	Hadronyche versuta (Blue mountains funnel-web spider)
16	IpTxa	GDCLPHLKRCKADNDCCGKCKRRGTNAEKRCR	P59868.1	Pandinus imperator (Emperor scorpion)
17	VSTX1	ECGKFMWCKKNSNDCKDLVCCSRWKCVLASPF	P60980.2	Grammostola rosea (Chilean rose tarantula)
18	HnTx-I	ECKGFGKSCVPGKNECCSGYACNSRDKWCKVLL	D2Y1X6.1	Haplopelma hainanum (Chinese bird spider)
19	Maurocalicine	GDCLPHLKLCKENKDCCSKCKRRGTNIEKRCR	P60254.1	Scorpio maurus palmatus (Chactoid scorpion)
20	HpTX3	ECGTLFSGCSTHADCCGFIKLCWCRYERTW	P58427.1	Heteropoda venatoria (Brown huntsman spider)
21	HNTX-VII	ECRYWLGTCSKTGDCCSHLSCSPKHGWCVDWTFRK	D2Y2C3.1	Haplopelma hainanum (Chinese bird spider)
22	JZTX F4-32.60	GCQKFFWTCHPGQPPCCSLACTWPTEICIDG	P0CH50.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
23	HnTx-III	GCKGFGDSCTPGKNECCPNYACSSKHKWKVYL	D2Y1X9.1	Haplopelma hainanum (Chinese bird spider)
24	Toxin_K	DDCGTLFSGCDTSKDCCEGYVCHLWCKYK	P61791.1	Heteropoda

	J1			venatoria (Brown huntsman spider)
25	ScTx1	DCTRMFGACRRSDCCPHLGCKPTSKYCAWDGTI	P60991.1	Stromatopelma calceatum (Featherleg baboon tarantula)
26	JZTX-50	RCIEEGKWCPKKAPCCGRLECKGPSPKQKKCTRP	B1P1B0.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
27	ProTx-1	ECRYWLGGCSAGQTCCKHLVCSRRHGWCVWDGTFS	P83480.1	Thrixopelma pruriens (Peruvian green velvet tarantula)
28	HmTx1	ECRYLFGGCSSTSDCKHLSCRSDWKYCAWDGTFS	P60992.1	Heteroscodra maculata (Togo starburst tarantula)
29	GxTx1E	EGECGGFWWKC GSGKPACCPKYVCSPKWGLCNFPMP	P84835.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
30	GxTx-1D	DGECGGFWWKC GSGKPACCPKYVCSPKWGLCNFPMP	P84836.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
31	Omega-AcTx-Hv1a	DDDCGWIMDDCTSDSDCCPNWVCSKTGFVKNICKYEM	P56207.1	Hadronyche versuta (Blue mountains funnel-web spider)
32	JZTX F7-15.33	LCSREGEFCYKLRKCCAGFYCKAFVLHCYRN	P0CH55.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
33	Tx2-9	SFCIPFKPCKSDENCKKFKCKTTGIVKLCRW	AAB32862.1	Brachypelma smithii (Mexican red knee tarantula)
34	GxTx-2	ECRKMFGGCSVDS DCCAHLGCKPTLK YCAWDGT	P84837.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
35	HpTX1	DCGTIWHYCGTDQSECEGWKCSRQLCKYVIDW	P58425.1	Heteropoda venatoria (Brown huntsman spider)
36	SHLP-I	GCLGDKCDYNNGCCSGYVCSRTWKWCVLAGPWR	Q86C51.1	Haplopelma schmidtii (Chinese bird spider)
37	JZTX-VII	GCGGLMAGCDGKSTFCCSGYNCSPTWKWCYARP	P0C2X7.2	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
38	JZTX-29	ECRKMFGGCSVHSDCCAHLGCKPTLK YCAWDGTF	B1P1E4.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
39	JZTX-12.1	GCGGLMDGCDGKSTFCCSGFNCSPWKWCYARP	B1P1C4.1	Chilobrachys guangxiensis (Chinese earth

				tiger tarantula)
40	Toxin_A U2	DDCGGLFSGCDSNADCCGYVCRLWCKYKL	P61792.1	Heteropoda venatoria (Brown huntsman spider)
41	HaTx1	ECRYLFGGCKTTSDCCKHLGCKFRDKYCAWDFTFS	P56852.1	Grammostola rosea (Chilean rose tarantula)
42	HaTx2	ECRYLFGGCKTTADCCCKHLGCKFRDKYCAWDFTFS	P56853.1	Grammostola rosea (Chilean rose tarantula)
43	VaTx1	SECRWFMGGCDSTLDCCCKHLSCKMGLYYCAWDGTF	P0C244.1	Psalmopoeus cambridgei (Trinidad chevron tarantula)
44	JzTx-XI	ECRMFGGCSVSDCCAHLGCKPTLKYCAWDGTFGK	P0C247.2	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
45	HmTx2	ECRYFWGECNDEMVCCEHLVCKEKWPITYKICVWDRT F	P60993.1	Heteroscodra maculata (Togo starburst tarantula)
46	JzTx- III	DGECGGFWWKCGRGKPPCCKGYACSKTWGCAVEAP	P62520.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula) (Chilobrachys jingzhao)
47	PcTx1	EDCIPKWKGCVNRHGDCCEGLECWKRRRSFVCPKT PKT	P60514.1	Psalmopoeus cambridgei (Trinidad chevron tarantula)
48	Ageleni n	GGCLPHNRFNCNALS GPRCCSGLKCKELSIWDSRCL	P31328.1	Allagelena opulenta (Funnel weaving spider)
49	JZTX-13	QCGEFMWKCGAGKPTCCSGYDCSPTWKVCVLKSPGRR	B1P1C9.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
50	JZTX-15	TCYDIGELCSDKPCCSGYCSPRWGWCYSTRGGR	B1P1D4.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
51	Omega- AcTx- Hv1f	SAVCIPSGQPCPYSKYCCSGSCTYKTENGENSVQRCD	P81599.1	Hadronyche versuta (Blue mountains funnel-web spider)
52	HNTX- XIX	CAAEGIPCDPNPVKDLPCCSGLACLKPTLHGIWYKHH YCYTQ	D2Y299.1	Haplopelma hainanum (Chinese bird spider)
53	lamda- MeuTx	GCNRKNKKCNSDADCCRYGERCISTKVNYCRPDRGP	P86399.2	Mesobuthus eupeus (Lesser Asian scorpion)
54	JZTX-24	VCRGYGLPCTPEKNDCCQRLYCSQHRLCSVKA	B1P1F0.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
55	HwTx-X	KCLPPGKPCYGATQKIPCCGVCSHNKCT	P68424.2	Haplopelma

				schmidtii (Chinese bird spider)
56	JZTX-21	CGGWMAKCADSDCCETFHCTRFNVCCK	B1P1E6.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
57	magi-11	SCKLTFWRCKKDKCECGWNICTGLCIPP	Q75WH2.1	Macrothele gigas (Spider)
58	SGTx1	TCRYLFGGCKTTADCCCKHLACRSDGKYCAWDGTF	P56855.1	Stromatopelma calceatum griseipes (Feather leg baboon tarantula)
59	JZTX-44	ECKWYLGDCKAHEDCCEHLRCHSRWDWCIWDGTF	B1P1G8.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
60	HNTX-VI	ECKYLWGTCEKDEHCCEHLGCNKKHGWCGWDGTF	P0CH70	Haplopelma hainanum (Chinese bird spider)
61	GsAF_I	YCQKWLWTCDSERKCCEDMVCRLWCKKRL	P61408.1	Grammostola rosea (Chilean rose tarantula)
62	GrTx1	YCQKWMWTCDSKRKCCEDMVCQLWCKKRL	P85117.1	Grammostola rosea (Chilean rose tarantula)
63	PaTx1	YCQKWMWTCDSARKCCEGLVCRLWCKKII	P61230.1	Paraphysa scrofa (Chilean copper tarantula)
64	Magi-5	GCKLTFWKCKNKKECCGWNACALGICMPR	P83561.2	Macrothele gigas (Spider)
65	HwTx-V	ECRWYLGCGSQDGDCCCKHLQCHSNYEWCVWDGTF SK	P61104.2	Haplopelma schmidtii (Chinese bird spider)
66	VaTx2	GACRWFLGGCKSTSDCCEHLSCKMGLDYCAWDGTF	P0C245.1	Psalmopoeus cambridgei (Trinidad chevron tarantula)
67	SNX482	GVDKAGCRYMFGGCSVNDDCCPRLGCHSLFSYCAWDL TFSD	P56854.1	Hysterochrates gigas (African tarantula)
68	PnVIIA	DCTSWFGRCTVNSECCSNSCDQTYCELYAFPSFGA	P56711.2	Conus pennaceus (Feathered cone)
69	PNTx27C ₄	IACAPRFSLCNSDKECKGLRCQSRIANMWPTFCSQ	P83996.2	Phoneutria nigriventer (Brazilian armed spider)
70	PRTx27C ₃	IACAPRGLLCFRDKECKGLTCKGRFVNTWPTFCLV	P83892.1	Phoneutria reidyi (Brazilian Amazonian armed spider)
71	JZTX-36	DCRKMFGGCSKHEDCCAHLACKRTFNCAWDGFSK	B1P1D7.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
72	JZTX-38	ECRWLFGGCEKSDCCEHLGCRRAKPSWCGWDFTV	B1P1G2.1	Chilobrachys guangxiensis

				(Chinese earth tiger tarantula)
73	JZTX-39	ECRWLFGGCEKSDCCEHLGCRRAPSWCGWDFTF	B1P1G4.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
74	PRTx26A n0C3	IACAPRFSICNSDKECKGLRCQSRIANMWPTFCLV	P86418.1	Phoneutria nigriventer (Brazilian armed spider)
75	HNTX-XVI	CIGEGVPCDENDPRCCSGLVCLKPTLHGIWYKSYCY KK	D2Y253.1	Haplopelma hainanum (Chinese bird spider)
76	HNTX-VIII	DCAGYMRECKEKLCCSGYVCSSRWKCVLPAPWRR	D2Y240.1	Haplopelma hainanum (Chinese bird spider)
77	HNTX-IX	ECRWYLGGSQDGDCKHLQCHSNYEWCIWDGTF SK	D2Y236.1	Haplopelma hainanum (Chinese bird spider)
78	F5-21.66	ECKKLFGGCTTSSECCAHLGCKQKWPFCYAWDSF	P0CH51.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
79	Hm-2	GCIPSFGECAWFSGESCTGICKWVFFTSKFCRRVW GKD	P85506.1	Heriaeus melloteei (Crab spider)
80	HdCa	SEKDCIKHLQRCRENKDCCSKKCSRRGTNPEKRCR	B8QG00.1	Hadrurus gertschi (Scorpion)
81	ProTx-2	YCQKWMWTCDSERKCCEGMVCRLWCKKKLW	P83476.1	Thrixopelma pruriens (Peruvian green velvet tarantula)
82	JzTx-V	YCQKWMWTCDSKRACCEGLRCKLWCRKIIG	Q2PAY4.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
83	HpTX2	DDCGKLFSGCDTNADCEGYVCRLWCKLDW	P58426.1	Heteropoda venatoria (Brown huntsman spider)
84	GsAF_II	YCQKWMWTCDEERKCCEGLVCRLWCKKKIEW	P61409.2	Grammostola rosea (Chilean rose tarantula) (Grammostola spatulata)
85	MrvIB	ACSKKWEYCIVPILGFVYCCPGLICGPFVCV	AAB34194.1	Conus marmoreus (Marble cone)
86	GsMTx-2	YCQKWMWTCDEERKCCEGLVCRLWCKRIINM	P60273.1	Grammostola rosea (Chilean rose tarantula)
87	VSTX2	YCQKWMWTCDEERKCCEGLVCRLWCKKKIEEG	P0C2P4.1	Grammostola rosea (Chilean rose tarantula)
88	JZTX-2	GCGTMWSPCSTEKPCDNFSCQPAIKWCIWSP	B1P1B9.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
89	VaTx3	ECRWYLGCKEDSECCEHLQCHSYWEWCLWDGSF	P0C246.1	Psalmopoeus

				cambridgei (Trinidad chevron tarantula)
90	CcoTx3	GVDKEGCRKLLGGCTIDDDCCPHLGCNKKYWHCGWDGTF	P84509.1	Ceratogyrus marshalli (Straighthorned baboon tarantula)
91	JZTX-IV	ECTKFLGGCSEDSECCPHLGCKDVLYYCAWDGTFGK	P0CH56.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
92	JzTx-IX	ECTKLLGGCTKDSECCPHLGCRKKWPYHCGWDGTF	B1P1F5.2	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
93	AcTx-Hv1	SPTCIPSGQPCPYNENCCSQSCTFKENENGNTVKRCD	P56207.1	Hadronyche versuta (Blue mountains funnel-web spider) (Atrax versutus)
94	JZTX-34	ACREWLGGSCKDADCCAHLECRKKWPYHCVWDWTV	B1P1F7.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
95	Omega-AcTx-Ar1a	SVCIPSGQPCPYNEHCCSGSCTYKENENGNTVQRCD	P83580.2	Atrax robustus
96	Omega-hexatoxin-Hv1b	SSTCIPSGQPCPYNENCCSQSCTYKENENGNTVKRCD	P81595	Hadronyche versuta (Blue mountains funnel-web spider)
97	Omega-AcTx-Hi1a	STCTPTDQPCPYHESCCSGSCTYKANENGNTVQRCD	P0C2L4.1	Hadronyche formidabilis (Northern tree funnel-web spider)
98	Omega-AcTx-Hf1a	SPTCIRSGQPCPYNENCCSQSCTFKTNENGNTVKRCD	P0C2L4.1	Hadronyche formidabilis (Northern tree funnel-web spider)
99	Omega-AcTx-Hi1b	SPTCIPTGQPCPYNENCCSQSCTYKANENGNTVQRCD	P0C2L6.1	Hadronyche infensa (Fraser island funnel-web spider)
100	Omega-AcTx-Hi1c	SSTCIRTDQPCPYNESCCSGSCTYKANENGNTVQRCD	P0C2L7.1	Hadronyche infensa (Fraser island funnel-web spider)
101	Omega-AcTx-Hv1c	SSTCIPSGQPCPYNENCCSQSCTFKENENGNTVKRCD	P81596.1	Hadronyche versuta (Blue mountains funnel-web spider)
102	Omega-AcTx-Hv1d	SPTCIPSGQPCPYNENCCSKSCTYKENENGNTVQRCD	P81597.1	Hadronyche versuta (Blue mountains funnel-web spider)
103	Omega-AcTx-Hv1e	SPTCIPSGQPCPYNENCCSQSCTYKENENGNTVKRCD	P81598.1	Hadronyche versuta (Blue mountains funnel-web spider)
104	magi-1	CMGYDIHCTDRLPCCFGLCVKTSYGYWYKKTYYCRRKS	P83557.1	Macrothele gigas (Spider)
105	Omega-MSTX-	SPVCTPSGQPCQPNTQPCCNNAEEETINCNGNTVYRCA	P83588.1	Missulena bradleyi (Eastern mouse)

	Mb1a			spider)
106	F3-24.71	SPVCTPSGQPCQPNTQPCCNNAEEETINCNGNTVYRCA	P0CH70.1	Haplopelma hainanum (Chinese bird spider)
107	JzTx-XII	YCQKWMWTCDSERKCCEGYVCELWCKYNL	P0C5X7.2	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
108	JZTX-1.2	ACGQFWWKCCEGKPPCCANFACKIGLYLCIWSP	B1P1B7.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)
109	GrTx-SIA	DCVRFWKGCSQTSDDCPHLACKSKWPRNICVWDGSV	P60590.2	Grammostola rosea (Chilean rose tarantula)
110	JZTX-35	DCRALYGGCTKDEDCCKHLACRRTLPTYCAWDLTFP	B1P1F9.1	Chilobrachys guangxiensis (Chinese earth tiger tarantula)

[93] In some embodiments, a wild-type toxin sequence can be a predicted wild-type toxin sequence. In some embodiments, a predicted wild-type toxin sequence is identified in public protein databases. In some embodiments, a sequence element found in a wild-type toxin sequence is identified by isolating an amino acid sequence delineated by six conserved cysteine residues that form disulfide bridges in an ICK motif of a wild-type toxin sequence. In some embodiments, a known amino acid sequence of a wild-type toxin sequence can be used as a template to align amino acid sequences from public protein databases and identify predicted wild type-toxin sequences. In one example, the amino acid sequence of the Peruvian green velvet tarantula (*Thrixopelma pruriens*) is used as a template to identify predicted wild-type toxin sequences using basic local alignment search tools in public protein databases.

[94] In some embodiments, a polypeptide component has an amino acid sequence that is substantially identical to a sequence set forth in Table 2. In some embodiments, a polypeptide component has an amino acid sequence that is substantially identical to a sequence set forth in Table 2 and an activating or inhibiting effect on Hv1 as set forth in Table 2B. In some embodiments, a polypeptide component has an amino acid sequence that is encoded by a nucleotide sequence that is substantially identical to a sequence set forth in Table 2C.

