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(54) **MECHANICALLY-ACTIVATED CATION CHANNELS**

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(57) **ABSTRACT**

Methods of screening for agents that modulate the activity of a mechanically-activated cation channel are provided. Also provided are compositions and methods for ameliorating pain by antagonizing or inhibiting mechanically-activated cation channels.

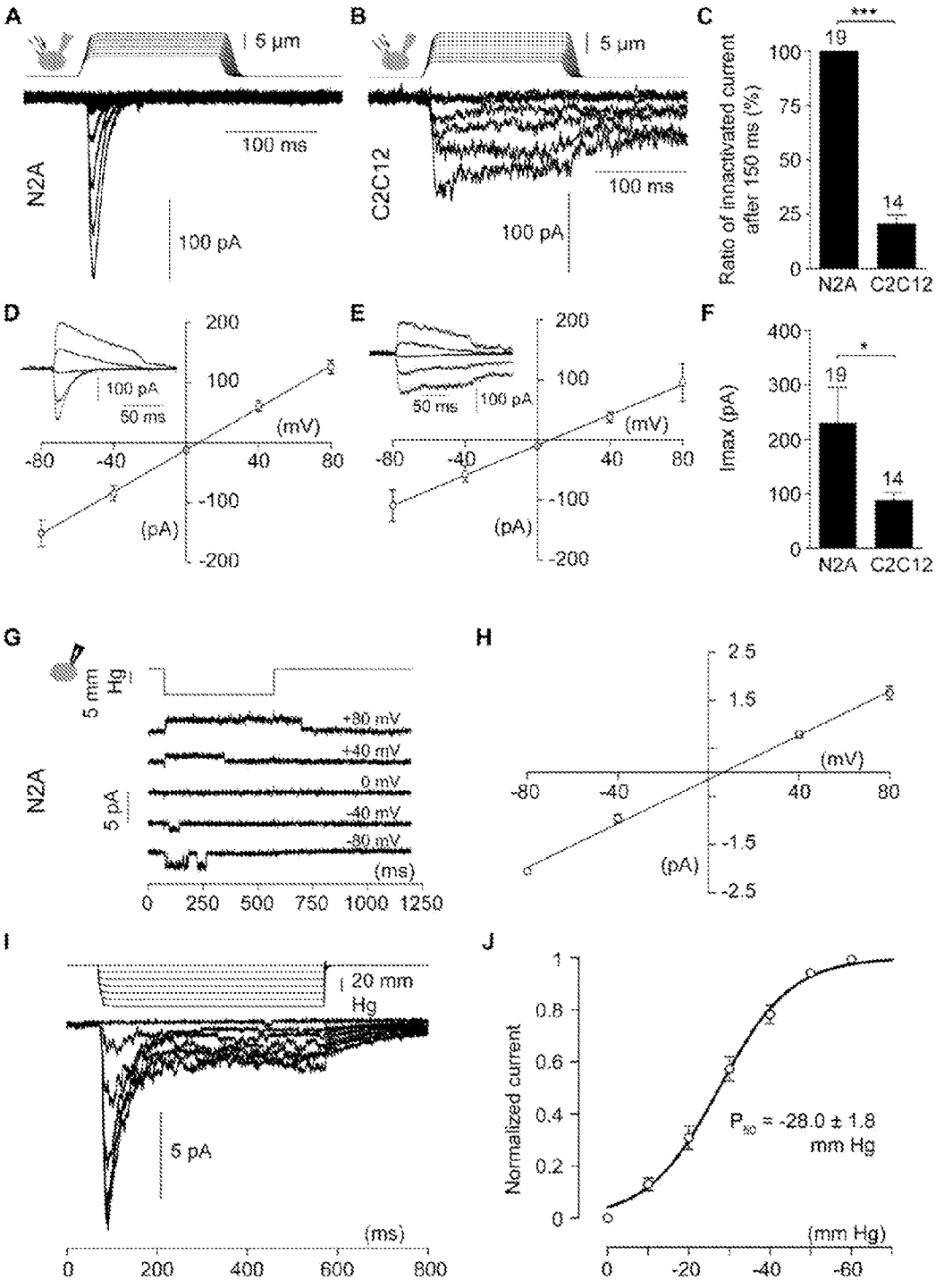
A
5 µm
100 ms
N2A
100 pA
B
5 µm
C2C12
100 ms
100 pA
C
***
19
14
Ratio of inactivated current after 150 ms (%)
100
75
50
25
0
N2A C2C12
D
200
100
100 pA
50 ms
(mV)
-80 -40 40 80
-100
(pA)
-200
E
200
100
50 ms
100 pA
(mV)
-80 -40 40 80
-100
(pA)
-200
F
*
19
14
Imax (pA)
400
300
200
100
0
N2A C2C12
G
5 mm Hg
N2A
5 pA
+80 mV
+40 mV
0 mV
-40 mV
-80 mV
(ms)
0 250 500 750 1000 1250
H
2.5
1.5
(mV)
-80 -40 40 80
-1.5
(pA)
-2.5
I
20 mm Hg
5 pA
(ms)
0 200 400 600 800
J
Normalized current
1
0.8
0.6
0.4
0.2
0
P50 = -28.0 ± 1.8 mm Hg
(mm Hg)
0 -20 -40 -60

FIG. 1

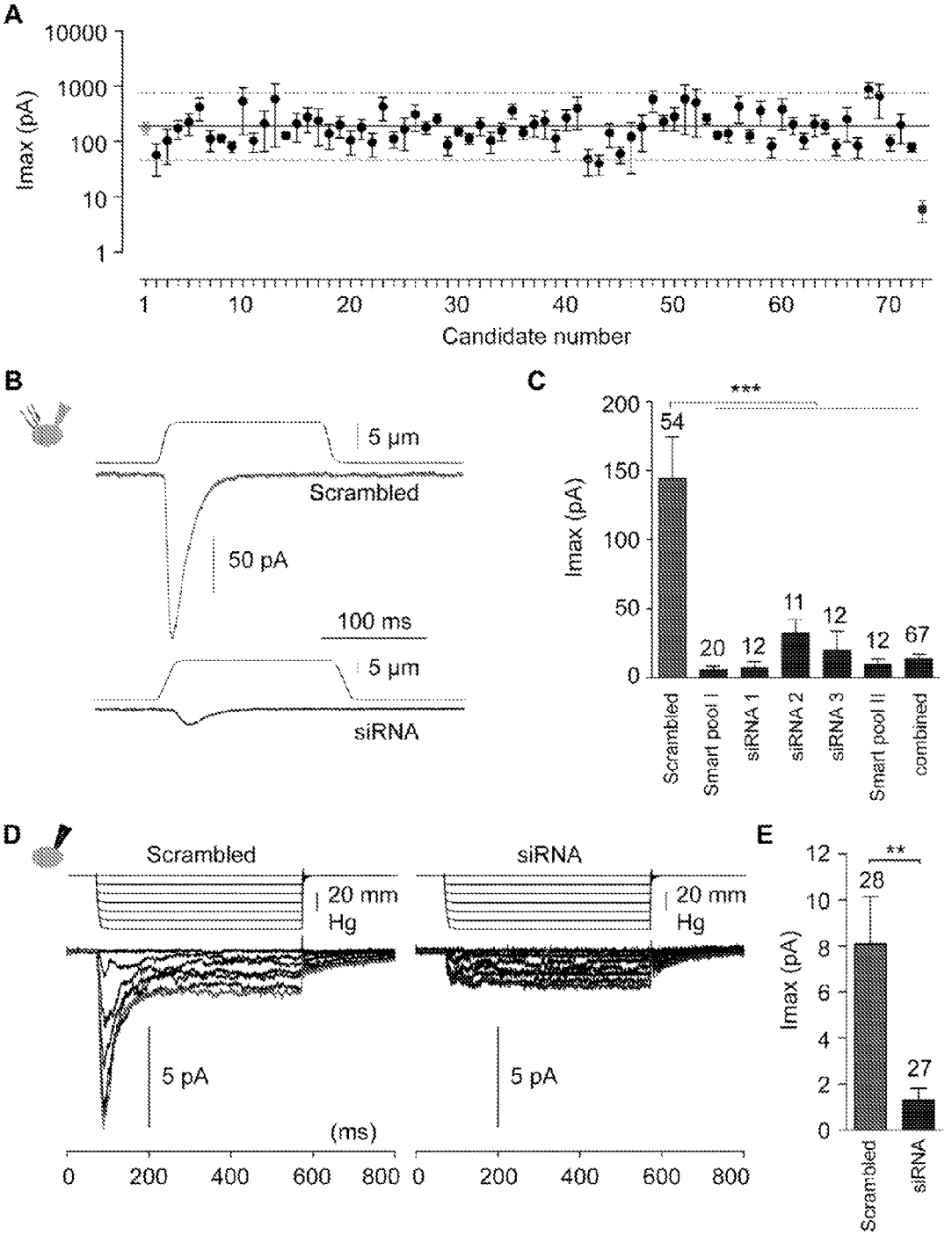
A
Imax (pA)
10000
1000
100
10
1
1
10
20
30
40
50
60
70
Candidate number
B
5 µm
Scrambled
50 pA
100 ms
5 µm
siRNA
C
***
Imax (pA)
200
150
100
50
0
54
20
12
11
12
12
67
Scrambled
Smart pool I
siRNA 1
siRNA 2
siRNA 3
Smart pool II
combined
D
Scrambled
siRNA
20 mm Hg
20 mm Hg
5 pA
5 pA
(ms)
0
200
400
600
800
0
200
400
600
800
E
**
Imax (pA)
12
10
8
6
4
2
0
28
27
Scrambled
siRNA

FIG. 2

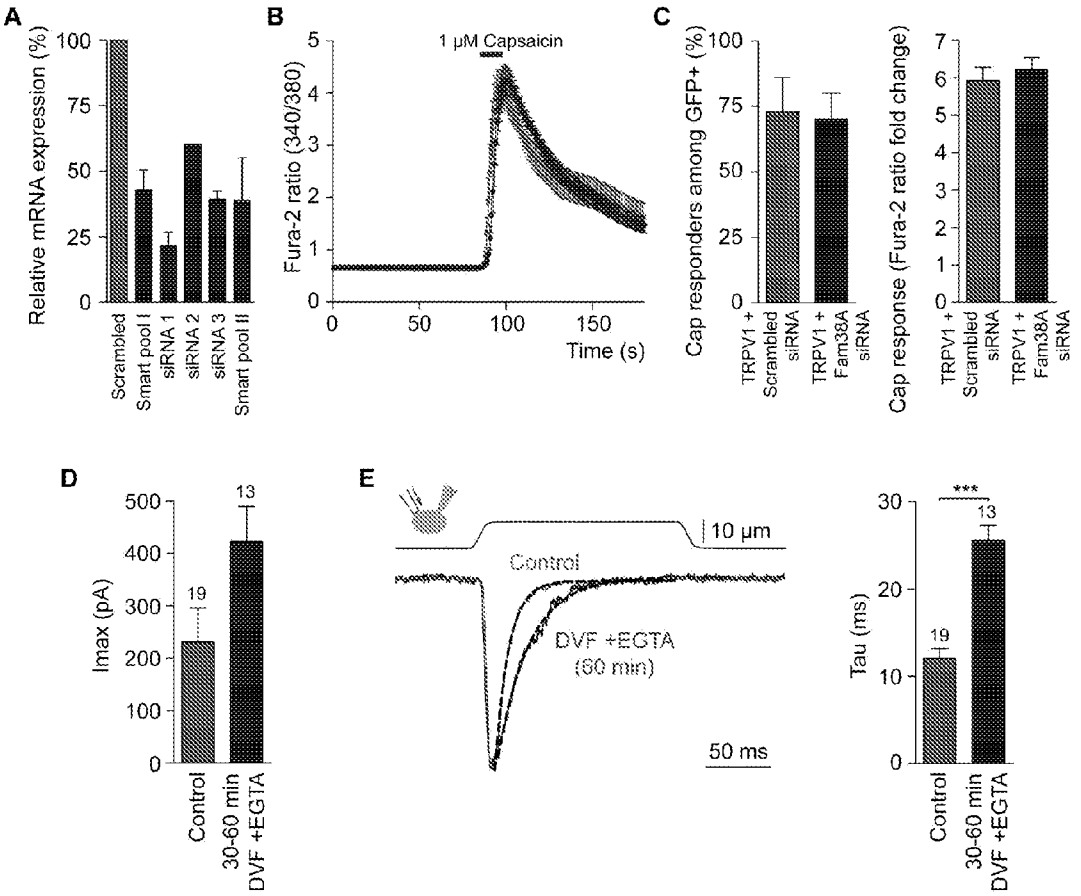
A
Relative mRNA expression (%)
100
75
50
25
0
Scrambled
Smart pool I
siRNA 1
siRNA 2
siRNA 3
Smart pool II
B
1 μM Capsaicin
Fura-2 ratio (340/380)
5
4
3
2
1
0
0
50
100
150
Time (s)
C
Cap responders among GFP+ (%)
100
75
50
25
0
TRPV1 + Scrambled siRNA
TRPV1 + Fam38A siRNA
Cap response (Fura-2 ratio fold change)
7
6
5
4
3
2
1
0
TRPV1 + Scrambled siRNA
TRPV1 + Fam38A siRNA
D
Imax (pA)
500
400
300
200
100
0
19
13
Control
30-60 min DVF +EGTA
E
10 μm
Control
DVF +EGTA (60 min)
50 ms
Tau (ms)
30
20
10
0
***
19
13
Control
30-60 min DVF +EGTA

FIG. 3

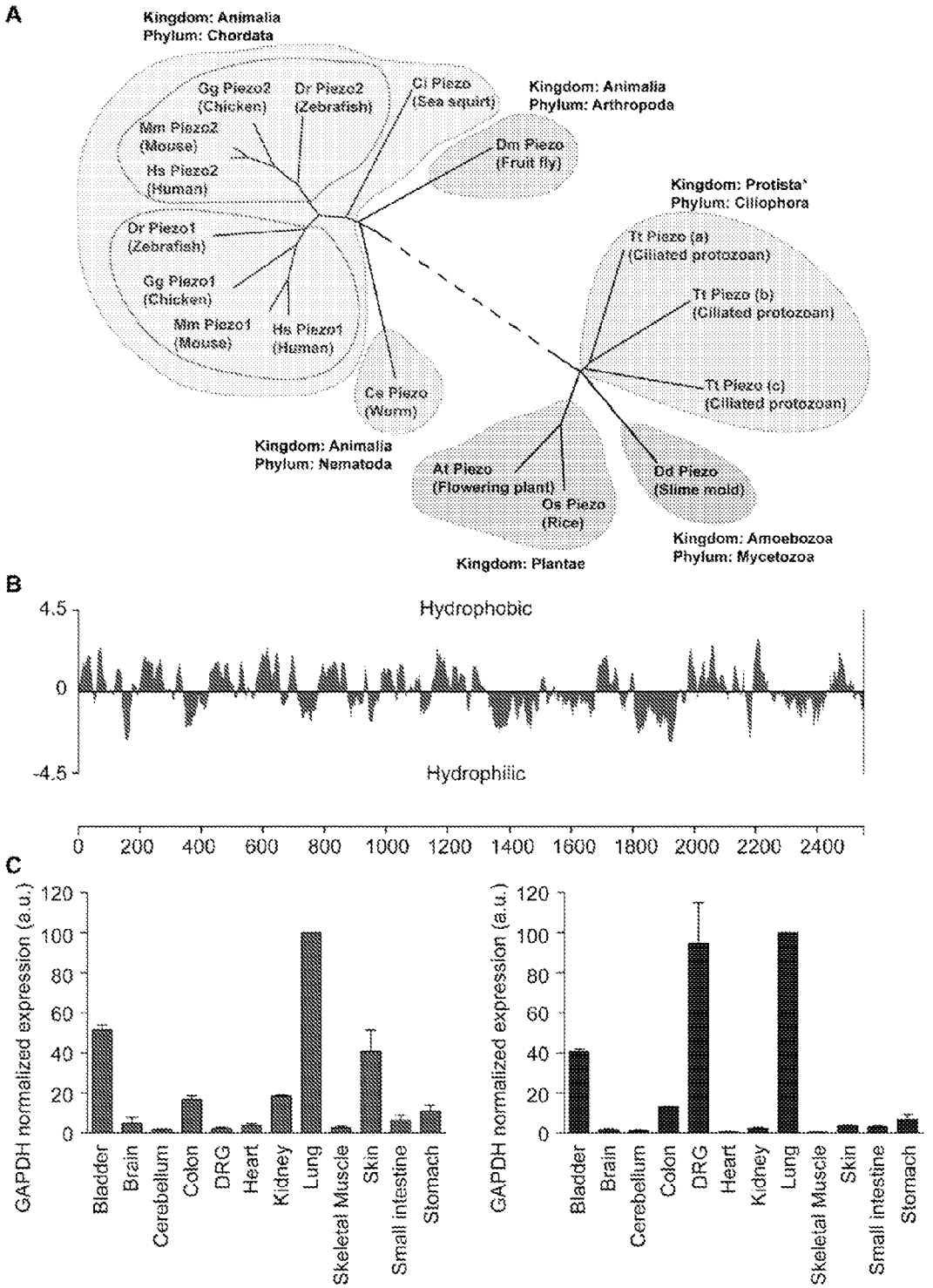
A
Kingdom: Animalia
Phylum: Chordata
Gg Piezo2 (Chicken)
Dr Piezo2 (Zebrafish)
Ci Piezo (Sea squirt)
Kingdom: Animalia
Phylum: Arthropoda
Mm Piezo2 (Mouse)
Hs Piezo2 (Human)
Dm Piezo (Fruit fly)
Kingdom: Protista*
Phylum: Ciliophora
Dr Piezo1 (Zebrafish)
Tt Piezo (a) (Ciliated protozoan)
Gg Piezo1 (Chicken)
Tt Piezo (b) (Ciliated protozoan)
Mm Piezo1 (Mouse)
Hs Piezo1 (Human)
Tt Piezo (c) (Ciliated protozoan)
Ce Piezo (Worm)
Kingdom: Animalia
Phylum: Nematoda
At Piezo (Flowering plant)
Dd Piezo (Slime mold)
Os Piezo (Rice)
Kingdom: Amoebozoa
Phylum: Mycetozoa
Kingdom: Plantae
B
Hydrophobic
4.5
0
-4.5
Hydrophilic
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400
C
GAPDH normalized expression (a.u.)
0 20 40 60 80 100 120
Bladder
Brain
Cerebellum
Colon
DRG
Heart
Kidney
Lung
Skeletal Muscle
Skin
Small intestine
Stomach
GAPDH normalized expression (a.u.)
0 20 40 60 80 100 120
Bladder
Brain
Cerebellum
Colon
DRG
Heart
Kidney
Lung
Skeletal Muscle
Skin
Small intestine
Stomach

FIG. 4

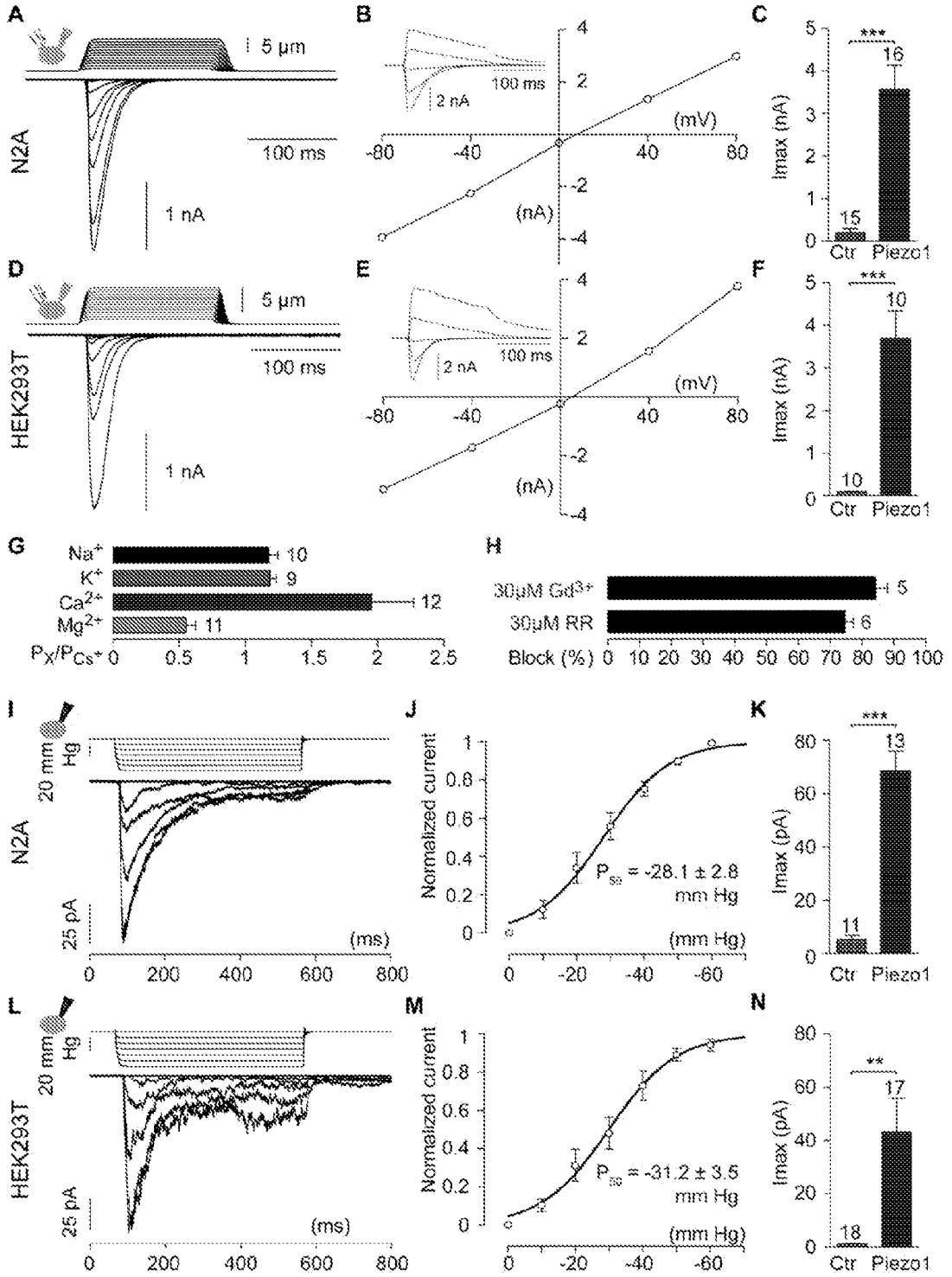
A
5 µm
N2A
100 ms
1 nA
B
100 ms
2 nA
(mV)
(nA)
C
Imax (nA)
Ctr
Piezo1
D
HEK293T
E
F
G
Na+
K+
Ca2+
Mg2+
PX/PCs+
H
30µM Gd3+
30µM RR
Block (%)
I
20 mm Hg
25 pA
(ms)
J
Normalized current
P50 = -28.1 ± 2.8 mm Hg
(mm Hg)
K
Imax (pA)
L
P50 = -31.2 ± 3.5 mm Hg
M
N

FIG. 5

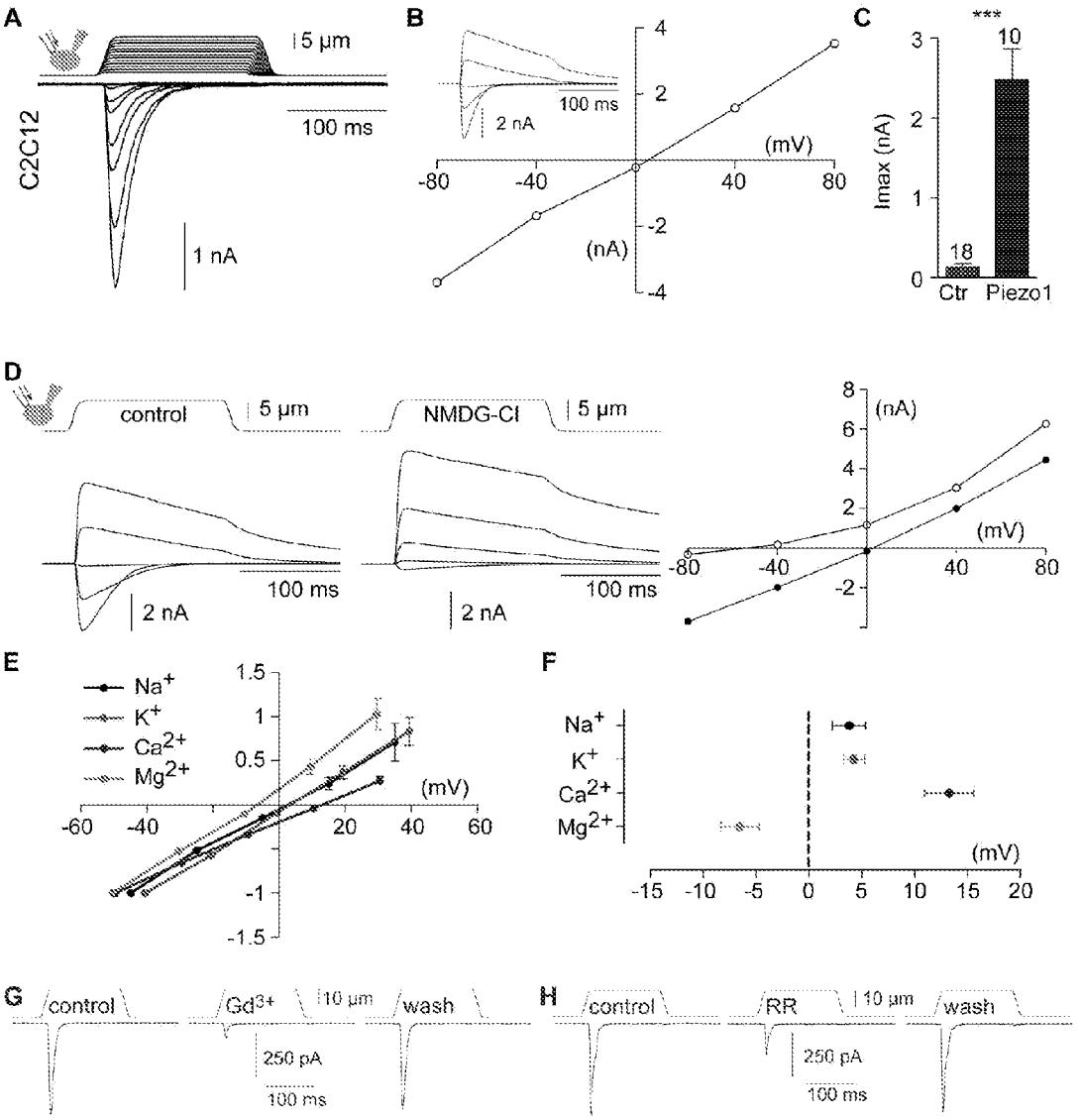
A
5 µm
C2C12
100 ms
1 nA
B
100 ms
2 nA
(mV)
(nA)
C
***
10
18
Imax (nA)
Ctr
Piezo1
D
control
5 µm
NMDG-Cl
5 µm
100 ms
2 nA
(nA)
(mV)
E
Na+
K+
Ca2+
Mg2+
(mV)
F
Na+
K+
Ca2+
Mg2+
(mV)
G
control
Gd3+
10 µm
wash
250 pA
100 ms
H
control
RR
10 µm
wash
250 pA
100 ms

FIG. 6

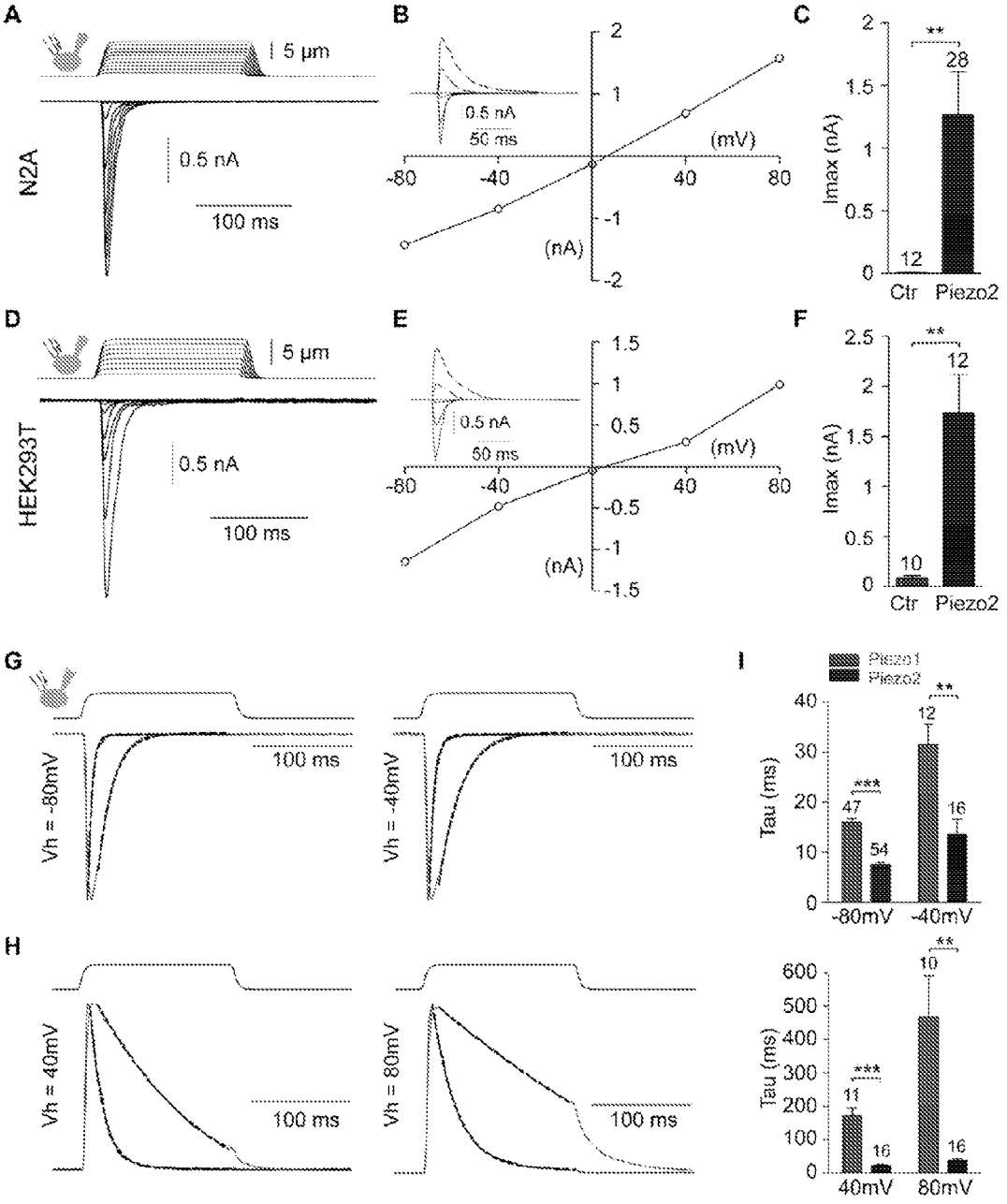
A
5 μm
N2A
0.5 nA
100 ms
B
0.5 nA
50 ms
(mV)
-80
-40
40
80
2
1
-1
-2
(nA)
C
**
28
12
Imax (nA)
2
1.5
1
0.5
0
Ctr
Piezo2
D
5 μm
HEK293T
0.5 nA
100 ms
E
0.5 nA
50 ms
(mV)
-80
-40
40
80
1.5
1
0.5
-0.5
-1
-1.5
(nA)
F
**
12
10
Imax (nA)
2.5
2
1.5
1
0.5
0
Ctr
Piezo2
G
Vh = -80mV
100 ms
Vh = -40mV
100 ms
H
Vh = 40mV
100 ms
Vh = 80mV
100 ms
I
Piezo1
Piezo2
**
***
12
47
54
16
Tau (ms)
40
30
20
10
0
-80mV
-40mV
**
***
10
11
16
16
Tau (ms)
600
500
400
300
200
100
0
40mV
80mV

FIG. 7

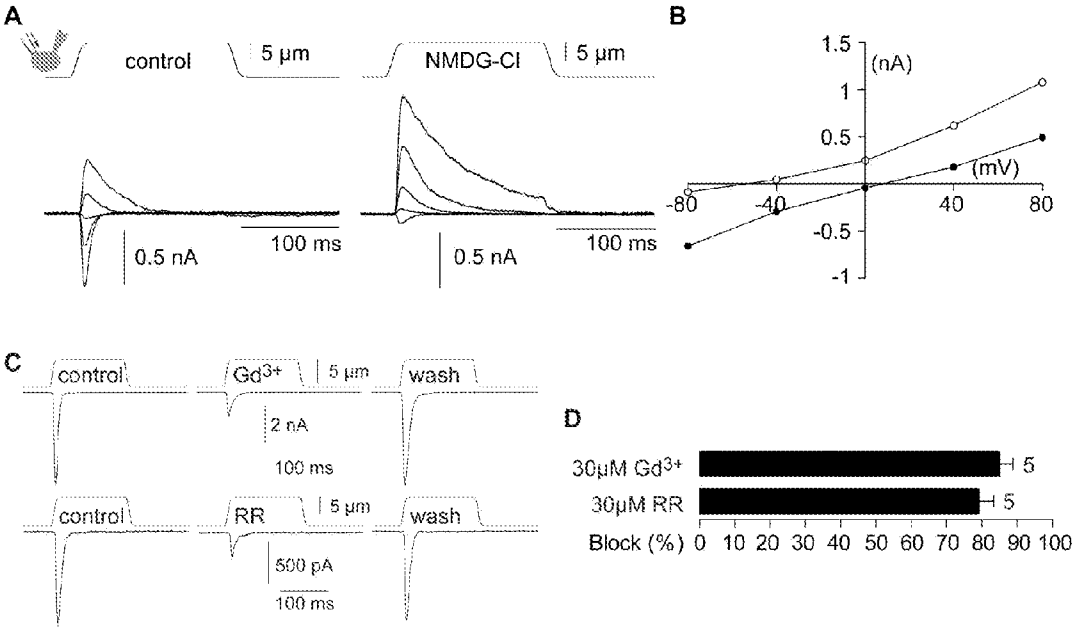
A
control
5 μm
NMDG-Cl
5 μm
0.5 nA
100 ms
0.5 nA
100 ms
B
1.5
(nA)
1
0.5
(mV)
-80
-40
40
80
-0.5
-1
C
control
Gd3+
5 μm
wash
2 nA
100 ms
control
RR
5 μm
wash
500 pA
100 ms
D
30μM Gd3+
5
30μM RR
5
Block (%) 0 10 20 30 40 50 60 70 80 90 100

FIG. 8

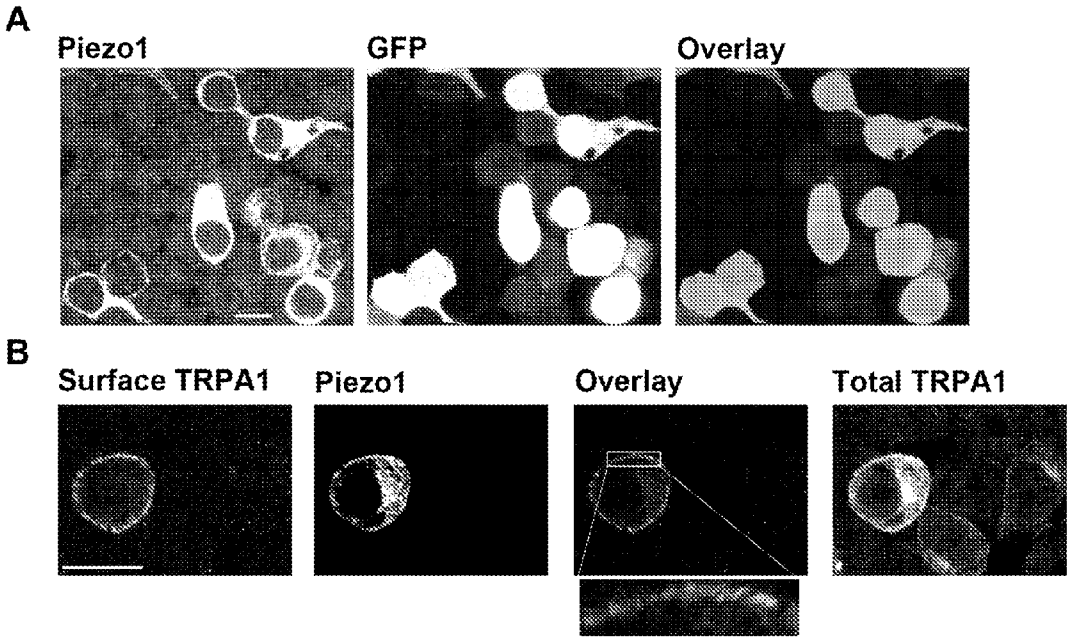
A
Piezo1
GFP
Overlay
B
Surface TRPA1
Piezo1
Overlay
Total TRPA1

FIG. 9

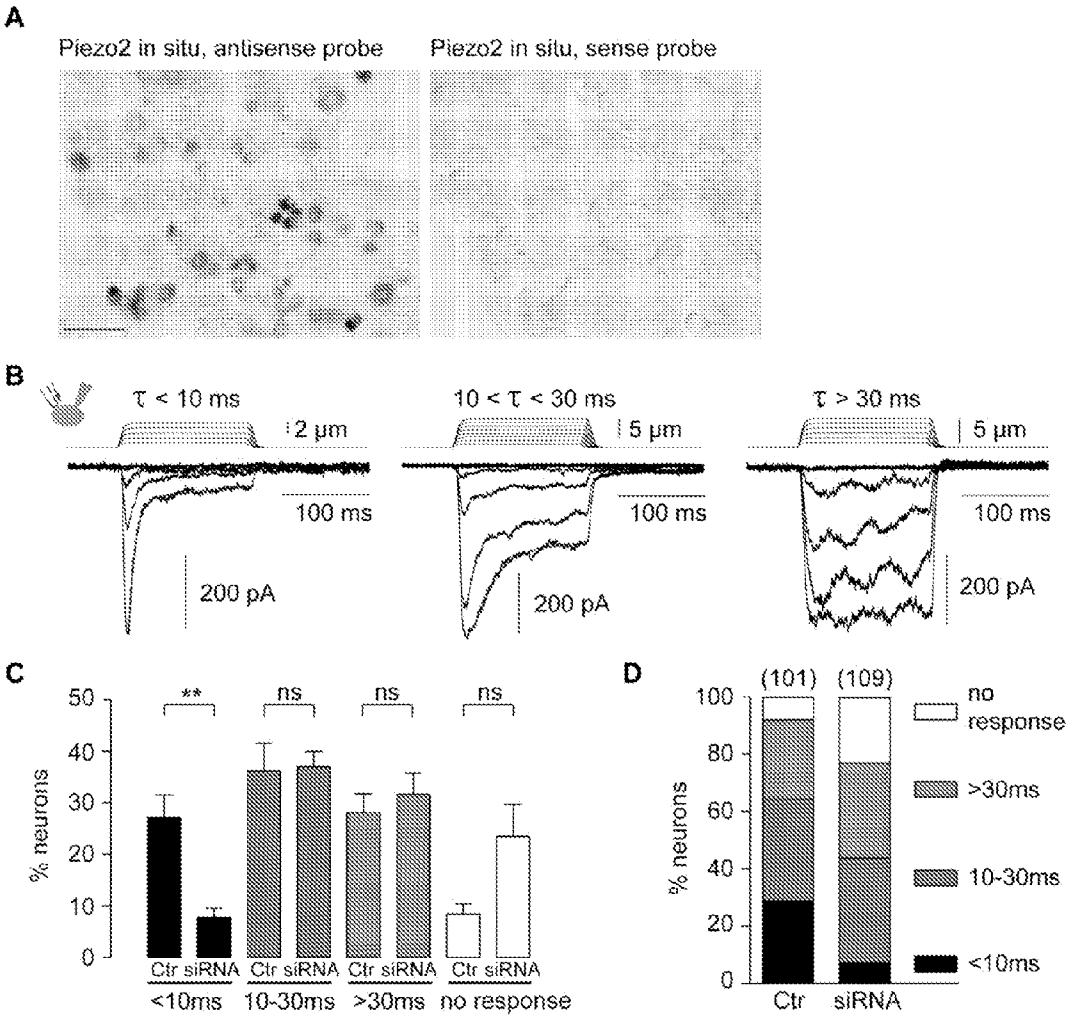
A
Piezo2 in situ, antisense probe
Piezo2 in situ, sense probe
B
τ < 10 ms
2 µm
100 ms
200 pA
10 < τ < 30 ms
5 µm
100 ms
200 pA
τ > 30 ms
5 µm
100 ms
200 pA
C
% neurons
50
40
30
20
10
0
**
ns
ns
ns
Ctr siRNA Ctr siRNA Ctr siRNA Ctr siRNA
<10ms 10-30ms >30ms no response
D
% neurons
100
80
60
40
20
0
(101)
(109)
no response
>30ms
10-30ms
<10ms
Ctr
siRNA

FIG. 10

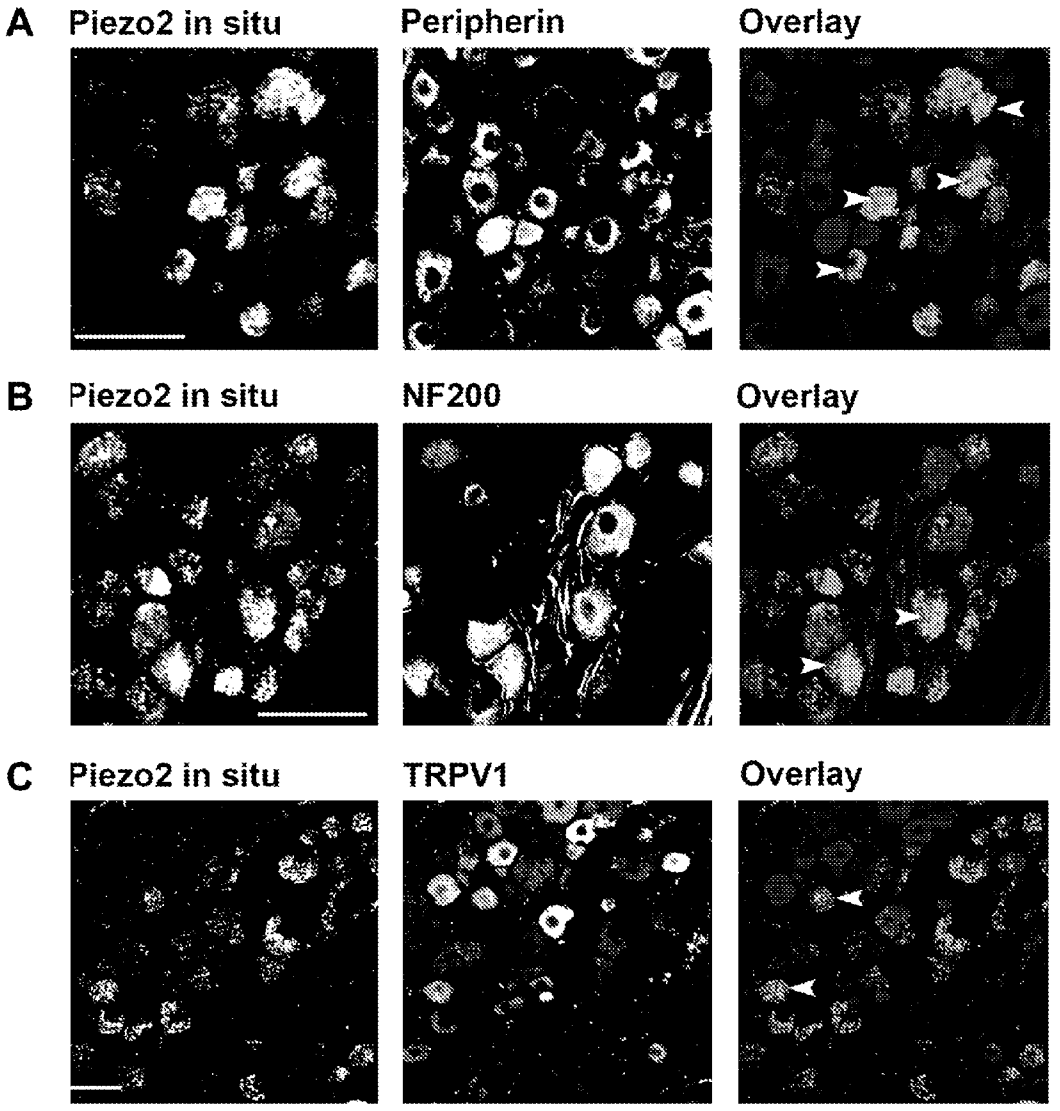
A
Piezo2 in situ
Peripherin
Overlay
B
Piezo2 in situ
NF200
Overlay
C
Piezo2 in situ
TRPV1
Overlay

FIG. 11

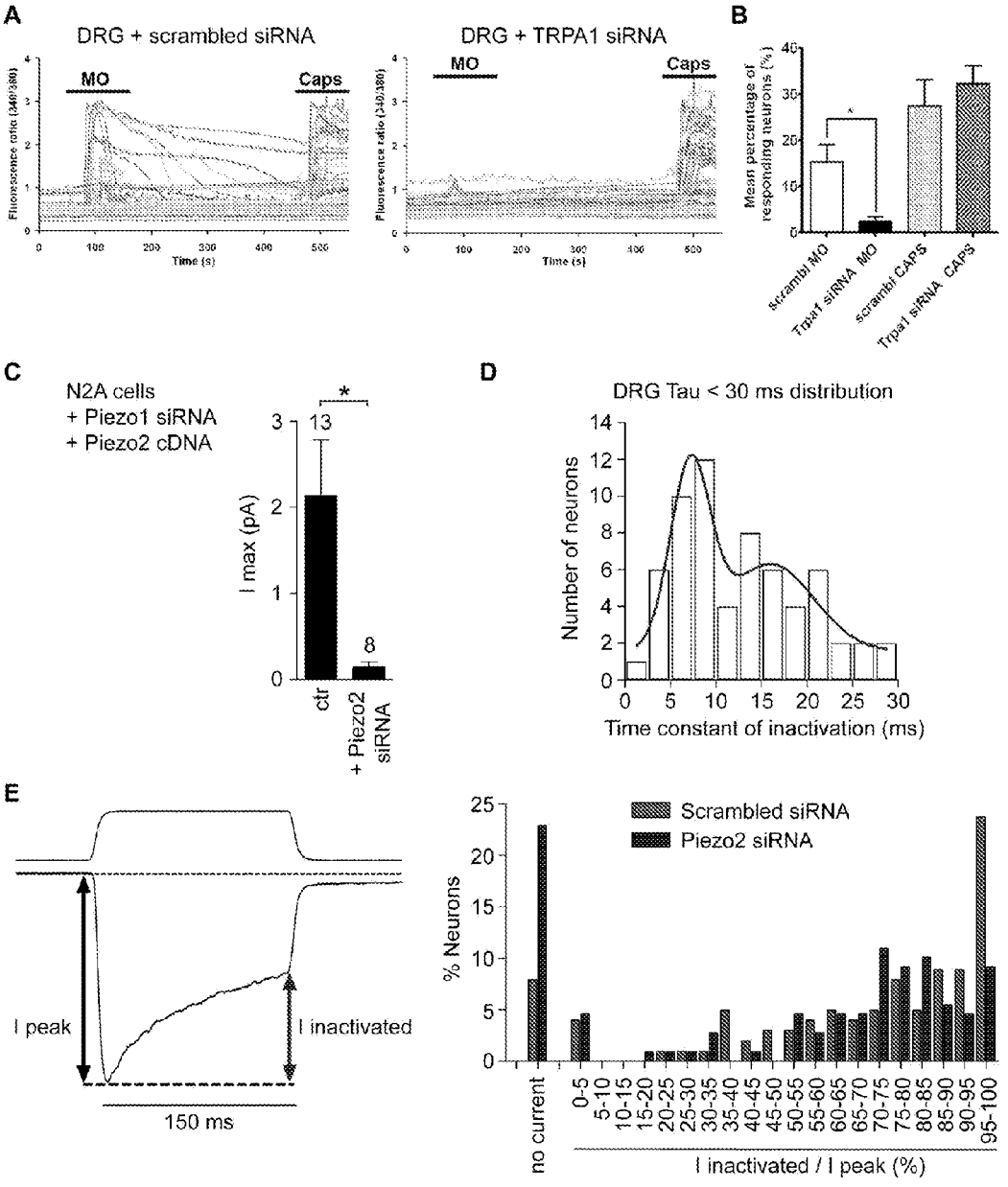
A
DRG + scrambled siRNA
DRG + TRPA1 siRNA
MO
Caps
Time (s)
B
Mean percentage of responding neurons (%)
Scramb MO
Trpa1 siRNA MO
scramb CAPS
Trpa1 siRNA CAPS
C
N2A cells
+ Piezo1 siRNA
+ Piezo2 cDNA
I max (pA)
13
8
ctr
+ Piezo2 siRNA
D
DRG Tau < 30 ms distribution
Number of neurons
Time constant of inactivation (ms)
E
I peak
I inactivated
150 ms
Scrambled siRNA
Piezo2 siRNA
% Neurons
no current
I inactivated / I peak (%)

FIG. 12

MECHANICALLY-ACTIVATED CATION CHANNELS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/376,182, filed Aug. 23, 2010. This priority application is hereby incorporated by reference in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with Government support under Grant No. DE016927 and NS046303 awarded by the National Institutes of Health. The government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Mechanotransduction, the conversion of mechanical force into biological signals, has crucial roles in physiology. In mammals, embryonic development, touch, pain, proprioception, hearing, adjustment of vascular tone and blood flow, flow sensing in kidney, lung growth and injury, bone and muscle homeostasis as well as metastasis are all regulated by mechanotransduction (M. Chalfie, *Nat. Rev. Mol. Cell. Biol.* 10, 44 (January 2009); O. P. Hamill, B. Martinac, *Physiol Rev* 81, 685 (April 2001)). In plants, mechanical force strongly impacts morphogenesis, for example in lateral root formation (G. B. Monshausen, S. Gilroy, *Trends Cell Biol.* 19, 228 (May 2009). Even unicellular organisms such as ciliates sense touch and change direction in response to a tactile stimulus (K. Iwatsuki, T. Hirano, *Comp. Biochem. Physiol. A. Physiol.* 110, 167 (February 1995)).

[0004] Electrophysiological recordings from vertebrate inner ear hair cells show that mechanotransduction is extremely rapid, implicating an ion channel directly activated by force D. P. Corey, A. J. Hudspeth, *Biophys. J.* 26, 499 (June 1979). Indeed, calcium-permeable mechanically-activated (MA) cationic currents have been described in various mechanosensitive cells, including in dorsal root ganglion (DRG) neurons (G. C. McCarter, D. B. Reichling, J. D. Levine, *Neurosci. Lett.* 273, 179), in kidney primary cilium (H. A. Praetorius, K. R. Spring, J Membr Biol 184, 71 (Nov. 1, 2001)), and in plants (G. B. Monshausen, S. Gilroy, *Trends Cell Biol.* 19, 228 (May 2009)). However, few MA channels have been identified to date.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides methods of screening for an agent that modulates the activity of a mechanically-activated cation channel. In some embodiments, the method comprises: contacting a mechanically-activated cation channel polypeptide having at least 70% amino acid sequence identity to one of SEQ ID NOs:2, 4, 18, or 20 with an agent; and selecting the agent that modulates the activity of the mechanically-activated cation channel polypeptide.

[0006] In some embodiments, the polypeptide is expressed in a cell and the contacting comprises contacting the cell with the agent. In some embodiments, the polypeptide is heterologous to the cell. In some embodiments, the cell comprises a heterologous expression cassette comprising a promoter operably linked to a polynucleotide encoding the mechanically-activated cation channel polypeptide. In some embodiments, the polynucleotide comprises SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:17, or SEQ ID NO:19. In other embodiments, the polypeptide is endogenous to the cell. In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a neuron.

[0007] In some embodiments, the activity of the mechanically-activated cation channel polypeptide is determined by measuring an electrophysiological change mediated by the polypeptide. In certain embodiments, the electrophysiological change is a change in membrane potential, a change in current, or an influx of a cation. In some embodiments, the membrane potential is measured with a membrane potential dye assay. In some embodiments, the electrophysiological change is measured with a patch-clamp assay. In certain embodiments, the measuring comprises measuring a mechanically-activated electrophysiological change.

[0008] In some embodiments, the method further comprises testing an agent identified as modulating the activity of the mechanically-activated cation channel polypeptide for the ability to modulate a mechanically-activated electrophysiological change.

[0009] In some embodiments, the selected agent reduces or inhibits the electrophysiological change mediated by the polypeptide. In some embodiments, the selected agent increases the electrophysiological change mediated by the polypeptide.

[0010] In some embodiments, the cell is in an animal. In some embodiments, the animal is a mouse. In some embodiments, the method further comprises administering the agent to the animal and determining the effect of the agent on pain sensitivity.

[0011] In some embodiments, the polypeptide comprises SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20.

[0012] The present invention also provides antibodies that antagonize the activity of a mechanically-activated cation channel.

[0013] In some embodiments, the antibody selectively binds to a mechanically-activated cation channel polypeptide having at least 70% amino acid sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20. In certain embodiments, the polypeptide comprises SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20.

[0014] In some embodiments, the antibody is a monoclonal antibody. In certain embodiments, the antibody is a chimeric antibody. In other embodiments, the antibody is a humanized antibody.

[0015] The present invention also provides methods of ameliorating pain in a subject. In some embodiments, the method comprises administering to the subject an antibody that selectively binds to a mechanically-activated cation channel polypeptide having at least 70% amino acid sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20.

[0016] In some embodiments, the method comprises administering an antibody that selectively binds to a mechanically-activated cation channel polypeptide comprising SEQ ID NO:4 or SEQ ID NO:20. In some embodiments, the polypeptide is expressed in bladder, colon, kidney, lung, or skin. In some embodiments, the polypeptide is expressed in a dorsal root ganglion neuron.

[0017] In some embodiments, the subject is a mammal. In certain embodiments, the subject is a human.

[0018] In some embodiments, the pain is selected from the group consisting of acute mechanical pain, chronic mechanical pain, mechanical hyperalgesia, mechanical allodynia, arthritis, inflammation, dental pain, cancer pain, and labor pain.

[0019] The present invention also provides isolated antisense oligonucleotides or small interfering RNAs (siRNAs) complementary to at least 15 contiguous nucleotides of a polynucleotide that is at least 70% identical to SEQ ID NOs: 1, 3, 17, or 19 and encoding a mechanically-activated cation channel polypeptide, wherein the antisense oligonucleotide or siRNA inhibits production of the mechanically-activated cation channel polypeptide.

[0020] In some embodiments, the antisense oligonucleotide or small interfering RNA (siRNA) is complementary to at least 15 contiguous nucleotides of SEQ ID NOs: 1, 3, 17, or 19. In some embodiments, the antisense oligonucleotide or siRNA comprises any of SEQ ID NOs:5-16.

[0021] The present invention also provides expression cassettes comprising a promoter operably linked to a polynucleotide comprising the antisense oligonucleotide or siRNA complementary to at least 15 contiguous nucleotides of a polynucleotide that is at least 70% identical to SEQ ID NOs: 1, 3, 17, or 19 and encoding a mechanically-activated cation channel polypeptide, wherein the antisense oligonucleotide or siRNA inhibits production of the mechanically-activated cation channel polypeptide. The present invention also provides vectors comprising said expression cassettes and cells comprising said expression cassettes and/or said vectors.

[0022] The present invention also provides methods of ameliorating pain in a subject, the method comprising administering to the subject an antisense oligonucleotide or small interfering RNA (siRNA) complementary to at least 15 contiguous nucleotides of a polynucleotide that is at least 70% identical to SEQ ID NOs:1, 3, 17, or 19 and encoding a mechanically-activated cation channel polypeptide, wherein the antisense oligonucleotide or siRNA inhibits production of the mechanically-activated cation channel polypeptide.

[0023] In some embodiments, the antisense oligonucleotide or siRNA inhibits production of the mechanically-activated cation channel in bladder, colon, kidney, lung, or skin. In certain embodiments, the antisense oligonucleotide or siRNA inhibits production of the mechanically-activated cation channel in a dorsal root ganglion neuron.

[0024] In some embodiments, the subject is a mammal. In certain embodiments, the subject is a human.

[0025] In some embodiments, the pain is selected from the group consisting of acute mechanical pain, chronic mechanical pain, mechanical hyperalgesia, mechanical allodynia, arthritis, inflammation, dental pain, cancer pain, and labor pain.

DEFINITIONS

[0026] As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0027] The term "mechanically-activated cation channel" refers to an ion channel that opens to allow passage of positively charged ions (i.e. cations) into and out of a cell in response to mechanical force or pressure being applied, e.g., to a cell expressing the channel. As used herein, the term also includes polypeptide components of mechanically-activated cation channels, e.g., subunits of a cation channel. In some embodiments, the mechanically-activated cation channels of the present invention are substantially identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20. In some embodiments, the mechanically-activated cation channels of the present invention are involved in sensory transduction, such as pain transduction, including but not limited to, cells such as neurons.

[0028] "Inhibitors," "activators," and "modulators" of mechanically-activated cation channel polypeptide activity are used interchangeably herein to refer to inhibitory, activating, or modulating molecules identified using in vitro and in vivo assays for sensory (e.g., pain or somatosensory) transduction, e.g., ligands, agonists, antagonists, and their homologs and mimetics. The term "modulator" encompasses inhibitors and activators. Inhibitors are compounds that, e.g., bind to, partially or totally block stimulation, decrease, prevent, delay activation, inactivate, desensitize, or down regulate signal transduction, e.g., antagonists. Activators are compounds that, e.g., bind to, stimulate, increase, open, activate, facilitate, enhance activation, sensitize, or up regulate signal transduction, e.g., agonists. Modulators include naturally occurring and synthetic ligands, antagonists, agonists, small chemical molecules and the like. Such assays for inhibitors and activators include, e.g., expressing a mechanically-activated cation channel polypeptide in cells or cell membranes, applying putative modulator compounds, and then determining the functional effects on ion flux, membrane potential, electrophysiology, or mechanical activation. Samples or assays comprising a mechanically-activated cation channel polypeptide that is treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of modulation. Control samples (untreated with inhibitors) are assigned a relative mechanically-activated cation channel polypeptide activity value of 100%. Inhibition of a mechanically-activated cation channel polypeptide is achieved when the mechanically-activated cation channel polypeptide activity value relative to the control is about 80%, optionally 75%, 50%, or 25-0%. Activation of the mechanically-activated cation channel polypeptide is achieved when the mechanically-activated cation channel polypeptide activity value relative to the control is 110%, optionally 125%, optionally 150%, optionally 200-500%, or 1000-3000% higher.

[0029] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0030] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka et al., *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini et al.,

Mol. Cell. Probes 8:91-98 (1994)). The term "nucleic acid" encompasses the terms gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0031] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0032] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, .gamma.-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0033] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG, and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0034] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0035] The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);

[0036] 2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);

7) Serine (S), Threonine (T); and

[0037] 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, *Proteins* (1984)).

[0038] The terms "isolated," "purified," or "biologically pure" refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least 85% pure, optionally at least 95% pure, and optionally at least 99% pure.

[0039] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed, or not expressed at all.

[0040] The term "heterologous" when used with reference to a protein's or nucleic acid's relationship to a cell indicates that the protein or nucleic acid is not found in the same relationship to the cell (e.g., not expressed in the cell) in nature. The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

[0041] A "promoter" is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand

base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0042] An "expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression cassette can be part of a plasmid, virus, or nucleic acid fragment. Typically, the expression cassette includes a nucleic acid to be transcribed operably linked to a promoter.

[0043] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. Sequences are "substantially identical" of they have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 70% identity, optionally 75%, 80%, 85%, 90%, or 95% identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region (a specified length, or when not specified, the entire length) as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. The present invention provides sequences substantially identical to, e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, or SEQ ID NO:20. Optionally, the identity exists over a region that is at least about 15 amino acids or nucleotides in length, or over a region that is at least about 18 amino acids or nucleotides in length, about 20 amino acids or nucleotides in length, about 22 amino acids or nucleotides in length, about 25-50 amino acids or nucleotides in length, or about 75-100 amino acids or nucleotides in length or more.

[0044] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0045] A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection (see, e.g., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Ausubel et al., eds. 1995 supplement)).

[0046] Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al.; *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul et al., *J. Mol. Biol.* 215:403-410 (1990), respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always>0) and N penalty score for mismatching residues; always<0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=−4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=−4, and a comparison of both strands.

[0047] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0048] The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

[0049] The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic

acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10° C. lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C. for short probes (e.g., 10 to 50 nucleotides) and at least about 60° C. for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, optionally 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5×SSC, and 1% SDS, incubating at 42° C., or, 5×SSC, 1% SDS, incubating at 65° C., with wash in 0.2×SSC, and 0.1% SDS at 65° C.

[0050] Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37° C., and a wash in 1×SSC at 45° C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

[0051] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0052] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0053] Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_{H1} by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (see FUNDAMENTAL IMMUNOLOGY (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (see, e.g., McCafferty et al., *Nature* 348:552-554 (1990)).

[0054] For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (see, e.g., Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor et al., *Immunology Today* 4:72 (1983); Cole et al., pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy* (1985)). Techniques for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., *Nature* 348:552-554 (1990); Marks et al., *Biotechnology* 10:779-783 (1992)).

[0055] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

[0056] A "humanized antibody" is an antibody that retains the reactivity of a non-human antibody while being less immunogenic in humans. This can be achieved, for instance, by retaining the non-human CDR regions and replacing the remaining parts of the antibody with their human counterparts. See, e.g., Morrison et al., *Proc. Natl. Acad. Sci. USA,* 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.,* 44:65-92 (1988); Verhoeyen et al., *Science,* 239:1534-1536 (1988); Padlan, *Molec. Immun.,* 28:489-498 (1991); Padlan, *Molec. Immun.,* 31(3):169-217 (1994).

[0057] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised

to Piezo1 or Piezo2 from specific species such as rat, mouse, or human can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with Piezo1 or Piezo2 and not with other proteins, except for polymorphic variants and alleles of Piezo1 or Piezo2. This selection may be achieved by subtracting out antibodies that cross-react with Piezo1 or Piezo2 molecules from other species. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

[0058] A "subject" or "individual" refers to an animal, including a human, non-human primate, mouse, rat, rabbit, dog, or other mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

[0059] FIG. **1**. Neuro2A and C2C12 cells display different types of mechanically-activated currents. (A, B) Representative traces of mechanically-activated (MA) inward currents expressed in Neuro2A (N2A, A) and C2C12 (B) cells. The cells were subjected to a series of mechanical steps in 1 μm increments using a stimulation pipette (inset drawing, arrow) in the whole-cell patch configuration at a holding potential of −80 mV. (C) Ratio of inactivated current at the end of a mechanical step (150 ms duration) relative to the peak current (mean±SEM, number of cells above the bars) in N2A and C2C12 cells for currents elicited at a holding potential of −80 mV. ***, P<0.001. (D, E) Average current-voltage relationships of MA currents in N2A (D, n=11) and C2C12 (E, n=4) cells. Inset, representative MA currents evoked at holding potentials ranging from −80 to +40 mV (applied 0.7 sec prior to the mechanical step). (F) Average maximal amplitude of MA inward currents elicited in N2A and C2C12 cells at a holding potential of −80 mV (mean±SEM, the number of cells tested is shown above the bars). *, P<0.05. (G) Single-channel currents (cell attached patch configuration) induced by negative pipette pressure (inset drawing, arrow) at holding potentials ranging from −80 mV to +80 mV in a N2A cell. (H) Average current-voltage relationships of stretch-activated single channels in N2A cells (n=4, mean±SEM). Single channel conductance is calculated from the slope of the linear regression line of each cell giving γ=22.9±1.4 pS (mean±SEM). Single channel amplitude was determined as the amplitude difference in Gaussian fits of full trace histograms. (I) Representative currents (averaged traces) induced by negative pipette pressure (0 to −60 mm Hg, Δ 10 mm Hg) in a N2A cell. (J) Normalized current-pressure relationship of stretch activated currents at −80 mV fitted with a Boltzmann equation (n=21). P_{50} is the average value of P_{50}s from individual cells.