Table 2A

SEQ ID NO:	Name	Sequence
115	A1	DCAGYMRCKKDKBCCGWNICNRKHKWKYKWLW
116	A2	GCQMTFWKCNALDHNCCHGYAACGCKKIIVSARIA
117	A4	GGCLPHNRFCNPSNDQCCCKSANLVORLWCKKKIEGDP
118	A6, G2	GCKGFGDSCADSDCCETFHCKWVFFTSKFMCRRVWGKD
119	B1	GCLGDKCADSDCCETFHCKWVFFTSKFMCRRVWGKD
120	B2	SPTCIPSGQPCADSDCCETFHCKWVFFTSKFMCRRVWGKD
121	B3	DEDCQPPGNFCXNTSDCCCEHLXCPTTPEFPYLCQYXMG
122	B4	GACRWFLGGCTPEKNDCCQRLYCGPFVGV
123	B5	SPVCTPSGQPCRENKDCCKSKKCTTGIVKLCRW
124	B6	ACSKKWEYCTKDSGCCPHLGWKRERSFEVCPKTPKT
125	C1	RCIEEGKWETKDEDCCKHLACNRKHKWKYKWLW
126	C2, F2	SPTCIRSGQPCADSDCCETFHCKWVFFTSKFMCRRVWGKD
127	C3	STCTPTDQPCADSDCCETFHCKWVFFTSKFMCRRVWGKD
128	C5	GCKWYLGDCADSDCCETFHCKWVFFTSKFMCRRVWGKD
129	C6, D5	SSTCIPSGQPCADSDCCETFHCKWVFFTSKFMCRRVWGKD
130	D3	ACSKKWEYCKEKLCCSGYVCKRRGTNIEKRORG
131	D4	ACGQFWWKCTSDSDCCPNWVORLWCKYKL
132	D6, E2	CRYWLGGCSQDGDCCCKHLQCSRWGWCIIYSTRGGR
133	E1	DGCTIWHYCTPEKNDCCQRLYCSRWRLVHL
134	E3	IACAPRPSICDPKNDKCCPNRVCSDKHKWKWKWL
135	E4	SSTCIPSGQPCRENKDCCKSKKCSDKHKWKWKWL
136	E5	DGECGGFWWKCKNSNDCCDLVCKEKWPITYKICVWDRTF
137	E6	IACAPRPSLCDTSKDCCEGYVCNRKHKWKYKWLW
138	F4	ECKGFGKSCADSDCCETFHCKWVFFTSKFMCRRVWGKD
139	F5	SPVCTPSGQPCADSDCCETFHCKWVFFTSKFMCRRVWGKD
140	F6	DDCGGLFSGCTPGKNECCPNRVCKIGLYLCIWS
141	G1	GCLGDKCADSDCCETFHCKWVFFTSKFMCRRVWGKD
142	G3	CRYLFGGCAWFSGESCCTGICSPRWGWCIIYSTRGGR
143	G4	GDCLPHLKLCPNDKCCRPKLKCSRGTNPEKROR
144	G6	DDCGTLFSGCPYSKYCCSGSCKRRGTNIEKROR
145	H4	AAEGCLCDRCXHSQDCCEDPHCTCEFFNM

Table 2B

SEQ ID NO:	Name	Sequence	Activator/Inhibitor
118	A6	GCKGFGDSCADSDCCETFHCKWVFFTSKFMCRRVWGKD	ACTIVATOR
119	B1	GCLGDKCADSDCCETFHCKWVFFTSKFMCRRVWGKD	ACTIVATOR
128	C5	GCKWYLGDCADSDCCETFHCKWVFFTSKFMCRRVWGKD	ACTIVATOR
129	C6	SSTCIPSGQPCADSDCCETFHCKWVFFTSKFMCRRVWGKD	INHIBITOR
129	D5	SSTCIPSGQPCADSDCCETFHCKWVFFTSKFMCRRVWGKD	INHIBITOR
126	F2	SPTCIRSGQPCADSDCCETFHCKWVFFTSKFMCRRVWGKD	ACTIVATOR
118	G2	GCKGFGDSCADSDCCETFHCKWVFFTSKFMCRRVWGKD	ACTIVATOR

Table 2C

SEQ ID NO:	Name	Nucleotide Sequences
146	A1	GATTGCGCGGGCTATATGCGCGAATGTAAAAAGATAAAGAATGCTGCGGCTGGAACATTTGCAACCGCAAA CATAAATGGTGCAAATATAAACTGTGG
147	A2	GGCTGCCAAATGACCTTTTGGAAATGTAAACGCGCTGGATCACAACCTGCTGCCATGGCTATGCCGCTGTGGA TGCAAAAAAATTATTGTATCCGCGAGAATCGCG
148	A4	GGCGGCTGCTGCCGCATAACCGCTTTTGTAAACCGAGCAACGATCAGTGCTGCAAAAGCGCGAACCTGGTG TGCCGCTGTGGTGCAAAAAAATTGAAGGGGATCCG
149	A6, G2	GGCTGCAAAGGCTTTGGCGATAGCTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAATGGGTG TTTTTTACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT
150	B1	GGCTGCCTGGGCGATAAATGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAATGGGTGTTTTTT ACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT
151	B2	AGCCCGACCTGCATTCCGAGCGGCCAGCCGTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAA TGGGTGTTTTTTACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGATGGAT
152	B3	GACGAAGATTGCCAACCGCCGGGCAACTTTGTANCAACACCAGCGATTGCTGCGAACATCTGNNCTGCCCG ACCACCCCCGCTTTCCCTATCTGTGCCAATACNCATGGGA
153	B4	AGGCGCGTGCCGCTGGTTTCTGGGCGGCTGTACCCCGGAAAAAAACGATTGCTGCCAGCGCCTGTATTGCGG CCCGTTTGTGTGCGTG
154	B5	AGCCCGGTGTGCACCCCGAGCGGCCAGCCGTGTGCGAAAAACAAAGATTGCTGCAGCAAAAAATGCAAAACC ACCGGCATTGTGAACTGTGCCGCTGG
155	B6	GCGTGCAACAAAAATGGGAATATTGTACCAAGATAGCGAATGCTGCCCGCATCTGGGCTGCTGGAAACGC CGCCGCAGCTTTGAAGTGTGCGTGCCGAAAAACCCGAAAAAC
156	C1	CGCTGCATTGAAGAAGGCAAATGGTGTACCAAGATGAAGATTGCTGCAAAACATCTGGCGTGCAACCGCAAA CATAAATGGTGCAAATATAAACTGTGG
157	C2, F2	AGCCCGACCTGCATTGCGAGCGGCCAGCCGTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAA TGGGTGTTTTTTACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT
158	C3	AGCACCTGCACCCCGACCGATCAGCCGTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAATGG GTGTTTTTTACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT
159	C4	AAATGCCGCTGGCTGTTTGGCGGGGTACCCCGGGCAAAACGAATGCTGGCCGAACATATGCGTGCCATAGCT ATTGGGAATGGGGCCTGTGGGATGGCAGCTTTGGATCCG
160	C5	GGCTGCAAATGGTATCTGGGCGATTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAATGGGTG TTTTTTACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT
161	C6, D5	AGCAGCACCTGCATTCCGAGCGGCCAGCCGTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAA TGGGTGTTTTTTACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT
162	D3	GCGTGCAACAAAAATGGGAATATTGTAAAGAAAACTGTGCTGCAGCGGCTATGTGTGCAAACGCCGCGGC ACCAACATTGAAAAACGCTGCCGCGGA
163	D4	GCGTGCGGCCAGTTTTGGTGGAATGTACCAGCGATAGCGATTGCTGCCCGAACTGGGTGTGCCGCTGTGG TGCAAATATAAACTG
164	D6, E2	TGCCGCTATTGGCTGGGCGGCTGTAGCCAGGATGGCGATTGCTGCAACATCTGCAGTGCAAGCCGCGCTGG GGCTGGTGCAATTATAGCACCCGCGCGCGCCG
165	E1	GATTGCGGCACCATTTGGCATTATTGTACCCCGGAAAAAACGATTGCTGCCAGCGCCTGTATTGCAGCCCG CGCTGGAGGCTGGTGCAATTA
166	E3	ATTGCGTGCGCGCCGCGCTTTAGCATTTTGTGATCCGAAAAACGATAAATGCTGCCCGAACCGCGTGTGCAGC GATAAACATAAATGGTGCAAATGGAACTG
167	E4	AGCAGCACCTGCATTCCGAGCGGCCAGCCGTGTGCGAAAAACAAAGATTGCTGCAGCAAAAAATGCAGCGAT AAACATAAATGGTGCAAATGGAACTGGGA
168	E5	GATGGCGAATGCGGCGGCTTTTGGTGGAATGTAAAAACAGCAACGATTGCTGCAAGATCTGGTGTGCAAA GAAAAATGGCCGATTACCTATAAAATTTGCGTGTGGGATCGCACCTTT
169	E6	ATTGCGTGCGCGCCGCGCTTTAGCCTGTGTGATACCAGCAAAAGATTGCTGCGAAGGCTATGTGTGCAACCGC AAACATAAATGGTGCAAATATAAACTGTGG
170	F4	GAATGCAAAGGCTTTGGCAAAAGCTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAATGGGTG TTTTTTACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT
171	F5	AGCCCGGTGTGCACCCCGAGCGGCCAGCCGTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAA TGGGTGTTTTTTACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT

172	F6	GATGATTGCGGCGGCTGTTTAGCGGCTGTACCCCGGGCAAAAACGAATGCTGCCCGAACCGCGTGTGCAAA ATTGGCCTGTATCTGTGCATTGGAGCCCG
173	G1	GGCTGCCTGGGCGATAAATGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAATGGGTGTTTTTT ACCAGCAAATTTATGTGCCGCCGCGTGTGGGGCAAAGAT
174	G3	TGCCGCTATCTGTTTGGCGGCTGTGCGTGGTTTAGCGGCGGAAAGCTGCTGCACCGGCATTTCAGCCCGCGC TGGGGCTGGTGCATTATAGCACCCGCGGCGGCCGC
175	G4	GGCGATTGCCTGCCGCATCTGAAACTGTGTAACCCGAACGATGATAAATGCTGCCGCCGAAACTGAAATGC AGCCGCCGCGGCACCAACCCGGAACGCTGCCGC
176	G6	GATGATTGCGGCACCTGTTTAGCGGCTGTCCGTATAGCAAATATTGCTGCAGCGGCAGCTGCAACGCCGC GGCACCACATTGAAAAACGCTGCCGC
177	H4	GCTGCCTGTGCGATAGATGTGTNCATAGCGGTGATTGTTGCGAAGACTTTCATTGCACCTGCGAGTTTTTTA ACATGTAATTTATG

[95] In some embodiments, a polypeptide component is composed of one or more polypeptide elements, each of which has an amino acid sequence that is substantially identical to a reference sequence element A, B, or C as set forth in Table 3A. In some embodiments, one or more A, B, C reference sequence elements is or comprises a wild type toxin sequence element. In some embodiments, a polypeptide component has an amino acid sequence that comprises or consists of a single sequence element corresponding to an A reference sequence element, a single sequence element corresponding to a B reference sequence element, and a single sequence element corresponding to a C reference sequence element. In some such embodiments, the single sequence elements are arranged in a linear order as follows: A-B-C. Examples of Hv1 modulating agents having A-B-C sequence elements are depicted in FIG. 2 and Table 2. Examples of nucleotide sequences encoding polypeptide sequence elements A, B, and C are set forth in Table 3B.

Table 3A

SEQ ID NO:	Element A	SEQ ID NO:	Element B	SEQ ID NO:	Element C
178	AACLGMFESC	273	ADSDDCCETFHC	377	ALGICMPR
179	ACGQFWKWC	274	AWFSGESCCTGIC	378	ATTGRFRYLCKYQI
180	ACKGVFDAC	275	DEERKCCGLVC	379	DRRDQWCKWNPW
181	ACREWLGGC	276	DENDPRCCSGLVC	380	ELWCKYNL
182	ADCGWLFHSC	277	DGKSTFCCSGFNC	381	ERSSPWCKIDIW
183	CAAEGIPC	278	DGKSTFCCSGYNC	382	HLWCKYK
184	CGGWMAKC	279	DPDNDKCCGYKC	383	HSLFSYCAWDLTFSD
185	CIGEGVPC	280	DPKNDKCKNYTC	384	HSNYEWCWIDGTFSK
186	CMGYDIHC	281	DPKNDKCCPNRVC	385	HSNYEWCWIDGT
187	DCAGYMREC	282	DPNNDKCCPNREC	386	HSRWDCWIDGT
188	DCGTIWHYC	283	DPNNDKCCPNRVC	387	HSYEWCLWDGSF
189	DCLGLFWKC	284	DPNPVKDLPCCSGLAC	388	ICSGXNWK
190	DCLGLFWIC	285	DSARKCCGLVC	389	ISTKVNYYCRPDRGP
191	DCLGWFKGC	286	DSEKCCEDMVC	390	KAFVLHCYRN

192	DCLGWFKSC	287	DSERKCCCEGMVC	391	KDVLYYCAWDGTF
193	DCRALYGGC	288	DSERKCCCEGYVC	392	KEKWPITYKICVWDRTF
194	DCRKMFGGC	289	DSKRACCEGLRC	393	KELSIWDSRCL
195	DCTRMFGAC	290	DSKRKCCCEDMVC	394	KFRDKYCAWDFTFS
196	DCVRFWGGC	291	DSNADCCCEGYVC	395	KGPSPKQKKCTRP
197	DDCGGLFSGC	292	DSTLDCCCKHLSC	396	KGRFVNTWPTFCLV
198	DDCGKLFSGC	293	DTNADCCCEGYVC	397	KIGLYLCIWSP
199	DDCGTLFSGC	294	DTSKDCCEGYVC	398	KLWCRKIIG
200	DDCLGMFSSC	295	DYNGCCSGYVC	399	KLWCRYERTW
201	DDDCGWIMDDC	296	EKDEHCCEHLGC	400	KMGLDYCAWDGTF
202	DGECGGFWWKC	297	EKDSDCCEHLGC	401	KMGLYYCAWDGTF
203	ECGKFMWKC	298	ESNADCCENWAC	402	KPTLKYCAWDGT
204	ECGTLFSGC	299	FRDKECCCKGLTC	403	KPTLKYCAWDGTF
205	ECKGFGKSC	300	GAGKPTCCSGYDC	404	KPTSKYCAWDGTI
206	ECKKLFGGC	301	GEGKPPCCANFAC	405	KQKWPFYCAWDWSF
207	ECKWYLGDC	302	GRGKPPCCGYAC	406	KRRGTNAEKRCR
208	ECKYLWGTC	303	GSGKPACCPKYVC	407	KRRGTNIEKRCR
209	ECLEIFKAC	304	GTDQSECCGWKC	408	KRTFNYCAWDGSFSK
210	ECLGFGKGC	305	HPGQPPCCSGLAC	409	KSKWPRNICVWDGVS
211	ECRKMFGGC	306	KADNDCCGKKC	410	KTTGIVKLCRW
212	ECRWLFGGC	307	KAHEDCCEHLRC	411	KWVFFTSKFMCRRWGKD
213	ECRWYLGGC	308	KEDSECCEHLQC	412	LKPTLHGIWYKHHYCYTQ
214	ECRYFWGEC	309	KEKLCCSGYVC	413	LKPTLHGIWYKSYCYCKK
215	ECRYLFGGC	310	KENKDCCSKKC	414	NGNTVYRCA
216	ECRYWLGGC	311	KKDKECCGWNIC	415	NKKHGWCGWDGTF
217	ECRYWLGTC	312	KNKKECCGWNAC	416	NKKYWHCGWDGTF
218	ECTKFLGGC	313	KNSNDCCCKDLVC	417	NRKHKWKYKYLW
219	ECTKLLGGC	314	KSDENCCCKFKC	418	NRRDKWKYKYLW
220	EDCIPKWKGC	315	KSTDCCCEHLSC	419	NSRDKWCKVLL
221	EGECGGFWWKC	316	KTTADCCCKHLAC	420	QLWCKKRL
222	GACRWFLGGC	37	KTTADCCCKHLGC	421	QPAIKWCIWSP
223	GCANAYKSC	318	KTTSDCCCKHLGC	422	QSRIANMWPTFCLV
224	GCGGLMAGC	319	NALSGPRCCSGLKC	423	QSRIANMWPTFCSQ
225	GCGGLMDGC	320	NDEMVCCEHLVC	424	RKKWPYHCGWDGTF
226	GCGTMWSPC	321	NGPHTCCWGYNGYKKAC	425	RKKWPYHCVWDWTV
227	GCIPSFGE	322	NPDNDKCCCEGRKC	426	RLWCKKII
228	GCKGFGDSC	323	NPNDKCCRPKLKC	427	RLWCKKKIEEG
229	GCKLTFWKC	324	NPSNDKCCRPNLVC	428	RLWCKKKIEW
230	GCLFVWKC	325	NPSNDQCCKSANLVC	429	RLWCKKKLW
231	GCLGDKC	326	NPSNDQCCKSSKLVC	430	RLWCKKRL
232	GCNRKNKKC	327	NPSNDQCCKSSNLVC	431	RLWCKLDW
233	GCQKFFWTC	328	NSDADCCRYGERC	432	RLWCKRIINM
234	GDCLPHLKLC	329	NSDKECCCKGLRC	433	RLWCKYKL
235	GDCLPHLKRC	330	NYMDDKCCPGYKC	434	RRAKPSWCGWDFTF
236	GGCLPHNRFC	331	PKKAPCCGRLEC	435	RRAKPSWCGWDFTV
237	GVDKAGCRYMFGGC	332	PYHESCCSGSC	436	RRTLPTYCAWDLTFP
238	GVDKEGCRKLLGGC	333	PYNEHCCSGSC	437	RSDGKYCAWDGTF

239	IACAPRFSIC	334	PYNENCCSKSC	438	RSDWKYCAWDGTFS
240	IACAPRFSLC	335	PYNENCCSQSC	439	RSRDQWCKYKLW
241	IACAPRGLLC	336	PYNESCCSGSC	440	RVRDQWCKYKLW
242	KCLPPGKPC	337	PYSKYCCSGSC	441	SDKHKWCKWKL
243	LCSREGEFC	338	QPNTQPCCNAAEEQTINC	442	SHNKCT
244	QCGEFMWKC	339	RENKDCCSKKC	443	SKLFLCNCFSF
245	RCIEEGKWC	340	RRDSDCCPHLGC	444	SKTGfVKNICKYEM
246	SAVCIPSGQPC	341	SAGQTCCKHLVC	445	SKTWGCAVEAP
247	SCKLTFWRC	342	SEDECCPHLGC	446	SPKHGWCVDWTFRK
248	SECRWFMGGC	343	SKDADCCAHLEC	447	SPKWGLCNFPMP
249	SEKDCIKHLQRC	344	SKHEDCCAHLAC	448	SPRWGWCYISTRGGR
250	SFCIPFKPC	345	SKTGDCSHLSC	449	SPTWKWCVLKSPGRR
251	SPTCIPSGQPC	346	SDQGDCCKHLQC	450	SPTWKWCVYARP
252	SPTCIPTGQPC	347	SQTSDDCCPHLAC	451	SQHRLCSVKA
253	SPTCIRSGQPC	348	SSDKPCCSGYYC	452	SRKDKWCKYQI
254	SPVCTPSGQPC	349	SSTDCCCKHLSC	453	SRKRWCKYEI
255	SSTCIPSGQPC	350	STEKPCDNFSC	454	SRKTRWCKYQI
256	SSTCIRTDQPC	351	STHADCCGFC	455	SRQLCKYVIDW
257	STCTPTDQPC	352	SVDSDDCAHLGC	456	SRRDRWCKYDL
258	SVCIPSGQPC	353	SVHSDCCAHLGC	457	SRRDRWCKYYL
259	TCRYLFGGC	354	SVNDCCCPRLGC	458	SRRGTNPEKRCR
260	TCYDIGELC	355	TDRLPCCFGLGC	459	SRRHGWCVDWGTFS
261	VCRGYGLPC	356	TIDDDCCPHLGC	460	SRTWKWCVLAGPW
262	YCQKWLWTC	357	TKDEDCCKHLAC	461	SSKHKWCCKYYL
263	YCQKWMWTC	358	TKDSECCPHLGC	462	SSRWKWCVLASPF
264	CKQADEPC	359	TPEKNDCCQRLYC	463	SSRWKWCVLAPW
265	ACRKKWEYC	360	TPGKNECCPNRVC	464	TFKENENGNTVKRCD
266	DDDCPEPPGNFC	361	TPGKNECCPNYAC	465	TFKTENENGNTVKRCD
267	VKPCRKEGQLC	362	TSDSDCCPNWVC	466	TGLCIPP
268	WCKQSGEMC	363	TTSSECCAHLGC	467	TRFNVCCK
269	CLSGGEVC	364	VNRHGDCCEGLEC	468	TWPTICID
270	GKPCHEEGCQL	365	VPKNECCSGYAC	469	TYKANENGNTVKRCD
271	CIPFLHPC	366	YGATQKIPCCGVC	470	TYKENENGNTVKRCD
272	ACSKKWEYC	367	YKLRKCCAGFYC	471	TYKENENGNTVQRCD
		368	DVFSLDCCGTIC	472	TYKTENENGNTSVQRCD
		369	IVPIIGFIYCCPGLIC	473	VKTSGYWYKKTTCRRKS
		370	GMIKIGPPCCSGWC	474	WKRRTSFVCCVPKTPKT
		371	DPIFQNCRCGWNC	475	LGVCMM
		372	NVLDQNCDDGYC	476	FFACA
		373	DFLFPKCCNYC	477	VLFCV
		374	DPFLQNCCLGWNC	478	IVFVCT
		375	TFFFPDCCNSIC	479	ILLFCS
		376	IVPILGFVYCCPGLIC	480	VFVCI
				481	AQFICL
				482	GPFVCV