[0060] FIG. **2**. Suppression of mechanically-activated currents by Piezo1 (Fam38A) siRNA. (A) Average maximal amplitude of MA inward currents elicited at a holding potential of −80 mV in N2A cells transfected with scrambled siRNA (far left dot, n=56), Piezo1 (Fam38A) siRNA (bottom right dot, n=20) or siRNA directed against other candidates tested (black dots, list of candidates available as Table S2). For each candidate, black circle and error bar represents the mean±SEM, n=4-27 each. The black line represents the average value of all cells tested (n=807), and the two dashed lines represent 4-fold decrease or increase of this value. (B) Representative traces of MA inward currents expressed in N2A cells transfected with scrambled siRNA (top trace) or Piezo1 (Fam38A) siRNA (bottom trace) at a holding potential of −80 mV. (C) Average maximal amplitude of MA inward currents elicited at a holding potential of −80 mV in N2A cells transfected either with scrambled siRNA (left bar) or different Piezo1 (Fam38A) siRNAs. siRNA 1, 2, and 3 are siRNAs of smart-pool I tested individually. (D) Representative currents (averaged traces) induced by negative pipette pressure (0 to −60 mm Hg, Δ 10 mm Hg, cell attached) in a N2A cell transfected with scrambled siRNA (left panel) or Piezo1 siRNA (right panel). Traces of current elicited by −60 mm Hg are highlighted. (E) Average maximal amplitude of stretch-activated currents elicited at a holding potential of −80 mV in N2A cells transfected with scrambled siRNA (left bar) or Piezo1 siRNA (right bar). Bars represent the mean±SEM, and the number of cells tested is shown above the bars. **, P<0.01. ***, P<0.001.

[0061] FIG. **3**. Piezo1 siRNA qPCR and cell viability control, and N2A MA currents after disruption of integrin function. (A) siRNA-induced down-regulation of Piezo1 mRNA in N2A cells. Transfected and untransfected cells are unsorted and thus these differences are underestimated. (B) Representative ratiometric calcium imaging experiment of capsaicin stimulated N2A cells co-transfected with TRPV 1 and GFP, together with either scrambled siRNA or Piezo1 siRNA (mean±SEM of GFP-positive cell traces). (C) Percentage of GFP-positive cells responding to capsaicin (left panel, mean±SEM of two experiments) and Fura-2 340/380 ratio fold change of capsaicin responding cells (mean±SEM). (D) Maximal current amplitude of whole cell MA currents elicited in N2A cells at a holding potential of −80 mV in control conditions or after 30-60 minutes perfusion with a divalent free solution containing 5 mM EGTA. (E) Representative traces of MA currents normalized to peak in control conditions or after 30-60 minutes perfusion with a divalent free solution containing 5 mM EGTA (left panel). Inactivation of currents is fitted with a mono-exponential equation. In the absence of divalent cations, the time constant for inactivation is higher than with control solution (right panel). ***, P<0.001, unpaired t-test.

[0062] FIG. **4**. Evolutionary conservation, hydrophobicity plot, and expression profile of Piezo1 and Piezo2. (A) Unrooted phylogenetic tree showing sequence relationship of different members of the Piezo family of proteins. The alignments were generated using Megalign and DrawTree programs. The dotted line represents an artificially extended line to accommodate fit. Hs, *Homo Sapiens*; Mm, *Mouse musculus*; Gg, *Gallus gallus*, Dr, *Danio Rerio*; Ci, *Ciona intestinalis*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Tt, *Tetrahymena thermophila* (accession numbers are provided in Methods). (B) Hydrophobicity analysis of mouse Piezo1. The Kyte-Doolittle pattern (19 residues window) shows succession of hydrophobic and hydrophilic regions. 30 transmembrane domains are predicted by TMHMM2. (C) mRNA expression profiles of Piezo1 (left panel) and Piezo2 (right panel) determined by qPCR from various adult mouse tissues. GAPDH was used as

the reference gene and lung as the tissue calibrator using the $2^{-\Delta\Delta CT}$ method. Each bar is the mean±SEM of the average of two separate experiments.

[0063] FIG. **5**. Piezo1 induces large, mechanically-activated nonselective cationic currents. (A-F) MA currents of Piezo1-expressing N2A (A-C) and HEK293T (D-F) cells recorded in the whole-cell configuration. (A, D) Representative traces of MA inward currents expressed in different cell types transfected with Piezo1. The cell was subjected to a series of mechanical steps in 1 μm (A) or 0.5 μm (D) increments using glass probe stimulation and at a holding potential of −80 mV. (B, E) Representative current-voltage relationships of MA currents expressed in different cell types transfected with Piezo1. Inset, MA currents evoked at holding potentials ranging from −80 to +40 mV. (C, F) Average maximal amplitude of MA inward currents elicited at a holding potential of −80 mV in Piezo1-transfected (right bar) or mock-transfected (left bar) cells. Bars represent the mean±SEM, and the number of cells tested is shown above the bars. ***, P<0.001. (G) P_X/P_{Cs} ion selectivity ratios of MA currents in Piezo1-expressing cells. (H) Percent block of MA currents in Piezo1-expressing cells by 30 μM gadolinium or ruthenium red. (I-N) Stretch-activated currents of mouse Piezo1-expressing N2A (I-K) and HEK293T (L-N) cells in cell-attached configuration. Representative averaged currents induced by negative pipette pressure (0 to −60 mm Hg, A 10 mm Hg) in N2A (I) and HEK293T (L) cells transfected with Piezo1. Normalized current-pressure relationship of stretch-activated currents elicited at −80 mV in Piezo1-transfected N2A (J, n=12) and HEK293T (M, n=11) cells and fitted with a Boltzmann equation. P_{50} is the average value of all P_{50}s determined for individual cells. Average maximal amplitude of stretch-activated currents elicited at a holding potential of −80 mV in N2A (K) and HEK293T (N) cells mock-transfected (left bar) or transfected with Piezo1 (right bar). Bars represent the mean±SEM, and the number of cells tested is shown above the bars. ***, P<0.001. **, P<0.01.

[0064] FIG. **6**. Piezo1-induced MA currents are cationic non-selective currents blocked by gadolinium and ruthenium red. (A-C) MA currents of Piezo1-expressing C2C12 cells recorded in the whole-cell configuration. (A) Representative traces of MA inward currents expressed in Piezo1-transfected cells. The cell is subjected to a series of mechanical steps in 1 μm increments using glass probe stimulation and at a holding potential of −80 mV. (B) Representative current-voltage relationships of MA currents expressed in Piezo1-transfected cells. Inset, MA currents evoked at holding potentials ranging from −80 to +40 mV. (C) Average maximal amplitude of MA inward currents elicited at a holding potential of −80 mV in Piezo1-transfected (right bar) or mock-transfected (left bar) cells. Bars represent the mean±SEM, and the number of cells tested is shown above the bars. ***, P<0.001, unpaired t-test with Welch's correction. (D) Whole-cell MA current traces elicited in a Piezo1 transfected cell bathed in control solution (left panel) or after perfusion with 150 mM NMDG-Cl (middle panel) solution. Currents are elicited from −80 mV to +80 mV in 40 mV steps. Right panel shows the MA current-voltage relationship from the same cell. Note that inward currents present in control condition (filled symbols) are suppressed with external NMDG-Cl solution (open symbol). (E-F) Average current-voltage relationship of MA currents elicited in Piezo1 transfected HEK293T cells and recorded with CsCl-based internal solution and 150 mM NaCl—, 150 mM KCl—, 100 mM $CaCl_2$- or 100 mM $MgCl_2$-based extracellular solutions. (E) I-V relationships from individual cells were normalized to the value at −40 mV before liquid junction potentials were corrected. (F) Average of reversal potential values determined for each recording conditions and for individual cells (mean±SEM). (G-H) Representative current traces of MA currents elicited in Piezo1 transfected cells before, during and after perfusion of 30 μM gadolinium (E) or ruthenium red (F).

[0065] FIG. **7**. Piezo2 induces large mechanically-activated currents kinetically distinct from Piezo1-induced currents. (A-F) MA currents of Piezo2-expressing N2A (A-C) and HEK293T (D-F) cells in whole-cell configuration. In N2A cells, Piezo2 or vector only were co-transfected with Piezo1 siRNA to suppress endogenous Piezo1-dependent MA currents. (A, D) Representative traces of MA inward currents expressed in different cell types transfected with Piezo2. The cell was subjected to a series of mechanical steps in 1 μm increments using glass probe stimulation at a holding potential of −80 mV. (B, E) Representative current-voltage relationships of MA currents expressed in different cell types transfected with Piezo2. Inset, MA currents evoked at holding potentials ranging from −80 to +40 mV. (C, F) Average maximal amplitude of MA inward currents elicited at a holding potential of −80 mV in Piezo1-transfected (right bar) or mock-transfected (left bar) cells. (G-H) Representative traces of mechanically-activated inward (G) or outward (H) currents expressed in cells transfected with Piezo1 (right trace) or Piezo2 (left trace) at the specified holding potentials. Traces are normalized to peak, and dashed lines represent fits of inactivation with a mono-exponential equation. (I) Time-constant of inactivation of Piezo1 (left bar) and Piezo2 (right bar) at negative (−80 and −40 mV, upper panel) and positive (40 and 80 mV, lower panel) holding potentials. Bars represent the mean±SEM and the numbers above bars the number of cells. **, P<0.01. ***, P<0.001.

[0066] FIG. **8**. Piezo2-induced MA currents are cationic non-selective. (A) Whole-cell MA current traces elicited in a Piezo2 transfected cell bathed in control solution (left panel) and after perfusion with NMDG-Cl (right panel) solution. Currents are elicited at −80, −40, 0, +40 and +80 mV. (B) MA current-voltage relationships from the same cell. Note that inward currents present in control condition (filled symbols) were suppressed with NMDG-Cl solution (open symbol). (C) Representative current traces of MA currents elicited in Piezo2-transfected cells before, during and after perfusion of 30 μM gadolinium (upper panels) or ruthenium red (lower panels). (D) Percent block of MA currents in Piezo2-expressing cells by 30 μM gadolinium and ruthenium red. Bars represent the mean±SEM, and the number of cells tested is shown above the bars.

[0067] FIG. **9**. Piezo1 antibodies detect Piezo1 in transfected HEK293T cells. (A) Representative images of Piezo1 labeling (red) in Piezo1-IRES-EGFP transfected cells (green). Note, GFP-negative, hence untransfected, cells are devoid of labeling. (B) A proportion of Piezo1 is expressed near or at the plasma membrane of TRPA1 and Piezo1 co-transfected HEK293T cells. Cells were live-labeled with TRPA1 antibodies (green) to delineate the plasma membrane, fixed, permeabilized, and stained for Piezo1 (red) and MYC (total TRPA1). Inset, higher magnification of boxed area in overlay image. Scale bars=20 μm.

[0068] FIG. **10**. siRNA-knockdown of Piezo2 in DRG neurons selectively reduces fast-inactivating MA currents. (A) Representative images of colorimetric in situ hybridization

for Piezo2 in Dorsal Root Ganglia (DRG) neurons using antisense (left panel) and sense (right panel) probes. (B) Representative traces of three typical MA inward currents expressed in DRG neurons are characterized by distinct inactivation kinetics. The neurons are subjected to a series of mechanical steps in 1 μm increments at a holding potential of −80 mV. Current inactivation is fitted with a bi-exponential equation giving fast time-constant (t) of 7.3 ms and slow time-constant>100 ms (left panel), or with a mono-exponential equation giving a time constant of 27 ms (middle panel). Some currents with t>30 ms are too slow to be efficiently fitted during the 150 ms lasting step stimulation (right panel). (C-D) Frequency histograms indicating the proportion of neurons transfected with scrambled siRNA (Ctr) or Piezo2 siRNA (siRNA) that respond to mechanical stimulation with MA currents characterized by their inactivation kinetic. Bars represent the mean±SEM of the proportion of neurons from seven separate experiments (B, n=12-19 neurons per condition and per experiment) or the proportion from all neurons pooled from all seven experiments (C); the numbers above bars in C represent the number of neurons. **, P<0.01. ns, not significantly different.

[0069] FIG. **11**. Piezo2 mRNA is expressed in a subset of DRG neurons. Combined Piezo2 fluorometric in situ hybridization (left panels) with Peripherin (A, middle panel) and Neurofilament 200 (NF200) (B, middle planel) immunostaining in mouse DRG and (C) with TRPV1 (middle panel) immunostaining in rat DRG show that among Piezo2-positive neurons, 60% also expressed Peripherin (n=204 for Piezo2 and n=555 for Peripherin of which 23% express Piezo2, out of 1188 total neurons); 28%, NF200 (n=277 for Piezo2, and n=368 for NF200 of which 19% express Piezo2, out of 1203 total neurons), and 24%, TRPV1 (n=233 for Piezo2 and n=394 for TRPV1 of which 15% express Piezo2, out of 975 total neurons). Arrowheads show examples of neurons expressing Piezo2 and respective markers (A-C, right panels). Scale bars=50 μm.

[0070] FIG. **12**. DRG and Piezo2 siRNA control experiments and comparison of MA current inactivation of DRG neurons. (A-B) siRNA-mediated knockdown of TRPA1 in cultured DRG neurons. (A) Representative traces of ratiometric calcium-imaging on cultured DRG neurons transfected with scrambled siRNA control (n=415 neurons, left panel) and TRPA1 siRNA (n=467 neurons, right panel). (B) Average percentage of neurons responding to mustard oil (MO, agonist of TRPA1 channels) and capsaicin (CAPS, agonist of TRPV1 channels) from 2 independent transfections were assayed 48 to 72 hours after transfection. While the percentage of responders to MO (100 μM) is significantly reduced upon siRNA treatment (scrambled: 15.34±3.69%; siRNA: 2.48±0.96%; P=0.0286, Mann Whitney test), responses to CAPS (0.5 μM) are unaffected by TRPA1 siRNA treatment (scrambled: 27.43±5.81%; siRNA: 32.28±3.84%; ns). Bars represent the mean±SEM. (C) Average maximal amplitude of Piezo2-induced MA currents in the presence or absence of Piezo2 siRNA. N2A cells transfected with Piezo1 siRNA to suppress endogenous MA currents were co-transfected with Piezo2 cDNA or Piezo2 cDNA+Piezo2 siRNA. (D) Histogram of time-constant of inactivation of MA currents recorded in scrambled siRNA transfected DRG neurons. Numbers of neurons expressing MA currents with time constant of inactivation ≦30 ms were plotted using a bin of 2.5 ms (the inactivation kinetic of currents with >30 ms time constant is too slow to be accurately fitted over 150 ms). Fit with double Gaussian equation shows two peaks centered at 7.2±0.5 ms and 16.0±2.1 ms, respectively. (E) Example trace of MA current showing comparison between the amount of current inactivated after 150 ms of mechanical stimulation (I inactivated, right double arrow) and the amount of current at the peak (I peak, left double arrow) (left panel). Percentage of DRG neurons with different degrees of current inactivation at 150 ms (I inactivated/I peak in 5% increments where 100% is completely inactivated) are compared between scrambled or Piezo2 siRNA transfected conditions (right panel, left and right bars in each pair, respectively).

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0071] The present invention identifies a role for multipass transmembrane proteins, called "Piezo" proteins, in mechanotransduction in cells. One or more Piezo proteins have been identified in animal, plant, and other eukaryotic species, including but not limited to, vertebrates (e.g., mammals such as humans and mice, birds such as chickens, and fish such as zebrafish), invertebrates (e.g., *Ciona, Drosophila, Annopheles*, and *C. elegans*), *Arabidopsis*, rice, and ciliates, although functional characterization of these Piezo proteins has not previously been reported. Piezo proteins have moderately conserved secondary structure and overall length, generally from about 2100 amino acids to about 4700 amino acids, with about 24-36 transmembrane domains located throughout the putative protein. It is demonstrated here for the first time that overexpression of Piezo induces robust mechanically-activated currents, while inhibition of Piezo expression reduces mechanically-activated currents. Without intending to limit the scope of the invention, it is believed that Piezos participate in mechanotransduction in cells as components of mechanically-activated cation channels.

[0072] Accordingly, the present invention provides methods of screening for agents that modulate the activity of mechanically-activated cation channel polypeptides by contacting the agents with polypeptides that are substantially identical to Piezo proteins. The present invention also provides antibodies against Piezo proteins that antagonize the activity of mechanically-activated cation channels and methods of ameliorating pain in a subject by administering said antibodies. The present invention further provides antisense oligonucleotides or siRNAs that inhibit the production of Piezo proteins and methods of ameliorating pain in a subject by administering said antisense oligonucleotides or siRNAs. The present invention further provides kits for practicing said methods.

II. Assays for Modulators of Mechanically-Activated Cation Channel Activity

[0073] In one aspect, the present invention provides a method of screening for agents that modulate the activity of a mechanically-activated cation channel, the method comprising: contacting a mechanically-activated cation channel polypeptide with an agent; and selecting the agent that modulates the activity of the mechanically-activated cation channel polypeptide.

[0074] A. Expression of Mechanically-Activated Cation Channel Polypeptides

[0075] The mechanically-activated cation channel polypeptides, and the polynucleotides encoding said

polypeptides, are substantially identical to members of the Piezo family of transmembrane proteins. In some embodiments, the mechanically-activated cation channel polypeptide is substantially identical to (e.g., at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, or 99% identical to) any one of SEQ ID NOs:2, 4, 18, or 20. In some embodiments, the mechanically-activated cation channel polypeptide comprises any of SEQ ID NOs:2, 4, 18, or 20.

[0076] In some embodiments, the method of screening for agents that modulate the activity of a mechanically-activated cation channel comprises contacting a cell comprising the mechanically-activated cation channel polypeptide that is substantially identical to (e.g., having at least 70% identity to) any one of SEQ ID NOs:2, 4, 18, or 20 with an agent, and selecting the agent that modulates the activity of the mechanically-activated cation channel polypeptide. In some embodiments, the cell endogenously expresses the mechanically-activated cation channel polypeptide. In some embodiments, the mechanically-activated cation channel polypeptide is heterologous to the cell.

[0077] Any cell that endogenously expresses a mechanically-activated cation channel polypeptide having at least 70% identity to any of SEQ ID NOs:2, 4, 18, or 20 at a detectable level may be used in the screening methods of the present invention. Whether a cell endogenously expresses the mechanically-activated cation channel polypeptide at a detectable level may be determined by any method of nucleic acid or protein expression known in the art. Nucleic acid may be detected using routine techniques such as Northern analysis, reverse-transcriptase polymerase chain reaction (RT-PCR), microarrays, sequence analysis, or any other methods based on hybridization to a nucleic acid sequence that is complementary to a portion of the marker coding sequence (e.g., slot blot hybridization). Protein may be detected using routine antibody-based techniques, for example, immunoassays such as ELISA, Western blotting, flow cytometry, immunofluorescence, and immunohistochemistry. Examples of cells that endogenously express mechanically-activated cation channel polypeptide having at least 70% identity to any one of SEQ ID NOs:2, 4, 18, or 20 at a detectable level include, but are not limited to, Neuro2A.

[0078] Alternatively, a mechanically-activated cation channel polypeptide can be heterologously expressed in a cell of interest using an expression cassette. An expression cassette, comprising a promoter operably linked to a polynucleotide encoding a mechanically-activated cation channel polypeptide as described herein, is generated using techniques that are known in the art.

[0079] In some embodiments, a polynucleotide encoding the mechanically-activated cation channel polypeptide is substantially identical to (e.g., at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, or 99% identical to) any one of SEQ ID NOs:1, 3, 17, or 19. In some embodiments, the polynucleotide encoding the mechanically-activated cation channel polypeptide comprises any one of SEQ ID NOs:1, 3, 17, or 19. The polynucleotides of the disclosure may be synthesized by chemical methods or prepared by techniques well known in the art. See, for example, Creighton, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., New York, N.Y. (1983). Nucleotide sequences encoding the mechanically-activated cation channel polypeptides of the disclosure may be synthesized and/or cloned, and expressed according to techniques well known to those of ordinary skill in the art. See, for example, Sambrook, et al., *Molecular Cloning, A Laboratory Manual*, Vols. 1-3, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989).

[0080] The polynucleotide sequences encoding the mechanically-activated cation channels can be cloned from cDNA and genomic DNA libraries by hybridization with a probe, or isolated using amplification techniques with oligonucleotide primers. For example, mechanically-activated cation channel polynucleotides sequences can be isolated from mammalian nucleic acid (genomic or cDNA) libraries by hybridizing with a nucleic acid probe, the sequence of which can be derived from SEQ ID NOs:1, 3, 17, or 19. Methods for making and screening cDNA libraries are well known (see, e.g., Gubler & Hoffman, *Gene* 25:263-269 (1983); Sambrook et al., supra; Ausubel et al., supra). Suitable tissues from which mechanically-activated cation channel polypeptide RNA and cDNA can be isolated include, but are not limited to, dorsal root ganglia, nerve, neurons, bladder, colon, kidney, lung, and skin.

[0081] An alternative method of isolating mechanically-activated cation channel polynucleotides combines the use of synthetic oligonucleotide primers and amplification of an RNA or DNA template (see U.S. Pat. Nos. 4,683,195 and 4,683,202; PCR Protocols: A Guide to Methods and Applications (Innis et al., eds, 1990)). Methods such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) can be used to amplify nucleic acid sequences of mechanically-activated cation channel directly from mRNA, from cDNA, from genomic libraries, or from cDNA libraries. Amplification techniques are known in the art, see, e.g., Sambrook, et al., 1989, *Molecular Cloning, A Laboratory Manual*, Vols. 1-3, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. Primers can be prepared using the polynucleotide sequences that are available in publicly available databases. Genes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector containing a selectable marker for propagation in a host. Such markers include but are not limited to dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline, ampicillin, or kanamycin resistance genes for culturing in *E. coli* and other bacteria.

[0082] To obtain high level expression of a cloned gene or nucleic acid, such as those cDNAs encoding the mechanically-activated cation channel, one typically subclones the mechanically-activated cation channel into an expression vector that contains a strong promoter to direct transcription, a transcription/translation terminator, and if for a nucleic acid encoding a protein, a ribosome binding site for translational initiation. Suitable bacterial promoters are well known in the art and described, e.g., in Sambrook et al. and Ausubel et al. Bacterial expression systems for expressing a mechanically-activated cation channel polypeptide are available in, e.g., *E. coli, Bacillus* sp., and *Salmonella* (Palva et al., *Gene* 22:229-235 (1983); Mosbach et al., *Nature* 302:543-545 (1983). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available.

[0083] The promoter used to direct expression of a heterologous nucleic acid depends on the particular application. The promoter is optionally positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

[0084] In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the mechanically-activated cation channel encoding nucleic acid in host cells. A typical expression cassette thus contains a promoter operably linked to the nucleic acid sequence encoding the mechanically-activated cation channel and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. The nucleic acid sequence encoding the mechanically-activated cation channel may typically be linked to a cleavable signal peptide sequence to promote secretion of the encoded protein by the transformed cell. Such signal peptides would include, among others, the signal peptides from tissue plasminogen activator, insulin, and neuron growth factor, and juvenile hormone esterase of *Heliothis virescens*. Additional elements of the cassette may include enhancers and, if genomic DNA is used as the structural gene, introns with functional splice donor and acceptor sites.

[0085] In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

[0086] The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic or prokaryotic cells may be used. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion expression systems such as GST and LacZ. Epitope tags can also be added to recombinant proteins to provide convenient methods of isolation, e.g., c-myc.

[0087] Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A^+, pMTO10/A^+, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV40 early promoter, SV40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

[0088] Some expression systems have markers that provide gene amplification such as—thymidine kinase, hygromycin B phosphotransferase, and dihydrofolate reductase. Alternatively, high yield expression systems not involving gene amplification are also suitable, such as using a baculovirus vector in insect cells, with a mechanically-activated cation channel encoding sequence under the direction of the polyhedrin promoter or other strong baculovirus promoters.

[0089] The elements that are typically included in expression vectors also include a replicon that functions in *E. coli*, a gene encoding antibiotic resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The particular antibiotic resistance gene chosen is not critical, any of the many resistance genes known in the art are suitable. The prokaryotic sequences are optionally chosen such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

[0090] Recombinant expression vectors comprising a mechanically-activated cation channel coding sequence driven by a heterologous promoter may be introduced into the genome of the desired host cell using any of a variety of well known procedures. These procedures include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (see, e.g., Sambrook et al., supra). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing the mechanically-activated cation channel.

[0091] B. Screening for Modulation of Cation Channel Activity

[0092] 1. Cation Channel Activity Assays

[0093] Piezos are components of mechanically-activated cation channels. The activity of mechanically-activated cation channels comprising polypeptides that are substantially identical to Piezos can be assessed using a variety of in vitro and in vivo assays, e.g., measuring electrophysiological changes such as changes in current (both in mechanically-sensitive assays and assays independent of mechanical stimulation), measuring second messengers and transcription levels, measuring ligand binding, measuring cation influx, and using voltage-sensitive dyes, ion-sensitive dyes (e.g., Ca^{2+}), and the like. Furthermore, such assays can be used to test for inhibitors and activators of mechanically-activated cation channels. Such modulators are useful for treating various disorders involving mechanically-activated cation channels.

[0094] In some embodiments, agents that modulate (activate or inhibit) the activity of the mechanically-activated cation channel are identified in an initial screen using an assay that measures an aspect independent of mechanical stimulation, e.g., voltage-clamp or patch-clamp assay, voltage-sensitive dye, ion-sensitive dye, cation influx assay, etc. Agents that are identified as agonizing or inhibiting the activity of the mechanically-activated cation channel using such an assay can then be screened for the ability to modulate the activity of the mechanically-activated cation channel in a mechanically-dependent manner by testing the agonistic or antagonistic effects of the agent in a mechanically-sensitive assay, e.g, using a piezoelectrically-driven pressure assay or membrane stretch assay.

[0095] In some embodiments, agents that modulate the activity of the mechanically-activated cation channel are identified in an initial screen using a mechanically-sensitive assay as described herein.

[0096] Modulators are tested using a biologically active mechanically-activated cation channel polypeptide that is substantially identical to Piezo, either recombinant or naturally occurring. The mechanically-activated cation channel polypeptide can be isolated, co-expressed or expressed in a cell, or expressed in a membrane derived from a cell. Modulation is tested using one of the in vitro or in vivo assays described above. Samples or assays that are treated with a potential mechanically-activated cation channel inhibitor or activator are compared to control samples without the test compound, to examine the extent of modulation. Control samples (untreated with activators or inhibitors) are assigned

a relative mechanically-activated cation channel activity value of 100. Inhibition of the mechanically-activated cation channel is achieved when the mechanically-activated cation channel activity value relative to the control is about less than 90%, e.g., less than 75%, less than 50%, or less than 25%. Activation of the mechanically-activated cation channel is achieved when the mechanically-activated cation channel activity value relative to the control is more than 110%, more than 125%, more than 150%, or more than 200% higher. Compounds that increase the flux of ions will cause a detectable increase in the ion current density by increasing the probability of a mechanically-activated cation channel being open, by decreasing the probability of it being closed, by increasing conductance through the channel, and/or by allowing the passage of ions.

[0097] Changes in ion flux may be assessed by determining changes in polarization (i.e., electrical potential) of the cell or membrane expressing the mechanically-activated cation channel. A method to determine changes in cellular polarization is 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 are conveniently determined using the standard methodology (see, e.g., Hamill et al., Pflugers. Archiv. 391:85-100 (1981)). Other known assays include: radiolabeled ion flux assays and fluorescence assays using voltage-sensitive or ion-sensitive dyes (see, e.g., Vestergarrd-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)). Assays for compounds capable of inhibiting or increasing cation flux through the mechanically-activated cation channels can be performed by application of the compounds to a bath solution in contact with and comprising cells having a channel of the present invention (see, e.g., Blatz et al., Nature 323:718-720 (1986); Park, J. Physiol. 481:555-570 (1994)). Generally, the compounds to be tested are present in the range from 1 pM to 100 mM.

[0098] The effects of the test compounds upon the function of the channels can be measured by changes in the electrical currents or ionic flux or by the consequences of changes in currents and flux. Changes in electrical current or ionic flux are measured by either increases or decreases in flux of ions such as cations (e.g., calcium, sodium, potassium, or magnesium ions). The ions can be measured in a variety of standard ways. They can be measured directly by concentration changes of the ions, e.g., changes in intracellular concentrations, or indirectly by membrane potential or by radio-labeling of the ions. Consequences of the test compound on ion flux can be quite varied. Accordingly, any suitable physiological change can be used to assess the influence of a test compound on the channels of this invention.

[0099] In some embodiments, the mechanically-activated cation channel polypeptide that is used in the assay will have the sequence displayed in the following GenBank accession numbers: human Piezo1—NP_001136336.2; mouse Piezo1—NP_001032375.1; chicken Piezo1—XP_414209.2 or XP_423106.2; zebrafish Piezo1—XP_696355.4; human Piezo2—NP_071351.2; mouse Piezo2—NP_001034574.3; chicken Piezo2—XP_419138.2; zebrafish Piezo2—XP_00266625.1; or a conservatively modified variant, ortholog, and/or substantially identical variant thereof. Generally, the amino acid sequence identity will be at least 65%, e.g., at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least 99%.

[0100] Piezo orthologs, alleles, polymorphic variants, and conservatively modified variants will generally confer substantially similar properties on a mechanically-activated cation channel as described above. In some embodiments, the cell placed in contact with a compound that is suspected to be a Piezo homolog is assayed for increasing or decreasing ion flux in a eukaryotic cell, e.g., an oocyte of *Xenopus* (e.g., *Xenopus laevis*) or a mammalian cell such as a CHO or HeLa cell or as assayed in binding studies using similar cell types. Channels that are affected by compounds in ways similar to Piezo are considered homologs or orthologs of Piezo.

[0101] 2. Mechanically-Sensitive Cation Channel Activity Assays

[0102] In some embodiments, agents are screened for the ability to modulate mechanically-activated electrophysiological changes in a channel comprising a polypeptide that is substantially identical to Piezo. Mechanically-sensitive assays are known in the art and include, for example, piezo-driven pressure, patch membrane stretch, shear stress, osmotic challenges, and amphipathic compounds.

[0103] As a non-limiting example, the ability of an agent to modulate mechanically-activated electrophysiological changes in a cell can be assayed using whole cell recordings measuring stimulation by a piezo-electrically driven mechanical probe. Methods of assaying piezo-driven pressure have been described, see, e.g., Hu and Lewin, J. Physiol. 577:815-828 (2006)). Briefly, a fire-polished glass probe is typically positioned close to the cell surface at an angle ≧45°. The probe is driven toward the cell at a controlled velocity and for a controlled length of time using a Clampex (Molecular Devices, Sunnyvale, Calif.)-controlled piezo-electric crystal microstage and mechanically-activated inward current is recorded. One of skill in the art will recognize that it may be useful to vary parameters such as voltage and the intensity, velocity, and duration of the mechanical stimulus.

[0104] As another non-limiting example, the ability of an agent to modulate mechanically-activated electrophysiological changes in a cell can be assayed by stretch of the plasma membrane through a patch pipette in cell-attached mode. Methods of assaying patch membrane stretch have been described, see, e.g., Gil et al., Proc. Natl. Acad. Sci. USA 96:14594-14599 (1999)). Briefly, on-cell, or cell-attached, patches are formed by pressing the tip of a heat-polished patch pipette against the membrane of the cell and then applying slight negative pressure to the patch pipette using a Clampex-controlled pressure clamp (e.g., HSPC-1 pressure clamp, ALA Scientific Instruments, Westbury, N.Y.) and mechanically-activated inward current is recorded. One of skill in the art will recognize that it may be useful to vary parameters such as voltage and the amount and duration of pressure applied.

[0105] 3. Solid State and High Throughput Assays

[0106] In the high throughput assays of the invention, it is possible to screen up to several thousand, ten thousand, hundred thousand or more different modulators or ligands in a single day. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 100 (e.g., 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100- about 1500 different

compounds. It is possible to assay several different plates per day; assay screens for up to about 6,000-20,000 or more different compounds is possible using the integrated systems of the invention. More recently, microfluidic approaches to reagent manipulation have been developed, e.g., by Caliper Technologies (Palo Alto, Calif.), and can be used.

[0107] As a non-limiting example, potential modulators can be screened for effect on mechanically-activated cation channels using a high throughput electrophysiological screening system such as IonWorks™ HT (Molecular Devices, Sunnyvale, Calif.). Briefly, the IonWorks™ HT system measures whole-cell current from multiple cells simultaneously using a 384-well plate. Cells expressing a voltage-gated ion channel of interest are dispensed into individual wells in parallel with an onboard fluidics system and a single cell is subsequently positioned over a single small aperture within each well, the aperture separating two isolated fluid-filled upper and lower chambers, each containing buffered solutions and separate electrodes. The positioned cells form stable seals over the apertures, impeding electrical flow between the two chambers. A cell membrane pore-forming agent (e.g., amphotericin B) is introduced into the lower chamber, creating an electrical pathway through the portion of the cell membrane exposed through the small aperture in each of the wells. An electronics head containing 48 electrodes is positioned into the upper chamber clamping the cell membrane potential and subsequently recording ionic currents from 48 cells in parallel. Compounds are aspirated from 96- or -384 well microplates and dispensed in parallel with a 12-channel fluidics head pipettor.

[0108] The molecule of interest can be bound to the solid state component, directly or indirectly, via covalent or non covalent linkage, e.g., via a tag. The tag can be any of a variety of components. In general, a molecule which binds the tag (a tag binder) is fixed to a solid support, and the tagged molecule of interest (e.g., a mechanically-activated cation channel polypeptide) is attached to the solid support by interaction of the tag and the tag binder.

[0109] A number of tags and tag binders can be used, based upon known molecular interactions well described in the literature. For example, where a tag has a natural binder, for example, biotin, protein A, or protein G, it can be used in conjunction with appropriate tag binders (avidin, streptavidin, neutravidin, the Fc region of an immunoglobulin, etc.) Antibodies to molecules with natural binders such as biotin are also widely available and appropriate tag binders; see, SIGMA Immunochemicals 1998 catalogue SIGMA, St. Louis Mo.).

[0110] Similarly, any haptenic or antigenic compound can be used in combination with an appropriate antibody to form a tag/tag binder pair. Thousands of specific antibodies are commercially available and many additional antibodies are described in the literature. For example, in one common configuration, the tag is a first antibody and the tag binder is a second antibody which recognizes the first antibody. In addition to antibody-antigen interactions, receptor-ligand interactions are also appropriate as tag and tag-binder pairs. For example, agonists and antagonists of cell membrane receptors (e.g., cell receptor-ligand interactions such as transferrin, c-kit, viral receptor ligands, cytokine receptors, chemokine receptors, interleukin receptors, immunoglobulin receptors and antibodies, the cadherein family, the integrin family, the selectin family, and the like; see, e.g., Pigott & Power, The Adhesion Molecule Facts Book I (1993). Similarly, toxins and venoms, viral epitopes, hormones (e.g., opiates, steroids, etc.), intracellular receptors (e.g. which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and cyclic polymer configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

[0111] Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

[0112] Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly-Gly sequences of between about 5 and 200 amino acids. Such flexible linkers are known to persons of skill in the art. For example, poly(ethylene glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Ala. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

[0113] Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent which fixes a chemical group to the surface which is reactive with a portion of the tag binder. For example, groups which are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature. See, e.g., Merrifield, J. Am. Chem. Soc. 85:2149-2154 (1963) (describing solid phase synthesis of, e.g., peptides); Geysen et al., J. Immun. Meth. 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank & Doring, *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor et al., Science, 251:767-777 (1991); Sheldon et al., Clinical Chemistry 39(4):718-719 (1993); and Kozal et al., Nature Medicine 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.

[0114] 4. Validation

[0115] Agents that are initially identified by any of the foregoing screening methods can be further tested to validate the apparent activity and/or determine other biological effects of the agent. In some cases, the identified agent is administered to an animal (e.g., a non-human mammal such as a mouse) to determine the effect of the agent on pain sensitivity.

[0116] C. Agents that Modulate Cation Channel Activity

[0117] The compounds tested as modulators of the mechanically-activated cation channels can be any small chemical compound, or a biological entity, such as a protein, sugar, nucleic acid or lipid. Typically, test compounds will be small chemical molecules and peptides. Essentially any chemical compound can be used as a potential modulator or ligand in the assays of the invention, although most often compounds can be dissolved in aqueous or organic (especially DMSO-based) solutions are used. The assays are designed to screen large chemical libraries by automating the

assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays). It will be appreciated that there are many suppliers of chemical compounds, including Sigma (St. Louis, Mo.), Aldrich (St. Louis, Mo.), Sigma-Aldrich (St. Louis, Mo.), Fluka Chemika-Biochemica Analytika (Buchs Switzerland) and the like.

[0118] In one embodiment, high throughput screening methods involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (potential modulator or ligand compounds). Such "combinatorial chemical libraries" or "ligand libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

[0119] A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

[0120] Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Pat. No. 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton et al., *Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (e.g., PCT Publication No. WO 91/19735), encoded peptides (e.g., PCT Publication WO 93/20242), random bio-oligomers (e.g., PCT Publication No. WO 92/00091), benzodiazepines (e.g., U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., *Proc. Nat. Acad. Sci. USA* 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara et al, *J. Amer. Chem. Soc.* 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschrnann et al., *J. Amer. Chem. Soc.* 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen et al., *J. Amer. Chem. Soc.* 116:2661 (1994)), oligocarbamates (Cho et al., *Science* 261:1303 (1993)), and/or peptidyl phosphonates (Campbell et al., *J. Org. Chem.* 59:658 (1994)), nucleic acid libraries (see Ausubel, Berger and Sambrook, all supra), peptide nucleic acid libraries (see, e.g., U.S. Pat. No. 5,539,083), antibody libraries (see, e.g., Vaughn et al., *Nature Biotechnology,* 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al., *Science,* 274:1520-1522 (1996) and U.S. Pat. No. 5,593,853), small organic molecule libraries (see, e.g., benzodiazepines, Baum C&EN, January 18, page 33 (1993); isoprenoids, U.S. Pat. No. 5,569,588; thiazolidinones and metathiazanones, U.S. Pat. No. 5,549,974; pyrrolidines, U.S. Pat. Nos. 5,525,735 and 5,519,134; morpholino compounds, U.S. Pat. No. 5,506,337; benzodiazepines, U.S. Pat. No. 5,288,514, and the like).

[0121] Devices for the preparation of combinatorial libraries are commercially available (see, e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville Ky., Symphony, Rainin, Woburn, Mass., 433A Applied Biosystems, Foster City, Calif., 9050 Plus, Millipore, Bedford, Mass.). In addition, numerous combinatorial libraries are themselves commercially available (see, e.g., ComGenex, Princeton, N.J., Tripos, Inc., St. Louis, Mo., 3D Pharmaceuticals, Exton, Pa., Martek Biosciences, Columbia, Md., etc.).

III. Inhibiting Expression of Mechanically-Activated Cation Channels

[0122] A. Antibodies

[0123] In another aspect, the present invention provides antibodies that specifically bind to the mechanically-activated cation channels. Such antibodies are useful, e.g., for ameliorating or treating pain or itch in a subject. Suitable antibodies include, but are not limited to, monoclonal antibodies, humanized antibodies, chimeric antibodies, and antibody fragments (i.e., Fv, Fab, $(Fab')_2$, or scFv).

[0124] In some embodiments, the antibody selectively binds to a mechanically-activated cation channel polypeptide having at least 70% amino acid sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20. In some embodiments, the antibody selectively binds to a polypeptide comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20.

[0125] Monoclonal antibodies are obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (see, for example, Kohler & Milstein, *Eur. J. Immunol.* 6: 511-519 (1976)). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse et al., *Science* 246: 1275-1281 (1989).

[0126] Monoclonal antibodies are collected and titered against the immunogen in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at least about 1 μM, and can often be designed to bind with a K_d of 1 nM or less.

[0127] In an exemplary embodiment, an animal, such as a rabbit or mouse is immunized with a mechanically-activated cation channel polypeptide, or an nucleic acid construct encoding such a polypeptide. The antibodies produced as a result of the immunization can be isolated using standard methods.

[0128] The immunoglobulins, including binding fragments and other derivatives thereof, of the present invention may be produced readily by a variety of recombinant DNA techniques, including by expression in transfected cells (e.g., immortalized eukaryotic cells, such as myeloma or hybridoma cells) or in mice, rats, rabbits, or other vertebrate

capable of producing antibodies by well known methods. Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection (Catalogue of Cell Lines and Hybridomas, Fifth edition (1985) Rockville, Md.).

[0129] In some embodiments, the antibody is a humanized antibody, i.e., an antibody that retains the reactivity of a non-human antibody while being less immunogenic in humans. This can be achieved, for instance, by retaining the non-human CDR regions that are specific for mechanically-activated cation channel, and replacing the remaining parts of the antibody with their human counterparts. See, e.g., Morrison et al., PNAS USA, 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.,* 44:65-92 (1988); Verhoeyen et al., *Science,* 239:1534-1536 (1988); Padlan, *Molec. Immun.,* 28:489-498 (1991); Padlan, *Molec. Immun.,* 31(3):169-217 (1994). Techniques for humanizing antibodies are well known in the art and are described in e.g., U.S. Pat. Nos. 4,816,567; 5,530,101; 5,859,205; 5,585,089; 5,693,761; 5,693,762; 5,777,085; 6,180,370; 6,210,671; and 6,329,511; WO 87/02671; EP Patent Application 0173494; Jones et al. (1986) *Nature* 321:522; and Verhoyen et al. (1988) *Science* 239:1534. Humanized antibodies are further described in, e.g., Winter and Milstein (1991) *Nature* 349:293. For example, polynucleotides comprising a first sequence coding for humanized immunoglobulin framework regions and a second sequence set coding for the desired immunoglobulin complementarity determining regions can be produced synthetically or by combining appropriate cDNA and genomic DNA segments. Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells. The CDRs for producing the immunoglobulins of the present invention will be similarly derived from monoclonal antibodies capable of specifically binding to a mechanically-activated cation channel.

[0130] In some cases, transfer of a CDR to a human framework leads to a loss of specificity for the humanized antibody. In these cases, back mutation can be introduced into the framework regions of the human portion of the antibody. Methods of making back mutations are well known in the art and are described in, e.g., Co et al., *PNAS USA* 88; 2269-2273 (1991) and WO 90/07861.

[0131] The mechanically-activated cation channel—specific antibody can also be chimeric, so that all or most of the variable region is retained, but the constant region replaced. For example, a murine variable region that possesses mechanically-activated cation channel binding activity may be combined with human constant regions, or constant regions from another mammal for use in veterinary treatments.

[0132] In some embodiments, the antibodies are antibody fragments such as Fab, $F(ab')_2$, Fv or scFv. The antibody fragments can be generated using any means known in the art including, chemical digestion (e.g., papain or pepsin) and recombinant methods. Methods for isolating and preparing recombinant nucleic acids are known to those skilled in the art (see, Sambrook et al., *Molecular Cloning. A Laboratory Manual* (2d ed. 1989); Ausubel et al., *Current Protocols in Molecular Biology* (1995)). The antibodies can be expressed in a variety of host cells, including *E. coli*, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO, and HeLa cells lines and myeloma cell lines.

[0133] B. siRNA and Antisense Oligonucleotides

[0134] In another aspect, the present invention provides oligonucleotide and polynucleotide sequences that inhibit production of a mechanically-activated cation channel polypeptide. Such inhibitory nucleic acid sequences are useful, e.g., for ameliorating or treating pain or itch in a subject. Suitable oligonucleotides and polynucleotides include, but are not limited to, siRNA and antisense oligonucleotides.

[0135] In some embodiments, the oligonucleotide or polynucleotide is complementary to at least 15 contiguous nucleotides of a polynucleotide that is at least 70% identical to SEQ ID NOs:1, 3, 17, or 19. In some embodiments, the oligonucleotide or polynucleotide is complementary to at least 18, at least 20, at least 22, at least 25, at least 30, at least 40, or at least 50 contiguous nucleotides of a polynucleotide that is at least 70% identical to SEQ ID NOs:1, 3, 17, or 19. In some embodiments, the oligonucleotide or polynucleotide comprises any of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16.

[0136] 1. siRNA

[0137] Double stranded siRNA that corresponds to a gene encoding a mechanically-activated cation channel polypeptide can be used to silence the transcription and/or translation of the mechanically-activated cation channel polypeptide by inducing degradation of mRNA transcripts, and thus ameliorate or treat pain or itch by preventing expression of the mechanically-activated cation channel polypeptide. The siRNA is typically about 5 to about 100 nucleotides in length, more typically about 10 to about 50 nucleotides in length, most typically about 15 to about 30 nucleotides in length. siRNA molecules and methods of generating them are described in, e.g., Bass, 2001, *Nature,* 411, 428-429; Elbashir et al., 2001, *Nature,* 411, 494-498; WO 00/44895; WO 01/36646; WO 99/32619; WO 00/01846; WO 01/29058; WO 99/07409; and WO 00/44914. A DNA molecule that transcribes dsRNA or siRNA (for instance, as a hairpin duplex) also provides RNAi. DNA molecules for transcribing dsRNA are disclosed in U.S. Pat. No. 6,573,099, and in U.S. Patent Application Publication Nos. 2002/0160393 and 2003/0027783, and Tusch1 and Borkhardt, *Molecular Interventions,* 2:158 (2002). For example, dsRNA oligonucleotides that specifically hybridize to the nucleic acid sequences set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:17, or SEQ ID NO:19 can be used in the methods of the present invention. A decrease in the severity of pain symptoms in comparison to symptoms detected in the absence of the interfering RNA can be used to monitor the efficacy of the siRNA.

[0138] siRNA can be delivered to the subject using any means known in the art, including by injection, inhalation, or oral ingestion of the siRNA. Another suitable delivery system for siRNA is a colloidal dispersion system such as, for example, macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. Nucleic acids, including RNA and DNA within liposomes and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.,* 6:77, 1981). Liposomes can be targeted to specific cell types or tissues using any means known in the art.

[0139] 2. Antisense Oligonucleotides

[0140] Antisense oligonucleotides that specifically hybridize to nucleic acid sequences encoding mechanically-activated cation channel polypeptides can also be used to silence the transcription and/or translation of the mechanically-activated cation channel polypeptide, and thus ameliorate or treat pain or itch. For example, antisense oligonucleotides that specifically hybridize to the nucleic acid sequences set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:17, or SEQ ID NO:19 can be used in the methods of the present invention. A decrease in the severity of pain symptoms in comparison to symptoms detected in the absence of the antisense nucleic acids can be used to monitor the efficacy of the antisense nucleic acids.

[0141] Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (see, e.g., Weintraub, *Scientific American,* 262:40 (1990)). Typically, synthetic antisense oligonucleotides are generally between 15 and 25 bases in length. Antisense nucleic acids may comprise naturally occurring nucleotides or modified nucleotides such as, e.g., phosphorothioate, methylphosphonate, and -anomeric sugar-phosphate, backbone-modified nucleotides.

[0142] In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target nucleotide mutant producing cell. The use of antisense methods to inhibit the in vitro translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.,* 172:289, (1988)). Less commonly, antisense molecules which bind directly to the DNA may be used.

[0143] Delivery of antisense polynucleotides specific for a gene encoding a mechanically-activated cation channel can be achieved using any means known in the art including, e.g., direct injection, inhalation, or ingestion of the polynucleotides. In addition, antisense polynucleotides can be delivered using a recombinant expression vector (e.g., a viral vector based on an adenovirus, a herpes virus, a vaccinia virus, or a retrovirus) or a colloidal dispersion system (e.g., liposomes) as described herein.

IV. Methods of Treating Mechanically-Activated Cation Channel-Mediated Diseases

[0144] In yet another aspect, the present invention provides compositions comprising antagonists of mechanically-activated cation channels. The compositions of the invention can be provided to ameliorate or treat diseases or conditions which involve pain transmitted via mechanically-activated cation channels.

[0145] Mechanically-activated cation channels are implicated in the transmission of various sensations such as touch, pressure, vibration, proprioception, and pain. Accordingly, antagonists of mechanically-activated cation channels may be administered to a subject having a disease or condition characterized by alterations in the transmission of these sensations, e.g., alterations in touch or pain pathways that result in acute or chronic pain, heightened sensitivity to pain or touch, or heightened intensity of pain or touch.

[0146] In one embodiment, the compositions of the invention (e.g., the antibodies that selectively bind to mechanically-activated cation channels or the oligonucleotides or polynucleotides that inhibit production of a mechanically-activated cation channel polypeptide) can be provided to a subject having pain selected from the group consisting of acute mechanical pain, chronic mechanical pain, mechanical hyperalgesia, mechanical allodynia, arthritis, inflammation, dental pain, cancer pain, and labor pain.

[0147] The compositions of the invention can be administered in a single dose, multiple doses, or on a regular basis (e.g., daily) for a period of time (e.g., 2, 3, 4, 5, 6, days or 1-3 weeks or more).

[0148] The compositions of the invention can be administered directly to the mammalian subject to block mechanically-activated cation channel activity using any route known in the art, including e.g., by injection (e.g., intravenous, intraperitoneal, subcutaneous, intramuscular, or intradermal), inhalation, transdermal application, rectal administration, or oral administration.

[0149] The pharmaceutical compositions of the invention may comprise a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (see, e.g., Remington's Pharmaceutical Sciences, 17th ed., 1989).

[0150] The compositions of the invention, alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[0151] Formulations suitable for administration include aqueous and non-aqueous solutions, isotonic sterile solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, orally, nasally, topically, intravenously, intraperitoneally, or intrathecally. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials. Solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. The modulators can also be administered as part a of prepared food or drug.

[0152] Formulations suitable for oral administration can comprise: (a) liquid solutions, such as an effective amount of the packaged nucleic acid suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g., sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and

acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

[0153] The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial response in the subject over time, e.g., a reduction in pulmonary capillary hydrostatic pressure, a reduction in fluid in the lungs, a reduction in the rate of fluid accumulation in the lungs, or a combination thereof. The optimal dose level for any patient will depend on a variety of factors including the efficacy of the specific modulator employed, the age, body weight, physical activity, and diet of the patient, on a possible combination with other drugs, and on the severity of the pain. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound or vector in a particular subject.

[0154] In determining the effective amount of the antagonists of mechanically-activated cation channels to be administered a physician may evaluate circulating plasma levels of the antagonist and antagonist toxicity. In general, the dose equivalent of an antagonist is from about 1 ng/kg to 10 mg/kg for a typical subject.

V. Kits

[0155] The present invention also provides for kits for screening for modulators of mechanically-activated cation channels and for treating pain in a subject. Such kits can be prepared from readily available materials and reagents. For example, a kit for screening for modulators of mechanically-activated cation channels can comprise any one or more of the following materials: a mechanically-activated cation channel polypeptide, reaction tubes, and instructions for testing mechanically-activated cation channel activity. A kit for treating pain in a subject can comprise any one or more of the following materials: an antibody or inhibitory oligonucleotide or polynucleotide composition as described herein and instructions for administering the composition to a subject. A wide variety of kits and components can be prepared according to the present invention, depending upon the intended user of the kit and the particular needs of the user.

EXAMPLES

[0156] The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1

Materials and Methods

[0157] This example provides a description of particular materials and methods used in the following examples. One of skill in the art would readily understand that various modifications of substitutions in the described methods may be used.

[0158] Cell culture and transient transfection. Neuro2A cells were grown in Eagle's Minimum Essential Medium containing 4.5 $mg.ml^{-1}$ glucose, 10% fetal bovine serum, 50 $units.ml^{-1}$ penicillin and 50 $\mu g.ml^{-1}$ streptomycin. C2C12 or Human Embryonic Kidney 293T (HEK293T) cells were grown in Dulbecco's Modified Eagle Medium containing 4.5 $mg.ml^{-1}$ glucose, 10% fetal bovine serum, 50 $units.ml^{-1}$ penicillin and 50 $\mu g.ml^{-1}$ streptomycin. Cells were plated onto 35 mm dishes or 12-mm round glass coverslips placed in 24-well plates and transfected using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. For Piezo1 overexpression experiment, 500 to 1000 $ng.ml^{-1}$ of Piezo1-IRES-GFP or vector only were transfected, and cells were recorded 12-48 hours later. For Piezo2 overexpression experiments, 600 to 1000 $ng.ml^{-1}$ of mPiezo2 or vector were co-transfected with 300 $ng.ml^{-1}$ GFP to identify transfected cells and cells were recorded 12-48 hours later.

[0159] For siRNA experiment, 20 nM total siRNA and 300 or 500 $ng.ml^{-1}$ GFP to identify transfected cells were co-transfected and cells were used 3 days after transfection. siRNA of Smartpool I directed against mPiezo1 were purchased from Qiagen (Target sequences: CACCGGCATCTACGTCAAATA (siRNA1) (SEQ ID NO:5), ACCAAGAAATACAACCATCTA (siRNA2) (SEQ ID NO:6), TCGGCGCTTGCTAGAACTTCA (siRNA3) (SEQ ID NO:7), and CGGAATCCTGCTGCTGCTATA (siRNA4) (SEQ ID NO:8)) and used at 5 nM each together in Smartpool I or at 20 nM separately. siRNA4 was toxic at 20 nM, as it caused cell detachment and subsequent death 3 days after transfection. Smartpool II siRNA was a pool of 4 different siRNA purchased from Dharmacon (Target sequences: GAAAGAGATGTCACCGCTA (SEQ ID NO:9), GCATCAACTTCCATCGCCA (SEQ ID NO:10), AAAGACAGATGAAGCGCAT (SEQ ID NO:11), GGCAGGATGCAGTGAGCGA (SEQ ID NO:12)). For the Piezo2 siRNA experiment in N2A cells, 600 $ng.ml^{-1}$ of mPiezo2 and 300 $ng.ml^{-1}$ GFP were co-transfected with 20 nM Piezo1 siRNA1 only or together with 20 nM Piezo2 siRNA. Cells were recorded 3 days after transfection. siRNA directed against mPiezo2 was a pool of 4 different siRNA purchased from Dharmacon (Target sequences: GAATGTAATTGGACAGCGA (SEQ ID NO:13), TCATGAAGGTGCTGGGTAA (SEQ ID NO:14), GATTATCCATGGAGATTTA (SEQ ID NO:15), GAAGAAAGGCATGAGGTAA (SEQ ID NO:16)).

[0160] DRG Culture and siRNA.

[0161] Preparation and culture of mouse dorsal root ganglion neurons (from male C57B16 mice) were performed as described previously (M. Chalfie, *Nat Rev Mol Cell Biol* 10, 44 (January, 2009)) with the following modifications: Growth medium was supplemented with 100 ng/ml nerve growth factor (NGF), 50 ng/ml GDNF, 50 ng/ml BDNF, 50 ng/ml NT-3, 50 ng/ml NT-4. Small interference RNA (siRNA)-mediated knockdown was achieved by nucleofection of siRNA into freshly isolated DRG neurons using the SCN nucleofector kit with the nucleofector device according to the manufacturer's instructions (SCN Basic Neuro program 6; Lonza AG). DRG neurons isolated from one mouse were used per siRNA tested. siRNAs were used at 150 nM-250 nM for TRPA1 (smartpool, Qiagen) and 250 nM for Piezo2 (smartpool, Qiagen), concentrations of scrambled controls (Qiagen) were adjusted accordingly. After nucleofection, neurons were allowed to recover in RPMI medium for 10 min at 37° C., growth medium (without antibiotics and without AraC) was added and neurons were plated on poly-D-lysine coated coverslips, previously coated with laminin (2 $\mu g.ml^{-1}$). 2-4 hrs after transfection, half of the growth medium was exchanged with fresh medium, and neurons were grown for 48-72 hours.

[0162] Electrophysiology.

[0163] Patch-clamp experiments were performed in standard whole-cell or cell attached recordings using an Axopatch 200B amplifier (Axon Instruments). Patch pipettes had resistance of 2-3 MΩ when filled with an internal solution consisting of (in mM) 133 CsCl, 10 HEPES, 5 EGTA, 1 $CaCl_2$, 1

$MgCl_2$, 4 MgATP, and 0.4 Na_2GTP (pH adjusted to 7.3 with CsOH). The extracellular solution consisted of (in mM) 127-130 NaCl, 3 KCl, 1 $MgCl_2$, 10 HEPES, 2.5 $CaCl_2$, 10 glucose (pH adjusted to 7.3 with NaOH). NMDG solution consisted of (in mM) 150 NMDG, 10 HEPES (pH 7.5). For ion selectivity experiments, internal solution consisted of (in mM) 150 CsCl, 10 Hepes (pH 7.3 with CsOH), monovalent external solutions consisted of (in mM) 150 NaCl or KCl, 10 HEPES (pH 7.3 with NaOH or KOH) and divalent external solutions consisted of (in mM) 100 $CaCl_2$ or $MgCl_2$, 10 HEPES (pH 7.3 with CsOH). For cell attached recordings, pipette were filled with a solution consisting of (in mM) 130 NaCl, 5 KCl, 10 HEPES, 1 $CaCl_2$, 1 $MgCl_2$, 10 TEA-C1 (pH 7.3 with NaOH) and external solution used to zero the membrane potential consisted of (in mM) 140 KCl, 10 HEPES, 1 $MgCl_2$, 10 glucose (pH 7.3 with KOH). All experiments were done at room temperature. Currents were sampled at 20 kHz and filtered at 2 kHz. Voltages were not corrected for a liquid junction potential excepted for ion selectivity experiments were LJP were calculated using Clampex 10.1 software (Axon Instruments). Leak currents before mechanical stimulations were subtracted off-line from the current traces. 10 mM ruthenium red stock solution was prepared in DMSO; 100 mM gadolinium stock solution, in water.

[0164] Mechanical Stimulation.

[0165] For whole-cell recordings mechanical stimulation was achieved using a fire-polished glass pipette (tip diameter 3-4 μm) positioned at an angle of 80° to the cell being recorded. Downward movement of the probe toward the cell was driven by a Clampex controlled piezo-electric crystal microstage (E625 LVPZT Controller/Amplifier; Physik Instrumente). The probe was typically positioned approximately 2 μm from the cell body. The piezoelectrically driven stimulus intensity used to measure the threshold of MA current activation was defined as the distance traveled beyond that which touched the cell. The probe had a velocity of 1 μm/ms during the ramp segment of the command for forward motion and the stimulus was applied for 150 ms. To assess the mechanical sensitivity of a cell, a series of mechanical steps in 1 μm increments were applied every 10 s, which allowed full recovery of mechanosensitive currents. Inward MA currents were recorded at a holding potential of −80 mV. For I-V relationship recordings, voltage steps were applied 0.7 s before the mechanical stimulation from a holding potential of −60 mV. For recordings of MA currents in DRG neurons, the inactivation kinetics at a holding potential of −80 mV of traces of currents reaching at least 75% of the maximal amplitude of current elicited per cell were fitted with mono-exponential equation (or in some case bi-exponential equation for the rapidly-adapting currents, accordingly to previous reports (O. P. Hamill, B. Martinac, *Physiol Rev* 81, 685 (April, 2001)) and using the fast time constant for analysis, see FIG. **5**A left panel) giving a value of τ_{inac} per responsive neuron that was used for analysis.

[0166] For cell-attached recordings, membrane patches were stimulated with brief negative pressure pulses through the recording electrode using a Clampex controlled pressure clamp HSPC-1 device (ALA-scientific). Otherwise stated, stretch-activated channels were recorded at a holding potential of −80 mV with pressure steps from 0 to −60 mm Hg (−10 mm Hg increments), and 3-10 recording traces were averaged per cell for analysis. Current-pressure relationships were fitted with a Boltzmann equation of the form: $I(P)=[1+\exp(-(P-P_{50})/s)]^{-1}$, where I is the peak of stretch-activated current at a given pressure, P is the applied patch pressure (in mm Hg), P_{50} is the pressure value that evoked a current value which is 50% of Imax, and s reflects the current sensitivity to pressure.

[0167] Determination of permeability ratios. Reversal potentials for each cell in each solution were determined by interpolation of the respective current-voltage data. In these experiments, I-V relationships were performed from a holding potential of −60 mV with voltage steps ranging from −60 mV to +60 mV (before liquid junction potential correction) in 20 mV increments.

[0168] The ratio of permeabilities, P_X/P_{Cs}, was determined for each test cation X for each cell from the reversal potential of the MA activated whole-cell current when that cation was the major external cation. The Goldman-Hodgkin-Katz (GHK) equation (G. B. Monshausen, S. Gilroy, *Trends Cell Biol* 19, 228 (May, 2009)), simplified for a single permeant cation on each side of the membrane, was employed:

$$? = \frac{HT}{z?}\ln\frac{F_X?}{??}$$

? indicates text missing or illegible when filed

where RT/zF has the value of 25.5 at 23° C. For the divalent cations the appropriately modified equation was used:

$$? = \frac{RT}{?}\ln\left(\sqrt{\frac{4??}{??}+\frac{1}{4}}-\frac{1}{2}\right)$$

? indicates text missing or illegible when filed

Ratio P_X/P_{Cs} were presented for each cation as mean±SEM.

[0169] Ratiometric calcium imaging. Intracellular Ca^{2+} imaging experiments were performed by washing cells three times with Ca^{2+} imaging buffer [1×Hanks Balanced Salt Solution (HBSS, 1.3 mM Ca^{2+}) supplemented with 10 mM HEPES], then loaded with ratiometric Ca^{2+} indicator dye Fura-2/AM (Molecular Probes) for 30 minutes at room temperature, according to the manufacturer's recommendations. Cells were washed three times prior to imaging on an inverted microscope. Fura-2 fluorescence was measured by illuminating the cells with an alternating 340/380 nm light. Fluorescence intensity was measured at 510 nm. The intracellular Ca^{2+} concentration is expressed as the 340/380 ratio.

[0170] Ratiometric calcium imaging of cultured DRG neurons was performed essentially as described [1]. Experiments were conducted at 37° C. 48-72 hrs after plating. Threshold for activation was set at 40% above the averaged baseline from 5 time points immediately before addition of MO (100 μM). Capsaicin (CAPS, 0.5 μM) was added at the end of each experiment to control for siRNA specificity, neuronal health and responsiveness. All experimental groups to be compared were processed in parallel using the same DRG culture preparation (2 independent preparations were used).

[0171] Generation of Piezo1 Antisera.

[0172] Custom polyclonal antibodies to the synthetic peptide RQRRERARQERAEQ (amino acids 1454-1467 of SEQ ID NO:2) were prepared in rabbit by standard methods and affinity purified (Thermo Fisher Scientific, Openbiosystems).