Table 3B

SEQ ID NO.	Element A	SEQ ID NO.	Element B	SEQ ID NO.	Element C
483	GCGGCGTGCCTGG GCATGTTTGAAAGC TGT	578	GCGGATAGCGATGATTGCTGC GAAACCTTTCATTGC	682	GCGCTGGGCATTTGCAT GCCGCGC
484	GCGTGCGGCCAGT TTTGGTGGAATGT	579	GCGTGGTTTAGCGGCGAAAGC TGCTGCACCGGCATTGTC	683	GCGACCACCGGCCGCTT TCGCTATCTGTGCAAATA TCAGATT
485	GCGTGCAAAGGCG TGTTTGATGCGTGT	580	GATGAAGAACGCAAATGCTGC GAAGGCCTGGTGTGC	684	GATCGCCGCGATCAGTG GTGCAAATGGAACCCGT GG
486	GCGTGCCGCGAAT GGCTGGGCGGCTG T	581	GATGAAAACGATCCGCGCTGC TGCAGCGGCCTGGTGTGC	685	GAAGTGTGGTGCAAATA TAACCTG
487	GCGGATTGCGGCT GGCTGTTTCATAGC TGT	582	GATGGCAAAAGCACCTTTTGCT GCAGCGGCTTTAACTGC	686	GAACGCAGCAGCCCGTG GTGCAAAATTGATATTTG G
488	TGCGCGGCGGAAG GCATTCGCTGT	583	GATGGCAAAAGCACCTTTTGCT GCAGCGGCTATAACTGC	687	CATCTGTGGTGCAAATAT AAA
489	TGCGGCGGCTGGA TGCGGAAATGT	584	GATCCGGATAACGATAAATGCT GCGAAGGCTATAAATGC	688	CATAGCCTGTTTAGCTAT TGCGCGTGGGATCTGAC CTTTAGCGAT
490	TGCATTGGCGAAGG CGTGCCGTGT	585	GATCCGAAAAACGATAAATGCT GCAAAAACATACCTGC	689	CATAGCAACTATGAATG GTGCATTTGGGATGGCA CCTTTAGCAAA
491	TGCATGGGCTATGA TATTCATTGT	586	GATCCGAAAAACGATAAATGCT GCCCGAACC GCGTGTGC	690	CATAGCAACTATGAATG GTGCGTGTGGGATGGCA CC
492	GATTGCGCGGGCT ATATGCGCGAATGT	587	GATCCGAACAACGATAAATGCT GCCCGAACC GCGAATGC	691	CATAGCCGCTGGGATTG GTGCATTTGGGATGGCA CCTTT
493	GATTGCGGCACCAT TTGGCATTATTGT	588	GATCCGAACAACGATAAATGCT GCCCGAACC GCGTGTGC	692	CATAGCTATTGGGAATG GTGCCTGTGGGATGGCA GCTTT
494	GATTGCCTGGGCTT TCTGTGGAATGT	589	GATCCGAACCCGGTGAAAGAT CTGCCGTGCTGCAGCGGCCTG GCGTGC	693	ATTTGCAGCGGCAACTG GAAA

495	GATTGCCTGGGCCT GTTTTGGATTGT	590	GATAGCGCGCGCAAATGCTGC GAAGGCCTGGTGTGC	694	ATTAGCACCAAAGTGAA CTATTATCGCCCGATC GCGGCCCG
496	GATTGCCTGGGCTG GTTTAAAGGCTGT	591	GATAGCGAACGCAAATGCTGC GAAGATATGGTGTGC	695	AAAGCGTTTGTGCTGCA TTGCTATCGCAAC
497	GATTGCCTGGGCTG GTTTAAAGCTGT	592	GATAGCGAACGCAAATGCTGC GAAGGCATGGTGTGC	696	AAAGATGTGCTGTATTAT TGC GCGTGGGATGGCAC CTTT
498	GATTGCCGCGCGC TGTATGGCGGCTGT	593	GATAGCGAACGCAAATGCTGC GAAGGCTATGTGTGC	697	AAAGAAAAATGGCCGAT TACCTATAAAATTTGCGT GTGGGATCGCACCTTT
499	GATTGCCGCAAAAT GTTTGGCGGCTGT	594	GATAGCAAACGCGCGTGTGC GAAGGCCTGCGCTGC	698	AAAGAACTGAGCATTTG GGATAGCCGCTGCCTG
500	GATTGCACCCGCAT GTTTGGCGCGTGT	595	GATAGCAAACGCAAATGCTGC GAAGATATGGTGTGC	699	AAATTTGCGGATAAATAT TGCGCGTGGGATTTTAC CTTAGC
501	GATTGCGTGCCTT TTGGGGCAAATGT	596	GATAGCAACGCGGATTGTGC GAAGGCTATGTGTGC	700	AAAGGCCCCGAGCCCGAA ACAGAAAAATGCACCC GCCCC
502	GATGATTGCGGCG GCCTGTTTAGCGGC TGT	597	GATAGCACCTTGGATTGTGC AAACATCTGAGCTGC	701	AAAGGCGCTTTGTGAA CACCTGGCCGACCTTTT GCCTGGTG
503	GATGATTGCGGCAA ACTGTTTAGCGGCT GT	598	GATACCAACGCGGATTGTGC GAAGGCTATGTGTGC	702	AAAATTGGCCTGTATCTG TGCATTTGGAGCCCG
504	GATGATTGCGGCAC CCTGTTTAGCGGCT GT	599	GATACCAGCAAAGATTGTGC GAAGGCTATGTGTGC	703	AAACTGTGGTGCCGCAA AATTATTGGC
505	GATGATTGCCTGGG CATGTTTAGCAGCT GT	600	GATTATAACAACGCTGCTGCA GCGGCTATGTGTGC	704	AAACTGTGGTGCCGCTA TGAACGCACCTGG
506	GATGATGATTGCGG CTGGATTATGGATG ATTGT	601	GAAAAAGATGAACATTGCTGCG AACATCTGGGCTGC	705	AAAATGGCCTGGATTA TTGCGCGTGGGATGGCA CCTTT
507	GATGGCGAATGCG GCGGCTTTTGGTGG AAATGT	602	GAAAAAGATAGCGATTGTGC GAACATCTGGGCTGC	706	AAAATGGCCTGTATTAT TGCGCGTGGGATGGCAC CTTT
508	GAATGCGGCAAAAT TATGTGAAATGT	603	GAAAGCAACGCGGATTGTGC GAAACTGGGCGTGC	707	AAACCGACCCTGAAATAT TGCGCGTGGGATGGCAC

					C
509	GAATGCGGCACCCT GTTTAGCGGCTGT	604	TTTCGCGATAAAGAATGCTGCA AAGGCCTGACCTGC	708	AAACCGACCCTGAAATAT TGC GCGTGGGATGGCAC CTTT
510	GAATGCAAAGGCTT TGGCAAAAGCTGT	605	GGCGCGGGCAAACCGACCTGC TGCAGCGGCTATGATTGC	709	AAACCGACCAGCAAATA TTGCGCGTGGGATGGCA CCATT
511	GAATGCAAAAACT GTTTGGCGGCTGT	606	GGCGAAGGCAAACCGCCGTGC TGC GCGA A C T T T G C G T G C	710	AAACAGAAATGGCCGTT TTATTGCGCGTGGGATT GGAGCTTT
512	GAATGCAAATGGTA TCTGGGCGATTGT	607	GGCCGCGGCAAACCGCCGTGC TGCA AAGGCTATGCGTGC	711	AAACGCCGCGCACCAA CGCGAAAAACGCTGCC GC
513	GAATGCAAATATCT GTGGGGCACCTGT	608	GGCAGCGGCAAACCGGCGTGC TGCCCGAAATATGTGTGC	712	AAACGCCGCGCACCAA CATTGAAAAACGCTGCC GC
514	GAATGCCTGGAAAT TTTTAAAGCGTGT	609	GGCACCGATCAGAGCGAATGC TGC G A A G C T G G A A T G C	713	AAACGCACCTTTAACTAT TGC G C G T G G G A T G G C A G CTTTAGCAAA
515	GAATGCCTGGGCTT TGGCAAAGGCTGT	610	CATCCGGGCCAGCCGCCGTGC TGCAGCGGCCTGGCGTGC	714	AAAAGCAAATGGCCGCG CAACATTTGCGTGTGGG ATGGCAGCGTG
516	GAATGCCGCAAAAT GTTTGGCGGCTGT	611	AAAGCGGATAACGATTGCTGC GGCAAAAAATGC	715	AAAACACCGGCATTGT GAAACTGTGCCGCTGG
517	GAATGCCGCTGGCT GTTTGGCGGTGT	612	AAAGCGCATGAAGATTGCTGC GAACATCTGCGCTGC	716	AAATGGGTGTTTTTTACC AGCAAATTTATGTGCCG CCGCGTGTGGGGCAAAG AT
518	GAATGCCGCTGGTA TCTGGGCGGCTGT	613	AAAGAAGATAGCGAATGCTGC GAACATCTGCAGTGC	717	CTGAAACCGACCCTGCA TGGCATT T G G T A T A A A C A TCATTATTGCTATACCCA G
519	GAATGCCGCTATTT TTGGGGCGAATGT	614	AAAGAAAACTGTGCTGCAGC GGCTATGTGTGC	718	CTGAAACCGACCCTGCA TGGCATT T G G T A T A A A A G CTATTATTGCTATAAAAA A
520	GAATGCCGCTATCT GTTTGGCGGCTGT	615	AAAGAAAACAAAGATTGCTGCA GCAAAAAATGC	719	AACGGCAACACCGTGTA TCGCTGCGCG
521	GAATGCCGCTATTG	616	AAAAAAGATAAAGAATGCTGCG	720	AACAAAAACATGGCTG GTGCGGCTGGGATGGCA

	GCTGGGCGGCTGT		GCTGGAACATTGTC		CCTTT
522	GAATGCCGCTATTG GCTGGGCACCTGT	617	AAAAACAAAAAGAATGCTGCG GCTGGAACGCGTGC	721	AACAAAAATATTGGCAT TGC GGCTGGGATGGCAC CTTT
523	GAATGCACCAAATT TCTGGGCGGCTGT	618	AAAAACAGCAACGATTGCTGCA AAGATCTGGTGTGC	722	AACCGCAACATAAATG GTGCAAAATATAAACTGTG G
524	GAATGCACCAAAT GCTGGGCGGCTGT	619	AAAAGCGATGAAAATGCTGCA AAAAATTAAATGC	723	AACCGCCGCGATAAATG GTGCAAAATATAAACTGTG G
525	GAAGATTGCATTCC GAAATGGAAAGGCT GT	620	AAAAGCACCAGCGATTGCTGC GAACATCTGAGCTGC	724	AACAGCCGCGATAAATG GTGCAAAGTCTGCTG
526	GAAGGCGAATGCG GCGGCTTTTGGTGG AAATGT	621	AAAACCACCGCGGATTGCTGC AAACATCTGGCGTGC	725	CAGCTGTGGTGCAAAAA ACGCCTG
527	GGCGCGTGCCGCT GGTTTCTGGGCGG CTGT	622	AAAACCACCGCGGATTGCTGC AAACATCTGGGCTGC	726	CAGCCGGCGATTAAATG GTGCATTGGAGCCCG
528	GGCTGCGCGAACG CGTATAAAAGCTGT	623	AAAACCACCGCGATTGCTGCA AACATCTGGGCTGC	727	CAGAGCCGCATTGCGAA CATGTGGCCGACCTTTT GCCTGGTG
529	GGCTGCGGCGGCC TGATGGCGGGCTG T	624	AACGCGCTGAGCGGCCCGCGC TGCTGCAGCGGCTGAAATGC	728	CAGAGCCGCATTGCGAA CATGTGGCCGACCTTTT GCAGCCAG
530	GGCTGCGGCGGCC TGATGGATGGCTGT	625	AACGATGAAATGGTGTGCTGC GAACATCTGGTGTGC	729	CGCAAAAAATGGCCGTA TCATTGCGGCTGGGATG GCACCTTT
531	GGCTGCGGCACCA TGTGGAGCCCGTGT	626	AACGGCCCGCATACCTGCTGC TGGGCTATAACGGCTATAAAA AAGCGTGC	730	CGCAAAAAATGGCCGTA TCATTGCGTGTGGGATT GGACCGTG
532	GGCTGCATTCCGAG CTTTGGCGAATGT	627	AACCCGATAACGATAAATGCT GCGAAGGCCGCAATGC	731	CGCCTGTGGTGCAAAAA AATTATT
533	GGCTGCAAAGGCTT TGGCGATAGCTGT	628	AACCCGAACGATGATAAATGCT GCCGCCCGAACTGAAATGC	732	CGCCTGTGGTGCAAAAA AAAAATTGAAGAAGGC
534	GGCTGCAAACGAC CTTTTGAAATGT	629	AACCCGAGCAACGATAAATGCT GCCGCCCGAACCTGGTGTGC	733	CGCCTGTGGTGCAAAAA AAAAATTGAATGG
535	GGCTGCCTGGAATT	630	AACCCGAGCAACGATCAGTGC TGCAAAAGCGCAACCTGGTG	734	CGCCTGTGGTGCAAAAA

	TTGGTGGAATGT		TGC		AAAACTGTGG
536	GGCTGCCTGGGCG ATAAATGT	631	AACCCGAGCAACGATCAGTGC TGCAAAAGCAGCAAACTGGTGT GC	735	CGCCTGTGGTGCAAAAA ACGCCTG
537	GGCTGCAACCGCA AAAACAAAAATGT	632	AACCCGAGCAACGATCAGTGC TGCAAAAGCAGCAACCTGGTG TGC	736	CGCCTGTGGTGCAAACT GGATTGG
538	GGCTGCCAGAAATT TTTTTGACCTGT	633	AACAGCGATGCGGATTGCTGC CGCTATGGCGAACGCTGC	737	CGCCTGTGGTGCAAACG CATTATTAACATG
539	GGCGATTGCTGCTGC CGCATCTGAAACTG TGT	634	AACAGCGATAAAGAATGCTGCA AAGGCCTGCGCTGC	738	CGCCTGTGGTGCAAATA TAAACTG
540	GGCGATTGCTGCTGC CGCATCTGAAACGC TGT	635	AACTATATGGATGATAAATGCT GCCCGGGCTATAAATGC	739	CGCCGCGCGAAACCGA GCTGGTGCGGCTGGGAT TTACCTTT
541	GGCGGCTGCCTGCTGC CGCATAACCGCTTT TGT	636	CCGAAAAAGCGCCGTGCTGC GGCCGCTGGAATGC	740	CGCCGCGCGAAACCGA GCTGGTGCGGCTGGGAT TTACCGTG
542	GGCGTGGATAAAG CGGGCTGCCGCTA TATGTTTGGCGGCT GT	637	CCGTATCATGAAAGCTGCTGCA GCGGCAGCTGC	741	CGCCGCACCCTGCCGAC CTATTGCGCGTGGGATC TGACCTTTCCG
543	GGCGTGGATAAAGA AGGCTGCCGCAAA CTGCTGGGCGGCT GT	638	CCGTATAACGAACATTGCTGCA GCGGCAGCTGC	742	CGCAGCGATGGCAAATA TTGCGCGTGGGATGGCA CCTTT
544	ATTGCGTGCGCGC CGCGCTTTAGCATT TGT	639	CCGTATAACGAAAAGCTGCTGCA GCAAAAGCTGC	743	CGCAGCGATTGGAAATA TTGCGCGTGGGATGGCA CCTTTAGC
545	ATTGCGTGCGCGC CGCGCTTTAGCCTG TGT	640	CCGTATAACGAAAAGCTGCTGCA GCCAGAGCTGC	744	CGCAGCCGCGATCAGTG GTGCAAATATAAACTGTG G
546	ATTGCGTGCGCGC CGCGCGGCCTGCT GTGT	641	CCGTATAACGAAAAGCTGCTGCA GCGGCAGCTGC	745	CGCGTGCGCGATCAGTG GTGCAAATATAAACTGTG G
547	AAATGCCTGCCGCC GGGCAAAACCGTGT	642	CCGTATAGCAAATATTGCTGCA GCGGCAGCTGC	746	AGCGATAAACATAAATG GTGCAAATGGAACCTG
548	CTGTGCAGCCGCG AAGGCGAATTT	643	CAGCCGAACCCAGCCGTGC TGCAACAACGCGGAAGAAGAA	747	AGCCATAACAAATGCAC C

			CAGACCATTAAGTGC		
549	CAGTGC GGCGAATT TATGTGGAAATGT	644	CGCGAAAACAAAGATTGCTGCA GCAAAAAATGC	748	AGCAAACTGTTTAAACTG TGCAACTTTAGCTTT
550	CGCTGCATTGAAGA AGGCAAATGGTGT	645	CGCCGCGATAGCGATTGCTGC CCGCATCTGGGCTGC	749	AGCAAAACCGGCTTTGT GAAAAACATTTGCAAATA TGAAATG
551	AGCGCGGTGTGCA TTCCGAGCGGCCA GCCGTGT	646	AGCGCGGGCCAGACCTGCTGC AAACATCTGGTGTGC	750	AGCAAAACCTGGGGCTG GTGCGCGGTGGAAGCG CCG
552	AGCTGCAAACTGAC CTTTTGCGCTGT	647	AGCGAAGATAGCGAATGCTGC CCGCATCTGGGCTGC	751	AGCCCGAAACATGGCTG GTGCGTGTGGATTGGA CCTTTCGCAA
553	AGCGAATGCCGCT GGTTTATGGGCGG CTGT	648	AGCAAAGATGCGGATTGCTGC GCGCATCTGGAATGC	752	AGCCCGAAATGGGGCCT GTGCAACTTCCGATGC CG
554	AGCGAAAAAGATTG CATTAAACATCTGC AGCGCTGT	649	AGCAAACATGAAGATTGCTGCG CGCATCTGGCGTGC	753	AGCCCGCGCTGGGGCT GGTGCAATTATAGCACC CGCGCGGGCCGC
555	AGCTTTTGCAATCC GTTTAAACCGTGT	650	AGCAAAACCGCGATTGCTGC AGCCATCTGAGCTGC	754	AGCCCGACCTGGAAATG GTGCGTGTGAAAAGCC CGGGCCGCCGC
556	AGCCCGACCTGCAT TCCGAGCGGCCAG CCGTGT	651	AGCCAGGATGGCGATTGCTGC AAACATCTGCAGTGC	755	AGCCCGACCTGGAAATG GTGCGTGTATGCGCGCC CG
557	AGCCCGACCTGCAT TCCGACCGGCCAG CCGTGT	652	AGCCAGACCAGCGATTGCTGC CCGCATCTGGCGTGC	756	AGCCAGCATCGCCTGTG CAGCGTGAAAGCG
558	AGCCCGACCTGCAT TCGAGCGGCCAG CCGTGT	653	AGCAGCGATAAACCGTGTGCTGC AGCGCTATTATTGC	757	AGCCGCAAAGATAAATG GTGCAAATATCAGATT
559	AGCCCGGTGTGCA CCCCGAGCGGCCA GCCGTGT	654	AGCAGCACCAGCGATTGCTGC AAACATCTGAGCTGC	758	AGCCGCAAACATCGCTG GTGCAAATATGAAATT
560	AGCAGCACCTGCAT TCCGAGCGGCCAG CCGTGT	655	AGCACCGAAAAACCGTGTGCTGC GATAACTTTAGCTGC	759	AGCCGCAAACCCGCTG GTGCAAATATCAGATT
561	CGGCTGGCCGCTC GGAATGCAGGTGCT GCTTGT	656	AGCACCCATGCGGATTGCTGC GAAGGCTTTATTGCTGC	760	AGCCGCCAGCTGTGCAA ATATGTGATTGATTGG