[0173] TRPA1 Live-Labeling and Immunocytochemistry.

[0174] TRPA1 live-labeling and immunocytochemistry on HEK 293T cells were performed essentially as described (Schmidt et al., 2009) with the following modifications: For assessment of the specificity of Piezo1 antisera, cells were transfected with a Piezo1-IRES-EGFP construct and used 36 hrs later for immunocytochemistry as outlined below. Piezo1 antisera were used at 1:100 and detected by secondary antibodies conjugated to Alexa Fluor 546 (Invitrogen). For assessment of membrane expression of Piezo1, cells were co-transfected with a murine Trpa1-MYC/His construct and Piezo1 and used for live-labeling 36 hrs after transfection. Surface TRPA1 was labeled by incubating live HEK293T cells with TRPA1 antibodies (1:50) followed by incubation with Alexa Fluor 488 F(ab')2 fragment of goat-anti-rabbit (1:200, Invitrogen). Cells were fixed with 2% paraformaldehyde (PFA) in PBS for 20 min, washed and incubated with an excess of non-labeled goat-anti-rabbit IgG for 1 hour to block binding sites on any remaining unbound TRPA1 antibody. Cells were then permeabilized in PBS containing 0.4% Triton X-100, blocked with normal goat serum (10% serum in PBS), and incubated with primary antibodies against Piezo1 (1:100) and c-MYC (1:100, 9E11, mouse, Santa Cruz Biotechnology), followed by secondary antibodies (Alexa Fluor 568 goat-anti-rabbit IgG and Alexa Fluor 633 goat-anti-mouse IgG; 1:200; Invitrogen).

[0175] Immunocytochemistry experiments were imaged using an Olympus (Tokyo, Japan) Fluoview 500 confocal microscope by sequential illumination using the 488 nm line of an argon laser, a HeNe green 543 nm laser and a HeNe red 633 nm laser. Merge stacked images were created using a 40× and 60×PlanAPO oil-immersion objective, the latter with a zoom of 1,5.

[0176] Real Time qPCR.

[0177] Dorsal root ganglia were freshly isolated from adult C57BL/6J wildtype mice and snap frozen on dry ice. Total RNA from DRG or siRNA transfected N2A cells (3 days after GFP co-transfection, same conditions then the one used for recordings) was extracted using Trizol treatment. Total RNA from all other tissues were purchased from Zyagen (San Diego). 500 ng total RNA was used to generate 1^{st} strand cDNA using the Quantitect reverse transcription kit (Qiagen). Real time Taqman PCR assays for mPiezo1 and mPiezo2 (assay id: Mm01241570_g1 and Mm01262433_m1) were purchased from Applied Biosystems with a FAM reporter dye and a non-fluorescent quencher. Universal TaqMan PCR master mix (20×) without AmpErase UNG (Applied Biosystems) was used. The reaction was run in the ABI 7900HT fast real time system using 1 μA of the cDNA in a 20 μA reaction according to the manufacturer's instructions in triplicate.

[0178] Calibrations and normalizations were done using the $2^{-\Delta\Delta CT}$ method [4], where $\Delta\Delta C_T$=((C_T(target gene)−C_T(reference gene))−(C_T(calibrator)−C_T(reference gene)). For the analysis of mRNA expression in different tissues (FIG. **4**C), the target gene was mPiezo1 or mPiezo2, while the reference gene was GAPDH and the calibrator was the lung tissue. For the analysis of siRNA induced down-regulation of mRNA expression (FIG. **3**A), β-actin was used as the reference gene and Scrambled siRNA transfected N2A cells was the calibrator.

[0179] In Situ Hybridization and Immunohistochemistry.

[0180] In situ hybridization and immunohistochemistry were performed as described previously (M. Chalfie, *Nat Rev Mol Cell Biol* 10, 44 (January, 2009)). In brief, adult male C57BL/6J mice, ages 6-16 weeks, or adult male Sprague Dawley rats, were perfused with 4% PFA and dorsal root ganglia were quickly dissected. Following post-fixation and cryoprotection in 30% sucrose, single DRG were embedded in OCT and sectioned with a cryostat at 10 μm thickness. Four different, 1000 bps cRNA sense and anti-sense probes were generated corresponding to bases-3822-4886; 4837-5849; 5922-7019 and 7102-8171. All probes were in vitro-transcribed and labeled with digoxigenin (Roche Diagnostics). For fluorometric in situ hybridizations, a peroxidase-conjugated anti-digoxygenin-POD antibody (1:500) and tyramide signal amplification (TSA; NEN) were used to detect and visualize the hybridized probes. Immunohistochemistry was performed after in situ hybridization and TSA detection. Chicken anti-NF-200 (1:1000; Abcam) and chicken anti-Peripherin (1:100; Abcam) were used on mouse DRG, while guinea pig anti-TRPV1 (1:1000; Abcam) primary antibodies were used on rat DRG (this antibody did not perform on mouse DRG). Primary antibodies were detected by secondary antibodies conjugated to Alexa Fluor 568. For colorimetric in situ hybridizations, an alkaline phosphatase anti-DIG-AP antibody (Roche; 1:500) was used followed by incubation with NBT/BCIP liquid substrate system (Sigma) for development of the dark purple color. Sections were mounted in Slow fade Gold reagent (Invitrogen) and imaged using a AX70 microscope (Olympus).

[0181] Fluorometric in situ hybridizations were used for quantitation and were imaged using an Olympus (Tokyo, Japan) Fluoview 500 confocal microscope by sequential illumination using the 488 nm line of an argon laser and the HeNe green 543 nm laser. Merge stacked images were created using a 20× and a 40×PlanAPO oil-immersion objective. Images for all experimental groups were taken using identical acquisition parameters and raw images were used for analysis with Image J (NIH). Neurons were considered Piezo2-positive if the mean fluorescence intensity (measured in arbitrary units) was higher than the mean background fluorescence plus 4 times the standard deviation measured from at least 10 random unstained cells. Only sections being at least 50 μm apart were considered to avoid double counting neurons. Neurons were considered NF200- or Peripherin-positive if the mean fluorescence intensity (measured in arbitrary units) was higher than the mean background fluorescence plus 3 times the standard deviation measured from at least 10 random unstained cells. Colorimetric in situ hybridizations of Piezo2 gave similar results (26.9% Piezo2-positive neurons, data not shown), but were not used for quantitation due to variability depending on the length of development with substrate. Only for presentation purposes brightness, contrast and levels of images were adjusted.

[0182] Molecular Cloning of Piezo1.

[0183] Primers were designed from cDNA sequence of mPiezo1 from the NCBI database (NM_001037298). A 7.644 kb fragment was amplified from cDNA libraries generated from Neuro2A total RNA using primers mPiezo1 fwd (5' atggagccgcacgtgctg 3' (SEQ ID NO:21)) and mPiezo1 rev (5' ctactccctctcacgtgtcca 3'(SEQ ID NO:22)) and cloned into pcDNA3.1 (−) (Invitrogen) with NotI and HindIII restriction sites. Fully sequencing this construct revealed insertion of Q at residue 156, and three amino acid changes (R147G, V228I and M1571V) compared to the NCBI sequence.

[0184] This vector was further modified to include 3' AscI and FseI restriction sites and an IRES-GFP PCR fragment from pIRES2-EGFP (Clontech) was then inserted using these sites. The protein sequence of Piezo1 that was cloned from N2A cells (SEQ ID NO:2) is:

MEPHVLGAGLYWLLLPCTLLAASLLRFNALSLVYLLFLLLLPWLPGPSRHSIPGHTGRLLRALLCLSLLFLV

AHLAFQICLHTVPHLDQFLGQNGSLWVKVSQHIGVTRLDLKDIFNTTRLVAPDLGVLLASSLCLGLCGRLT

RKAGQSRRTQELQDDDDDDDDDDEDIDAAPAVGLKGAPALATKRRLWLASRFRVTAHWLLMTSGRTLVI

VLLALAGIAHPSAFSSIYLVVFLAICTWWSCHFPLSPLGFNTLCVMVSCFGAGHLICLYCYQTPFIQDMLPPG

NIWARLFGLKNFVDLPNYSSPNALVLNTKHAWPIYVSPGILLLLYYTATSLLKLHKSCPSELRKETPREDEE

HELELDHLEPEPQARDATQGEMPMTTEPDLDNCTVHVLTSQSPVRQRPVRPRLAELKEMSPLHGLGHLIM

DQSYVCALIAMMVWSIMYHSWLTFVLLLWACLIWTVRSRHQLAMLCSPCILLYGLTLCCLRYVWAMELP

ELPTTLGPVSLHQLGLEHTRYPCLDLGAMLLYLLTFWLLLRQFVKEKLLKKQKVPAALLEVTVADTEPTQT

QTLLRSLGELVTGIYVKYWIYVCAGMFIVVSFAGRLVVYKIVYMFLFLLCLTLFQVYYTLWRKLLRVFWW

LVVAYTMLVLIAVYTFQFQDFPTYWRNLTGFTDEQLGDLGLEQFSVSELFSSILIPGFFLLACILQLHYFHRP

FMQLTDLEHVPPPGTRHPRWAHRQDAVSEAPLLEHQEEEEVFREDGQSMDGPHQATQVPEGTASKWGLV

ADRLLDLAASFSAVLTRIQVFVRRLLELHVFKLVALYTVWVALKEVSVMNLLLVVLWAFALPYPRFRPMA

SCLSTVWTCIIIVCKMLYQLKIVNPHEYSSNCTEPFPNNTNLQPLEINQSLLYRGPVDPANWFGVRKGYPNL

GYIQNHLQILLLLVFEAVVYRRQEHYRRQHQQAPLPAQAVCADGTRQRLDQDLLSCLKYFINFFFYKFGLE

ICFLMAVNVIGQRMNFMVILHGCWLVAILTRRRREAIARLWPNYCLFLTLFLLYQYLLCLGMPPALCIDYP

WRWSKAIPMNSALIKWLYLPDFFRAPNSTNLISDFLLLLCASQQWQVFSAERTEEWQRMAGINTDHLEPLR

GEPNPIPNFIHCRSYLDMLKVAVFRYLFWLVLVVVFVAGATRISIFGLGYLLACFYLLLFGTTLLQKDTRAQ

LVLWDCLILYNVTVIISKNMLSLLSCVFVEQMQSNFCWVIQLFSLVCTVKGYYDPKEMMTRDRDCLLPVEE

AGIIWDSICFFFLLLQRRIFLSHYFLHVSADLKATALQASRGFALYNAANLKSINFHRQIEEKSLAQLKRQMK

RIRAKQEKYRQSQASRGQLQSKDPQDPSQEPGPDSPGGSSPPRRQWWRPWLDHATVIHSGDYFLFESDSEE

EEEALPEDPRPAAQSAFQMAYQAWVTNAQTVLRQRRERARQERAEQLASGGDLNPDVEPVDVPEDEMAG

RSHMMQRVLSTMQFLWVLGQATVDGLTRWLRAFTKHHRTMSDVLCAERYLLTQELLRVGEVRRGVLDQ

LYVGEDEATLSGPVETRDGPSTASSGLGAEEPLSSMTDDTSSPLSTGYNTRSGSEEIVTDAGDLQAGTSLHG

SQELLANARTRMRTASELLLDRRLHIPELEEAERFEAQQGRTLRLLRAGYQCVAAHSELLCYFIIILNHMVT

ASAASLVLPVLVFLWAMLTIPRPSKRFWMTAIVFTEVMVVTKYLFQFGFFPWNSYVVLRRYENKPYFPPRI

LGLEKTDSYIKYDLVQLMALFFHRSQLLCYGLWDHEEDRYPKDHCRSSVKDREAKEEPEAKLESQSETGT

GHPKEPVLAGTPRDHIQGKGSIRSKDVIQDPPEDLKPRHTRHISIRFRRRKETPGPKGTAVMETEHEEGEGKE

TTERKRPRHTQEKSKFRERMKAAGRRLQSFCVSLAQSFYQPLQRFFHDILHTKYRAATDVYALMFLADIVD

IIIIIFGFWAFGKHSAATDIASSLSDDQVPQAFLFMLLVQFGTMVIDRALYLRKTVLGKLAFQVVLVVAIHIW

MFFILPAVTERMFSQNAVAQLWYFVKCIYFALSAYQIRCGYPTRILGNFLTKKYNHLNLFLFQGFRLVPFLV

ELRAVMDWVWTDTTLSLSNWMCVEDIYANIFIIKCSRETEKKYPQPKGQKKKKIVKYGMGGLIILFLIAIIW

FPLLFMSLIRSVVGVVNQPIDVTVTLKLGGYEPLFTMSAQQPSIVPFTPQAYEELSQQFDPYPLAMQFISQYS

PEDIVTAQIEGSSGALWRISPPSRAQMKQELYNGTADITLRFTWNFQRDLAKGGTVEYTNEKHTLELAPNST

ARRQLAQLLEGRPDQSVVIPHLFPKYIRAPNGPEANPVKQLQPDEEEDYLGVRIQLRREQVGTGASGEQAG

TKASDFLEWWVIELQDCKADCNLLPMVIFSDKVSPPSLGFLAGYGIVGLYVSIVLVVGKFVRGFFSEISHSI

MFEELPCVDRILKLCQDIFLVRETRELELEEELYAKLIFLYRSPETMIKWTRERE

[0185] Molecular Cloning of Piezo2. Primers were designed from cDNA sequence of Piezo2 from the NCBI database (NM_001039485). An 8.469 kb fragment was amplified from cDNA libraries generated from adult C57BL/6J DRG total RNA using primers mPiezo2 fwd (5' atggcttcg-gaagtggtgtgc 3' (SEQ ID NO:23)) and mPiezo2 rev (5' tcagtttgtttttctctagtccac 3' (SEQ ID NO:24)) and cloned into pCMV-Sport6 (Invitrogen) with KpnI and NotI restriction sites. Sequencing of the cloned mPiezo2 gene from DRG revealed differences from the NCBI annotation where three

regions of amino acid insertions were not correctly assigned as exons (628E, 14 residues at 833 and 56 residues at 1751). The protein sequence of Piezo2 that we cloned from mouse DRG (SEQ ID NO:4) is:

MASEVVCGLIFRLLLPICLAVACAFRYNGLSFVYLIYLLLIPLFSEPTKATMQGHTGRLLQSLCITSLSFLLLHI

IFHITLASLEAQHRITPAYNCSTWEKTFRQIGFESLKGADAGNGIRVFVPDIGMFIASLTIWLVCRTIVKKPDT

EEIAQLNSECENEELAGGEKMDSEEALIYEEDLDGEEGMEGELEESTKLKILRRFASVASKLKEFIGNMITTA

GKVVVTILLGSSGMMLPSLTSAVYFFVFLGLCTWWSWCRTFDPLLFGCLCVLLAIFTAGHLIGLYLYQFQFF

QEAVPPNDYYARLFGIKSVIQTDCASTWKIIVNPDLSWYHHANPILLLVMYYTLATLIRIWLQEPLVQEEMA

KEDEGALDCSSNQNTAERRRSLWYATQYPTDERKLLSMTQDDYKPSDGLLVTVNGNPVDYHTIHPSLPIEN

GPAKTDLYTTPQYRWEPSEESSEKKEEEEDKREDSEGEGSQEEKRSVRMHAMVAVFQFIMKQSYICALIAM

MAWSITYHSWLTFVLLIWSCTLWMIRNRRKYAMISSPFMVVYANLLLVLQYIWSFELPEIKKVPGFLEKKE

PGELASKILFTITFWLLLRQHLTEQKALREKEALLSEVKIGSQELEEKEDEELQDVQVEGEPTEKEEEEEEEI

KEERHEVKKEEEEEVEEDDDQDIMKVLGNLVVALFIKYWIYVCGGMFFFVSFEGKIVMYKIIYMVLFLFCV

ALYQVHYEWWRKILKYFWMSVVIYTMLVLIFIYTYQFENFPGLWQNMTGLKKEKLEDLGLKQFTVAELFT

RIFIPTSFLLVCILHLHYFHDRFLELTDLKSIPSKEDNTIYSHAKVNGRVYLIINRLAHPEGSLPDLAIMNMTAS

LDKPEVQKLAESGEERPEECVKKTEKGEAGKDSDESEEEEDEEEESEEEESSDLRNKWHLVIDRLTVLFLKF

LEYFHKLQVFMWWILELHIIKIVSSYIIWVTVKEVSLFNYVFLISWAFALPYAKLRRAASSVCTVWTCVIIVC

KMLYQLQTIKPENFSVNCSLPNENQTNIPLHELNKSLLYSAPVDPTEWVGLRKSSPLLVYLRNNLLMLAILA

FEVTVYRHQEYYRGRNNLTAPVSKTIFHDITRLHLDDGLINCAKYFVNYFFYKFGLETCFLMSVNVIGQRM

DFYAMIHACWLIGVLYRRRRKAIAEVWPKYCCFLACIITFQYFVCIGIPPAPCRDYPWRFKGAYFNDNIIKW

LYFPDFIVRPNPVFLVYDFMLLLCASLQRQIFEDENKAAVRIMAGDNVEICMNLDAASFSQHNPVPDFIHCR

SYLDMSKVIIFSYLFWFVLTIIFITGTTRISIFCMGYLVACFYFLLFGGDLLLKPIKSILRYWDWLIAYNVFVIT

MKNILSIGACGYIGALVRNSCWLIQAFSLACTVKGYQMPEDDSRCKLPSGEAGIIWDSICFAFLLLQRRVFM

SYYFLHVVADIKASQILASRGAELFQATIVKAVKARIEEEKKSMDQLKRQMDRIKARQQKYKKGKERMLS

LTQESGEGQDIQKVSEEDDEREADKQKAKGKKKQWWRPWVDHASMVRSGDYYLFETDSEEEEEEELKKE

DEEPPRKSAFQFVYQAWITDPKTALRQRRKEKKKLAREEQKERRKGSGDGPVEWEDREDEPVKKKSDGPD

NIIKRIFNILKFTWVLFLATVDSFTTWLNSISREHIDISTVLRIERCMLTREIKKGNVPTRESIHMYYQNHIMNL

SRESGLDTIDEHSGAGSRAQAAHRMDSLDSRDSISSCYTEATLLISRQSTLDDLDGQDPVPKTSERARPRLR

KMFSLDMSSSSADSGSVASSEPTQCTMLYSRQGTTETIEEVEAEAEEEVVEGLEPELHDAEEKEYAAEYEA

GVEEISLTPDEELPQFSTDDCEAPPSYSKAVSFEHLSFASQDDSGAKNHMVVSPDDSRTDKLESSILPPLTHE

LTASDLLMSKMFHDDELEESEKFYVDQPRFLLLFYAMYNTLVARSEMVCYFVIILNHMTSASIITLLLPILIF

LWAMLSVPRPSRRFWMMAIVYTEVAIVVKYFFQFGFFPWNKDLEIYKERPYFPPNIIGVEKKEGYVLYDLI

QLLALFFHRSILKCHGLWDEDDIVDSNTDKEGSDDELSLDQGRRGSSDSLKSINLAASVESVHVTFPEQPAA

IRRKRSCSSSQISPRSSFSSNRSKRGSTSTRNSSQKGSSVLSLKQKSKRELYMEKLQEHLIKAKAFTIKKTLQI

-continued

YVPIRQFFYDLIHPDYSAVTDVYVLMFLADTVDFIIIVFGFWAFGKHSAAADITSSLSEDQVPGPFLVMVLIQ

FGTMVVDRALYLRKTVLGKVIFQVILVFGIHFWMFFILPGVTERKFSQNLVAQLWYFVKCVYFGLSAYQIR

CGYPTRVLGNFLTKSYNYVNLFLFQGFRLVPFLTELRAVMDWVWTDTTLSLSSWICVEDIYAHIFILKCWR

ESEKRYPQPRGQKKKKAVKYGMGGMIIVLLICIVWFPLLFMSLIKSVAGVINQPLDVSVTITLGGYQPIFTM

SAQQSQLKVMDNSKYNEFLKSFGPNSGAMQFLENYEREDVTVAELEGNSNSLWTISPPSKQKMIQELTDPN

SCFSVVFSWSIQRNMTLGAKAEIATDKLSFPLAVATRNSIAKMIAGNDTESSNTPVTIEKIYPYYVKAPSDSN

SKPIKQLLSENNFMNITIILFRDNVTKSNSEWWVLNLTGSRIFNQGSQALELVVFNDKVSPPSLGFLAGYGIM

GLYASVVLVIGKFVREFFSGISHSIMFEELPNVDRILKLCTDIFLVRETGELELEEDLYAKLIFLYRSPETMIK

WTREKTN

[0186] Phylogenic Analysis.

[0187] Accession numbers of Piezo sequences used to make the phylogenic analysis are given with the number of TM domain predicted using TMHMM2 program. For some species, multiple predicted gene sequences were fused to obtain a complete sequence.

Hs Piezo1 (*Homo Sapiens*): NP_001136336.2, 2521aa (31 TM)

[0188] Mm Piezo1 (*Mus musculus*): NP_001032375.1, 2546aa (30 TM)
Gg Piezo1 (*Gallus gallus*): XP_414209.2, 1718aa; XP_423106.2, 217aa (25 TM)

Dr Piezo1 (*Danio Rerio*): XP_696355.4, 2538aa (29 TM)

Hs Piezo2 (*Homo Sapiens*): NP_071351.2, 2752aa (35 TM)

[0189] Mm Piezo2 (*Mus musculus*): NP_001034574.3, 2753aa (34 TM)
Gg Piezo2 (*Gallus gallus*): XP_419138.2, 3080aa (33 TM)

Dr Piezo2 (*Danio Rerio*): XP_002666625., 2102aa (24 TM)

[0190] Ci Piezo (*Ciona intestinalis*): XP_002122901.1, 1669aa; XP_002128850.1, 591aa (33 TM)
Dm Piezo (*Drosophila melanogaster*): NP_001036346.3, 2671 as (36 TM)
Ce Piezo (*Caenorhabditis elegans*): NP_501648.2, 800a; NP_501647.2, 1843 (33 TM)
Dd Piezo (*Dictyostelium discoideum*): XP_640187, 3080 aa (35 TM)
At Piezo (*Arabidopsis thaliana*): NP_182327.5, 2440 aa (28 TM)
Os Piezo (*Oryza sativa-japonica* group): NP_001043105.1, 2196aa (24 TM)
Tt Piezo i (*Tetrahymena thermophila*): XP_976967.1, 4690aa (30 TM)
Tt Piezo ii (*Tetrahymena thermophila*): XP_001021704.1, 4136aa (29 TM)
Tt Piezo iii (*Tetrahymena thermophila*): XP_001017682.1, 2636aa (26 TM)

[0191] Data Analysis.

[0192] Data in all figures are shown as mean±SEM. Unless otherwise stated, statistical significance was evaluated using unpaired two-tailed Student's t-test for comparing difference between two samples. Unpaired two-tailed Student's t-test with Welch correction was used when variances where significantly different. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Example 2

Neuro2A Cells Express MA Currents

[0193] To identify proteins involved in mechanotransduction, a cell line was sought that expresses a robust MA current similar to those recorded from primary cells (B. Coste, M. Crest, P. Delmas, *J Gen Physiol* 129, 57 (January, 2007)). Several mouse and rat cell lines (Neuro2A, C2C12, NIH/3T3, Min-6, 50B11, F11, PC12) were screened by patch-clamp in the whole cell configuration using a mechanical stimulus consisting of a piezo-electrically driven glass probe (G. C. McCarter, D. B. Reichling, J. D. Levine, *Neurosci Lett* 273, 179 (Oct. 8, 1999); B. Coste, M. Crest, P. Delmas, *J Gen Physiol* 129, 57 (January, 2007); L. J. Drew, J. N. Wood, P. Cesare, *J Neurosci* 22, RC228 (Jun. 15, 2002)). Neuro2A (N2A) mouse neuroblastoma cell line expressed the most consistent MA currents with considerable kinetics of adaptation (FIG. **1**A). In comparison, as a representative of MA currents recorded in other cell lines, the C2C12 mouse myoblast cell line expressed MA currents with slower kinetics of inactivation (FIG. **1**B). All the N2A MA currents recorded at a holding potential of −80 mV are inactivated at the end of a 150 ms mechanical stimulation pulse, whereas the C2C12 MA currents are only partially inactivated (FIG. **1**C). These currents share kinetic properties with some currents expressed in DRG neurons, where rapidly-adapting and slowly-adapting MA currents are described (B. Coste et al., *J Gen Physiol* 129, 57 (January, 2007); L. J. Drew et al., *J Physiol* 556, 691 (May 1, 2004); L. J. Drew et al., *PLoS ONE* 2, e515 (2007); J. Hu, G. R. Lewin, *J Physiol* 577, 815 (Dec. 15, 2006); C. Wetzel et al., *Nature* 445, 206 (Jan. 11, 2007)) (Table 1 and see below). The difference in inactivation kinetics suggests that distinct components of the mechanotransduction apparatus are expressed in these cell lines; however, it is also possible that this difference in inactivation kinetics is due to indirect factors, such as membrane distensibility of the cell lines. Current-voltage relationships of N2A and C2C12 MA currents are linear between −80 and +80 mV with reversal potentials (E_{rev}) at +6.6 and +6.7 mV, respectively, and inward currents are suppressed with NMDG-chloride external solutions (data not shown), suggesting cationic non-selective permeability (FIG. **1**D-E). Finally, the amplitude of MA currents expressed in N2A cells is approximately two-fold larger than the C2C12 MA currents (FIG. **1**F).

TABLE 1

Kinetics of inactivation in untreated and Piezo1-treated cell lines

		Whole-cell mechanical stimulation			Cell-attached stretch	
		Threshold (μm)	Tau inac (ms)	Imax (−80 mV) (pA)	P50 (mm Hg)	Imax (−80 mV) (pA)
N2A	control	5.4 ± 0.3 (n = 71)	12.3 ± 0.6 (n = 74)	−194.3 ± 34.7 (n = 89)	−28.0 ± 1.8 (n = 21)	−8.1 ± 2.0 (n = 28)
	Piezo1 siRNA	n.d.	n.d.	−13.8 ± 3.2 (n = 67)	n.d.	−1.3 ± 0.5 (n = 27)
	Piezo1 cDNA	3.7 ± 0.7 (n = 16)	15.3 ± 1.5 (n = 16)	−3568 ± 567.6 (n = 16)	−28.1 ± 2.8 (n = 13)	−68.6 ± 7.3 (n = 13)
C2C12	control	3.3 ± 0.4 (n = 23)	129.8 ± 55.4 (n = 21)	−101.5 ± 19.7 (n = 25)	n.d.	n.d.
	Piezo1 cDNA	3.0 ± 0.3 (n = 10)	15.6 ± 0.9 (n = 10)	−2482 ± 384.4 (n = 10)	n.d.	n.d.
HEK293T	control	6.0 ± 0.9 (n = 9)	88.7 ± 26.0 (n = 7)	−88.5 ± 19.7 (n = 10)	n.d.	−1.1 ± 0.2 (n = 18)
	Piezo1 cDNA	2.6 ± 0.7 (n = 10)	16.5 ± 1.4 (n = 10)	−3696 ± 641.1 (n = 10)	−31.2 ± 3.5 (n = 11)	−43.0 ± 12.8 (n = 17)

[0194] The N2A MA currents were further characterized by using patch-membrane stretch stimulation in cell-attached mode (Besch et al., Pflugers Arch 445, 161 (October, 2002)). Brief negative pressure pulses evoked opening of endogenous channels (FIG. **1**G), with a single-channel conductance of 22.9±1.4 pS and E_{rev} of +6.2 mV (FIG. **1**H). Increasing the magnitude of pressure pulses induced gradual and reversible opening of these MA channels (FIG. **1**I). The current-pressure relationship is characterized by maximal opening at −60 mm Hg, with a pressure for half-maximal activation (P_{50}) of −28.0±1.8 mm Hg (FIG. **1**J). These conductance and P_{50} values are similar to the properties of reported stretch-activated channels (Cho et al., *Eur J Neurosci* 23, 2543 (May, 2006); Earley et al., *Circ Res* 95, 922 (Oct. 29, 2004); Sharif-Naeini et al., *J Mol Cell Cardiol* 48, 83 (January, 2010)).

Example 3

Piezo1 (Fam38A) is Required for MA Currents of N2A Cells

[0195] To generate a list of candidate MA ion channels in N2A, gene expression profiling was carried out on N2A and other mouse cell lines tested, and transcripts were focused on that are enriched in N2A cells using a combination of criteria. Proteins predicted to span the membrane at least two times (a characteristic shared by all ion channels) were selected. This list was prioritized by picking either known cation channels, or proteins with unknown function. Each candidate was tested using siRNA knockdown in N2A cells, measuring MA currents via piezo-driven pressure stimulation in the whole cell mode. Knockdown of Fam38A (Family with sequence similarity 38), the 73rd candidate, caused a pronounced decrease of MA currents (FIG. **2**A-B, Table 2). In follow-up experiments, robust attenuation of MA currents was observed with different siRNAs directed against this gene (FIG. **2**C). All the siRNAs tested significantly decreased target transcripts as assayed by qPCR (FIG. **3**A).

TABLE 2

Candidate proteins for siRNA knockdown

No:	siRNA:
1	Scrambled
2	2400010G15Rik
3	2410015B03Rik
4	4833424O15Rik
5	Tmem129
6	1500016O10Rik
7	2810432L12Rik
8	3632451O06Rik
9	4930500O05Rik
10	9330182L06Rik
11	A830020B06Rik
12	Accn2
13	Al848100
14	B430119L13Rik
15	BC042720
16	BC062109
17	Chrna3
18	Creld1
19	D630045J12Rik
20	Emb
21	Gpr173
22	Grik5
23	Htr3a
24	Htr3b
25	Josd2
26	Leprotl1
27	Lphn1
28	Mcam
29	Mfap3
30	Npal2
31	Npal3
32	Nrsn1-Vmp
33	P2X3
34	Pcdh1
35	Pcnxl2
36	PKD1L2
37	Prrt3
38	Punc
39	Reep2
40	Rom1
41	Sfxn5
42	Slc16a6
43	Slc47a1

TABLE 2-continued

Candidate proteins for siRNA knockdown

No:	siRNA:
44	Slc7a3
45	Slc7a7
46	Slc8a1
47	TM6sf1
48	Tmc6
49	Tmem108
50	Tmem136
51	Tmem161a
52	Tmem164
53	Tmem16f
54	TMEM176a
55	TMEM176b
56	Tmem181
57	Tmem20
58	Tmem41a
59	Tmem54
60	Tmem56
61	Tmem74
62	Tmhs
63	Tmtc1
64	Tmtc2
65	TRPM2
66	TRPML3
67	TRPV2
68	Tspan13
69	Tspan18
70	Tspan2
71	Tspan33
72	Xkr6
73	Fam38a

[0196] Given that Fam38A encodes a protein required for the expression of ion channels activated by pressure, this gene was named Piezo1, from the Greek "πιεση" (piesi) meaning pressure. To test whether knocking down Piezo1 impairs general cell signaling or viability, N2A cells were transfected with TRPV1 cDNA and either scrambled or Piezo1 siRNA. No differences were observed in capsaicin responses (FIG. **3**B-C). Next, experiments were conducted to determine if Piezo1 is also required for N2A MA currents elicited by patch membrane stretch (FIG. **2**D-E). Once again, strong knockdown of MA currents was observed with siRNA against Piezo1. This suggests that Piezo1 is required for the expression of the MA currents recorded using either of the two mechanostimulation protocols.