562	AGCACCTGCACCCC GACCGATCAGCCGT GT	657	AGCGTGGATAGCGATTGCTGC GCGCATCTGGGCTGC	761	AGCCGCCGCGATCGCTG GTGCAAATATGATCTG
563	AGCGTGTGCATTCC GAGCGGCCAGCCG TGT	658	AGCGTGCATAGCGATTGCTGC GCGCATCTGGGCTGC	762	AGCCGCCGCGATCGCTG GTGCAAATATTATCTG
564	ACCTGCCGCTATCT GTTTGGCGGCTGT	659	AGCGTGAACGATGATTGCTGC CCGCGCCTGGGCTGC	763	AGCCGCCGCGGCACCAA CCCGGAAAAACGCTGCC GC
565	ACCTGCTATGATAT TGCGAACTGTGT	660	ACCGATCGCCTGCCGTGCTGC TTTGGCCTGGAATGC	764	AGCCGCCGCCATGGCTG GTGCGTGTGGGATGGCA CCTTTAGC
566	GTGTGCCGCGGCT ATGGCCTGCCGTGT	661	ACCATTGATGATGATTGCTGCC CGCATCTGGGCTGC	765	AGCCGCACCTGGAAATG GTGCGTGTGCGGGC CCGTGG
567	TATTGCCAGAAATG GCTGTGGACCTGT	662	ACCAAAGATGAAGATTGCTGCA AACATCTGGCGTGC	766	AGCAGCAAACATAAATG GTGCAAAGTGATCTG
568	TATTGCCAGAAATG GATGTGGACCTGT	663	ACCAAAGATAGCGAATGCTGC CCGCATCTGGGCTGC	767	AGCAGCCGCTGGAAATG GTGCGTGTGCGGAGCC CGTTT
569	TGCAAACAGGCGG ATGAACCGTGT	664	ACCCCGGAAAAAACGATTGCT GCCAGCGCCTGTATTGC	768	AGCAGCCGCTGGAAATG GTGCGTGTGCGGCGC CCGTGG
570	GCGTGCCGCAAAAA ATGGGAATATTGT	665	ACCCCGGGCAAAAACGAATGC TGCCCGAACCGGTGTGC	769	ACCTTTAAAGAAACGAA AACGGCAACACCGTGAA ACGCTGCGAT
571	GATGATGATTGCGA ACCGCCGGGCAAC TTTTGT	666	ACCCCGGGCAAAAACGAATGC TGCCCGAATATGCGTGC	770	ACCTTTAAACCAACGAA AACGGCAACACCGTGAA ACGCTGCGAT
572	GTGAAACCGTGCC GCAAAGAAGGCCA GCTGTGT	667	ACCAGCGATAGCGATTGCTGC CCGAACCTGGGTGTGC	771	ACCGGCCTGTGCATTCC GCCG
573	TGGTGCAAACAGAG CGGCGAAATGTGT	668	ACCACCAGCAGCGAATGCTGC GCGCATCTGGGCTGC	772	ACCCGCTTTAACGTGTG CGGCAAA
574	TGCCTGAGCGGCG GCGAAGTGTGT	669	GTGAACCGCCATGGCGATTGC TGCGAAGGCCTGGAATGC	773	ACCTGGCCGACCGAAAT TTGCATTGAT
575	GGCAAACCGTGCC ATGAAGAAGGCCAG CTGTGT	670	GTGCCGGGCAAAAACGAATGC TGCAGCGGCTATGCGTGC	774	ACCTATAAAGCGAACGA AAACGGCAACCGGTGA AACGCTGCGAT

576	TGCATTCCGTTTCT GCATCCGTGT	671	TATGGCGCGACCCAGAAAATTC CGTGCTGCGGCGTGTGC	775	ACCTATAAGAAAAACGAA AACGGCAACACCGTGAA ACGCTGCGAT
577	GCGTGCAGCAAAAA ATGGGAATATTGT	672	TATAAACTGCGCAAATGCTGCG CGGGCTTTTATTGC	776	ACCTATAAGAAAAACGAA AACGGCAACACCGTGCA GCGCTGCGAT
		673	GATGTGTTTAGCCTGGATTGCT GCACCGGCATTTC	777	ACCTATAAAACCAACGAA AACGGCAACAGCGTGCA GCGCTGCGAT
		674	ATTGTGCCGATTATTGGCTTTA TTTATTGCTGCCCGGGCCTGAT TTGC	778	GTGAAAACAGCGGCTA TTGGTGGTATAAAAAAC CTATTGCCGCCGCAAAA GC
		675	GGCATGATTAATAATTGGCCCGC CGTGCTGCAGCGGCTGGTGC	779	TGGAACGCCGCCGCGAG CTTTGAAGTGTGCGTGC CGAAAACCCGAAAACC
		676	GATCCGATTTTTCAGAACTGCT GCCGCGGCTGGAAGTGC	780	CTGGGCGTGTGCATGTG G
		677	AACGTGCTGGATCAGAACTGCT GCGATGGCTATTGC	781	TTTTTTCGTGCGCG
		678	GATTTTCTGTTCCGAAATGCT GCAACTATTGC	782	GTGCTGTTTTCGTG
		679	GATCCGTTTCTGCAGAACTGCT GCCTGGGCTGGAAGTGC	783	ATTGTGTTTGTGTGCACC
		680	ACCTTTTTTTTTCCGGATTGCT GCAACAGCATTTC	784	ATTCTGCTGTTTTCAGC
		681	ATTGTGCCGATTCTGGGCTTTG TGTATTGCTGCCCGGGCCTGA TTTGC	785	GTGTTTGTGTGCATT
				786	GCGCAGTTTATTTCCT G
				787	GGCCCGTTTGTGTGCGT G

[96] In some embodiments, reference sequence element A has an amino acid sequence GCKWYLGDC (SEQ ID NO: 809). In some embodiments, reference sequence element A has an amino acid sequence SSTCIPSGQPC (SEQ ID NO: 255). In some embodiments, reference sequence element B has an amino acid sequence ADSDDCCETFHC (SEQ ID NO: 273). In some embodiments, reference sequence element C has an amino acid sequence KWVFFTSKFMCRRVWGKD (SEQ ID NO: 411).

[97] In some embodiments, a provided polypeptide component has an amino acid sequence that is or comprises GCKWYLGDCADSDDCCETFHC KWVFFTSKFMCRRVWGKD (SEQ ID NO: 128), as is found in the Hv1 modulating agent labeled as “C5” in Table 2A.

[98] In some embodiments, a provided polypeptide component has an amino acid sequence that is or comprises SSTCIPSGQPCADSDDCCETFHC KWVFFTSKFMCRRVWGKD (SEQ ID NO: 129), as is found in the Hv1 modulating agent labeled as “C6” in Table 2A.

[99] In some embodiments, a polypeptide component has an amino acid sequence that includes one or more cysteine residues at positions corresponding to those at which a cysteine residue is found in a relevant wild-type toxin (e.g., wild-type voltage sensor toxin) sequence or reference sequence element (e.g., as depicted in FIG. 2). In some embodiments, a polypeptide component has an amino acid sequence that includes all cysteine residues at positions corresponding to those at which cysteine residues are found in a relevant wild-type toxin sequence or reference sequence element. In some embodiments, a polypeptide component has an amino acid sequence that shares the same approximate relative position of cysteines (e.g., number of residues between them) with a relevant wild-type toxin sequence or reference sequence element.

[100] In some embodiments, a polypeptide component has an amino acid sequence that includes one or more sequence elements that are identical to or includes not more than 1, 2, 3, 4, or 5 sequence differences relative to a wild-type toxin sequence element or reference sequence element.

[101] In some embodiments, such sequence difference(s) are or comprise one or more insertions, deletions, substitutions, rearrangements (e.g., inversions) or combinations

thereof. In some embodiments, such sequence difference(s) do not include any insertions. In some embodiments, such sequence difference(s) do not include any deletions. In some embodiments, such sequence differences do not include any rearrangements (e.g., inversions). In some embodiments, such sequence difference(s) may include one or more random sequence alterations.

[102] In some embodiments, a polypeptide component has an amino acid sequence that includes one or more sequence elements that shares one or more cysteines with a sequence set forth in Table 1, Table 2, and/or Table 3. In some embodiments, such a sequence element has an amino acid sequence that shares all cysteines with a sequence set forth in Table 1, Table 2, and/or Table 3. In some embodiments, a sequence element shares the same approximate relative position of cysteines (e.g., number of residues between them) with a sequence set forth in Table 1, Table 2, and/or Table 3.

[103] In some embodiments, a reference sequence element has an amino acid sequence of an element found in a wild-type voltage toxin sequence that differs at residues that undergo posttranslational modifications.

[104] In some embodiments, a polypeptide component of an Hv1 modulating agent may include one or more pendant groups or other modifications, e.g., modifying or attached to one or more amino acid residues (e.g., to one or more amino acid side chains), at the polypeptide component's N-terminus, at the polypeptide component's C-terminus, or any combination thereof. In some embodiments, such pendant groups or modifications may be selected from the group consisting of acetylation, amidation, glycosylation, lipidation, methylation, pegylation, phosphorylation, etc., and combinations thereof.

[105] In some embodiments, exemplary Hv1 modulating agents comprise a polypeptide component whose amino acid sequence further comprises one or more tag elements (e.g., a detectable tag, a localizing tag, etc).

[106] In some embodiments, an Hv1 modulating agent may be a dimer or multimer of relevant entities (e.g., of a polypeptide component as described herein); in some embodiments, an Hv1 modulating agent may be or comprise heterodimer or heteromultimer. In some embodiments, an Hv1 modulating agent may be or comprise a homodimer or homomultimer. Exemplary Hv1 modulating agent dimers are presented in Table 4.

Table 4A: Dimer Sequences (with linkers)

SEQ ID NO:	Name	Sequence
788	HaTx-C6	ECRYLFGGCKTTSDCCKHLGCKFRDKYCAWDFTFSGNGNGNGSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD
789	C6-C6 with DkTx linker	SSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDDSSPYVPVTTSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD
790	C6-C6 with flexible linker	SSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDDSSGNGNGSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD
791	C6-C6 with long flexible linker	SSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDDSSGNGNGNGNGNGNGNGAAAGGNGNGNGNGNGNGSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD

Table 4B: Monomer C6 + signal AND dimer + signal

SEQ ID NO:	Name	Sequence
792	C6 with signal peptide (MSALLILALVGAAVA)	MSALLILALVGAAVASSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD
793	HaTx-C6	MSALLILALVGAAVAECRYLFGGCKTTSDCCKHLGCKFRDKYCAWDFTFSGNGNGNGSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD
794	C6-C6 with DkTx linker with signal peptide	MSALLILALVGAAVASSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDDSSPYVPVTTSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD
795	C6-C6 with flexible linker with signal peptide	MSALLILALVGAAVASSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDDSSGNGNGNGSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD
796	C6-C6 with long flexible linker with signal peptide	MSALLILALVGAAVASSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDDSSGNGNGNGNGNGNGNGAAAGGNGNGNGNGNGNGSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKD

Table 4C: Monomer + Myc tag, dimer + Myc tag, either/both plus signal and tag

SEQ ID NO:	Name	Sequence
797	C6 with signal peptide and Myc tag	MSALLILALVGAAVASSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDL
798	HaTx-C6 with signal peptide and Myc tag	MSALLILALVGAAVAECRYLFGGCKTTSDCCKHLGCKFRDKYCAWDFTFSGNGNGNGSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDL
799	C6-C6 with DkTx linker with signal peptide and Myc tag	MSALLILALVGAAVASSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDDSSPYVPVTTSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDL
800	C6-C6 with	MSALLILALVGAAVASSTCIPSGQPCADSDCCETFHCKWVFFTSKFCRRV

	flexible linker with signal peptide and Myc tag	WGKDDSSGNGNGNGSSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDL
801	C6-C6 with long flexible linker with signal peptide and Myc tag	MSALLILALVGAAVASSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDDSSGNGNGNGNGNGNGNGAAAGNGNGNGNGNGNGSSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDL

Table 4D: Monomer + GPI targeting, dimer + GPI targeting, either/both plus signal, tag and linker

SEQ ID NO:	Name	Sequence
802	C6 with signal peptide and Myc tag and GPI anchor	MSALLILALVGAAVASSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDLGALCNGAGFATPVTALVPALLATFWSLL
803	HaTx-C6 with signal peptide and Myc tag and GPI anchor	MSALLILALVGAAVAECRYLFGGCKTTSDCCKHLGCKFRDKYCAWDFTFSGNGNGNGSSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDLGALCNGAGFATPVTALVPALLATFWSLL
804	C6-C6 with DkTx linker with signal peptide and Myc tag and GPI anchor	MSALLILALVGAAVASSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDDSSPYVPVTTSSCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDLGALCNGAGFATPVTALVPALLATFWSLL
805	C6-C6 with flexible linker with signal peptide and Myc tag and GPI anchor	MSALLILALVGAAVASSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDDSSGNGNGNGSSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDLGALCNGAGFATPVTALVPALLATFWSLL
806	C6-C6 with long flexible linker with signal peptide and Myc tag and GPI anchor	MSALLILALVGAAVASSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDDSSGNGNGNGNGNGNGNGAAAGNGNGNGNGNGNGSSTCIPSGQPCADSDDCCETFHCKWVFFTSKFCRRVWGKDGEQKLISEEDLGALCNGAGFATPVTALVPALLATFWSLL

[107] Optionally, monomer components of a dimer or multimer agent may be or become covalently linked to one another. In some embodiments, such components may be or become covalently linked to one another via one or more disulfide bonds; in some embodiments, such components may be or become covalently linked to one another via one or more peptide bonds; in some embodiments, such components may be or become covalently linked to one another via a bond other than a peptide bond. In some

embodiments, such components may be or become covalently linked to one another via a linker (e.g., via a polypeptide linker). Those skilled in the art will appreciate that a linker may be comprised of any of a variety of chemical entities. In those embodiments in which a linker comprises one or a plurality of amino acids, it may be of any desired length. In some embodiments, a linker has a size (e.g., a length) that is smaller than that of one or more of the monomer components.

[108] In some embodiments, a polypeptide component of an Hv1 modulating agent may be connected directly or via a linker sequence to a signal peptide and/or a coat protein of a phage (for phage display methods) and/or to any other domain that may alter one or more of Hv1 modulating agent expression, binding, or function.

[109] In some embodiments, an Hv1 modulating agent may have structural modification(s). For example, an Hv1 modulating agent may be or comprise a cyclic structure, and/or may comprise a cyclic portion. For example, a polypeptide component of an Hv1 modulating agent may be cyclized such that its N-terminus is not part of the cyclic structure. In some embodiments, an Hv1 modulating agent is not cyclic and/or does not comprise any cyclic portion. In some embodiments, an Hv1 modulating agent is linear (e.g., one or more, or all, polypeptide components of an Hv1 modulating agent is/are linear polypeptide(s)). In some embodiments, an Hv1 modulating agent may be or comprise a stapled polypeptide.

[110] In some embodiments, a polypeptide component of an Hv1 modulating agent is incorporated into a framework or scaffold structure. For example, such a polypeptide component can be incorporated into an antibody framework. Alternatively or additionally, a polypeptide component may be incorporated into a beta-sheet framework. In some embodiments, an Hv1 modulating agent may be or comprise an antibody agent or fragment or component thereof (e.g., an antigen-binding fragment or component, such as a polypeptide including sufficient CDR sequences to bind antigen comparably to an antibody). In some embodiments, an Hv1 modulating agent may be or comprise a polypeptide component that includes one or more of an immunoglobulin domain or fragment thereof. In some embodiments, an Hv1 modulating agent may be or comprise a polypeptide component that includes a domain of an immunoglobulin heavy chain. Strategies for preparing such antibody fusions are known in the art (U.S. 14/152441).

[111] Alternatively or additionally, in some embodiments, an Hv1 modulating agent may be or comprise a nucleic acid, for example that may encode a polypeptide having Hv1 modulating agent activity and/or structure, as described herein. Exemplary nucleic acid sequences for Hv1 modulating agents are illustrated in Table 2C.

Production of Hv1 Modulating Agents

[112] Hv1 modulating agents can be produced by many methods. In some embodiments, an Hv1 modulating agent is produced by recombinant expression in a cell. In some embodiments, an Hv1 modulating agent is produced by peptide synthesis. In some embodiments, an Hv1 modulating agent is produced by *in vitro* translation.

[113] Exemplary methods of designing and producing Hv1 modulating agents are presented in Example 1.

[114] In some embodiments, an Hv1 modulating agent is presented on a replicable genetic package, e.g., in the form of a phage, yeast, ribosome, or nucleic acid-protein fusion.

[115] In some embodiments, an Hv1 modulating agent is provided and/or utilized in the context of an expression or display system.

[116] In some embodiments, Hv1 modulating agents are first synthesized as nucleic acids that encode polypeptide elements (e.g. elements A, B, and C in Table 3) and then annealed to produce nucleic acid sequences encoding polypeptide components (e.g. as in Table 2).

[117] In some embodiments, a nucleic acid sequence encoding an Hv1 modulating agent may be inserted into a phagemid or phage vector, in-frame, to form a leader-linker-Hv1 modulating agent-linker-coat protein construct (Clackson and Lowman, Phage display. Oxford University Press, 2004; Barbas et al., Phage display. A laboratory manual. Cold Spring Harbor Laboratory Press, 2001). Exemplary upstream leader and downstream amino acid sequences are MAAE and GSASSA, respectively. An exemplary phage vector is pAS62. An exemplary coat protein is protein III or its truncated version. Phages can be grown, prepared, titered and stored (Clackson and Lowman, Phage display. Oxford

University Press, 2004; Barbas et al., Phage display. A laboratory manual. Cold Spring Harbor Laboratory Press, 2001).

[118] In some embodiments, Hv1 modulating agents can be inserted into vectors for expression and/or library selection. In some embodiments, a library is presented in a polypeptide array. In some embodiments, a library is presented on a replicable genetic package, e.g., in the form of a phage display, yeast display, ribosome, or nucleic acid-protein fusion library. See, e.g., U.S. Pat. No. 5,223,409; Garrard et al. (1991) *Bio/Technology* 9:1373-1377; WO 03/029456; and U.S. Pat. No. 6,207,446. Binding members of such libraries can be obtained by selection and screened in a high throughput format. See, e.g., U.S. 2003-0129659.

[119] Hv1 modulating agent libraries for phage display can be generated by standard methods (Clackson and Lowman, Phage display. Oxford University Press, 2004; Barbas et al., Phage display. A laboratory manual. Cold Spring Harbor Laboratory Press, 2001). For example, Hv1 modulating agent libraries for phage display with a combinatorial arrangement of sequence elements are generated by designing overlapping or non-overlapping oligonucleotides corresponding to each individual element. These oligonucleotides are phosphorylated, annealed, mixed in a desired combination and concentration and ligated into a phagemid vector with or without linker sequences to create a library by standard methods (Sambrook et al., *Molecular Cloning: A Laboratory Manual*. Vols 1-3. Cold Spring Harbor Laboratory Press, 1989). Combinatorial arrangement of sequence elements to yield phage particles expressing a library of Hv1 modulating agents is demonstrated in Example 1A.

Identification and/or Characterization of Hv1 Modulating Agents

[120] Identification and/or characterization of Hv1 modulating agents can include determining effects of candidate agents on Hv1, including Hv1 that is naturally or recombinantly expressed. In some embodiments, Hv1 is expressed in cells. In some embodiments, Hv1 is immobilized (e.g., immobilized on a solid support, an artificial membrane, or a plasma membrane of a cell). In some embodiments, Hv1 is purified.

[121] Identification and/or characterization of Hv1 modulating agents can include the use of libraries of candidate agents.

[122] In some embodiments, an Hv1 modulating agent is identified from a candidate library incorporated into a phage display system. In phage display, candidate Hv1 modulating agents are functionally displayed on the surface of phage and nucleic acid sequences encoding candidate Hv1 modulating agents are enclosed inside phage particles. Functional display permits selection of Hv1 modulating agents that interact with a target or targets (e.g. Hv1 channels). Selection of Hv1 modulating agents from the library can be based on the Hv1 modulating agent type (e.g., toxin type) and/or target biochemistry, pharmacology, immunology and/or other physicochemical or biological property.

[123] A phage library can be transfected into *Escherichia coli* (*E. coli*) or other suitable bacterial species, propagated, and the phages purified. At this stage, Hv1 modulating agents or candidate agents can be functionally expressed on the surface of the phage and physically linked to their respective genes inside of the phage particle. A library is brought into contact with a target, such as Hv1 channels. For example, a phage library can be brought into contact with Hv1 channels that are immobilized on magnetic beads, as described in Example 2. After incubation with the target, those phages that express candidate Hv1 modulating agents with no or weak recognition for the target are washed away. The remaining Hv1 modulating agents that interact with the target are dissociated and can be (i) genotyped to establish the Hv1 modulating agent identity, or (ii) processed for one or more rounds of panning, or (iii) otherwise quantified and/or identified (e.g., ELISA, microbiological titering, functional testing).

[124] Panning may be performed by the binding of candidate modulating agents to Hv1, followed by washes and modulating agent recovery. Panning may be repeated until the desired enrichment is achieved. In addition, libraries can be pre-depleted on surfaces or cells that contain no Hv1 or on an Hv1 where the putative modulating agent binding domain may be directly or indirectly altered. Additionally, any and all conditions of panning may be varied, altered or changed to achieve optimal results, such as the isolation of a specific Hv1 modulating agent. Panning variations include, but are not limited to, the presence of competing polypeptide(s), presence of excess target(s), length and temperature of binding, pre-absorption of the library on one or more different receptor(s) or cells or surfaces, composition of binding solution (e.g., ionic strength), stringency of washing, and recovery procedures. Phages recovered from panning may be processed for further rounds of panning, functional analysis, and/or sequencing/genotyping to deduce the resulting Hv1

modulating agent's amino acid sequence or biological properties (Clackson and Lowman, Phage display. Oxford University Press, 2004; Barbas et al., Phage display. A laboratory manual. Cold Spring Harbor Laboratory Press, 2001).

[125] Following recovery after panning, Hv1 modulating agents of interest may be produced in native form by standard methods of peptide/protein synthesis/production (Sambrook et al., Molecular Cloning: A Laboratory Manual. Vols 1-3. Cold Spring Harbor Laboratory Press, 1989; Albericio, Solid-Phase Synthesis: A Practical Guide. CRC, 2000; Howl, Peptide Synthesis and Applications. Humana Press, 2005).

[126] In some embodiments, Hv1 modulating agents are tested for activity toward recombinant or functional Hv1. Samples that include functional channels (e.g., cells or artificial membranes) can be treated with an Hv1 modulating agent and compared to control samples (e.g., samples without the Hv1 modulating agent), to examine the extent of modulation. In some embodiments, Hv1 may be naturally expressed.

[127] In some embodiments, cells may be stably or transiently transfected with functional Hv1. For example, HEK-293T (mammalian human embryonic kidney) cells may be transfected with Hv1 (e.g. human Hv1 or human Hv1 tagged with a fluorescent protein) for transient expression. In one example, HEK-293T cells transiently expressing hHv1 tagged with teal fluorescent protein are used in patch clamp electrophysiology to determine effects of the Hv1 modulating agents C5 or C6 on proton currents.

[128] Changes in proton flux may be assessed by determining changes in polarization (i.e., electrical potential) of a cell or membrane expressing Hv1. In some embodiments, a change in cellular polarization is measured by measuring changes in current (thereby measuring changes in polarization) with voltage-clamp and patch-clamp techniques, e.g., the “cell-attached” mode, the “inside-out” mode, and the “whole cell” mode (see, e.g., Ackerman et al., New Engl. J. Med. 336:1575-1595, 1997). Whole cell currents can be determined using standard methodology (see, e.g., Hamil et al., Pflugers. Archiv. 391 :85, 1981). Other assays include radiolabeled rubidium flux assays and fluorescence assays using voltage sensitive dyes (see, e.g., Vestergaard-Bogind et al., J. Membrane Biol. 88:67-75, 1988; Daniel et al., J. Pharmacol. Meth. 25:185-193, 1991; Holevinsky et al., J. Membrane Biology 137:59-70, 1994). In some embodiments, candidate Hv1 modulating

agents are present in the range from 1 pM to 100 mM. Other methods for assessing Hv1 modulating agent effects on proton flux are described in the Examples herein.

[129] Hv1 modulating agents can also be identified or characterized by evaluating processes at the cellular, tissue and/or organism level. For example, Hv1 modulating agents can be evaluated for effects downstream of Hv1 activity or signaling. Various effects of Hv1 modulating agents that may be determined using intact cells or animals include transcriptional changes, changes in cell metabolism, and changes in intracellular second messengers.

[130] In some embodiments, Hv1 modulating agents can be evaluated for effects on human sperm. For example, Hv1 modulating agents can be evaluated for effects on sperm capacitation-related processes, including changes in sperm motility, decrease of cholesterol in the membranes, increase of tyrosine phosphorylation in several proteins, or maturation of the sperm response to progesterone. Capacitation of spermatozoa occurs along with an increase in the amplitude of voltage-gated proton current. Known Hv1 inhibitor, Zn^{2+} , reduces H^+ current in sperm cells. Accordingly, an Hv1 modulating agent can be evaluated for suppression of such sperm capacitation-related processes. Alternatively, an Hv1 modulating agent can be evaluated for enhancing sperm capacitation-related processes. Alternatively or additionally, an Hv1 modulating agent can be evaluated for non-capacitation-related processes that affect sperm activation, mobility, and/or fertilization.

[131] In some embodiments, Hv1 modulating agents can be evaluated for effects on cells that function in the immune system. For example, Hv1 is expressed in white blood cells (WBCs). Hv1 in WBCs has been shown to compensate charge buildup on the cell membrane during production of ROS. Hv1 knockout or inhibition impairs ROS production in these cells. Accordingly, an Hv1 modulating agent can be evaluated for suppression of ROS production in WBCs.

[132] Hv1 modulating agents can be selected for their potency and selectivity of modulation of Hv1. For example, an Hv1 modulating agent that demonstrates low IC_{50} value for Hv1, and a higher IC_{50} value for other ion channels within the test panel, is considered to be selective toward Hv1.

Compositions

[133] The present disclosure also features compositions that include and/or deliver Hv1 modulating agents.

[134] In some embodiments, a composition is a pharmaceutically acceptable composition that includes and/or delivers an Hv1 modulating agent described herein. For example, in some embodiments, a provided composition includes an Hv1 modulating agent polypeptide component. Alternatively or additionally, in some embodiments, a provided composition includes a nucleic acid that encodes an Hv1 modulating agent polypeptide component, a cell that expresses (or is adapted to express) an Hv1 modulating agent polypeptide component, etc. In some embodiments Hv1 modulating agents having any of the modifications of the present disclosure are included in pharmaceutical compositions.

[135] General considerations in the formulation and manufacture of pharmaceutical agents may be found, for example, in *Remington's Pharmaceutical Sciences*, 19th ed., Mack Publishing Co., Easton, PA, 1995.

[136] Pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, microemulsions, liposomes and suppositories. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. The preferred form of pharmaceutical composition depends on the intended mode of administration and therapeutic application. Typical compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for administration of antibodies to humans.

[137] The pharmaceutical composition can include a pharmaceutically acceptable carrier. For example, pharmaceutical compositions can include a therapeutic agent in addition to one or more inactive agents such as a sterile, biocompatible carrier.

[138] Exemplary carriers include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that

are physiologically compatible. For example, carriers may include sterile water, saline, buffered saline, or dextrose solution. Alternatively or additionally, the composition can contain any of a variety of additives, such as stabilizers, buffers, excipients (e.g., sugars, amino acids, etc.), or preservatives. Preferably, the carrier is suitable for oral, intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion).

[139] The pharmaceutical composition can include a pharmaceutically acceptable salt, e.g., a salt that retains the desired biological activity of the Hv1 modulating agent and does not impart any undesired toxicological effects (see e.g., Berge, S.M., et al., J. Pharm. Sci. 66:1-19, 1977).

[140] Depending on the route of administration, the Hv1 modulating agent may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

[141] In certain embodiments, a pharmaceutical composition can include a therapeutic agent that is encapsulated, trapped, or bound within a lipid vesicle, a bioavailable and/or biocompatible and/or biodegradable matrix, or other microparticles.

[142] In certain embodiments, an Hv1 modulating agent is prepared with a carrier that protects against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and poly lactic acid. Many methods for the preparation of such formulations are patented or generally known. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. Pharmaceutical formulation is a well-established art, and is further described in Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20^{sup}.th ed., Lippincott, Williams & Wilkins, 2000 (ISBN: 0683306472); Ansel et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, 7^{sup}.th Ed., Lippincott Williams & Wilkins Publishers, 1999 (ISBN: 0683305727); and Kibbe (ed.), Handbook of Pharmaceutical Excipients American Pharmaceutical Association, 3rd ed., 2000 (ISBN: 091733096X).

[143] In some embodiments, a provided pharmaceutical composition will include an Hv1 modulating agent that is not aggregated. For example, in some embodiments, less than 1%, 2%, 5%, 10%, 20%, or 30%, by dry weight or number, of Hv1 modulating agent is present in an aggregate.

[144] In some embodiments, a provided pharmaceutical composition will include an Hv1 modulating agent that is not denatured. For example, less than 1%, 2%, 5%, 10%, 20%, or 30%, by dry weight or number, of Hv1 modulating agents administered is denatured.

[145] In some embodiments, a provided pharmaceutical composition will include an Hv1 modulating agent that is not inactive. For example, less than 1%, 2%, 5%, 10%, 20%, or 30%, by dry weight or number, of Hv1 modulating agents administered is inactive.

[146] In some embodiments, pharmaceutical compositions are formulated to reduce immunogenicity of provided Hv1 modulating agents. For example, in some embodiments, a provided Hv1 modulating agent is associated with (e.g., bound to) an agent, such as polyethylene glycol and/or carboxymethyl cellulose, that masks its immunogenicity. In some embodiments, a provided binding agent has additional glycosylating that reduces immunogenicity.

Kits

[147] Also provided by the present disclosure are kits that include an Hv1 modulating agent described herein and instructions for use, e.g., treatment, prophylactic, or diagnostic use.

[148] In addition to the Hv1 modulating agent, the composition of the kit can include other ingredients, such as a solvent or buffer, a stabilizer or a preservative, and/or a second agent for treating a condition or disorder described herein. Alternatively, other ingredients can be included in the kit, but in different compositions or containers than the Hv1 modulating agent. In such embodiments, the kit can include instructions for admixing the Hv1 modulating agent and the other ingredients, or for using the Hv1 modulating agent together with the other ingredients.

[149] Alternatively or additionally, contents of kits may include, but are not limited to, expression plasmids containing nucleotides (or characteristic or biologically active portions) encoding Hv1 modulating agents of interest (or characteristic or biologically active portions). Alternatively or additionally, kits may contain expression plasmids that express Hv1 modulating agents of interest (or characteristic or biologically active portions). Alternatively or additionally, kits may contain isolated and stored Hv1 modulating agents.

[150] In certain embodiments, kits for use in accordance with the present invention may include, a reference sample, instructions for processing samples, performing tests on samples, instructions for interpreting the results, buffers and/or other reagents necessary for performing tests. In certain embodiments the kit can comprise a panel of antibodies.

[151] The present invention provides kits for administration of pharmaceutical compositions. For example, in some embodiments, the invention provides a kit comprising at least one dose of an Hv1 modulating agent. In some embodiments, the invention provides a kit comprising an initial unit dose and one or more subsequent unit doses of an Hv1 modulating agent. In some such embodiments, the initial unit dose is greater than the subsequent unit doses or wherein the all of the doses are equal.

Methods of Administration

[152] Pharmaceutical compositions may be administered in any dose appropriate to achieve a desired outcome. In some embodiments, the desired outcome is reduction in intensity, severity, and/or frequency, and/or delay of onset of one or more symptoms of an Hv1 associated disease or condition.

[153] A therapeutically effective amount of an Hv1 modulating agent composition can be administered, typically an amount which is effective, upon single or multiple dose administration to a subject, in treating a subject, e.g., curing, alleviating, relieving or improving at least one symptom of a disease or condition in a subject to a degree beyond that expected in the absence of such treatment. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition is outweighed by the therapeutically beneficial effects. A therapeutically

effective dosage preferably modulates a measurable parameter, favorably, relative to untreated subjects. The ability of an Hv1 modulating agent to inhibit a measurable parameter can be evaluated in an animal model system predictive of efficacy in a human disorder.

[154] In some embodiments, pharmaceutical compositions are administered in multiple doses. In some embodiments, pharmaceutical compositions are administered in multiple doses/day. In some embodiments, pharmaceutical compositions are administered according to a continuous dosing regimen, such that the subject does not undergo periods of less than therapeutic dosing interposed between periods of therapeutic dosing. In some embodiments, pharmaceutical compositions are administered according to an intermittent dosing regimen, such that the subject undergoes at least one period of less than therapeutic dosing interposed between two periods of therapeutic dosing.

[155] Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of ligand calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[156] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an Hv1 modulating agent described herein is 0.1-20 mg/Kg, more preferably 1-10 mg/Kg. In some embodiments, an agent can be administered by parenteral (e.g., intravenous or subcutaneous) infusion at a rate of less than 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 50 mg/m² or about 5 to 20 mg/m². It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions (e.g., the supervising physician), and that dosage ranges set forth herein are only exemplary.

[157] Pharmaceutical compositions of the present invention can be administered by a variety of routes, including oral, intravenous, intramuscular, intra-arterial, subcutaneous, intraventricular, transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, or drops), mucosal, nasal, buccal, enteral, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. For example, for therapeutic applications, an Hv1 modulating agent composition can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m² or 7 to 25 mg/m². Alternatively, the dose could be 100 µg/Kg, 500 µg/Kg, 1 mg/Kg, 5 mg/Kg, 10 mg/Kg, or 50 mg/Kg. The route and/or mode of administration will vary depending upon the desired results. In general the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (e.g., its stability in the environment of the gastrointestinal tract), the condition of the patient (e.g., whether the patient is able to tolerate oral administration), etc.

[158] A common mode of administration is parenteral (e.g., intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion). In one embodiment, the Hv1 modulating agent composition is administered by intravenous infusion or injection. In another embodiment, the Hv1 modulating agent composition is administered by intramuscular or subcutaneous injection. In another embodiment, the Hv1 modulating agent composition is administered orally. In some embodiments, the Hv1 modulating agent composition is administered topically. In some embodiments, the Hv1 modulating agent composition is administered transdermally. Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage.

[159] Hv1 modulating agents or pharmaceutical compositions of the present disclosure may be administered either alone or in combination with one or more other agents. In some embodiments, Hv1 modulating agents or pharmaceutical compositions of the present disclosure may be administered with one or more other Hv1 modulating agents. In some embodiments, Hv1 modulating agents or pharmaceutical compositions of the present disclosure may be administered with one or more other pharmaceutical agent including, but not limited to, small molecules, vaccines and/or antibodies. In some

embodiments, Hv1 modulating agents or pharmaceutical compositions may be administered in combination with an adjuvant.

[160] Combinations of agents may be administered at the same time or formulated for delivery together. Alternatively, each agent may be administered at a dose and on a time schedule determined for that agent. Additionally, the invention encompasses the delivery of the pharmaceutical compositions in combination with agents that may improve their bioavailability, reduce or modify their metabolism, inhibit their excretion, or modify their distribution within the body. Although the pharmaceutical compositions of the present invention can be used for treatment of any subject (e.g., any animal) in need thereof, they are most preferably used in the treatment of humans.

Uses

[161] As described herein, Hv1 channels have been reported to play a role in a variety of biological processes, and to impact various diseases, disorders, and conditions.

[162] The present disclosure encompasses treatment of Hv1 associated diseases or conditions. Hv1 modulating agents and/or Hv1 modulating agent compositions described herein can be administered, alone or in combination with, another agent to a subject, e.g., a patient, e.g., a patient who has a disorder (e.g., an Hv1-associated disease or condition, e.g. immune deficiency), a symptom of a disorder or a predisposition toward a disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. The treatment may also delay onset, e.g., prevent onset, or prevent deterioration of a condition.

[163] In some embodiments, Hv1 modulating agent pharmaceutical compositions are administered to a subject suffering from or susceptible to an Hv1 associated disease or condition. In some embodiments, a subject is considered to be suffering from an Hv1 associated disease or condition if the subject is displaying one or more symptoms commonly associated with said disease or condition. Hv1 modulating agents or Hv1 modulating agent compositions may be administered prior to or after development of one or more such symptoms.

[164] For example Hv1 modulating agents may be used to ameliorate inflammation, allergies, autoimmunity, cancer, asthma, brain damage from ischemic stroke, Alzheimer's disease, infertility, and numerous other conditions. In some embodiments, the desired outcome is reduction in intensity, severity, and/or frequency, and/or delay of onset of one or more of these conditions. Additionally or alternatively, Hv1 modulating agents may be used as a form of birth control by blocking sperm function.

[165] Additionally, Hv1 modulating agents may be used to change any of the functions of Hv1 channels described in the present disclosure to achieve a preferred or therapeutic outcome. As described herein, Hv1 channels transport protons across cell membranes and are expressed in a variety of cells and tissues. Functions of Hv1 channels differ depending on the cells in which they are expressed. Uses for Hv1 modulating agents can include increasing or decreasing proton current across cell membranes and/or increasing or decreasing pH in the cytosolic, extracellular, or intraluminal space of cells.

[166] In some embodiments, uses for Hv1 modulating agents may include effects on Hv1-related processes. For example, in some embodiments, Hv1 modulating agents may be used to increase or decrease the expression and/or function of NOX enzymes, including NOX1, NOX2, NOX3, and/or NOX4. In some embodiments, Hv1 modulating agents may be used to increase or decrease production of ROS.

[167] In some embodiments, uses for Hv1 modulating agents may include altering biological functions in specific cells. For example, the function of Hv1 channels in white blood cells includes extrusion of protons to facilitate ROS production via NOX activity in the phagosome. This process allows white blood cells to destroy bacteria and other pathogens. In some embodiments, uses of Hv1 modulating agents may include changing these functions in white blood cells. Alternatively, Hv1 channel function in human sperm has been associated with sperm capacitation, activation and mobility to achieve fertilization. In some embodiments, Hv1 modulating agent uses may include increasing or decreasing sperm function and/or fertilization ability.

[168] While various aspects and examples have been described, it will be apparent to those of ordinary skill in the art that many more examples and implementations are possible within the scope of this disclosure. Accordingly, the disclosure is not to be restricted except in light of the attached claims and their equivalents.

Exemplification

Example 1: Designing Hv1 Modulating Agents

[169] The present Example describes certain Hv1 modulating agents provided herein. Certain Hv1 modulating agents provided herein comprise a polypeptide component having an amino acid sequence including element(s) found in wild-type toxin polypeptides. In some embodiments, exemplary Hv1 modulating agents comprise a polypeptide component whose amino acid sequence further comprises one or more tag elements (e.g., a detectable tag, a localizing tag, etc). In some embodiments, exemplary Hv1 modulating agents have a structure that comprises both a polypeptide component and a non-polypeptide component (e.g., a modifying component such as a lipid-containing moiety, a saccharide-containing moiety, etc). Alternatively or additionally, in some embodiments, exemplary Hv1 modulating agents are multimeric in that their structure includes multiple (e.g., 2 or more) monomer components associated with one another. In some embodiments, all monomers in a multimer are structurally identical (or substantially identical) to one another. In some embodiments, a multimer may comprise 2 or more distinct monomers. In some embodiments, two or more monomers in a multimer may be covalently associated with one another (e.g., via a linker or cross-linker).

A. Toxin sequences

[170] As noted above, certain Hv1 modulating agents provided herein have an amino acid sequence including element(s) found in wild-type toxin polypeptides. Representative such agents were designed as described below.

[171] The amino acid sequence of the Peruvian green velvet tarantula (*Thrixopelma pruriens*) was used as a template to identify predicted wild-type toxin sequences using the basic local alignment search tool (BLAST) in the UniProt and Pfam databases. A total of 110 predicted wild-type toxin sequences were identified (Table 1). All of these sequences encode a polypeptide characterized as having an inhibitor cysteine knot (ICK) structural motif. The amino acid sequences of these 110 predicted wild-type toxins were aligned around six conserved cysteine residues of the ICK motif. Three sequence elements, A, B, and C were delineated by the second and fifth of the six conserved cysteine residues in each predicted wild-type toxin sequence, resulting in 95 A, 104 B, and 106 C

sequence elements (Table 3). Nucleotides were synthesized corresponding to these A, B, and C elements.