[0197] Very little is known about mammalian Piezo1 (KIAA0233, Fam38A, Mib). Its expression is induced in senile plaque-associated astrocytes (K. Satoh et al., *Brain Res* 1108, 19 (Sep. 7, 2006)), and the protein has been suggested to be involved in integrin activation (B. J. McHugh et al., *J Cell Sci* 123, 51 (Jan. 1, 2010)). Extracellular perfusion of divalent-free and 5 mM EGTA solution for 30-60 min, which disrupts integrin function (R. O. Hynes, *Cell* 110, 673 (Sep. 20, 2002)), did not suppress MA currents (FIG. **3**D-E). Thus it is unlikely that Piezo1 siRNA blocks MA currents through integrin modulation. However, it is possible that mechanical activation of Piezo1 could lead to integrin activation and various downstream consequences including cell adhesion, division, and migration.

Example 4

Piezos are Large Transmembrane Proteins Conserved Among Various Species

[0198] Piezo proteins are present in non-mammalian species, none reported as characterized. Many animal, plant, and other eukaryotic species contain a single Piezo (FIG. **4**A). Vertebrates (mammals, birds, fish) have two members, Piezo1 (Fam38A) and Piezo2 (Fam38B). However, the early chordate Ciona has a single member. Multiple Piezos are also present in the Ciliophora kingdom: *Tetrahymena thermophila* has three members; Paramecium Tetraurelia, six (not shown). No clear homologs were identified in yeast or bacteria. The secondary structure and overall length of Piezo proteins are moderately conserved, while homology to other proteins is minimal. As assayed by the TMHMM2 program, all have between 24-36 predicted transmembrane domains (with variability perhaps due to inaccurate cDNA or transmembrane prediction). The predicted proteins are 2100-4700 amino acids, and the transmembrane domains are located throughout the putative protein, as illustrated by the hydrophobicity plot of mouse Piezo1 (FIG. **4**B).

[0199] The expression profile of Piezo1 determined by qPCR (FIG. **4**C) includes robust expression in bladder, colon, kidney, lung and skin, and low expression in the other tissues tested including DRG sensory neurons. This pattern agrees with Northern blot expression analysis in rat (K. Satoh et al., *Brain Res* 1108, 19 (Sep. 7, 2006)). Bladder, colon, and lung undergo mechanotransduction related to visceral pain (G. Burnstock, *Mol Pain* 5, 69 (2009)), and primary cilia in the kidney sense urinary flux (L. Rodat-Despoix, P. Delmas, *Pflugers Arch* 458, 179 (May, 2009)). The low level of mRNA in DRG suggests that Piezo1 may not account for MA currents observed there (B. Coste, M. Crest, P. Delmas, *J Gen Physiol* 129, 57 (January, 2007); L. J. Drew, J. N. Wood, P. Cesare, *J Neurosci* 22, RC228 (Jun. 15, 2002); J. Hu, G. R. Lewin, *J Physiol* 577, 815 (Dec. 15, 2006)), but Piezo1 is expressed in the skin, another putative site of somatosensation. Piezo2 expression is observed in bladder, colon and lung as well, but less in kidney or skin. Interestingly, very strong expression of Piezo2 is observed in DRG sensory neurons, suggesting a potential role in somatosensory mechanotransduction (see below).

Example 5

Piezo1 Induces MA Currents in Various Cell Types

[0200] The full-length Piezo1 from N2A cells was cloned into the pIRES2-EGFP vector. Electrophysiological recordings of MA currents in the whole-cell mode were performed 12-48 hours after transfection. Piezo1 transfected cells, but not mock-transfected cells, showed large MA currents in N2A, HEK293T (FIG. **5**A-F) and C2C12 cell-lines (FIG. **6**A-C). In all cells overexpressing Piezo1, the MA current-voltage relationships were similar to endogenous N2A MA currents (FIGS. **5**B, **5**E, **6**B, and **1**D), with E, mV. The threshold of activation and the time constant for inactivation of MA currents elicited in Piezo1 overexpressing cells is similar in all three cell lines tested (see Table 1). Next, the ionic selectivity of Piezo1-induced MA currents was characterized. Substituting the non-permeant cation NMDG in the extracellular bathing solution suppressed inward MA currents, demonstrating that this channel activity is cationic in nature (FIG. **6**D). The ionic selectivity was further examined by recording with CsCl-only internal solutions and various cations in the bath. Na^+, K^+, Ca^{2+} and Mg^{2+} ions were all able to permeate, with a slight preference for Ca^{2+} (FIG. **5**G and FIG. **6**E-F). Moreover, 30 μM of ruthenium red and gadolinium, known blockers of many cationic MA currents (L. J. Drew, J. N. Wood, P. Cesare, *J Neurosci* 22, RC228 (Jun. 15, 2002); J.

Hao et al., in *Mechanosensitivity of the Nervous System*, I. K. e. A. Kamkin, Ed. (Springer Netherlands, 2008), vol. 2, pp. 51-67), blocked 74.6±2.5% (n=6) and 84.3±3.8% (n=5) of Piezo1-induced MA current, respectively (FIGS. **5**H and **6**G-H).

[0201] Next, patch-membrane stretch stimulation was used in cell-attached mode to assay Piezo1-transfected cells (FIG. **5**I-N). Overexpression of Piezo1 in N2A and HEK293T cells gave rise to large currents elicited by negative pressure pulses (FIGS. **5**I, **5**L). The MA current-pressure relationships in cells overexpressing Piezo1 and in endogenous N2A cells is similar, with P_{50} of −28.1±2.8 and −31.2±3.5 mm Hg in N2A and HEK293T overexpressing cells, respectively (FIGS. **5**J, **5**M, and **1**J). No channel activity similar to N2A endogenous MA channels was detected in vector-only transfected HEK293T cells (data not shown). Therefore, Piezo1 overexpression induces MA currents elicited both by piezoelectrically-driven glass probe stimulation and by patch-membrane stretch in all the cell lines tested. This is the first demonstration that these two different mechanostimulation protocols activate the same MA channels.

Example 6

Piezo2 Induces MA Currents Distinct from Piezo1-Induced Currents

[0202] The full-length Piezo2 was cloned from DRG neurons and its mechanosensitivity upon heterologous expression was tested. Piezo2/GFP co-transfected, but not mock-transfected cells, revealed large MA currents in N2A (co-transfected with Piezo1 siRNA to suppress endogenous MA currents) and HEK293T cells (FIG. **7**A-F). The Piezo2-induced MA current-voltage relationship is linear between −80 and +80 mV (FIGS. **7**B, **7**E), with a reversal potential (E_{rev}) of +6.3±0.4 mV (n=3) and +8.7±1.5 mV (n=7) in N2A and HEK293T cells, respectively, suggesting relatively non-selective cationic conductance. Similar to Piezo1, Piezo2-induced currents were suppressed by the non-permeant cation NMDG and inhibited by gadolinium and ruthenium red [85.0±3.7% (n=5) and 79.2±4.2% (n=5), respectively] (FIG. **8**). These characteristics fit the profile of MA currents recorded from DRG neurons (G. C. McCarter et al., *Neurosci Lett* 273, 179 (Oct. 8, 1999); L. J. Drew et al., *J Neurosci* 22, RC228 (Jun. 15, 2002); but see also J. Hu, G. R. Lewin, *J Physiol* 577, 815 (Dec. 15, 2006)).

[0203] The inactivation kinetics of heterologously expressed Piezo2-induced MA currents are best-fitted with a mono-exponential equation, and calculated time constants for inactivation (τ_{inac}) are relatively fast in both N2A (6.8±0.7 ms, n=27) and HEK293T (7.3±0.7, n=11) cells when measured at −80 mV. Furthermore, the kinetics of inactivation of Piezo2-induced MA currents are faster than Piezo1-induced MA currents, both for inward (FIG. **7**G) and outward (FIG. **7**H) currents, and at all holding potentials tested (FIG. **7**I). These differences are not due to higher Ca^{2+} permeability, since the Ca^{2+} selectivity of Piezo1- and Piezo2-induced MA currents are similar (data not shown). Therefore, Piezo1 and Piezo2 confer unique channel properties. Initial attempts to further characterize properties of Piezo2-induced MA currents using negative-pressure stimulations of membrane patches in cell-attached mode could not detect Piezo2-dependent channel activity (not shown, 21 patches).

Example 7

Piezo1 is detected at the plasma membrane

[0204] The results above suggest that Piezo1 and 2 are components of mechanotransduction complexes and therefore should be present at the plasma membrane. Previous reports have shown expression of Fam38A (Piezo1) in the endoplasmic reticulum (K. Satoh et al., Brain Res 1108, 19 (Sep. 7, 2006); B. J. McHugh et al., *J Cell Sci* 123, 51 (Jan. 1, 2010)). A peptide antibody against mouse Piezo1 was generated. This antibody specifically recognized Piezo1-transfected HEK293T cells, but not naive HEK293T cells (FIG. **9**A). In cells transfected with Piezo1 and TRPA1, an ion channel known to be expressed at the plasma membrane, co-staining of Piezo1 was observed with live-labelled surface TRPA1 (M. Schmidt, A. E. Dubin, M. J. Petrus, T. J. Earley, A. Patapoutian, *Neuron* 64, 498 (Nov. 25, 2009)), although the majority of Piezo1 expression (similar to TRPA1) is present inside the cell. This demonstrates that Piezo1 protein can be localized at or near the plasma membrane (FIG. **9**B). The antibody was not sensitive enough to detect endogenous N2A expression of Piezo1 (data not shown).

Example 8

Piezo2 is Required for DRG Rapidly-Adapting MA Currents

[0205] Piezo2 but not Piezo1 is expressed at relatively high levels in DRGs as assessed by qPCR (FIG. **4**C). To characterize Piezo2 expression within the heterogeneous population of neurons and glial cells of the DRGs, in situ hybridization was performed on adult mouse DRG sections (FIG. **10**A). Piezo2 mRNA expression was observed in 20% of DRG neurons (from 2391 total neurons—see methods section below). Piezo2 is expressed in a subset of DRG neurons also expressing peripherin (60%) and Neurofilament 200 (28%), markers present in mechanosensory neurons (FIG. **11**) (M. E. Goldstein et al., *J Neurosci Res* 30, 92 (September, 1991); S. N. Lawson, *Exp Physiol* 87, 239 (March, 2002); S, N. Lawson et al., *J Comp Neurol* 228, 263 (Sept. 10, 1984); H. Sann et al., *Cell Tissue Res* 282, 155 (October, 1995)). Some overlap with nociceptive marker TRPV1 (24%) further suggests a potential role of Piezo2 in noxious mechanosensation.

[0206] Next, the role of Piezo2 in MA currents of DRG neurons was directly examined using siRNA transfection. The RNAi approach was first validated on TRPA1, an ion channel expressed in DRG neurons and activated by mustard oil (MO) (M. Bandell et al., *Neuron* 41, 849 (Mar. 25, 2004); S. E. Jordt et al., *Nature* 427, 260 (Jan. 15, 2004)) (FIG. **12**A-B). The ability of siRNAs to block functional expression of Piezo2 was demonstrated in N2A cells co-transfected with both Piezo2 cDNA and Piezo2 siRNA (FIG. **12**C, 15-fold decrease). Then whole-cell MA currents were recorded from DRG neurons co-transfected with GFP and either scrambled or Piezo2 siRNA (n=101 for scrambled, and n=109 for Piezo2 siRNA). The recorded MA currents were grouped according to their inactivation kinetics, as previously described (FIG. **10**B) (B. Coste et al., *J Gen Physiol* 129, 57 (January, 2007);

L. J. Drew et al., *J Neurosci* 22, RC228 (Jun. 15, 2002); L. J. Drew et al., *J Physiol* 556, 691 (May 1, 2004); J. Hu, G. R. Lewin, *J Physiol* 577, 815 (Dec. 15, 2006); C. Wetzel et al., *Nature* 445, 206 (Jan. 11, 2007)). Four different classes of neurons were defined based on τ_{inac} distribution in scrambled siRNA transfected cells (FIG. **6**D): τ_{inac}<10 ms, 10<τ_{inac}<30, τ_{inac}>30, and non-responsive neurons. Piezo2-expressing neurons with T_{inac}~7 ms (FIG. **7**) would be expected within the τ_{inac}<10 ms DRG population. Remarkably, the proportion of neurons expressing MA currents with τ_{inac}<10 ms was specifically and significantly reduced in Piezo2 siRNA compared to scrambled siRNA transfected neurons (FIG. **10**C). 28.7% of scrambled siRNA transfected neurons had τ_{inac}<10 ms versus 7.3% in Piezo2 siRNA transfected neurons (FIG. **10**D). Neurons with MA currents with slower kinetics (τ_{inac} between 10 and 30 ms and τ_{inac}>30 ms) were present at normal populations in Piezo2 siRNA samples. A trend towards increased numbers of mechanically insensitive neurons was observed, as predicted if loss of Piezo2 converts rapidly adapting neurons into non-responders. These RNAi data also were analyzed according to the degree of current inactivation during the 150 ms test pulse and came to similar conclusions (FIG. **12**E). It is unknown whether the remaining MA currents with τ_{inac}<10 ms are due to incomplete Piezo2 knockdown or the presence of another channel complex. Regardless, Piezo2 is specifically required for the majority of the rapidly adapting MA ion channel activity in cultured DRGs.

Example 9

Therapeutic Benefit of Targeting of Piezo1 and Piezo2

[0207] Many studies have implicated calcium-permeable mechanically-activated (MA) cationic currents in vertebrate mechanotransduction (O. P. Hamill, B. Martinac, *Physiol Rev* 81, 685 (April, 2001)). Cell-based mechanical sensitivity was assayed here using two different, well-established methods: a piezoelectrically-driven pressure applied from a glass probe in whole-cell conformation, and stretch of the plasma membrane through a patch pipette in cell-attached mode (J. Hao et al., in *Mechanosensitivity of the Nervous System*, I. K. e. A. Kamkin, Ed. (Springer Netherlands, 2008), vol. 2, pp. 51-67). Using these assays, Piezo1 was found to be required for MA currents in Neuro2A cells, and Piezo2, for a subset of MA currents in DRG neurons. Moreover, overexpressing Piezo1 or Piezo2 in three different cell types gives rise to a remarkable 17-300 fold increase in MA currents. Therefore, Piezos are both necessary and sufficient for the expression of a MA current in various cell types. Notably, Piezo1 or Piezo2 overexpression confers unique adaptation properties of MA currents, arguing that they are components of distinct MA ion channels.

[0208] Piezo1 and Piezo2 sequences appear unique, not resembling known ion channels or other protein classes. The very large number of predicted transmembrane domains (30 and 34 transmembranes for mouse Piezo1 and Piezo2, respectively) is reminiscent of voltage-activated sodium channels with 24 transmembrane domains, composed of a 4-fold repeat of 6-transmembrane units (M. R. Hanlon, B. A. Wallace, *Biochemistry* 41, 2886 (Mar. 5, 2002)). However, pore-containing or repetitive domains have not initially been observed in Piezo proteins. It may be that Piezo proteins are non-conducting subunits of ion channels required for proper expression or for modulating channel properties, similar to beta subunits of voltage-gated channels (M. R. Hanlon, B. A. Wallace, *Biochemistry* 41, 2886 (Mar. 5, 2002)) or SUR subunits of ATP-sensitive K+ channels (S. J. Tucker, F. M. Ashcroft, *Curr Opin Neurobiol* 8, 316 (June, 1998)). This is unlikely, since it would imply that all the cell types used here express a silent conducting subunit of an MA channel that requires Piezos to function. Alternatively, Piezo proteins may define a novel class of ion channels, akin to Orail, a recently identified ion-conducting channel without significant homology to previously known channels (M. Prakriya et al., *Nature* 443, 230 (Sep. 14, 2006)). Piezo1/Fam38A has also been found in the endoplasmic reticulum (K. Satoh et al., *Brain Res* 1108, 19 (Sep. 7, 2006); B. J. McHugh et al., *J Cell Sci* 123, 51 (Jan. 1, 2010)), so Piezos may act at both the plasma membrane and in intracellular compartments. Indeed, the data here have shown that overexpressed Piezo1 can be observed at or near the plasma membrane.

[0209] Piezo1 is expressed in a variety of tissues involved in mechanotransduction, including in the kidney. Interestingly, stretch-activated channels with similar properties have been described in kidney-derived cells (R. Sharif-Naeini et al., *J Mol Cell Cardiol* 48, 83 (January, 2010); P. Gottlieb et al., *Pflugers Arch,* (Oct. 23, 2007)). Piezo1 expressed sequenced tags (ESTs) are also found in the inner ear. The conductance of MA channels of hair cells varies according to location in the cochlea, ranging from 80-163 pS (A. J. Ricci et al., *Neuron* 40, 983 (Dec. 4, 2003)). Although this range does not resemble that conducted via Piezo1, the variability in conductance suggests it may be modulated by yet unknown factors, and therefore a candidate should not be excluded on this basis.

[0210] Piezo2 is expressed in sensory neurons and is required for mechanically-activated currents. Mechanical hyperalgesia is a condition prevalent in many pain conditions including inflammatory and neuropathic pain. Therefore, Piezo2 can be a target for a variety of pain, itch, and inflammation indications. The targeting of Piezo1 and Piezo2 could also have therapeutic benefit in a variety of indications including hearing, adjustment of vascular tone and blood flow, urine flow sensing in kidney, lung growth and injury, as well as bone and muscle homeostasis, all of which are all regulated by mechanotransduction.

SEQUENCE LISTING

[0211] A sequence listing is submitted along with this application as an ASCII text file entitled 87396-817888_ST25.txt. This file was created on Aug. 23, 2011 and is 139 kilobytes.

[0212] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

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<213> ORGANISM: Mus musculus

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Cys Thr Leu Leu Ala Ala Ser Leu Leu Arg Phe Asn Ala Leu Ser Leu
            20                  25                  30

Val Tyr Leu Leu Phe Leu Leu Leu Leu Pro Trp Leu Pro Gly Pro Ser
        35                  40                  45

Arg His Ser Ile Pro Gly His Thr Gly Arg Leu Leu Arg Ala Leu Leu
    50                  55                  60

Cys Leu Ser Leu Leu Phe Leu Val Ala His Leu Ala Phe Gln Ile Cys
65                  70                  75                  80

Leu His Thr Val Pro His Leu Asp Gln Phe Leu Gly Gln Asn Gly Ser
                85                  90                  95

Leu Trp Val Lys Val Ser Gln His Ile Gly Val Thr Arg Leu Asp Leu
            100                 105                 110

Lys Asp Ile Phe Asn Thr Thr Arg Leu Val Ala Pro Asp Leu Gly Val
        115                 120                 125

Leu Leu Ala Ser Ser Leu Cys Leu Gly Leu Cys Gly Arg Leu Thr Arg
    130                 135                 140

Lys Ala Gly Gln Ser Arg Arg Thr Gln Glu Leu Gln Asp Asp Asp Asp
145                 150                 155                 160

Asp Asp Asp Asp Asp Asp Glu Asp Ile Asp Ala Ala Pro Ala Val Gly
                165                 170                 175
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Leu Lys Gly Ala Pro Ala Leu Ala Thr Lys Arg Arg Leu Trp Leu Ala
            180                 185                 190

Ser Arg Phe Arg Val Thr Ala His Trp Leu Leu Met Thr Ser Gly Arg
        195                 200                 205

Thr Leu Val Ile Val Leu Leu Ala Leu Ala Gly Ile Ala His Pro Ser
    210                 215                 220

Ala Phe Ser Ser Ile Tyr Leu Val Val Phe Leu Ala Ile Cys Thr Trp
225                 230                 235                 240

Trp Ser Cys His Phe Pro Leu Ser Pro Leu Gly Phe Asn Thr Leu Cys
                245                 250                 255

Val Met Val Ser Cys Phe Gly Ala Gly His Leu Ile Cys Leu Tyr Cys
            260                 265                 270

Tyr Gln Thr Pro Phe Ile Gln Asp Met Leu Pro Pro Gly Asn Ile Trp
        275                 280                 285

Ala Arg Leu Phe Gly Leu Lys Asn Phe Val Asp Leu Pro Asn Tyr Ser
    290                 295                 300

Ser Pro Asn Ala Leu Val Leu Asn Thr Lys His Ala Trp Pro Ile Tyr
305                 310                 315                 320

Val Ser Pro Gly Ile Leu Leu Leu Leu Tyr Tyr Thr Ala Thr Ser Leu
                325                 330                 335

Leu Lys Leu His Lys Ser Cys Pro Ser Glu Leu Arg Lys Glu Thr Pro
            340                 345                 350

Arg Glu Asp Glu Glu His Glu Leu Glu Leu Asp His Leu Glu Pro Glu
        355                 360                 365

Pro Gln Ala Arg Asp Ala Thr Gln Gly Glu Met Pro Met Thr Thr Glu
    370                 375                 380

Pro Asp Leu Asp Asn Cys Thr Val His Val Leu Thr Ser Gln Ser Pro
385                 390                 395                 400

Val Arg Gln Arg Pro Val Arg Pro Arg Leu Ala Glu Leu Lys Glu Met
                405                 410                 415

Ser Pro Leu His Gly Leu Gly His Leu Ile Met Asp Gln Ser Tyr Val
            420                 425                 430

Cys Ala Leu Ile Ala Met Met Val Trp Ser Ile Met Tyr His Ser Trp
        435                 440                 445

Leu Thr Phe Val Leu Leu Leu Trp Ala Cys Leu Ile Trp Thr Val Arg
    450                 455                 460

Ser Arg His Gln Leu Ala Met Leu Cys Ser Pro Cys Ile Leu Leu Tyr
465                 470                 475                 480

Gly Leu Thr Leu Cys Cys Leu Arg Tyr Val Trp Ala Met Glu Leu Pro
                485                 490                 495

Glu Leu Pro Thr Thr Leu Gly Pro Val Ser Leu His Gln Leu Gly Leu
            500                 505                 510

Glu His Thr Arg Tyr Pro Cys Leu Asp Leu Gly Ala Met Leu Leu Tyr
        515                 520                 525

Leu Leu Thr Phe Trp Leu Leu Leu Arg Gln Phe Val Lys Glu Lys Leu
    530                 535                 540

Leu Lys Lys Gln Lys Val Pro Ala Ala Leu Leu Glu Val Thr Val Ala
545                 550                 555                 560

Asp Thr Glu Pro Thr Gln Thr Gln Thr Leu Leu Arg Ser Leu Gly Glu
                565                 570                 575

Leu Val Thr Gly Ile Tyr Val Lys Tyr Trp Ile Tyr Val Cys Ala Gly
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           580                 585                 590

Met Phe Ile Val Val Ser Phe Ala Gly Arg Leu Val Val Tyr Lys Ile
        595                 600                 605

Val Tyr Met Phe Leu Phe Leu Leu Cys Leu Thr Leu Phe Gln Val Tyr
    610                 615                 620

Tyr Thr Leu Trp Arg Lys Leu Leu Arg Val Phe Trp Trp Leu Val Val
625                 630                 635                 640

Ala Tyr Thr Met Leu Val Leu Ile Ala Val Tyr Thr Phe Gln Phe Gln
                645                 650                 655

Asp Phe Pro Thr Tyr Trp Arg Asn Leu Thr Gly Phe Thr Asp Glu Gln
            660                 665                 670

Leu Gly Asp Leu Gly Leu Glu Gln Phe Ser Val Ser Glu Leu Phe Ser
        675                 680                 685

Ser Ile Leu Ile Pro Gly Phe Phe Leu Leu Ala Cys Ile Leu Gln Leu
    690                 695                 700

His Tyr Phe His Arg Pro Phe Met Gln Leu Thr Asp Leu Glu His Val
705                 710                 715                 720

Pro Pro Pro Gly Thr Arg His Pro Arg Trp Ala His Arg Gln Asp Ala
                725                 730                 735

Val Ser Glu Ala Pro Leu Leu Glu His Gln Glu Glu Glu Glu Val Phe
            740                 745                 750

Arg Glu Asp Gly Gln Ser Met Asp Gly Pro His Gln Ala Thr Gln Val
        755                 760                 765

Pro Glu Gly Thr Ala Ser Lys Trp Gly Leu Val Ala Asp Arg Leu Leu
    770                 775                 780

Asp Leu Ala Ala Ser Phe Ser Ala Val Leu Thr Arg Ile Gln Val Phe
785                 790                 795                 800

Val Arg Arg Leu Leu Glu Leu His Val Phe Lys Leu Val Ala Leu Tyr
                805                 810                 815

Thr Val Trp Val Ala Leu Lys Glu Val Ser Val Met Asn Leu Leu Leu
            820                 825                 830

Val Val Leu Trp Ala Phe Ala Leu Pro Tyr Pro Arg Phe Arg Pro Met
        835                 840                 845

Ala Ser Cys Leu Ser Thr Val Trp Thr Cys Ile Ile Ile Val Cys Lys
    850                 855                 860

Met Leu Tyr Gln Leu Lys Ile Val Asn Pro His Glu Tyr Ser Ser Asn
865                 870                 875                 880

Cys Thr Glu Pro Phe Pro Asn Asn Thr Asn Leu Gln Pro Leu Glu Ile
                885                 890                 895

Asn Gln Ser Leu Leu Tyr Arg Gly Pro Val Asp Pro Ala Asn Trp Phe
            900                 905                 910

Gly Val Arg Lys Gly Tyr Pro Asn Leu Gly Tyr Ile Gln Asn His Leu
        915                 920                 925

Gln Ile Leu Leu Leu Leu Leu Val Phe Glu Ala Val Val Tyr Arg Arg Gln
    930                 935                 940

Glu His Tyr Arg Arg Gln His Gln Gln Ala Pro Leu Pro Ala Gln Ala
945                 950                 955                 960

Val Cys Ala Asp Gly Thr Arg Gln Arg Leu Asp Gln Asp Leu Leu Ser
                965                 970                 975

Cys Leu Lys Tyr Phe Ile Asn Phe Phe Phe Tyr Lys Phe Gly Leu Glu
            980                 985                 990
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Ile Cys Phe Leu Met Ala Val Asn  Val Ile Gly Gln Arg  Met Asn Phe
        995                1000                1005

Met Val  Ile Leu His Gly Cys  Trp Leu Val Ala Ile  Leu Thr Arg
    1010                1015                1020

Arg Arg  Arg Glu Ala Ile Ala  Arg Leu Trp Pro Asn  Tyr Cys Leu
    1025                1030                1035

Phe Leu  Thr Leu Phe Leu Leu  Tyr Gln Tyr Leu Leu  Cys Leu Gly
    1040                1045                1050

Met Pro  Pro Ala Leu Cys Ile  Asp Tyr Pro Trp Arg  Trp Ser Lys
    1055                1060                1065

Ala Ile  Pro Met Asn Ser Ala  Leu Ile Lys Trp Leu  Tyr Leu Pro
    1070                1075                1080

Asp Phe  Phe Arg Ala Pro Asn  Ser Thr Asn Leu Ile  Ser Asp Phe
    1085                1090                1095

Leu Leu  Leu Leu Cys Ala Ser  Gln Gln Trp Gln Val  Phe Ser Ala
    1100                1105                1110

Glu Arg  Thr Glu Glu Trp Gln  Arg Met Ala Gly Ile  Asn Thr Asp
    1115                1120                1125

His Leu  Glu Pro Leu Arg Gly  Glu Pro Asn Pro Ile  Pro Asn Phe
    1130                1135                1140

Ile His  Cys Arg Ser Tyr Leu  Asp Met Leu Lys Val  Ala Val Phe
    1145                1150                1155

Arg Tyr  Leu Phe Trp Leu Val  Leu Val Val Val Phe  Val Ala Gly
    1160                1165                1170

Ala Thr  Arg Ile Ser Ile Phe  Gly Leu Gly Tyr Leu  Leu Ala Cys
    1175                1180                1185

Phe Tyr  Leu Leu Leu Phe Gly  Thr Thr Leu Leu Gln  Lys Asp Thr
    1190                1195                1200

Arg Ala  Gln Leu Val Leu Trp  Asp Cys Leu Ile Leu  Tyr Asn Val
    1205                1210                1215

Thr Val  Ile Ile Ser Lys Asn  Met Leu Ser Leu Leu  Ser Cys Val
    1220                1225                1230

Phe Val  Glu Gln Met Gln Ser  Asn Phe Cys Trp Val  Ile Gln Leu
    1235                1240                1245

Phe Ser  Leu Val Cys Thr Val  Lys Gly Tyr Tyr Asp  Pro Lys Glu
    1250                1255                1260

Met Met  Thr Arg Asp Arg Asp  Cys Leu Leu Pro Val  Glu Glu Ala
    1265                1270                1275

Gly Ile  Ile Trp Asp Ser Ile  Cys Phe Phe Phe Leu  Leu Leu Gln
    1280                1285                1290

Arg Arg  Ile Phe Leu Ser His  Tyr Phe Leu His Val  Ser Ala Asp
    1295                1300                1305

Leu Lys  Ala Thr Ala Leu Gln  Ala Ser Arg Gly Phe  Ala Leu Tyr
    1310                1315                1320

Asn Ala  Ala Asn Leu Lys Ser  Ile Asn Phe His Arg  Gln Ile Glu
    1325                1330                1335

Glu Lys  Ser Leu Ala Gln Leu  Lys Arg Gln Met Lys  Arg Ile Arg
    1340                1345                1350

Ala Lys  Gln Glu Lys Tyr Arg  Gln Ser Gln Ala Ser  Arg Gly Gln
    1355                1360                1365

Leu Gln  Ser Lys Asp Pro Gln  Asp Pro Ser Gln Glu  Pro Gly Pro
    1370                1375                1380
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Asp Ser  Pro Gly Gly Ser Ser  Pro Pro Arg Arg Gln  Trp Trp Arg
    1385                1390                1395

Pro Trp  Leu Asp His Ala Thr  Val Ile His Ser Gly  Asp Tyr Phe
    1400                1405                1410

Leu Phe  Glu Ser Asp Ser Glu  Glu Glu Glu Glu Ala  Leu Pro Glu
    1415                1420                1425

Asp Pro  Arg Pro Ala Ala Gln  Ser Ala Phe Gln Met  Ala Tyr Gln
    1430                1435                1440

Ala Trp  Val Thr Asn Ala Gln  Thr Val Leu Arg Gln  Arg Arg Glu
    1445                1450                1455

Arg Ala  Arg Gln Glu Arg Ala  Glu Gln Leu Ala Ser  Gly Gly Asp
    1460                1465                1470

Leu Asn  Pro Asp Val Glu Pro  Val Asp Val Pro Glu  Asp Glu Met
    1475                1480                1485

Ala Gly  Arg Ser His Met Met  Gln Arg Val Leu Ser  Thr Met Gln
    1490                1495                1500

Phe Leu  Trp Val Leu Gly Gln  Ala Thr Val Asp Gly  Leu Thr Arg
    1505                1510                1515

Trp Leu  Arg Ala Phe Thr Lys  His His Arg Thr Met  Ser Asp Val
    1520                1525                1530

Leu Cys  Ala Glu Arg Tyr Leu  Leu Thr Gln Glu Leu  Leu Arg Val
    1535                1540                1545

Gly Glu  Val Arg Arg Gly Val  Leu Asp Gln Leu Tyr  Val Gly Glu
    1550                1555                1560

Asp Glu  Ala Thr Leu Ser Gly  Pro Val Glu Thr Arg  Asp Gly Pro
    1565                1570                1575

Ser Thr  Ala Ser Ser Gly Leu  Gly Ala Glu Glu Pro  Leu Ser Ser
    1580                1585                1590

Met Thr  Asp Asp Thr Ser Ser  Pro Leu Ser Thr Gly  Tyr Asn Thr
    1595                1600                1605

Arg Ser  Gly Ser Glu Glu Ile  Val Thr Asp Ala Gly  Asp Leu Gln
    1610                1615                1620

Ala Gly  Thr Ser Leu His Gly  Ser Gln Glu Leu Leu  Ala Asn Ala
    1625                1630                1635

Arg Thr  Arg Met Arg Thr Ala  Ser Glu Leu Leu Leu  Asp Arg Arg
    1640                1645                1650

Leu His  Ile Pro Glu Leu Glu  Glu Ala Glu Arg Phe  Glu Ala Gln
    1655                1660                1665