[172] Complementary nucleotide pairs for each unique element A, B, or C, were synthesized to produce nucleotide duplexes. These nucleotide duplexes were phosphorylated using T4 Polynucleotide Kinase and annealed unidirectionally to produce polynucleotide components having an A-B-C sequence element pattern linked at cysteine residues. To achieve and monitor incorporation of the sequence elements, 104 separate reactions were performed to ligate the ABC inserts into the pAS62 phagemid vector in frame with phage particle coat protein pIII, resulting in phagemids having the ABC inserts (see, e.g., PCT/US2008/013385). Each reaction contained one B nucleotide duplex, 95 A nucleotide duplexes, and 106 C nucleotide duplexes. Ligation mixtures were transformed in SS320 electrocompetent cells (Lucigen, Middleton, WI). To verify unbiased insert utilization, 416 plaques were sequenced. The processes yielded phage particles expressing the original 110 predicted wild-type toxins and approximately 1,047,170 novel peptides. Exemplary novel peptides are listed in Table 2A and FIG. 1. Exemplary A, B and C sequence elements are listed in Table 3.

B. Linkers and Dimerization

[173] Certain exemplary Hv1 modulating agents were prepared by linking together two polypeptide components, each of which had an amino acid sequence comprising elements of wild-type toxin polypeptides as described above in Section A.

[174] For example, two monomers of the agent labeled as “C6” in Table 2A were joined together via a peptide linker. Several different peptide linkers were utilized. For example, each of a rigid linker of 10 amino acid residues, a flexible linker of 10 amino acid residues, or a long flexible linker of 38 amino acid residues was used. Table 4A lists amino acid sequences of exemplary polypeptides created through such linkage.

C. Signal Peptides

[175] In some embodiments, one or more signal peptides can be included in exemplary Hv1 modulating agents (Table 4B).

[176] For example, Hv1 modulating agents with an N-terminal trypsin secretory signal sequence can be prepared.

D. Detectable Tags

[177] In some embodiments, an Hv1 modulating agent may be modified with an epitope tag.

[178] For example, a c-Myc epitope tag can be added near the C-terminus of a polypeptide component in an Hv1 modulating agent (Table 4C).

E. Tethering Moieties

[179] In some embodiments, an Hv1 modulating agent may be modified with a tethering moiety that targets the Hv1 modulating agent to a specific surface.

[180] For example, a hydrophobic sequence may be added to the C-terminus of an Hv1 modulating agent that targets the Hv1 modulating agent for covalent tethering to glycosylphosphatidylinositol (GPI) anchors inserted in the extracellular leaflet of the plasma membrane (Table 4D).

[181] Annotated sequences of exemplary modified Hv1 modulating agents are presented in FIG. 3.

F. Generating Hv1 modulating agents with linkers and modifications

[182] Representative Hv1 modulating agents having signal peptides, detectable tags, and tethering moieties were generated as follows. The sequence encoding mammalian Lynx1, a toxin-like nicotinic acetylcholine receptor modulator, was replaced by cDNA of the Hv1 modulating agent C6, in-frame between the secretion signal and the Lynx1 hydrophobic sequence for GPI attachment. A flexible linker containing a glycine-asparagine repeat was inserted between the C6 sequence and the hydrophobic sequence for GPI attachment, and a c-Myc epitope tag was introduced in the middle of the linker. Hv1 modulating agents having these modifications are also called "T-toxins." Exemplary T-toxin sequences are depicted in FIGS. 3A-3E. The present disclosure appreciates that methods analogous to those described in Gui, J. et al., "A tarantula-venom peptide antagonizes the TRPA1 nociceptor ion channel by binding to the S1-S4 gating domain,"

Curr Biol. 24(5):473-83 (2014) can be employed to generate GPI-tethered toxins as described herein.

Example 2: Characterization of Hv1 Modulating Agents

[183] The present Example demonstrates a high-throughput assay for characterization of Hv1 modulating agents. Specifically, this assay characterizes whether Hv1 modulating agents are capable of binding human Hv1 (hHv1) protein.

[184] To characterize if an Hv1 modulating agent can bind to hHv1 channels, a phage display library expressing Hv1 modulating agents was generated. Phage particles from Example 1A were used to infect *Escherichia coli* (*E. coli*) XL1-Blue cells for 15 min at room temperature. The infected cells were grown overnight at 37 °C in 150 mL 2xYT in the presence of 1010/mL M13K07 helper phage, 100 g/mL ampicillin, and 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Cultures were centrifuged and the supernatant was precipitated with PEG/NaCl solution. The phage pellet was collected by centrifugation and dissolved in TBS. Phage particle titer was determined by serial dilution in TBS and infection of *E. coli* XL1-Blue followed by plating on LB plates with antibiotic and determination of colony forming units (cfu).

[185] hHv1 protein was biotinylated using sulfosuccinimidyl 2-(biotinamido)-ethyl-1,3-dithiopropionate (EZ-Link Sulfo-NHS-SS-Biotin, Thermo Scientific). Biotinylation was verified by a pull-down assay using streptavidin MagneSphere beads (Promega). Biotinylated hHv1 was adsorbed to 300 μl streptavidin MagneSphere paramagnetic particles, and free streptavidin-binding sites were blocked with biotin to prevent nonspecific binding.

[186] After manual washing of the magnetic beads, library phage particles (10^{11} cfu) were added in 300 μL TBSB (25 mM Tris-HCl, 140 mM NaCl, 3 mM KCl, 2 mM LPPG, 0.5% bovine serum albumin, pH 7.4) and incubated on a rocking shaker for 1 h. Poorly adherent phage particles were removed by washing 2-5 times with TBSTB (TBSB with 0.1% Tween 20). The captured phages on the magnetic beads were eluted with 100 mM DTT in 20 mM Tris, pH 8.0, for 10 min, and then used to infect *E. coli* XL1-Blue cells (Stratagene) for phage amplification. The phage particles captured in the first round were cycled through an additional five rounds of binding and selection using an automated

magnetic bead manipulator (KingFisher, Thermo Scientific). Phage particles were quantified by titring before and after selective library sorting and genotyped by DNA sequencing after six rounds of panning. Phage enrichment was observed with immobilized hHv1 as the target compared with the control target streptavidin. Exemplary Hv1 modulating agents enriched by this method are listed in Table 2A. In some instances, repeats of Hv1 modulating agent sequences after several rounds of panning can be observed, as demonstrated by agents labeled as A6 and G2, C2 and F2, C6 and D5, and D6 and E2. Without wishing to be bound by any particular theory, any repeat may be considered significant (since the library had more than 1 million peptides initially) and may demonstrate selection and functional convergence.

Example 3: Characterization of Hv1 modulating agents by T-toxin assay

[187] The present example demonstrates characterization of the effects of Hv1 modulating agents on hHv1 using T-toxins. Specifically, the present example demonstrates inhibition of hHv1 function as measured by tail current from *Xenopus laevis* oocytes expressing only hHv1 or both hHv1 and Hv1 modulating agents tethered to the oocyte plasma membrane.

[188] T-toxin cDNAs were cloned into the pCS2+ plasmid vector for *in vitro* transcription of T-toxin cRNA. Capped cRNAs were prepared by restriction enzyme linearization, followed by *in vitro* transcription reaction with SP6 (for T-toxins) and T7 (for hHv1) RNA polymerase (mMessenger mMachinE kit, Ambion). Concentrations of cRNAs were measured using NanoDrop 2000 (Thermo Scientific).

[189] cRNAs for T-toxins and hHv1 were mixed at 1:1 ratio (w/w) and injected into the *Xenopus laevis* oocytes. Currents were measured by Two Electrode Voltage Clamp (TEVC). Recording solution was (in mM): 90 NaCl, 1 MgCl₂, 2 CaCl₂, 120 HEPES, pH 7.3. Recordings were performed with constant gravity flow of solution at 2ml/min yielding chamber exchange in ~ 5 s. Currents were recorded 2-3 days after cRNA injection using an oocyte clamp amplifier OC-725C (Warner Instruments, Hamden, CT), and electrodes filled with 3 M KCl with resistance of 0.3 – 1 MΩ. Data were filtered at 1 kHz and digitized at 20 kHz using pClamp software and assessed with Clampfit v9.0 and Origin 6.0. Inhibition was studied by comparing tail current from oocytes expressing only hHv1 and those with both hHv1 and T-toxins. Inhibition was calculated as unblocked fractional current (FIG. 3F).

Example 4: Characterization of Hv1 modulating agents with a transmembrane link

[190] The present example demonstrates characterization of Hv1 modulating agent effects on hHv1 using Hv1 modulating agents expressed from cells via a transmembrane link.

[191] The Hv1 modulating agent C6 was tethered to cell surfaces using a transmembrane domain from the PDGF receptor, which links an internal mVenus fluorescent protein to an external C6 (FIG. 7). Unlike the tether in oocytes which attaches C6 to the outside of the cell (e.g. FIG. 3), this is a transmembrane link. To transiently express hHv1, 1 μ g of hHv1 tagged with teal fluorescence protein (hHv1-TFP) was transfected into HEK-293T cells using Lipofectamine 2000. Changes in the single exponential fit of fluorescence decay were indicative of FRET (FIG. 8). Changes in current density and shifts in the g_H - V were indicative of blocking of hHv1.

Example 5: Hv1 Modulating Agents Activate or Inhibit Hv1 proton current

[192] The present Example documents assessing activity of the voltage-gated proton channel Hv1 in response to Hv1 modulating agents. Activity of Hv1 was assessed by changes in proton currents.

[193] To transiently express hHv1, 1 μ g of hHv1 or hHv1 tagged with teal fluorescence protein (hHv1-TFP) was transfected into HEK-293T cells using Lipofectamine 2000. In some instances, green fluorescent protein was used as a transfection marker. Transfected cells were plated onto glass coverslips. Green or teal cells were selected for whole-cell patch clamp after 24 hours.

[194] Patch clamp recordings were performed with an external solution of 100mM HEPES, pH 7.5, 70mM NaCl, and 10 mM glucose. The pipette solution was 100mM Bis Tris buffer, pH 6.5, 70 mM NaCl and 10 mM glucose. Hv1 modulating agent was applied to these cells while pulsing to 40mV every 10 seconds and proton currents were measured using whole-cell patch clamp electrophysiology.

[195] FIG. 4 shows activation or inhibition of Hv1 proton currents by Hv1 modulating agent as compared to current without addition of modulating agent. Cells were

incubated with either 500 nM C5 (FIG. 4A) or 250 nM C6 (FIG. 4B). Activation of Hv1 channels by C5 increased proton current and slowed channel closing. Block of Hv1 channels by C6 decreased proton currents.

Example 6: Hv1 Modulating Agent Inhibits Effects of Progesterone on Sperm Capacitation

[196] The present example demonstrates effectiveness of certain Hv1 modulating agents in suppressing maturation of the sperm response to progesterone. This example shows that activity of Hv1 channels during capacitation is necessary for calcium rise and acrosomal reaction stimulation by physiological inducers required for fertilization.

[197] To test effects of Hv1 modulating agents on sperm capacitation in a blind study, human sperm was exposed to capacitating conditions in the presence or absence of Hv1 modulating agent C6 (Tx C) or a control peptide (Tx A) having the amino acid sequence GVEINVKCSGSPQCLKPCKDAGMDFGDCMNDKCHCTPK (SEQ ID NO: 810) (a mutant scorpion venom peptide; Takacs, Z., et al., "A designer ligand specific for Kv1.3 channels from a scorpion neurotoxin-based library," *Proc Natl Acad. Sci. USA*, 106(52): 22211–22216 (2009)). Sperm were incubated for 1 hour with the Tx C or Tx A (20 μ M) in a medium that does not promote capacitation. Cells were transferred to a capacitating medium with Tx C or Tx A. After 4 hours of incubation, parameters related to capacitation were analyzed (FIG. 5). Known protocols for such analyses are described in Pocognoni, C.A., et al., "Perfringolysin O as a useful tool to study human sperm physiology," *Fertility and Sterility*, 99(1): p. 99-106.e2 (2013).

[198] C6 did not affect the vitality (FIG. 5A), the protein tyrosine phosphorylation (FIG. 5F and FIG. 5G), or the cholesterol content of the membranes (FIG. 5H). C6 did not significantly alter the mobility of sperm (FIGS. 5B-5E). C6 did affect the response of sperm to progesterone. The increase of cytosolic calcium triggered by progesterone is diminished in the presence of the peptide modulator and the acrosome reaction induced by the hormone is inhibited (FIGS. 5I-5K). Moreover, when C6 was added after capacitation, both intracellular calcium and acrosomal reaction, triggered by progesterone, did not show any changes.

Example 7: Hv1 Modulating Agents Inhibit Production of Reactive Oxygen Species in Human Blood Cells

[199] The present example demonstrates effectiveness of certain Hv1 modulating agents in blocking ROS production by human blood cells.

[200] Whole blood was purchased in 10 mL tubes from Innovative Research, Inc, in accordance with FDA guidelines. Blood was used within 24-48 hours after being drawn. Upon arrival, blood cells were counted using a hemocytometer and diluted in Tyrode's solution to approximately 5×10^6 cells/mL. Twenty μ L of the dilute blood cells were added to each well of a 96-well plate with a total volume of 100 μ L in Tyrode's solution. Reactive oxygen species (ROS) were detected by fluorescence readout using 100 μ M Amplex Red with 2 units/mL horseradish peroxidase added to each well.

[201] Blood was incubated with the following treatment conditions for 1 hour at 37 C: control; 100 μ M zinc; 100 pM – 5 μ M C6; and 10 μ M MOKA toxin. Each treatment condition was added to wells in 5 repeats. After the incubation, 200 nM phorbol myristate acetate (PMA) was added to all wells except the control. PMA was used to stimulate ROS production. Fluorescence measurements were taken immediately after using excitation at 530 nM and emission at 590 nM. Measurements were repeated every 15 to 30 min for the next 2 hours. Relative fluorescence intensity was plotted versus time and used to calculate inhibition. FIG. 6 demonstrates that C6 blocks production of ROS in human blood cells in a dose-dependent manner. The known inhibitor of Hv1, zinc, blocks ROS production to background (control) levels. Two toxins that block potassium channels with nM affinity (Moka and KTX) had no effect.

Example 8: The Hv1 modulating agent C6 targets an S3-S4 external loop region of hHv1

[202] The present example demonstrates identification of regions in hHv1 that can bind and/or respond to modulation by the Hv1 modulating agent C6.

[203] The Hv1 modulating agent C6 did not inhibit proton current of *Ciona intestinalis* (*C. intestinalis*) Hv1 channels (CiHv1). Chimeric forms of ciHv1 were generated in which amino acids from human Hv1 (hHv1) corresponding to the S3-S4 external loop replaced the same region of CiHv1 (hS3-S4-ciHv1). The resulting hS3-S4-ciHv1 chimera comprised hHv1 amino acids I183 to L204: ILDIVLLFQEHQFEALGLLILL (SEQ ID NO: 111) and maintained characteristics of Hv1 currents. C6 blocked current for hS3-S4-ciHv1 (FIGS. 9A and 9B).

[204] Twelve residues in the S3-S4 external loop region of hHv1 (F190 to L201) were individually mutated to Cysteine. Current with 1 μ M C6 normalized to current without toxin (Itox/Ictr) was measured (FIG. 9C). Mutating hHv1 E192C increased normalized current with C6 compared to WT hHv1. Mutating hHv1 G199C or G199L increased inhibitory effects of C6 compared to WT hHv1.

Equivalents

[205] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

Claims

We claim:

1. An Hv1 modulating agent that is or comprises an engineered polypeptide component having an inhibitor cysteine knot (ICK)-like structural motif.
2. The Hv1 modulating agent of claim 1, wherein the polypeptide component is about 10 to about 100 amino acids in length.
3. The Hv1 modulating agent of claim 1, wherein the polypeptide component has an amino acid sequence that is composed of one or more toxin sequence elements, each of which has a length within a range of about 5 to about 18 amino acids in length.
4. The Hv1 modulating agent of claim 1, wherein the polypeptide component has 0, 1, 2, 3, 4, 5, or 6 conserved cysteine residues of an ICK structural motif.
5. The Hv1 modulating agent of claim 1, wherein the polypeptide component has 0, 1, 2, or 3 disulfide bridges.
6. The Hv1 modulating agent of claim 1, wherein the polypeptide component has 0, 1, 2, or 3 beta strands.
7. An Hv1 modulating agent that is or comprises an engineered polypeptide component that includes one or more toxin sequence elements, each of which has an amino acid sequence that is substantially identical to, but differs from, that of a corresponding element found in a wild-type toxin.
8. The Hv1 modulating agent of claim 7, wherein the polypeptide component is about 10 to about 100 amino acids in length.
9. The Hv1 modulating agent of claim 7, wherein the polypeptide component has an amino acid sequence that is substantially identical to, but differs from, that of a wild-type toxin.
10. The Hv1 modulating agent of claim 7, wherein each of the one or more toxin sequence elements has a length within a range of about 5 to about 18 amino acids.

11. The Hv1 modulating agent of claim 7, wherein two or more toxin sequence elements are assembled in a linear order such that the amino acid sequence shows overall correspondence to a full-length toxin.
12. The Hv1 modulating agent of claim 7, wherein the one or more toxin sequence elements has an amino acid sequence that comprises one or more insertions, deletions substitutions, rearrangements, or combinations thereof relative to a wild-type toxin sequence element and shows at least 40% sequence identity with the wild-type toxin.
13. The Hv1 modulating agent of claim 7, wherein the one or more toxin sequence elements has an amino acid sequence that differs at residues that undergo posttranslational modifications relative to a wild-type toxin sequence element.
14. The Hv1 modulating agent of claim 7, wherein the polypeptide component has an amino acid sequence that includes one or more cysteine residues at positions corresponding to those at which a cysteine residue is found in a wild-type toxin sequence.
15. The Hv1 modulating agent of claim 7, wherein the polypeptide component has an amino acid sequence that shares the same approximate relative position of cysteines with a wild-type toxin sequence.
16. The Hv1 modulating agent of claim 7, wherein the wild-type toxin has an amino acid sequence delineated by six conserved cysteine residues that form disulfide bridges in an inhibitor cysteine knot (ICK) motif.
17. The Hv1 modulating agent of claim 7, wherein the wild-type toxin is a venom toxin.
18. The Hv1 modulating agent of claim 7, wherein the wild-type toxin has an amino acid sequence that aligns with a sequence in Table 1.
19. An Hv1 modulating agent according to claims 1 or 7 that binds to the external surface of human Hv1.
20. The Hv1 modulating agent of claim 19 that binds to the S3-S4 external loop region of human Hv1.

21. The Hv1 modulating agent of claim 19 that binds to or within a region of human Hv1 having the amino acid sequence ILDIVLLFQEHQFEALGLLILL (SEQ ID NO: 111).
22. The Hv1 modulating agent of claim 19 that binds to the closed state of human Hv1.
23. The Hv1 modulating agent of claim 19 that binds to human Hv1 monomers and dimers.
24. The Hv1 modulating agent according to claims 1 or 7 that is or comprises a cyclic structure.
25. The Hv1 modulating agent according to claims 1 or 7 that is or comprises a stapled polypeptide.
26. The Hv1 modulating agent according to claims 1 or 7, wherein the polypeptide component includes one or more pendant groups or other modifications selected from the group consisting of acetylation, amidation, glycosylation, lipidation, methylation, pegylation, phosphorylation, including combinations thereof.
27. The Hv1 modulating agent according to claims 1 or 7, wherein the polypeptide component has an amino acid sequence that comprises one or more tag elements, such as a detectable tag or a localizing tag.
28. The Hv1 modulating agent according to claims 1 or 7, wherein the polypeptide component is connected to a signal peptide or a coat protein of a phage.
29. An Hv1 modulating agent that is or comprises a dimer or multimer of the Hv1 modulating agent of claims 1 or 7.
30. The Hv1 modulating agent of claim 29, wherein the dimer or multimer is a heterodimer or heteromultimer.
31. The Hv1 modulating agent of claim 29, wherein the dimer or multimer is a homodimer or homomultimer.
32. The Hv1 modulating agent of claim 29, wherein the dimer or multimer is linked via a peptide linker.