Gln Gly  Arg Thr Leu Arg Leu  Leu Arg Ala Gly Tyr  Gln Cys Val
    1670                1675                1680

Ala Ala  His Ser Glu Leu Leu  Cys Tyr Phe Ile Ile  Ile Leu Asn
    1685                1690                1695

His Met  Val Thr Ala Ser Ala  Ala Ser Leu Val Leu  Pro Val Leu
    1700                1705                1710

Val Phe  Leu Trp Ala Met Leu  Thr Ile Pro Arg Pro  Ser Lys Arg
    1715                1720                1725

Phe Trp  Met Thr Ala Ile Val  Phe Thr Glu Val Met  Val Val Thr
    1730                1735                1740

Lys Tyr  Leu Phe Gln Phe Gly  Phe Phe Pro Trp Asn  Ser Tyr Val
    1745                1750                1755

Val Leu  Arg Arg Tyr Glu Asn  Lys Pro Tyr Phe Pro  Pro Arg Ile
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    1760                1765                1770

Leu Gly  Leu Glu Lys Thr Asp  Ser Tyr Ile Lys Tyr  Asp Leu Val
    1775                1780                1785

Gln Leu  Met Ala Leu Phe Phe  His Arg Ser Gln Leu  Leu Cys Tyr
    1790                1795                1800

Gly Leu  Trp Asp His Glu Glu  Asp Arg Tyr Pro Lys  Asp His Cys
    1805                1810                1815

Arg Ser  Ser Val Lys Asp Arg  Glu Ala Lys Glu Glu  Pro Glu Ala
    1820                1825                1830

Lys Leu  Glu Ser Gln Ser Glu  Thr Gly Thr Gly His  Pro Lys Glu
    1835                1840                1845

Pro Val  Leu Ala Gly Thr Pro  Arg Asp His Ile Gln  Gly Lys Gly
    1850                1855                1860

Ser Ile  Arg Ser Lys Asp Val  Ile Gln Asp Pro Pro  Glu Asp Leu
    1865                1870                1875

Lys Pro  Arg His Thr Arg His  Ile Ser Ile Arg Phe  Arg Arg Arg
    1880                1885                1890

Lys Glu  Thr Pro Gly Pro Lys  Gly Thr Ala Val Met  Glu Thr Glu
    1895                1900                1905

His Glu  Glu Gly Glu Gly Lys  Glu Thr Thr Glu Arg  Lys Arg Pro
    1910                1915                1920

Arg His  Thr Gln Glu Lys Ser  Lys Phe Arg Glu Arg  Met Lys Ala
    1925                1930                1935

Ala Gly  Arg Arg Leu Gln Ser  Phe Cys Val Ser Leu  Ala Gln Ser
    1940                1945                1950

Phe Tyr  Gln Pro Leu Gln Arg  Phe Phe His Asp Ile  Leu His Thr
    1955                1960                1965

Lys Tyr  Arg Ala Ala Thr Asp  Val Tyr Ala Leu Met  Phe Leu Ala
    1970                1975                1980

Asp Ile  Val Asp Ile Ile Ile  Ile Ile Phe Gly Phe  Trp Ala Phe
    1985                1990                1995

Gly Lys  His Ser Ala Ala Thr  Asp Ile Ala Ser Ser  Leu Ser Asp
    2000                2005                2010

Asp Gln  Val Pro Gln Ala Phe  Leu Phe Met Leu Leu  Val Gln Phe
    2015                2020                2025

Gly Thr  Met Val Ile Asp Arg  Ala Leu Tyr Leu Arg  Lys Thr Val
    2030                2035                2040

Leu Gly  Lys Leu Ala Phe Gln  Val Val Leu Val Val  Ala Ile His
    2045                2050                2055

Ile Trp  Met Phe Phe Ile Leu  Pro Ala Val Thr Glu  Arg Met Phe
    2060                2065                2070

Ser Gln  Asn Ala Val Ala Gln  Leu Trp Tyr Phe Val  Lys Cys Ile
    2075                2080                2085

Tyr Phe  Ala Leu Ser Ala Tyr  Gln Ile Arg Cys Gly  Tyr Pro Thr
    2090                2095                2100

Arg Ile  Leu Gly Asn Phe Leu  Thr Lys Lys Tyr Asn  His Leu Asn
    2105                2110                2115

Leu Phe  Leu Phe Gln Gly Phe  Arg Leu Val Pro Phe  Leu Val Glu
    2120                2125                2130

Leu Arg  Ala Val Met Asp Trp  Val Trp Thr Asp Thr  Thr Leu Ser
    2135                2140                2145
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Leu Ser  Asn Trp Met Cys Val  Glu Asp Ile Tyr Ala  Asn Ile Phe
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Ile Ile  Lys Cys Ser Arg Glu  Thr Glu Lys Lys Tyr  Pro Gln Pro
    2165                2170                2175

Lys Gly  Gln Lys Lys Lys Lys  Ile Val Lys Tyr Gly  Met Gly Gly
    2180                2185                2190

Leu Ile  Ile Leu Phe Leu Ile  Ala Ile Ile Trp Phe  Pro Leu Leu
    2195                2200                2205

Phe Met  Ser Leu Ile Arg Ser  Val Val Gly Val Val  Asn Gln Pro
    2210                2215                2220

Ile Asp  Val Thr Val Thr Leu  Lys Leu Gly Gly Tyr  Glu Pro Leu
    2225                2230                2235

Phe Thr  Met Ser Ala Gln Gln  Pro Ser Ile Val Pro  Phe Thr Pro
    2240                2245                2250

Gln Ala  Tyr Glu Glu Leu Ser  Gln Gln Phe Asp Pro  Tyr Pro Leu
    2255                2260                2265

Ala Met  Gln Phe Ile Ser Gln  Tyr Ser Pro Glu Asp  Ile Val Thr
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Pro Ser  Arg Ala Gln Met Lys  Gln Glu Leu Tyr Asn  Gly Thr Ala
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Asp Ile  Thr Leu Arg Phe Thr  Trp Asn Phe Gln Arg  Asp Leu Ala
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Lys Gly  Gly Thr Val Glu Tyr  Thr Asn Glu Lys His  Thr Leu Glu
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Leu Ala  Pro Asn Ser Thr Ala  Arg Arg Gln Leu Ala  Gln Leu Leu
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Glu Gly  Arg Pro Asp Gln Ser  Val Val Ile Pro His  Leu Phe Pro
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Lys Tyr  Ile Arg Ala Pro Asn  Gly Pro Glu Ala Asn  Pro Val Lys
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Gln Leu  Gln Pro Asp Glu Glu  Glu Asp Tyr Leu Gly  Val Arg Ile
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Gln Leu  Arg Arg Glu Gln Val  Gly Thr Gly Ala Ser  Gly Glu Gln
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Ala Gly  Thr Lys Ala Ser Asp  Phe Leu Glu Trp Trp  Val Ile Glu
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Leu Gln  Asp Cys Lys Ala Asp  Cys Asn Leu Leu Pro  Met Val Ile
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Phe Ser  Asp Lys Val Ser Pro  Pro Ser Leu Gly Phe  Leu Ala Gly
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Tyr Gly  Ile Val Gly Leu Tyr  Val Ser Ile Val Leu  Val Val Gly
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Lys Phe  Val Arg Gly Phe Phe  Ser Glu Ile Ser His  Ser Ile Met
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Phe Glu  Glu Leu Pro Cys Val  Asp Arg Ile Leu Lys  Leu Cys Gln
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Asp Ile  Phe Leu Val Arg Glu  Thr Arg Glu Leu Glu  Leu Glu Glu
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Lys Ala Thr Met Gln Gly His Thr Gly Arg Leu Leu Gln Ser Leu Cys
    50                  55                  60

Ile Thr Ser Leu Ser Phe Leu Leu Leu His Ile Ile Phe His Ile Thr
65                  70                  75                  80

Leu Ala Ser Leu Glu Ala Gln His Arg Ile Thr Pro Ala Tyr Asn Cys
                85                  90                  95

Ser Thr Trp Glu Lys Thr Phe Arg Gln Ile Gly Phe Glu Ser Leu Lys
            100                 105                 110

Gly Ala Asp Ala Gly Asn Gly Ile Arg Val Phe Val Pro Asp Ile Gly
        115                 120                 125

Met Phe Ile Ala Ser Leu Thr Ile Trp Leu Val Cys Arg Thr Ile Val
    130                 135                 140

Lys Lys Pro Asp Thr Glu Glu Ile Ala Gln Leu Asn Ser Glu Cys Glu
145                 150                 155                 160

Asn Glu Glu Leu Ala Gly Gly Glu Lys Met Asp Ser Glu Glu Ala Leu
                165                 170                 175

Ile Tyr Glu Glu Asp Leu Asp Gly Glu Glu Gly Met Glu Gly Glu Leu
            180                 185                 190

Glu Glu Ser Thr Lys Leu Lys Ile Leu Arg Arg Phe Ala Ser Val Ala
        195                 200                 205

Ser Lys Leu Lys Glu Phe Ile Gly Asn Met Ile Thr Thr Ala Gly Lys
    210                 215                 220

Val Val Val Thr Ile Leu Leu Gly Ser Ser Gly Met Met Leu Pro Ser
225                 230                 235                 240

Leu Thr Ser Ala Val Tyr Phe Phe Val Phe Leu Gly Leu Cys Thr Trp
                245                 250                 255

Trp Ser Trp Cys Arg Thr Phe Asp Pro Leu Leu Phe Gly Cys Leu Cys
            260                 265                 270

Val Leu Leu Ala Ile Phe Thr Ala Gly His Leu Ile Gly Leu Tyr Leu
        275                 280                 285

Tyr Gln Phe Gln Phe Phe Gln Glu Ala Val Pro Pro Asn Asp Tyr Tyr
    290                 295                 300

Ala Arg Leu Phe Gly Ile Lys Ser Val Ile Gln Thr Asp Cys Ala Ser
305                 310                 315                 320

Thr Trp Lys Ile Ile Val Asn Pro Asp Leu Ser Trp Tyr His His Ala
                325                 330                 335

Asn Pro Ile Leu Leu Leu Val Met Tyr Tyr Thr Leu Ala Thr Leu Ile
            340                 345                 350

Arg Ile Trp Leu Gln Glu Pro Leu Val Gln Glu Glu Met Ala Lys Glu
        355                 360                 365

Asp Glu Gly Ala Leu Asp Cys Ser Ser Asn Gln Asn Thr Ala Glu Arg
    370                 375                 380

Arg Arg Ser Leu Trp Tyr Ala Thr Gln Tyr Pro Thr Asp Glu Arg Lys
385                 390                 395                 400

Leu Leu Ser Met Thr Gln Asp Asp Tyr Lys Pro Ser Asp Gly Leu Leu
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Val Thr Val Asn Gly Asn Pro Val Asp Tyr His Thr Ile His Pro Ser
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465                 470                 475                 480

Lys Arg Ser Val Arg Met His Ala Met Val Ala Val Phe Gln Phe Ile
                485                 490                 495

Met Lys Gln Ser Tyr Ile Cys Ala Leu Ile Ala Met Met Ala Trp Ser
            500                 505                 510

Ile Thr Tyr His Ser Trp Leu Thr Phe Val Leu Leu Ile Trp Ser Cys
        515                 520                 525

Thr Leu Trp Met Ile Arg Asn Arg Arg Lys Tyr Ala Met Ile Ser Ser
    530                 535                 540

Pro Phe Met Val Val Tyr Ala Asn Leu Leu Leu Val Leu Gln Tyr Ile
545                 550                 555                 560

Trp Ser Phe Glu Leu Pro Glu Ile Lys Lys Val Pro Gly Phe Leu Glu
                565                 570                 575

Lys Lys Glu Pro Gly Glu Leu Ala Ser Lys Ile Leu Phe Thr Ile Thr
            580                 585                 590

Phe Trp Leu Leu Leu Arg Gln His Leu Thr Glu Gln Lys Ala Leu Arg
        595                 600                 605

Glu Lys Glu Ala Leu Leu Ser Glu Val Lys Ile Gly Ser Gln Glu Leu
    610                 615                 620

Glu Glu Lys Glu Asp Glu Glu Leu Gln Asp Val Gln Val Glu Gly Glu
625                 630                 635                 640

Pro Thr Glu Lys Glu Glu Glu Glu Glu Glu Glu Ile Lys Glu Glu Arg
                645                 650                 655

His Glu Val Lys Lys Glu Glu Glu Glu Glu Val Glu Glu Asp Asp Asp
            660                 665                 670

Gln Asp Ile Met Lys Val Leu Gly Asn Leu Val Val Ala Leu Phe Ile
        675                 680                 685

Lys Tyr Trp Ile Tyr Val Cys Gly Gly Met Phe Phe Phe Val Ser Phe
    690                 695                 700

Glu Gly Lys Ile Val Met Tyr Lys Ile Ile Tyr Met Val Leu Phe Leu
705                 710                 715                 720

Phe Cys Val Ala Leu Tyr Gln Val His Tyr Glu Trp Trp Arg Lys Ile
                725                 730                 735

Leu Lys Tyr Phe Trp Met Ser Val Val Ile Tyr Thr Met Leu Val Leu
            740                 745                 750

Ile Phe Ile Tyr Thr Tyr Gln Phe Glu Asn Phe Pro Gly Leu Trp Gln
        755                 760                 765

Asn Met Thr Gly Leu Lys Lys Glu Lys Leu Glu Asp Leu Gly Leu Lys
    770                 775                 780

Gln Phe Thr Val Ala Glu Leu Phe Thr Arg Ile Phe Ile Pro Thr Ser
785                 790                 795                 800

Phe Leu Leu Val Cys Ile Leu His Leu His Tyr Phe His Asp Arg Phe
                805                 810                 815

Leu Glu Leu Thr Asp Leu Lys Ser Ile Pro Ser Lys Glu Asp Asn Thr
            820                 825                 830

Ile Tyr Ser His Ala Lys Val Asn Gly Arg Val Tyr Leu Ile Ile Asn
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Met Thr Ala Ser Leu Asp Lys Pro Glu Val Gln Lys Leu Ala Glu Ser
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Gly Glu Glu Arg Pro Glu Glu Cys Val Lys Lys Thr Glu Lys Gly Glu
                885                 890                 895

Ala Gly Lys Asp Ser Asp Glu Ser Glu Glu Glu Glu Asp Glu Glu Glu
            900                 905                 910

Glu Ser Glu Glu Glu Glu Ser Ser Asp Leu Arg Asn Lys Trp His Leu
        915                 920                 925

Val Ile Asp Arg Leu Thr Val Leu Phe Leu Lys Phe Leu Glu Tyr Phe
    930                 935                 940

His Lys Leu Gln Val Phe Met Trp Trp Ile Leu Glu Leu His Ile Ile
945                 950                 955                 960

Lys Ile Val Ser Ser Tyr Ile Ile Trp Val Thr Val Lys Glu Val Ser
                965                 970                 975

Leu Phe Asn Tyr Val Phe Leu Ile Ser Trp Ala Phe Ala Leu Pro Tyr
            980                 985                 990

Ala Lys Leu Arg Arg Ala Ala Ser  Ser Val Cys Thr Val  Trp Thr Cys
        995                 1000                1005

Val Ile  Ile Val Cys Lys Met  Leu Tyr Gln Leu Gln  Thr Ile Lys
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Pro Glu  Asn Phe Ser Val Asn  Cys Ser Leu Pro Asn  Glu Asn Gln
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Thr Asn  Ile Pro Leu His Glu  Leu Asn Lys Ser Leu  Leu Tyr Ser
    1040                1045                1050

Ala Pro  Val Asp Pro Thr Glu  Trp Val Gly Leu Arg  Lys Ser Ser
    1055                1060                1065

Pro Leu  Leu Val Tyr Leu Arg  Asn Asn Leu Leu Met  Leu Ala Ile
    1070                1075                1080

Leu Ala  Phe Glu Val Thr Val  Tyr Arg His Gln Glu  Tyr Tyr Arg
    1085                1090                1095

Gly Arg  Asn Asn Leu Thr Ala  Pro Val Ser Lys Thr  Ile Phe His
    1100                1105                1110

Asp Ile  Thr Arg Leu His Leu  Asp Asp Gly Leu Ile  Asn Cys Ala
    1115                1120                1125

Lys Tyr  Phe Val Asn Tyr Phe  Phe Tyr Lys Phe Gly  Leu Glu Thr
    1130                1135                1140

Cys Phe  Leu Met Ser Val Asn  Val Ile Gly Gln Arg  Met Asp Phe
    1145                1150                1155

Tyr Ala  Met Ile His Ala Cys  Trp Leu Ile Gly Val  Leu Tyr Arg
    1160                1165                1170

Arg Arg  Arg Lys Ala Ile Ala  Glu Val Trp Pro Lys  Tyr Cys Cys
    1175                1180                1185

Phe Leu  Ala Cys Ile Ile Thr  Phe Gln Tyr Phe Val  Cys Ile Gly
    1190                1195                1200

Ile Pro  Pro Ala Pro Cys Arg  Asp Tyr Pro Trp Arg  Phe Lys Gly
    1205                1210                1215

Ala Tyr  Phe Asn Asp Asn Ile  Ile Lys Trp Leu Tyr  Phe Pro Asp
    1220                1225                1230

Phe Ile  Val Arg Pro Asn Pro  Val Phe Leu Val Tyr  Asp Phe Met
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    1235                1240                1245

Leu Leu  Leu Cys Ala Ser Leu  Gln Arg Gln Ile Phe  Glu Asp Glu
    1250                1255                1260

Asn Lys  Ala Ala Val Arg Ile  Met Ala Gly Asp Asn  Val Glu Ile
    1265                1270                1275

Cys Met  Asn Leu Asp Ala Ala  Ser Phe Ser Gln His  Asn Pro Val
    1280                1285                1290

Pro Asp  Phe Ile His Cys Arg  Ser Tyr Leu Asp Met  Ser Lys Val
    1295                1300                1305

Ile Ile  Phe Ser Tyr Leu Phe  Trp Phe Val Leu Thr  Ile Ile Phe
    1310                1315                1320

Ile Thr  Gly Thr Thr Arg Ile  Ser Ile Phe Cys Met  Gly Tyr Leu
    1325                1330                1335

Val Ala  Cys Phe Tyr Phe Leu  Leu Phe Gly Gly Asp  Leu Leu Leu
    1340                1345                1350

Lys Pro  Ile Lys Ser Ile Leu  Arg Tyr Trp Asp Trp  Leu Ile Ala
    1355                1360                1365

Tyr Asn  Val Phe Val Ile Thr  Met Lys Asn Ile Leu  Ser Ile Gly
    1370                1375                1380

Ala Cys  Gly Tyr Ile Gly Ala  Leu Val Arg Asn Ser  Cys Trp Leu
    1385                1390                1395

Ile Gln  Ala Phe Ser Leu Ala  Cys Thr Val Lys Gly  Tyr Gln Met
    1400                1405                1410

Pro Glu  Asp Asp Ser Arg Cys  Lys Leu Pro Ser Gly  Glu Ala Gly
    1415                1420                1425

Ile Ile  Trp Asp Ser Ile Cys  Phe Ala Phe Leu Leu  Leu Gln Arg
    1430                1435                1440

Arg Val  Phe Met Ser Tyr Tyr  Phe Leu His Val Val  Ala Asp Ile
    1445                1450                1455

Lys Ala  Ser Gln Ile Leu Ala  Ser Arg Gly Ala Glu  Leu Phe Gln
    1460                1465                1470

Ala Thr  Ile Val Lys Ala Val  Lys Ala Arg Ile Glu  Glu Glu Lys
    1475                1480                1485

Lys Ser  Met Asp Gln Leu Lys  Arg Gln Met Asp Arg  Ile Lys Ala
    1490                1495                1500

Arg Gln  Gln Lys Tyr Lys Lys  Gly Lys Glu Arg Met  Leu Ser Leu
    1505                1510                1515

Thr Gln  Glu Ser Gly Glu Gly  Gln Asp Ile Gln Lys  Val Ser Glu
    1520                1525                1530

Glu Asp  Asp Glu Arg Glu Ala  Asp Lys Gln Lys Ala  Lys Gly Lys
    1535                1540                1545

Lys Lys  Gln Trp Trp Arg Pro  Trp Val Asp His Ala  Ser Met Val
    1550                1555                1560

Arg Ser  Gly Asp Tyr Tyr Leu  Phe Glu Thr Asp Ser  Glu Glu Glu
    1565                1570                1575

Glu Glu  Glu Glu Leu Lys Lys  Glu Asp Glu Glu Pro  Pro Arg Lys
    1580                1585                1590

Ser Ala  Phe Gln Phe Val Tyr  Gln Ala Trp Ile Thr  Asp Pro Lys
    1595                1600                1605

Thr Ala  Leu Arg Gln Arg Arg  Lys Glu Lys Lys Lys  Leu Ala Arg
    1610                1615                1620
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Glu Glu  Gln Lys Glu Arg Arg  Lys Gly Ser Gly Asp  Gly Pro Val
    1625                 1630                 1635

Glu Trp  Glu Asp Arg Glu Asp  Glu Pro Val Lys Lys  Lys Ser Asp
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Gly Pro  Asp Asn Ile Ile Lys  Arg Ile Phe Asn Ile  Leu Lys Phe
    1655                 1660                 1665

Thr Trp  Val Leu Phe Leu Ala  Thr Val Asp Ser Phe  Thr Thr Trp
    1670                 1675                 1680

Leu Asn  Ser Ile Ser Arg Glu  His Ile Asp Ile Ser  Thr Val Leu
    1685                 1690                 1695

Arg Ile  Glu Arg Cys Met Leu  Thr Arg Glu Ile Lys  Lys Gly Asn
    1700                 1705                 1710

Val Pro  Thr Arg Glu Ser Ile  His Met Tyr Tyr Gln  Asn His Ile
    1715                 1720                 1725

Met Asn  Leu Ser Arg Glu Ser  Gly Leu Asp Thr Ile  Asp Glu His
    1730                 1735                 1740

Ser Gly  Ala Gly Ser Arg Ala  Gln Ala Ala His Arg  Met Asp Ser
    1745                 1750                 1755

Leu Asp  Ser Arg Asp Ser Ile  Ser Ser Cys Tyr Thr  Glu Ala Thr
    1760                 1765                 1770

Leu Leu  Ile Ser Arg Gln Ser  Thr Leu Asp Asp Leu  Asp Gly Gln
    1775                 1780                 1785

Asp Pro  Val Pro Lys Thr Ser  Glu Arg Ala Arg Pro  Arg Leu Arg
    1790                 1795                 1800

Lys Met  Phe Ser Leu Asp Met  Ser Ser Ser Ser Ala  Asp Ser Gly
    1805                 1810                 1815

Ser Val  Ala Ser Ser Glu Pro  Thr Gln Cys Thr Met  Leu Tyr Ser
    1820                 1825                 1830

Arg Gln  Gly Thr Thr Glu Thr  Ile Glu Glu Val Glu  Ala Glu Ala
    1835                 1840                 1845

Glu Glu  Glu Val Val Glu Gly  Leu Glu Pro Glu Leu  His Asp Ala
    1850                 1855                 1860

Glu Glu  Lys Glu Tyr Ala Ala  Glu Tyr Glu Ala Gly  Val Glu Glu
    1865                 1870                 1875

Ile Ser  Leu Thr Pro Asp Glu  Glu Leu Pro Gln Phe  Ser Thr Asp
    1880                 1885                 1890

Asp Cys  Glu Ala Pro Pro Ser  Tyr Ser Lys Ala Val  Ser Phe Glu
    1895                 1900                 1905

His Leu  Ser Phe Ala Ser Gln  Asp Asp Ser Gly Ala  Lys Asn His
    1910                 1915                 1920

Met Val  Val Ser Pro Asp Asp  Ser Arg Thr Asp Lys  Leu Glu Ser
    1925                 1930                 1935

Ser Ile  Leu Pro Pro Leu Thr  His Glu Leu Thr Ala  Ser Asp Leu
    1940                 1945                 1950

Leu Met  Ser Lys Met Phe His  Asp Asp Glu Leu Glu  Glu Ser Glu
    1955                 1960                 1965

Lys Phe  Tyr Val Asp Gln Pro  Arg Phe Leu Leu Leu  Phe Tyr Ala
    1970                 1975                 1980

Met Tyr  Asn Thr Leu Val Ala  Arg Ser Glu Met Val  Cys Tyr Phe
    1985                 1990                 1995

Val Ile  Ile Leu Asn His Met  Thr Ser Ala Ser Ile  Ile Thr Leu
    2000                 2005                 2010
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Leu Leu  Pro Ile Leu Ile Phe  Leu Trp Ala Met Leu  Ser Val Pro
    2015                 2020                 2025

Arg Pro  Ser Arg Arg Phe Trp  Met Met Ala Ile Val  Tyr Thr Glu
    2030                 2035                 2040

Val Ala  Ile Val Val Lys Tyr  Phe Phe Gln Phe Gly  Phe Phe Pro
    2045                 2050                 2055

Trp Asn  Lys Asp Leu Glu Ile  Tyr Lys Glu Arg Pro  Tyr Phe Pro
    2060                 2065                 2070

Pro Asn  Ile Ile Gly Val Glu  Lys Lys Glu Gly Tyr  Val Leu Tyr
    2075                 2080                 2085

Asp Leu  Ile Gln Leu Leu Ala  Leu Phe Phe His Arg  Ser Ile Leu
    2090                 2095                 2100

Lys Cys  His Gly Leu Trp Asp  Glu Asp Asp Ile Val  Asp Ser Asn
    2105                 2110                 2115

Thr Asp  Lys Glu Gly Ser Asp  Asp Glu Leu Ser Leu  Asp Gln Gly
    2120                 2125                 2130

Arg Arg  Gly Ser Ser Asp Ser  Leu Lys Ser Ile Asn  Leu Ala Ala
    2135                 2140                 2145

Ser Val  Glu Ser Val His Val  Thr Phe Pro Glu Gln  Pro Ala Ala
    2150                 2155                 2160

Ile Arg  Arg Lys Arg Ser Cys  Ser Ser Ser Gln Ile  Ser Pro Arg
    2165                 2170                 2175

Ser Ser  Phe Ser Ser Asn Arg  Ser Lys Arg Gly Ser  Thr Ser Thr
    2180                 2185                 2190

Arg Asn  Ser Ser Gln Lys Gly  Ser Ser Val Leu Ser  Leu Lys Gln
    2195                 2200                 2205

Lys Ser  Lys Arg Glu Leu Tyr  Met Glu Lys Leu Gln  Glu His Leu
    2210                 2215                 2220

Ile Lys  Ala Lys Ala Phe Thr  Ile Lys Lys Thr Leu  Gln Ile Tyr
    2225                 2230                 2235

Val Pro  Ile Arg Gln Phe Phe  Tyr Asp Leu Ile His  Pro Asp Tyr
    2240                 2245                 2250

Ser Ala  Val Thr Asp Val Tyr  Val Leu Met Phe Leu  Ala Asp Thr
    2255                 2260                 2265

Val Asp  Phe Ile Ile Ile Val  Phe Gly Phe Trp Ala  Phe Gly Lys
    2270                 2275                 2280

His Ser  Ala Ala Ala Asp Ile  Thr Ser Ser Leu Ser  Glu Asp Gln
    2285                 2290                 2295

Val Pro  Gly Pro Phe Leu Val  Met Val Leu Ile Gln  Phe Gly Thr
    2300                 2305                 2310

Met Val  Val Asp Arg Ala Leu  Tyr Leu Arg Lys Thr  Val Leu Gly
    2315                 2320                 2325

Lys Val  Ile Phe Gln Val Ile  Leu Val Phe Gly Ile  His Phe Trp
    2330                 2335                 2340

Met Phe  Phe Ile Leu Pro Gly  Val Thr Glu Arg Lys  Phe Ser Gln
    2345                 2350                 2355

Asn Leu  Val Ala Gln Leu Trp  Tyr Phe Val Lys Cys  Val Tyr Phe
    2360                 2365                 2370

Gly Leu  Ser Ala Tyr Gln Ile  Arg Cys Gly Tyr Pro  Thr Arg Val
    2375                 2380                 2385

Leu Gly  Asn Phe Leu Thr Lys  Ser Tyr Asn Tyr Val  Asn Leu Phe
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    2390                2395                2400

Leu Phe  Gln Gly Phe Arg Leu  Val Pro Phe Leu Thr  Glu Leu Arg
    2405                2410                2415

Ala Val  Met Asp Trp Val Trp  Thr Asp Thr Thr Leu  Ser Leu Ser
    2420                2425                2430

Ser Trp  Ile Cys Val Glu Asp  Ile Tyr Ala His Ile  Phe Ile Leu
    2435                2440                2445

Lys Cys  Trp Arg Glu Ser Glu  Lys Arg Tyr Pro Gln  Pro Arg Gly
    2450                2455                2460

Gln Lys  Lys Lys Lys Ala Val  Lys Tyr Gly Met Gly  Gly Met Ile
    2465                2470                2475

Ile Val  Leu Leu Ile Cys Ile  Val Trp Phe Pro Leu  Leu Phe Met
    2480                2485                2490

Ser Leu  Ile Lys Ser Val Ala  Gly Val Ile Asn Gln  Pro Leu Asp
    2495                2500                2505

Val Ser  Val Thr Ile Thr Leu  Gly Gly Tyr Gln Pro  Ile Phe Thr
    2510                2515                2520

Met Ser  Ala Gln Gln Ser Gln  Leu Lys Val Met Asp  Asn Ser Lys
    2525                2530                2535

Tyr Asn  Glu Phe Leu Lys Ser  Phe Gly Pro Asn Ser  Gly Ala Met
    2540                2545                2550

Gln Phe  Leu Glu Asn Tyr Glu  Arg Glu Asp Val Thr  Val Ala Glu
    2555                2560                2565

Leu Glu  Gly Asn Ser Asn Ser  Leu Trp Thr Ile Ser  Pro Pro Ser
    2570                2575                2580

Lys Gln  Lys Met Ile Gln Glu  Leu Thr Asp Pro Asn  Ser Cys Phe
    2585                2590                2595

Ser Val  Val Phe Ser Trp Ser  Ile Gln Arg Asn Met  Thr Leu Gly
    2600                2605                2610

Ala Lys  Ala Glu Ile Ala Thr  Asp Lys Leu Ser Phe  Pro Leu Ala
    2615                2620                2625

Val Ala  Thr Arg Asn Ser Ile  Ala Lys Met Ile Ala  Gly Asn Asp
    2630                2635                2640

Thr Glu  Ser Ser Asn Thr Pro  Val Thr Ile Glu Lys  Ile Tyr Pro
    2645                2650                2655

Tyr Tyr  Val Lys Ala Pro Ser  Asp Ser Asn Ser Lys  Pro Ile Lys
    2660                2665                2670

Gln Leu  Leu Ser Glu Asn Asn  Phe Met Asn Ile Thr  Ile Ile Leu
    2675                2680                2685

Phe Arg  Asp Asn Val Thr Lys  Ser Asn Ser Glu Trp  Trp Val Leu
    2690                2695                2700

Asn Leu  Thr Gly Ser Arg Ile  Phe Asn Gln Gly Ser  Gln Ala Leu
    2705                2710                2715

Glu Leu  Val Val Phe Asn Asp  Lys Val Ser Pro Pro  Ser Leu Gly
    2720                2725                2730

Phe Leu  Ala Gly Tyr Gly Ile  Met Gly Leu Tyr Ala  Ser Val Val
    2735                2740                2745

Leu Val  Ile Gly Lys Phe Val  Arg Glu Phe Phe Ser  Gly Ile Ser
    2750                2755                2760

His Ser  Ile Met Phe Glu Glu  Leu Pro Asn Val Asp  Arg Ile Leu
    2765                2770                2775
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Lys Leu  Cys Thr Asp Ile Phe  Leu Val Arg Glu Thr  Gly Glu Leu
    2780                2785                2790