33. A nucleic acid encoding all or part of an Hv1 modulating agent of any of the above claims.
34. A vector including the nucleic acid of claim 33.
35. A cell including one or more of the Hv1 modulating agents of any of the above claims, the nucleic acid of claim 33, or the vector of claim 34.
36. An Hv1 modulating agent of any of the above claims that is incorporated into an antibody framework.
37. The Hv1 modulating agent of claim 36, wherein the agent is fused to an antibody sequence or fragment thereof.
38. The Hv1 modulating agent of claim 36, wherein the agent is attached or inserted into one or more of an immunoglobulin domain or fragment thereof.
39. An Hv1 modulating agent of any of the above claims that inhibits human Hv1 function.
40. The Hv1 modulating agent of claim 39, wherein the agent decreases or blocks proton current.
41. The Hv1 modulating agent of claim 39, wherein the agent reduces the number or likelihood of Hv1 channel opening.
42. The Hv1 modulating agent of claim 39, wherein the agent increases the rate of Hv1 channel closing.
43. The Hv1 modulating agent of claim 39, wherein the agent causes Hv1 channels to require stronger voltage to elicit proton current.
44. An Hv1 modulating agent of any of the above claims that activates human Hv1 function.
45. The Hv1 modulating agent of claim 44, wherein the agent increases proton current.
46. The Hv1 modulating agent of claim 44, wherein the agent increases the rate of Hv1 channel opening.

47. The Hv1 modulating agent of claim 44, wherein the agent slows the rate of Hv1 channel closing.
48. An Hv1 modulating agent of any of the above claims that changes transmembrane voltage of a cell.
49. The Hv1 modulating agent of claim 48, wherein the agent hyperpolarizes membrane potential.
50. The Hv1 modulating agent of claim 48, wherein the agent depolarizes membrane potential.
51. An Hv1 modulating agent of any of the above claims that changes the transmembrane pH gradient.
52. The Hv1 modulating agent of claim 51, wherein the agent increases or decreases cytosolic pH.
53. The Hv1 modulating agent of claim 51, wherein the agent increases or decreases extracellular or intraluminal pH.
54. An Hv1 modulating agent of any of the above claims that increases or decreases the activity of NOX enzymes.
55. The Hv1 modulating agent of claim 54 that increases or decreases ROS production.
56. An Hv1 modulating agent of any of the above claims that inhibits sperm capacitation.
57. An Hv1 modulating agent of any of the above claims that decreases ROS production in white blood cells.
58. An Hv1 modulating agent of any of the above claims that modulates the activity of one or more Hv1 isoforms.
59. A polypeptide comprising one or more of an amino acid sequence element that is substantially identical with a sequence in Table 3A and is different from each sequence in Table 1.

60. The polypeptide of claim 59 having a length of about 10 to about 100 amino acids.
61. The polypeptide of claim 59, wherein the sequence element has a length within a range of about 5 to about 18, comprising a single sequence element corresponding to an A reference element, a single sequence element corresponding to a B reference element, and a single sequence element corresponding to a C reference element set forth in Table 3A.
62. The polypeptide of claim 61 having an amino acid sequence composed of a linear order of reference elements A-B-C.
63. The polypeptide of claim 62, wherein the A reference element has a sequence according to SEQ ID NO: 809, the B element has a sequence according to SEQ ID NO: 273, and the C element has a sequence according to SEQ ID NO: 411.
64. The polypeptide of claim 62, wherein the A reference element has a sequence according to SEQ ID NO: 255, the B element has a sequence according to SEQ ID NO: 273, and the C element has a sequence according to SEQ ID NO: 411.
65. The polypeptide of claim 59 whose amino acid sequence has an element that shares one or more cysteines with a sequence in Table 3A.
66. The polypeptide of claim 59 whose amino acid sequence has an element that shares the same approximate relative position of cysteines with a sequence in Table 3A.
67. A nucleic acid comprising one or more of a sequence element that is substantially identical with a sequence in Table 3B.
68. A polypeptide having an amino acid sequence that is substantially identical with a sequence in Table 2A and is different from each sequence in Table 1.
69. The polypeptide of claim 68 having a length within a range of about 10 to about 100 amino acids.
70. The polypeptide of claim 68 having an amino acid sequence that shares one or more cysteines with a sequence in Table 2A.

71. The polypeptide of claim 68 having an amino acid sequence that shares the same approximate relative position of cysteines with a sequence in Table 2A.
72. The polypeptide of any of claims 68-71 that activates or inhibits human Hv1.
73. A polypeptide having an amino acid sequence that shares one or more cysteines with a sequence set forth in Table 1.
74. The polypeptide of claim 73 having a length within a range of about 10 to about 100 amino acids.
75. The polypeptide of claim 73 having an amino acid sequence that share the same approximate relative position of cysteines with a sequence in Table 1.
76. A polypeptide comprising an amino acid sequence
GCKWYLGDCADSDDCCETFHCKWVFFTSKFMCRRVWGKD.
77. A polypeptide comprising an amino acid sequence
SSTCIPSGQPCADSDDCCETFHCKWVFFTSKFMCRRVWGKD.
78. A nucleic acid comprising the sequence
GGCTGCAAATGGTATCTGGGCGATTGTGCGGATAGCGATGATTGCTGCG
AAACCTTTCATTGCAAATGGGTGTTTTTTACCAGCAAATTTATGTGCCGC
CGCGTGTGGGGCAAAGAT.
79. A nucleic acid comprising the sequence
AGCAGCACCTGCATTCCGAGCGGCCAGCCGTGTGCGGATAGCGATGATT
GCTGCGAAACCTTTCATTGCAAATGGGTGTTTTTTACCAGCAAATTTATG
TGCCGCCGCGTGTGGGGCAAAGAT.
80. A pharmaceutical composition comprising:
 - A polypeptide of any of the prior claims; and
 - A pharmaceutically acceptable carrier or excipient.
81. A method comprising the step of administering to a subject suffering from or susceptible to an Hv1 associated disease or condition a pharmaceutical composition of claim 80, such that the subject's Hv1 associated disease or condition is treated or risk of developing an Hv1 associated disease or condition is reduced.

82. The method of claim 81, wherein the Hv1 associated disease or condition is selected from the group consisting of: inflammation, allergies, autoimmunity, cancer, asthma, brain damage from ischemic stroke, Alzheimer's disease, or infertility.
83. The method of claim 82 wherein the cancer is a leukemia or solid tumor.
84. The method of claim 83 wherein the solid tumor is breast cancer.
85. A method comprising the steps of:
identifying a subject in need of modulating Hv1 activity; and
administering to the subject a pharmaceutical composition of claim 80.
86. The method of claim 85 wherein the subject is in need of blocking fertilization.
87. A method of making or manufacturing the pharmaceutical composition of claim 80, the method comprising a step of formulating the polypeptide with at least one pharmaceutically acceptable carrier or excipient to generate the pharmaceutical composition.
88. A method of making or manufacturing an Hv1 modulating agent, the method comprising steps of culturing a cell of claim 35 under conditions wherein the cell expresses the Hv1 modulating agent.
89. A method of making or manufacturing an Hv1 modulating agent the method comprising steps of:
synthesizing nucleotide duplexes encoding the toxin sequence elements of claim 3;
annealing the duplexes; and
ligating the annealed duplexes into a vector for expression of the Hv1 modulating agent.
90. A method of characterizing a composition having the structure of an Hv1 modulating agent, said method comprising the steps of:

Contacting the composition with a system comprising a human Hv1 channel;
and
recording proton current in the system.

91. The method of claim 90 wherein the system is *Xenopus laevis* oocytes.
92. The method of claim 90 wherein the system is HEK-293T cells.

FIG. 1

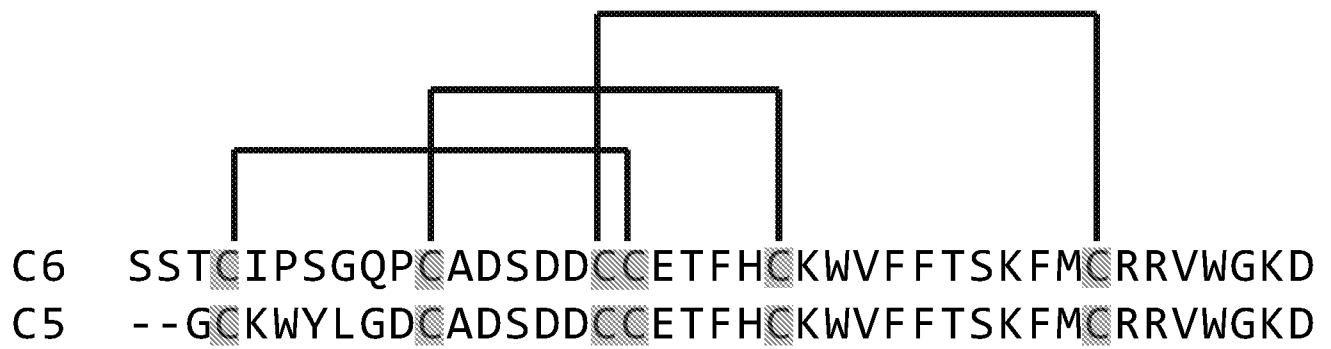


FIG. 2

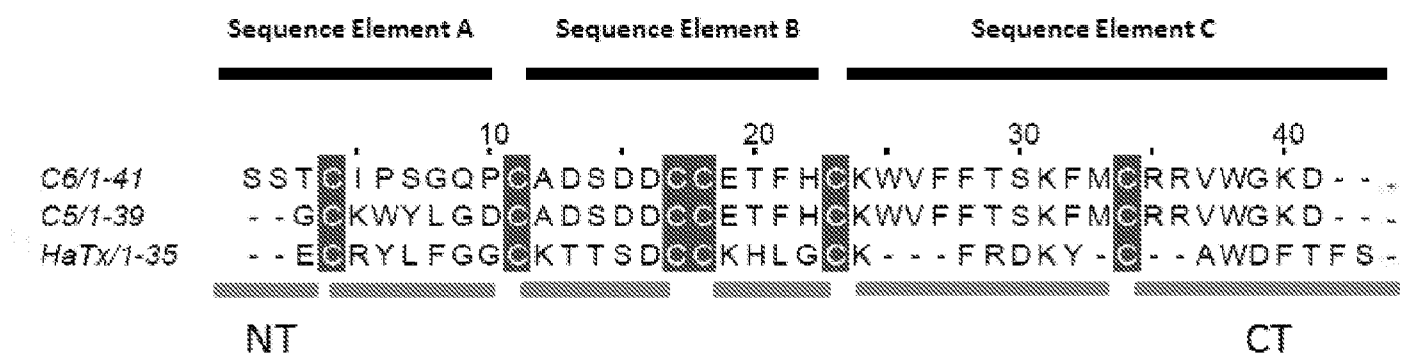


FIG. 3



KEY:

Gray = Signal peptide for T-toxin expression

Dark Gray = rigid linker from natural spider toxin DkTx

Dash with dots = c-Myc epitope

Underline = C6 SequenceDouble Underline = HaTx1 sequence

Boxed = hydrophobic sequence

Dashed = flexible linker

FIG. 3F

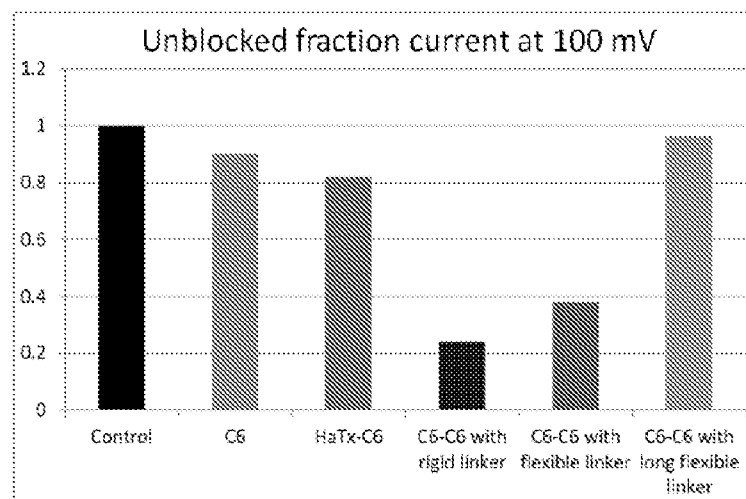


FIG. 4

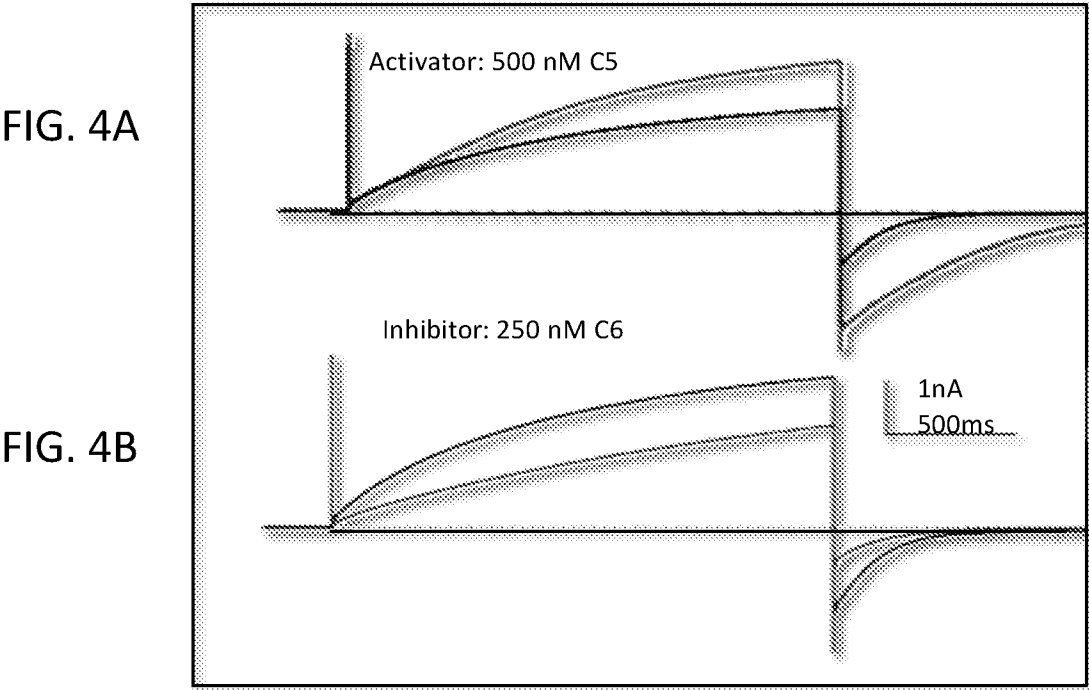


FIG. 5

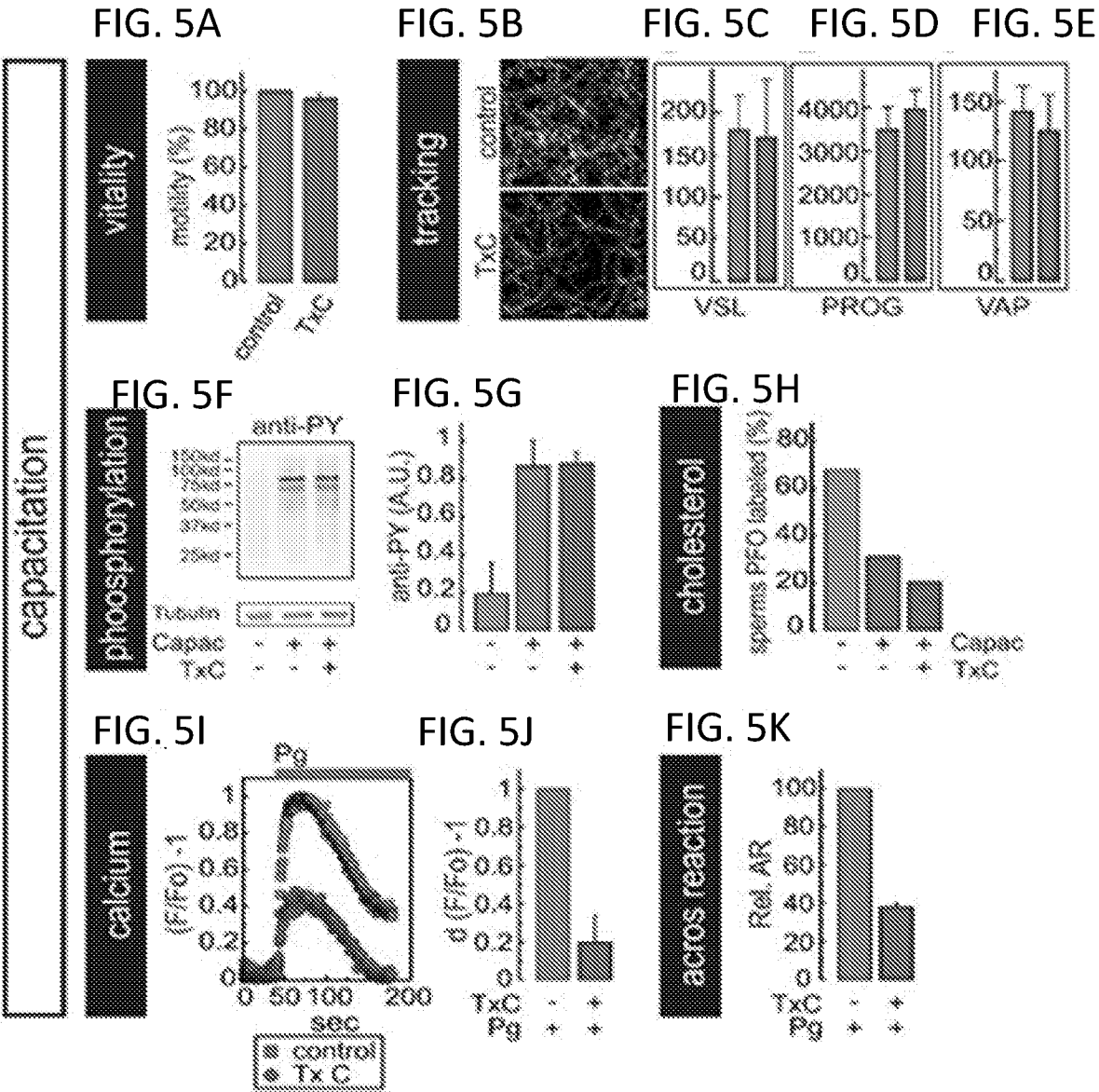


FIG. 6

FIG. 6A

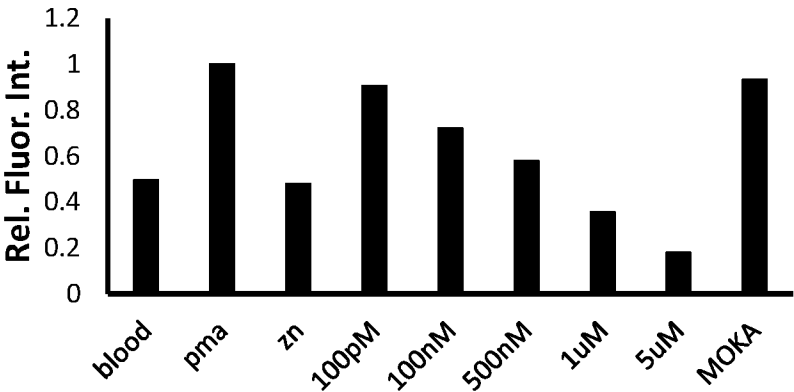


FIG. 6B

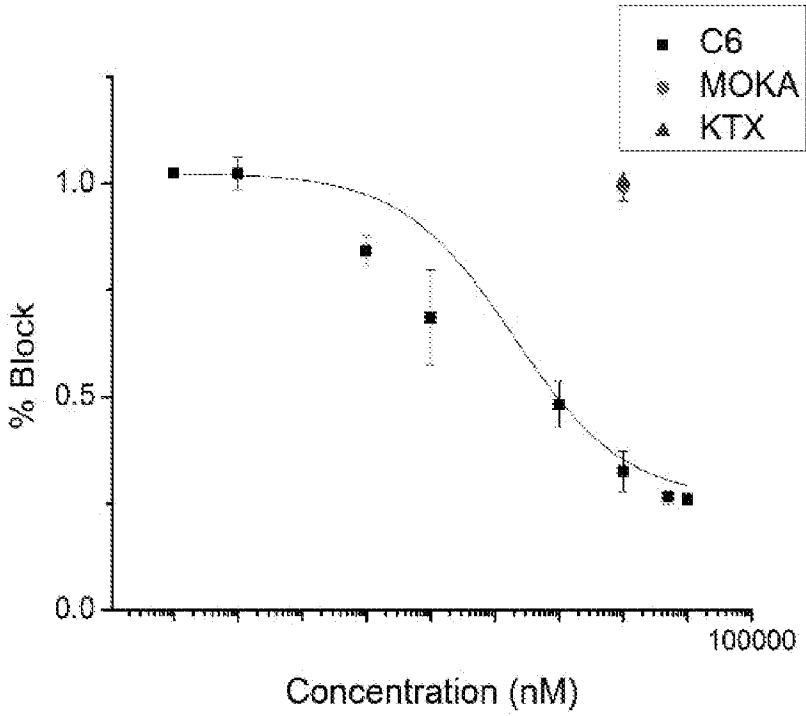


FIG. 7

FIG. 7A (SEQ ID NO: 807)

ATGTCTGCACTTCTGATCCTAGCTCTTGTGGAGCTGCAatgAGCAGCACCTGCATTCCGAGCGG
 CCAGCCGTGTGCGGATAGCGATGATTGCTGCGAAACCTTTCATTGCAAATGGGTGTTTTTACCA
 GCAAATTTATGTGCCGCGCGTGTGGGGCAAAGATaccggtGTTGCTATCGATTACAAAGACGATG
 ACGACAAGCTTGCGGCCGCTggtaacggaaatggcaacgggaatggcaacggtaacggaaacgggGATGTA
 CTCGagtTGTGTGGGCCAGGACACGCAGGAGGTCATCGTGGTGCCACACTCCTTGCCCTTTAA
 GGTGGTGGTGTCTCAGCCATCCTGGCCCTGGTGGTGTCTACCATCATCTCCCTTATCATCCTCAT
 CATGCTTTGGCAGAAGAAACCACGTAGGATCCACCGGCCGGTCGCCACCatggtgagcaagggcgag
 gagctgttcaccgggggtggtgcccacctggtcgagctggacggcgacgtaaaccggccacaagttcagcgtgtccggcgagg
 gcgagggcgatgccacctacggcaagctgaccctgaagctgatctgcaccaccggcaagctgccgtgccctggcccaccct
 cgtgaccaccctgggctacggccttcagtgccttcgcccgtacccccgaccacatgaagcagcacgacttcttcaagtccgcat
 gcccgaaggctacgtccaggagcgccacatcttcttcaaggacgacggcaactacaagacccgcgcccaggtgaagttcga
 gggcgacaccctggtgaaccgcatcgagctgaaggcgatcgacttcaaggaggacggcaacatcctggggcacaagctgg
 agtacaactacaacagccacaacgtctatatcaccgcccagacaagcagaagaacggcatcaaggccaactcaagatccgcc
 acaacatcgaggacggcggtgcagctcgccgaccactaccagcagaacacccccatcgggcgacggccccgtgctgctgc
 ccgacaaccactacctgagctaccagtccaaactgagcaaaagaccccaacgagaagcgcgatcacatggtcctgctggagtt
 cgtgaccgcccgggatcactctcggcacggacgagctgtacaagTAA

FIG. 7B (SEQ ID NO: 808)

MSALLILALVGAAMSSSTCIPSGQPCADSDCCETFHCKWVFFTSKFMCRVWGKDTGVAIDYKDDD
 DKLAAAGNGNGNGNGNGNGDGTAVAVGQDTQEVIVVPHSLPFKVVVISAILALVLTIIISLIILIML
 WQKKPRRIHRPVATMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKICTTGKLP
 VPWPTLVTTLYGLOCFARYPDHMKOHDFFKSAMPEGYVOERTIFFKDDGNYKTRAEVKFEGDTLV
 NRIELKGIDFKEDGNILGHKLEYNNSHNVIYITADKQKNGIKANFKIRHNIEDGGVOLADHYQONTPI
 GDGPVLLPDNHYLSYQSKLSKDPNEKRDHMLVLEFVTAAGITLGMDELYK

KEY:

Secretory Sequence

C6 sequence

Linker sequences

PDGFR transmembrane helix

Flag epitope

mVenus

FIG. 8

FIG. 8A

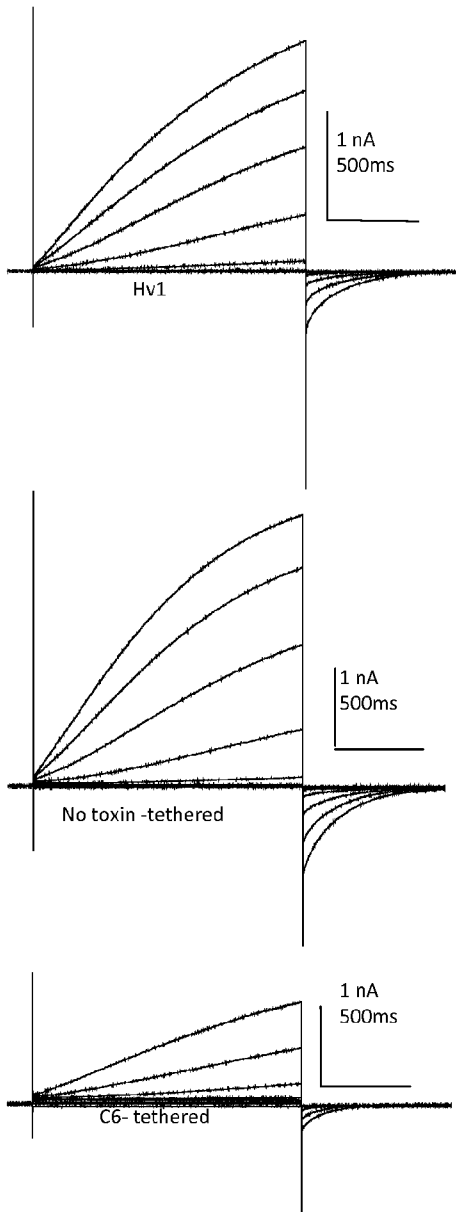


FIG. 8B

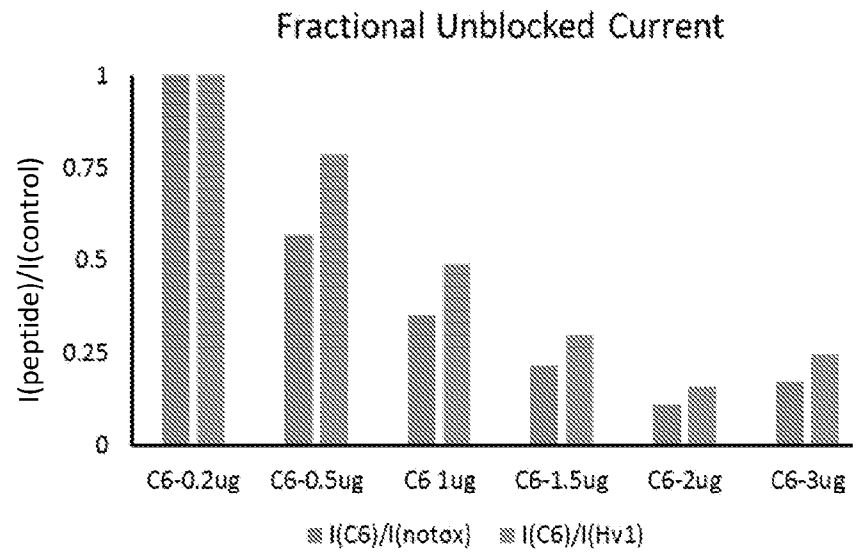


FIG. 8C

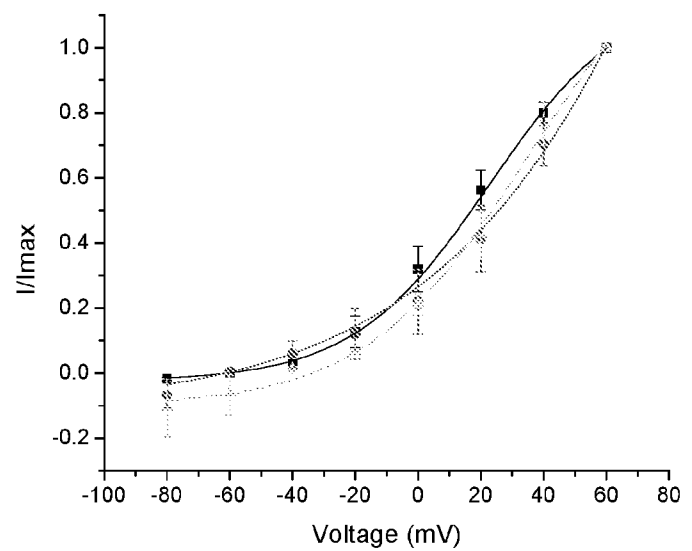


FIG. 8D

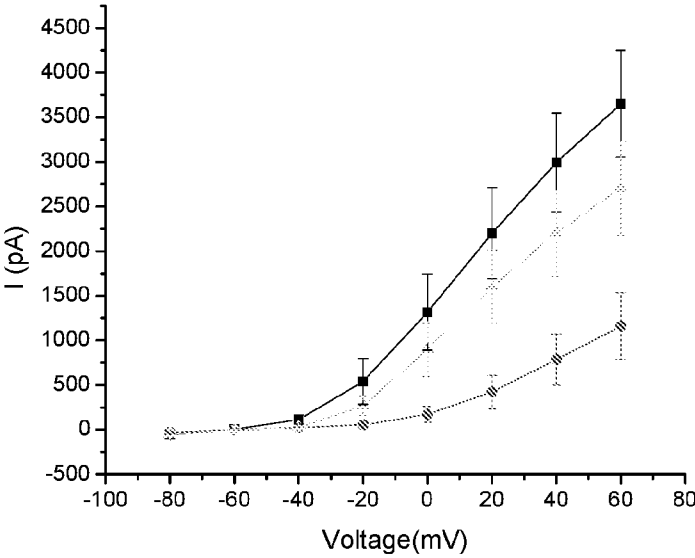


FIG. 8E

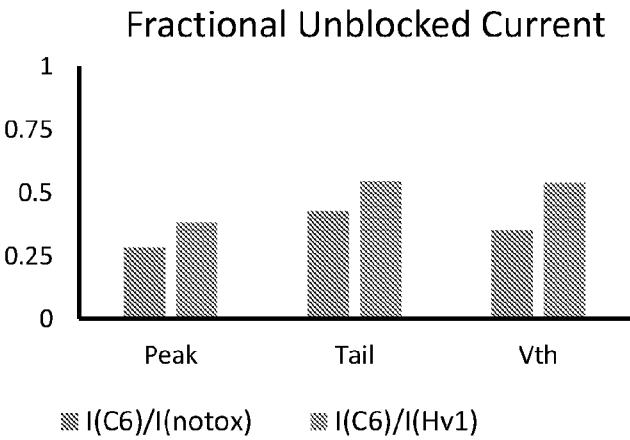


FIG. 8F

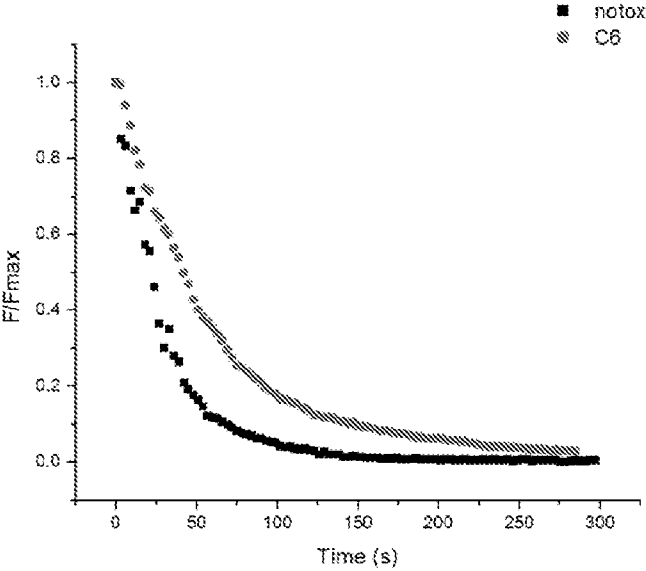


FIG. 8G

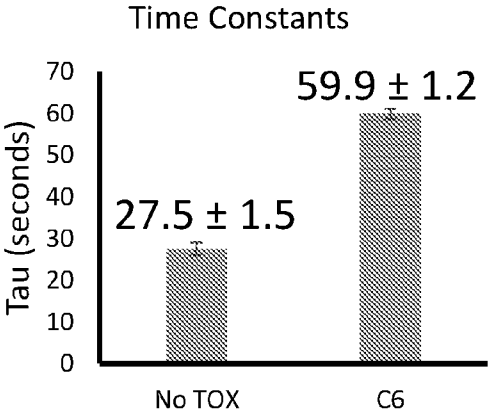


FIG. 9

Name	SEQ ID NO:	Sequence	Blocks
CiHv1	113	VVVVISFGVDIALIFVGESEALAAIGLLVILRLWRVFR	NO
hHv1	114	VVVVVSFILDIVLLFQ-E-HQFEALGLLILLRLWRVAR	YES
hS3S4CiHv1	811	VVVVISFILDIVLLFQ-E-HQFEALGLLILLRLWRVFR	YES
ciS3S4hHv1	816	VVVVVSFILDIVLLFVGESEALAAIGLLVILRLWRVAR	
shortN	817	VVVVISFGVDIALIFQ-E-HQFEALGLLILLRLWRVFR	
shortNC	818	VVVVISFGVDIALIFQ-E-HQFEALGLLILLRLWRVFR	
shortC	819	VVVVISFILDIVLLFQ-E-HQFEALGLLILLRLWRVFR	
ciS3S4hHv1-2	812	VVVVVSFGVDIALIFVGESEALAAIGLLVILRLWRVAR	NO
shortN-2	813	VVVVISFGVDIALLFQ-E-HQFEALGLLILLRLWRVFR	X
shortNC-2	814	VVVVISFGVDIALLFQ-E-HQFAAIGLLVILRLWRVFR	
shortC-2	815	VVVVISFILDIVLLFQ-E-HQFAAIGLLVILRLWRVFR	
shortNC-3	820	VVVVISFGVDIALLFQ-E-HQFEALGLLVILRLWRVFR	X
shortC-3	821	VVVVISFILDIVLLFQ-E-HQFEALGLLVILRLWRVFR	

FIG. 9A

FIG. 9B

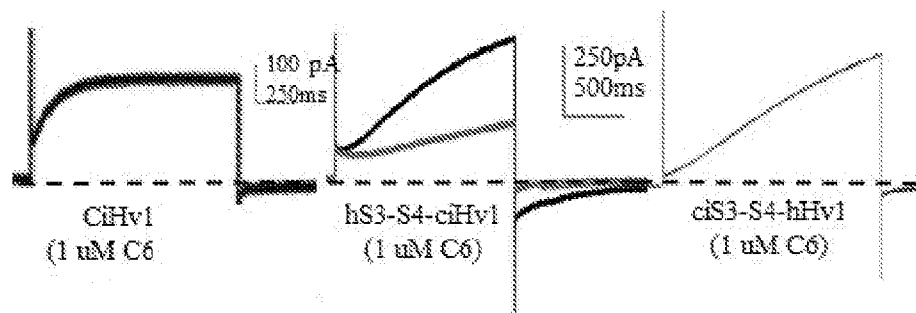
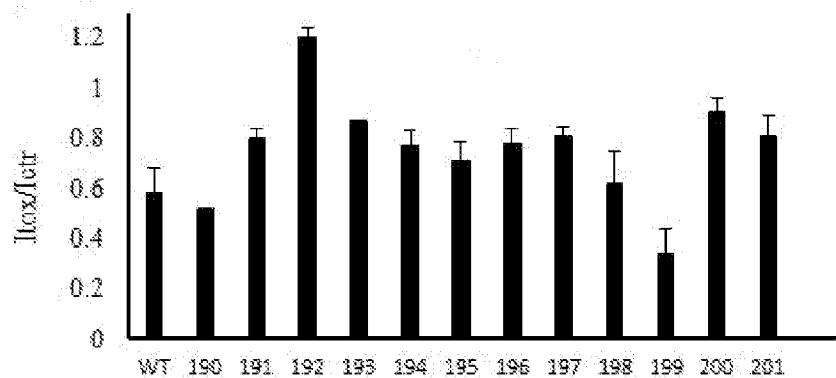


FIG. 9C



A. CLASSIFICATION OF SUBJECT MATTER**C07K 14/00(2006.01)i, C07K 7/06(2006.01)i, C07K 7/08(2006.01)i, A61K 38/00(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 14/00; C12N 5/071; G01N 33/487; G01N 27/26; G01N 33/68; C12N 1/19; C07K 7/06; C07K 7/08; A61K 38/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: voltage-gated proton channel, modulating agent, venom, inhibitor cysteine knot

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015-0087552 A1 (D.E. SHAW RESEARCH, LLC et al.) 26 March 2015 See claims 1, 21-23; paragraphs [0014], [0091], [0525]-[0530], [0644], [0671]; Table 4; and Examples 9-11.	7-13, 17-32, 90-92
Y		
A		1-6, 14-16, 89 59-79
Y	SOKOLOV, STANISLAV et al., 'Inhibition of sodium channel gating by trapping the domain II voltage sensor with protoxin II', Molecular Pharmacology, 2008, Vol.73, No.3, pp.1020-1028 See abstract; and page 1021.	1-6, 14-16, 89
A	US 2012-0270236 A1 (RAMSEY, IAN S. et al.) 25 October 2012 See the whole document.	1-32, 59-79, 89-92
A	US 2015-0203812 A1 (TOMBOLA, FRANCESCO) 23 July 2015 See the whole document.	1-32, 59-79, 89-92
A	US 7132505 B1 (LAZDUNSKI, MICHEL et al.) 07 November 2006 See the whole document.	1-32, 59-79, 89-92

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

23 April 2018 (23.04.2018)

Date of mailing of the international search report

23 April 2018 (23.04.2018)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

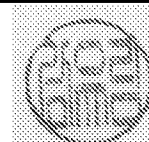
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Authorized officer

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Telephone No. +82-42-481-3516



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/068896

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	KENNEDY, KELLEIGH et al., `(2042-Pos) A designer peptide toxin isolated by phage display that inhibits the human voltage-gated proton channel, hHv1', Biophysical Journal, February 2017, Vol.112, No.3, Supplement 1, page 415a See the whole document.	1-32, 89-92

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/068896

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

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

1. ☒ Claims Nos.: 81-86
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 81-86 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.
2. ☒ Claims Nos.: 34,37,38,40-43,45-47,49,50,52,53,55,81-88
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 34, 37, 38, 40-43, 45-47, 49, 50, 52, 53, 55 and 81-88 directly or indirectly refer to one of unsearchable claims which do not comply with PCT Rule 6.4(a).
3. ☒ Claims Nos.: 33,35,36,39,44,48,51,54,56-58,80
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2017/068896

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