Glu Leu  Glu Glu Asp Leu Tyr  Ala Lys Leu Ile Phe  Leu Tyr Arg
    2795                2800                2805

Ser Pro  Glu Thr Met Ile Lys  Trp Thr Arg Glu Lys  Thr Asn
    2810                2815                2820
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

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Cys Ala Leu Leu Ala Ala Cys Leu Leu Arg Phe Ser Gly Leu Ser Leu
            20                  25                  30

Val Tyr Leu Leu Phe Leu Leu Leu Leu Pro Trp Phe Pro Gly Pro Thr
        35                  40                  45

Arg Cys Gly Leu Gln Gly His Thr Gly Arg Leu Leu Arg Ala Leu Leu
    50                  55                  60

Gly Leu Ser Leu Leu Phe Leu Val Ala His Leu Ala Leu Gln Ile Cys
65                  70                  75                  80

Leu His Ile Val Pro Arg Leu Asp Gln Leu Leu Gly Pro Ser Cys Ser
                85                  90                  95

Arg Trp Glu Thr Leu Ser Arg His Ile Gly Val Thr Arg Leu Asp Leu
            100                 105                 110

Lys Asp Ile Pro Asn Ala Ile Arg Leu Val Ala Pro Asp Leu Gly Ile
        115                 120                 125

Leu Val Val Ser Ser Val Cys Leu Gly Ile Cys Gly Arg Leu Ala Arg
    130                 135                 140

Asn Thr Arg Gln Ser Pro His Pro Arg Glu Leu Asp Asp Asp Glu Arg
145                 150                 155                 160

Asp Val Asp Ala Ser Pro Thr Ala Gly Leu Gln Glu Ala Ala Thr Leu
                165                 170                 175

Ala Pro Thr Arg Arg Ser Arg Leu Ala Ala Arg Phe Arg Val Thr Ala
            180                 185                 190

His Trp Leu Leu Val Ala Ala Gly Arg Val Leu Ala Val Thr Leu Leu
        195                 200                 205
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Ala Leu Ala Gly Ile Ala His Pro Ser Ala Leu Ser Ser Val Tyr Leu
    210                 215                 220

Leu Leu Phe Leu Ala Leu Cys Thr Trp Trp Ala Cys His Phe Pro Ile
225                 230                 235                 240

Ser Thr Arg Gly Phe Ser Arg Leu Cys Val Ala Val Gly Cys Phe Gly
                245                 250                 255

Ala Gly His Leu Ile Cys Leu Tyr Cys Tyr Gln Met Pro Leu Ala Gln
            260                 265                 270

Ala Leu Leu Pro Pro Ala Gly Ile Trp Ala Arg Val Leu Gly Leu Lys
        275                 280                 285

Asp Phe Val Gly Pro Thr Asn Cys Ser Ser Pro His Ala Leu Val Leu
    290                 295                 300

Asn Thr Gly Leu Asp Trp Pro Val Tyr Ala Ser Pro Gly Val Leu Leu
305                 310                 315                 320

Leu Leu Cys Tyr Ala Thr Ala Ser Leu Arg Lys Leu Arg Ala Tyr Arg
                325                 330                 335

Pro Ser Gly Gln Arg Lys Glu Ala Ala Lys Gly Tyr Glu Ala Arg Glu
            340                 345                 350

Leu Glu Leu Ala Glu Leu Asp Gln Trp Pro Gln Glu Arg Glu Ser Asp
        355                 360                 365

Gln His Val Val Pro Thr Ala Pro Asp Thr Glu Ala Asp Asn Cys Ile
    370                 375                 380

Val His Glu Leu Thr Gly Gln Ser Ser Val Leu Arg Arg Pro Val Arg
385                 390                 395                 400

Pro Lys Arg Ala Glu Pro Arg Glu Ala Ser Pro Leu His Ser Leu Gly
                405                 410                 415

His Leu Ile Met Asp Gln Ser Tyr Val Cys Ala Leu Ile Ala Met Met
            420                 425                 430

Val Trp Ser Ile Thr Tyr His Ser Trp Leu Thr Phe Val Leu Leu Leu
        435                 440                 445

Trp Ala Cys Leu Ile Trp Thr Val Arg Ser Arg His Gln Leu Ala Met
    450                 455                 460

Leu Cys Ser Pro Cys Ile Leu Leu Tyr Gly Met Thr Leu Cys Cys Leu
465                 470                 475                 480

Arg Tyr Val Trp Ala Met Asp Leu Arg Pro Glu Leu Pro Thr Thr Leu
                485                 490                 495

Gly Pro Val Ser Leu Arg Gln Leu Gly Leu Glu His Thr Arg Tyr Pro
            500                 505                 510

Cys Leu Asp Leu Gly Ala Met Leu Leu Tyr Thr Leu Thr Phe Trp Leu
        515                 520                 525

Leu Leu Arg Gln Phe Val Lys Glu Lys Leu Leu Lys Trp Ala Glu Ser
    530                 535                 540

Pro Ala Ala Leu Thr Glu Val Thr Val Ala Asp Thr Glu Pro Thr Arg
545                 550                 555                 560

Thr Gln Thr Leu Leu Gln Ser Leu Gly Glu Leu Val Lys Gly Val Tyr
                565                 570                 575

Ala Lys Tyr Trp Ile Tyr Val Cys Ala Gly Met Phe Ile Val Val Ser
            580                 585                 590

Phe Ala Gly Arg Leu Val Val Tyr Lys Ile Val Tyr Met Phe Leu Phe
        595                 600                 605

Leu Leu Cys Leu Thr Leu Phe Gln Val Tyr Tyr Ser Leu Trp Arg Lys
    610                 615                 620
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Leu Leu Lys Ala Phe Trp Trp Leu Val Val Ala Tyr Thr Met Leu Val
625                 630                 635                 640

Leu Ile Ala Val Tyr Thr Phe Gln Phe Gln Asp Phe Pro Ala Tyr Trp
                645                 650                 655

Arg Asn Leu Thr Gly Phe Thr Asp Glu Gln Leu Gly Asp Leu Gly Leu
            660                 665                 670

Glu Gln Phe Ser Val Ser Glu Leu Phe Ser Ser Ile Leu Val Pro Gly
        675                 680                 685

Phe Phe Leu Leu Ala Cys Ile Leu Gln Leu His Tyr Phe His Arg Pro
    690                 695                 700

Phe Met Gln Leu Thr Asp Met Glu His Val Ser Leu Pro Gly Thr Arg
705                 710                 715                 720

Leu Pro Arg Trp Ala His Arg Gln Asp Ala Val Ser Gly Thr Pro Leu
                725                 730                 735

Leu Arg Glu Glu Gln Gln Glu His Gln Gln Gln Gln Gln Glu Glu Glu
            740                 745                 750

Glu Glu Glu Glu Asp Ser Arg Asp Glu Gly Leu Gly Val Ala Thr Pro
        755                 760                 765

His Gln Ala Thr Gln Val Pro Glu Gly Ala Ala Lys Trp Gly Leu Val
    770                 775                 780

Ala Glu Arg Leu Leu Glu Leu Ala Ala Gly Phe Ser Asp Val Leu Ser
785                 790                 795                 800

Arg Val Gln Val Phe Leu Arg Arg Leu Leu Glu Leu His Val Phe Lys
                805                 810                 815

Leu Val Ala Leu Tyr Thr Val Trp Val Ala Leu Lys Glu Val Ser Val
            820                 825                 830

Met Asn Leu Leu Leu Val Val Leu Trp Ala Phe Ala Leu Pro Tyr Pro
        835                 840                 845

Arg Phe Arg Pro Met Ala Ser Cys Leu Ser Thr Val Trp Thr Cys Val
    850                 855                 860

Ile Ile Val Cys Lys Met Leu Tyr Gln Leu Lys Val Val Asn Pro Gln
865                 870                 875                 880

Glu Tyr Ser Ser Asn Cys Thr Glu Pro Phe Pro Asn Ser Thr Asn Leu
                885                 890                 895

Leu Pro Thr Glu Ile Ser Gln Ser Leu Leu Tyr Arg Gly Pro Val Asp
            900                 905                 910

Pro Ala Asn Trp Phe Gly Val Arg Lys Gly Phe Pro Asn Leu Gly Tyr
        915                 920                 925

Ile Gln Asn His Leu Gln Val Leu Leu Leu Leu Val Phe Glu Ala Ile
    930                 935                 940

Val Tyr Arg Arg Gln Glu His Tyr Arg Arg Gln His Gln Leu Ala Pro
945                 950                 955                 960

Leu Pro Ala Gln Ala Val Phe Ala Ser Gly Thr Arg Gln Gln Leu Asp
                965                 970                 975

Gln Asp Leu Leu Gly Cys Leu Lys Tyr Phe Ile Asn Phe Phe Phe Tyr
            980                 985                 990

Lys Phe Gly Leu Glu Ile Cys Phe  Leu Met Ala Val Asn  Val Ile Gly
        995                 1000                1005

Gln Arg  Met Asn Phe Leu Val  Thr Leu His Gly Cys  Trp Leu Val
    1010                1015                1020

Ala Ile  Leu Thr Arg Arg His  Arg Gln Ala Ile Ala  Arg Leu Trp
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    1025                1030                1035

Pro Asn  Tyr Cys Leu Phe Leu  Ala Leu Phe Leu Leu  Tyr Gln Tyr
    1040                1045                1050

Leu Leu  Cys Leu Gly Met Pro  Pro Ala Leu Cys Ile  Asp Tyr Pro
    1055                1060                1065

Trp Arg  Trp Ser Arg Ala Val  Pro Met Asn Ser Ala  Leu Ile Lys
    1070                1075                1080

Trp Leu  Tyr Leu Pro Asp Phe  Phe Arg Ala Pro Asn  Ser Thr Asn
    1085                1090                1095

Leu Ile  Ser Asp Phe Leu Leu  Leu Leu Cys Ala Ser  Gln Gln Trp
    1100                1105                1110

Gln Val  Phe Ser Ala Glu Arg  Thr Glu Glu Trp Gln  Arg Met Ala
    1115                1120                1125

Gly Val  Asn Thr Asp Arg Leu  Glu Pro Leu Arg Gly  Glu Pro Asn
    1130                1135                1140

Pro Val  Pro Asn Phe Ile His  Cys Arg Ser Tyr Leu  Asp Met Leu
    1145                1150                1155

Lys Val  Ala Val Phe Arg Tyr  Leu Phe Trp Leu Val  Leu Val Val
    1160                1165                1170

Val Phe  Val Thr Gly Ala Thr  Arg Ile Ser Ile Phe  Gly Leu Gly
    1175                1180                1185

Tyr Leu  Leu Ala Cys Phe Tyr  Leu Leu Leu Phe Gly  Thr Ala Leu
    1190                1195                1200

Leu Gln  Arg Asp Thr Arg Ala  Arg Leu Val Leu Trp  Asp Cys Leu
    1205                1210                1215

Ile Leu  Tyr Asn Val Thr Val  Ile Ile Ser Lys Asn  Met Leu Ser
    1220                1225                1230

Leu Leu  Ala Cys Val Phe Val  Glu Gln Met Gln Thr  Gly Phe Cys
    1235                1240                1245

Trp Val  Ile Gln Leu Phe Ser  Leu Val Cys Thr Val  Lys Gly Tyr
    1250                1255                1260

Tyr Asp  Pro Lys Glu Met Met  Asp Arg Asp Gln Asp  Cys Leu Leu
    1265                1270                1275

Pro Val  Glu Glu Ala Gly Ile  Ile Trp Asp Ser Val  Cys Phe Phe
    1280                1285                1290

Phe Leu  Leu Leu Gln Arg Arg  Val Phe Leu Ser His  Tyr Tyr Leu
    1295                1300                1305

His Val  Arg Ala Asp Leu Gln  Ala Thr Ala Leu Leu  Ala Ser Arg
    1310                1315                1320

Gly Phe  Ala Leu Tyr Asn Ala  Ala Asn Leu Lys Ser  Ile Asp Phe
    1325                1330                1335

His Arg  Arg Ile Glu Glu Lys  Ser Leu Ala Gln Leu  Lys Arg Gln
    1340                1345                1350

Met Glu  Arg Ile Arg Ala Lys  Gln Glu Lys His Arg  Gln Gly Arg
    1355                1360                1365

Val Asp  Arg Ser Arg Pro Gln  Asp Thr Leu Gly Pro  Lys Asp Pro
    1370                1375                1380

Gly Leu  Glu Pro Gly Pro Asp  Ser Pro Gly Gly Ser  Ser Pro Pro
    1385                1390                1395

Arg Arg  Gln Trp Trp Arg Pro  Trp Leu Asp His Ala  Thr Val Ile
    1400                1405                1410
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His Ser  Gly Asp Tyr Phe Leu  Phe Glu Ser Asp Ser  Glu Glu Glu
    1415                1420                1425

Glu Glu  Ala Val Pro Glu Asp  Pro Arg Pro Ser Ala  Gln Ser Ala
    1430                1435                1440

Phe Gln  Leu Ala Tyr Gln Ala  Trp Val Thr Asn Ala  Gln Ala Val
    1445                1450                1455

Leu Arg  Arg Arg Gln Gln Glu  Gln Glu Gln Ala Arg  Gln Glu Gln
    1460                1465                1470

Ala Gly  Gln Leu Pro Thr Gly  Gly Gly Pro Ser Gln  Glu Val Glu
    1475                1480                1485

Pro Ala  Glu Gly Pro Glu Glu  Ala Ala Ala Gly Arg  Ser His Val
    1490                1495                1500

Val Gln  Arg Val Leu Ser Thr  Ala Gln Phe Leu Trp  Met Leu Gly
    1505                1510                1515

Gln Ala  Leu Val Asp Glu Leu  Thr Arg Trp Leu Gln  Glu Phe Thr
    1520                1525                1530

Arg His  His Gly Thr Met Ser  Asp Val Leu Arg Ala  Glu Arg Tyr
    1535                1540                1545

Leu Leu  Thr Gln Glu Leu Leu  Gln Gly Gly Glu Val  His Arg Gly
    1550                1555                1560

Val Leu  Asp Gln Leu Tyr Thr  Ser Gln Ala Glu Ala  Thr Leu Pro
    1565                1570                1575

Gly Pro  Thr Glu Ala Pro Asn  Ala Pro Ser Thr Val  Ser Ser Gly
    1580                1585                1590

Leu Gly  Ala Glu Glu Pro Leu  Ser Ser Met Thr Asp  Asp Met Gly
    1595                1600                1605

Ser Pro  Leu Ser Thr Gly Tyr  His Thr Arg Ser Gly  Ser Glu Glu
    1610                1615                1620

Ala Val  Thr Asp Pro Gly Glu  Arg Glu Ala Gly Ala  Ser Leu Tyr
    1625                1630                1635

Gln Gly  Leu Met Arg Thr Ala  Ser Glu Leu Leu Leu  Asp Arg Arg
    1640                1645                1650

Leu Arg  Ile Pro Glu Leu Glu  Glu Ala Glu Leu Phe  Ala Glu Gly
    1655                1660                1665

Gln Gly  Arg Ala Leu Arg Leu  Leu Arg Ala Val Tyr  Gln Cys Val
    1670                1675                1680

Ala Ala  His Ser Glu Leu Leu  Cys Tyr Phe Ile Ile  Ile Leu Asn
    1685                1690                1695

His Met  Val Thr Ala Ser Ala  Gly Ser Leu Val Leu  Pro Val Leu
    1700                1705                1710

Val Phe  Leu Trp Ala Met Leu  Ser Ile Pro Arg Pro  Ser Lys Arg
    1715                1720                1725

Phe Trp  Met Thr Ala Ile Val  Phe Thr Glu Ile Ala  Val Val Val
    1730                1735                1740

Lys Tyr  Leu Phe Gln Phe Gly  Phe Phe Pro Trp Asn  Ser His Val
    1745                1750                1755

Val Leu  Arg Arg Tyr Glu Asn  Lys Pro Tyr Phe Pro  Pro Arg Ile
    1760                1765                1770

Leu Gly  Leu Glu Lys Thr Asp  Gly Tyr Ile Lys Tyr  Asp Leu Val
    1775                1780                1785

Gln Leu  Met Ala Leu Phe Phe  His Arg Ser Gln Leu  Leu Cys Tyr
    1790                1795                1800
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Gly Leu  Trp Asp His Glu Glu  Asp Ser Pro Ser Lys  Glu His Asp
    1805                 1810                 1815

Lys Ser  Gly Glu Glu Glu Gln  Gly Ala Glu Glu Gly  Pro Gly Val
    1820                 1825                 1830

Pro Ala  Ala Thr Thr Glu Asp  His Ile Gln Val Glu  Ala Arg Val
    1835                 1840                 1845

Gly Pro  Thr Asp Gly Thr Pro  Glu Pro Gln Val Glu  Leu Arg Pro
    1850                 1855                 1860

Arg Asp  Thr Arg Arg Ile Ser  Leu Arg Phe Arg Arg  Arg Lys Lys
    1865                 1870                 1875

Glu Gly  Pro Ala Arg Lys Gly  Ala Ala Ala Ile Glu  Ala Glu Asp
    1880                 1885                 1890

Arg Glu  Glu Glu Glu Gly Glu  Glu Glu Lys Glu Ala  Pro Thr Gly
    1895                 1900                 1905

Arg Glu  Lys Arg Pro Ser Arg  Ser Gly Gly Arg Val  Arg Ala Ala
    1910                 1915                 1920

Gly Arg  Arg Leu Gln Gly Phe  Cys Leu Ser Leu Ala  Gln Gly Thr
    1925                 1930                 1935

Tyr Arg  Pro Leu Arg Arg Phe  Phe His Asp Ile Leu  His Thr Lys
    1940                 1945                 1950

Tyr Arg  Ala Ala Thr Asp Val  Tyr Ala Leu Met Phe  Leu Ala Asp
    1955                 1960                 1965

Val Val  Asp Phe Ile Ile Ile  Ile Phe Gly Phe Trp  Ala Phe Gly
    1970                 1975                 1980

Lys His  Ser Ala Ala Thr Asp  Ile Thr Ser Ser Leu  Ser Asp Asp
    1985                 1990                 1995

Gln Val  Pro Glu Ala Phe Leu  Val Met Leu Leu Ile  Gln Phe Ser
    2000                 2005                 2010

Thr Met  Val Val Asp Arg Ala  Leu Tyr Leu Arg Lys  Thr Val Leu
    2015                 2020                 2025

Gly Lys  Leu Ala Phe Gln Val  Ala Leu Val Leu Ala  Ile His Leu
    2030                 2035                 2040

Trp Met  Phe Phe Ile Leu Pro  Ala Val Thr Glu Arg  Met Phe Asn
    2045                 2050                 2055

Gln Asn  Val Val Ala Gln Leu  Trp Tyr Phe Val Lys  Cys Ile Tyr
    2060                 2065                 2070

Phe Ala  Leu Ser Ala Tyr Gln  Ile Arg Cys Gly Tyr  Pro Thr Arg
    2075                 2080                 2085

Ile Leu  Gly Asn Phe Leu Thr  Lys Lys Tyr Asn His  Leu Asn Leu
    2090                 2095                 2100

Phe Leu  Phe Gln Gly Phe Arg  Leu Val Pro Phe Leu  Val Glu Leu
    2105                 2110                 2115

Arg Ala  Val Met Asp Trp Val  Trp Thr Asp Thr Thr  Leu Ser Leu
    2120                 2125                 2130

Ser Ser  Trp Met Cys Val Glu  Asp Ile Tyr Ala Asn  Ile Phe Ile
    2135                 2140                 2145

Ile Lys  Cys Ser Arg Glu Thr  Glu Lys Lys Tyr Pro  Gln Pro Lys
    2150                 2155                 2160

Gly Gln  Lys Lys Lys Lys Ile  Val Lys Tyr Gly Met  Gly Gly Leu
    2165                 2170                 2175

Ile Ile  Leu Phe Leu Ile Ala  Ile Ile Trp Phe Pro  Leu Leu Phe
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    2180                2185                2190

Met Ser  Leu Val Arg Ser Val  Val Gly Val Val Asn  Gln Pro Ile
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Asp Val  Thr Val Thr Leu Lys  Leu Gly Gly Tyr Glu  Pro Leu Phe
    2210                2215                2220

Thr Met  Ser Ala Gln Gln Pro  Ser Ile Ile Pro Phe  Thr Ala Gln
    2225                2230                2235

Ala Tyr  Glu Glu Leu Ser Arg  Gln Phe Asp Pro Gln  Pro Leu Ala
    2240                2245                2250

Met Gln  Phe Ile Ser Gln Tyr  Ser Pro Glu Asp Ile  Val Thr Ala
    2255                2260                2265

Gln Ile  Glu Gly Ser Ser Gly  Ala Leu Trp Arg Ile  Ser Pro Pro
    2270                2275                2280

Ser Arg  Ala Gln Met Lys Arg  Glu Leu Tyr Asn Gly  Thr Ala Asp
    2285                2290                2295

Ile Thr  Leu Arg Phe Thr Trp  Asn Phe Gln Arg Asp  Leu Ala Lys
    2300                2305                2310

Gly Gly  Thr Val Glu Tyr Ala  Asn Glu Lys His Met  Leu Ala Leu
    2315                2320                2325

Ala Pro  Asn Ser Thr Ala Arg  Arg Gln Leu Ala Ser  Leu Leu Glu
    2330                2335                2340

Gly Thr  Ser Asp Gln Ser Val  Val Ile Pro Asn Leu  Phe Pro Lys
    2345                2350                2355

Tyr Ile  Arg Ala Pro Asn Gly  Pro Glu Ala Asn Pro  Val Lys Gln
    2360                2365                2370

Leu Gln  Pro Asn Glu Glu Ala  Asp Tyr Leu Gly Val  Arg Ile Gln
    2375                2380                2385

Leu Arg  Arg Glu Gln Gly Ala  Gly Ala Thr Gly Phe  Leu Glu Trp
    2390                2395                2400

Trp Val  Ile Glu Leu Gln Glu  Cys Arg Thr Asp Cys  Asn Leu Leu
    2405                2410                2415

Pro Met  Val Ile Phe Ser Asp  Lys Val Ser Pro Pro  Ser Leu Gly
    2420                2425                2430

Phe Leu  Ala Gly Tyr Gly Ile  Met Gly Leu Tyr Val  Ser Ile Val
    2435                2440                2445

Leu Val  Ile Gly Lys Phe Val  Arg Gly Phe Phe Ser  Glu Ile Ser
    2450                2455                2460

His Ser  Ile Met Phe Glu Glu  Leu Pro Cys Val Asp  Arg Ile Leu
    2465                2470                2475

Lys Leu  Cys Gln Asp Ile Phe  Leu Val Arg Glu Thr  Arg Glu Leu
    2480                2485                2490

Glu Leu  Glu Glu Glu Leu Tyr  Ala Lys Leu Ile Phe  Leu Tyr Arg
    2495                2500                2505

Ser Pro  Glu Thr Met Ile Lys  Trp Thr Arg Glu Lys  Glu
    2510                2515                2520


<210> SEQ ID NO 19
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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Val Tyr Leu Ile Tyr Leu Leu Leu Ile Pro Leu Phe Ser Glu Pro Thr
        35                  40                  45

Lys Thr Thr Met Gln Gly His Thr Gly Arg Leu Leu Lys Ser Leu Cys
    50                  55                  60

Phe Ile Ser Leu Ser Phe Leu Leu Leu His Ile Ile Phe His Ile Thr
65                  70                  75                  80

Leu Val Ser Leu Glu Ala Gln His Arg Ile Ala Pro Gly Tyr Asn Cys
                85                  90                  95

Ser Thr Trp Glu Lys Thr Phe Arg Gln Ile Gly Phe Glu Ser Leu Lys
            100                 105                 110

Gly Ala Asp Ala Gly Asn Gly Ile Arg Val Phe Val Pro Asp Ile Gly
        115                 120                 125

Met Phe Ile Ala Ser Leu Thr Ile Trp Leu Leu Cys Arg Asn Ile Val
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Gln Lys Pro Val Thr Asp Glu Ala Ala Gln Ser Asn Pro Glu Phe Glu
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Asn Glu Glu Leu Ala Glu Gly Glu Lys Ile Asp Ser Glu Glu Ala Leu
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Ile Tyr Glu Glu Asp Phe Asn Gly Gly Asp Gly Val Glu Gly Glu Leu
            180                 185                 190

Glu Glu Ser Thr Lys Leu Lys Met Phe Arg Arg Leu Ala Ser Val Ala
        195                 200                 205

Ser Lys Leu Lys Glu Phe Ile Gly Asn Met Ile Thr Thr Ala Gly Lys
    210                 215                 220

Val Val Val Thr Ile Leu Leu Gly Ser Ser Gly Met Met Leu Pro Ser
225                 230                 235                 240

Leu Thr Ser Ser Val Tyr Phe Phe Val Phe Leu Gly Leu Cys Thr Trp
                245                 250                 255

Trp Ser Trp Cys Arg Thr Phe Asp Pro Leu Leu Phe Ser Cys Leu Cys
            260                 265                 270

Val Leu Leu Ala Ile Phe Thr Ala Gly His Leu Ile Gly Leu Tyr Leu
        275                 280                 285

Tyr Gln Phe Gln Phe Phe Gln Glu Ala Val Pro Pro Asn Asp Tyr Tyr
    290                 295                 300

Ala Arg Leu Phe Gly Ile Lys Ser Val Ile Gln Thr Asp Cys Ser Ser
305                 310                 315                 320

Thr Trp Lys Ile Ile Val Asn Pro Asp Leu Ser Trp Tyr His His Ala
                325                 330                 335

Asn Pro Ile Leu Leu Leu Val Met Tyr Tyr Thr Leu Ala Thr Leu Ile
            340                 345                 350

Arg Ile Trp Leu Gln Glu Pro Leu Val Gln Asp Glu Gly Thr Lys Glu
        355                 360                 365

Glu Asp Lys Ala Leu Ala Cys Ser Pro Ile Gln Ile Thr Ala Gly Arg
    370                 375                 380

Arg Arg Ser Leu Trp Tyr Ala Thr His Tyr Pro Thr Asp Glu Arg Lys
385                 390                 395                 400

Leu Leu Ser Met Thr Gln Asp Asp Tyr Lys Pro Ser Asp Gly Leu Leu
                405                 410                 415

Val Thr Val Asn Gly Asn Pro Val Asp Tyr His Thr Ile His Pro Ser
            420                 425                 430

Leu Pro Met Glu Asn Gly Pro Gly Lys Ala Asp Leu Tyr Ser Thr Pro
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Gln Tyr Arg Trp Glu Pro Ser Asp Glu Ser Ser Glu Lys Arg Glu Glu
    450                 455                 460

Glu Glu Glu Glu Lys Glu Glu Phe Glu Glu Glu Arg Ser Arg Glu Glu
465                 470                 475                 480

Lys Arg Ser Ile Lys Val His Ala Met Val Ser Val Phe Gln Phe Ile
                485                 490                 495

Met Lys Gln Ser Tyr Ile Cys Ala Leu Ile Ala Met Met Ala Trp Ser
            500                 505                 510

Ile Thr Tyr His Ser Trp Leu Thr Phe Val Leu Leu Ile Trp Ser Cys
        515                 520                 525

Thr Leu Trp Met Ile Arg Asn Arg Arg Lys Tyr Ala Met Ile Ser Ser
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Pro Phe Met Val Val Tyr Gly Asn Leu Leu Leu Ile Leu Gln Tyr Ile
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Trp Ser Phe Glu Leu Pro Glu Ile Lys Lys Val Pro Gly Phe Leu Glu
                565                 570                 575

Lys Lys Glu Pro Gly Glu Leu Ala Ser Lys Ile Leu Phe Thr Ile Thr
            580                 585                 590

Phe Trp Leu Leu Leu Arg Gln His Leu Thr Glu Gln Lys Ala Leu Gln
        595                 600                 605

Glu Lys Glu Ala Leu Leu Ser Glu Val Lys Ile Gly Ser Gln Glu Asn
    610                 615                 620

Glu Glu Lys Asp Glu Glu Leu Gln Asp Ile Gln Val Glu Gly Glu Pro
625                 630                 635                 640

Lys Glu Glu Glu Glu Glu Glu Ala Lys Glu Glu Lys Gln Glu Arg Lys
                645                 650                 655

Lys Val Glu Gln Glu Glu Ala Glu Glu Glu Asp Glu Gln Asp Ile Met
            660                 665                 670

Lys Val Leu Gly Asn Leu Val Val Ala Met Phe Ile Lys Tyr Trp Ile
        675                 680                 685

Tyr Val Cys Gly Gly Met Phe Phe Phe Val Ser Phe Glu Gly Lys Ile
    690                 695                 700

Val Met Tyr Lys Ile Ile Tyr Met Val Leu Phe Leu Phe Cys Val Ala
705                 710                 715                 720

Leu Tyr Gln Val His Tyr Glu Trp Trp Arg Lys Ile Leu Lys Tyr Phe
                725                 730                 735

Trp Met Ser Val Val Ile Tyr Thr Met Leu Val Leu Ile Phe Ile Tyr
            740                 745                 750

Thr Tyr Gln Phe Glu Asn Phe Pro Gly Leu Trp Gln Asn Met Thr Gly
        755                 760                 765

Leu Lys Lys Glu Lys Leu Glu Asp Leu Gly Leu Lys Gln Phe Thr Val
    770                 775                 780

Ala Glu Leu Phe Thr Arg Ile Phe Ile Pro Thr Ser Phe Leu Leu Val
785                 790                 795                 800

Cys Ile Leu His Leu His Tyr Phe His Asp Arg Phe Leu Glu Leu Thr
                805                 810                 815

Asp Leu Lys Ser Ile Pro Ser Lys Glu Asp Asn Thr Ile Tyr Arg Leu
            820                 825                 830

Ala His Pro Glu Gly Ser Leu Pro Asp Leu Thr Met Met His Leu Thr
        835                 840                 845

Ala Ser Leu Glu Lys Pro Glu Val Arg Lys Leu Ala Glu Pro Gly Glu
    850                 855                 860

Glu Lys Leu Glu Gly Tyr Ser Glu Lys Ala Gln Lys Gly Asp Leu Gly
865                 870                 875                 880

Lys Asp Ser Glu Glu Ser Glu Glu Asp Gly Glu Glu Glu Glu Glu Ser
                885                 890                 895

Glu Glu Glu Glu Glu Thr Ser Asp Leu Arg Asn Lys Trp His Leu Val
            900                 905                 910

Ile Asp Arg Leu Thr Val Leu Phe Leu Lys Phe Leu Glu Tyr Phe His
        915                 920                 925

Lys Leu Gln Val Phe Met Trp Trp Ile Leu Glu Leu His Ile Ile Lys
    930                 935                 940

Ile Val Ser Ser Tyr Ile Ile Trp Val Ser Val Lys Glu Val Ser Leu
945                 950                 955                 960
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Lys Leu Arg Arg Leu Ala Ser Ser Val Cys Thr Val Trp Thr Cys Val
           980                 985                 990

Ile Ile Val Cys Lys Met Leu Tyr  Gln Leu Gln Thr Ile  Lys Pro Glu
       995                 1000                1005

Asn Phe  Ser Val Asn Cys Ser  Leu Pro Asn Glu Asn  Gln Thr Asn
    1010                1015                1020

Ile Pro  Phe Asn Glu Leu Asn  Lys Ser Leu Leu Tyr  Ser Ala Pro
    1025                1030                1035

Ile Asp  Pro Thr Glu Trp Val  Gly Leu Arg Lys Ser  Ser Pro Leu
    1040                1045                1050

Leu Val  Tyr Leu Arg Asn Asn  Leu Leu Met Leu Ala  Ile Leu Ala
    1055                1060                1065

Phe Glu  Val Thr Ile Tyr Arg  His Gln Glu Tyr Tyr  Arg Gly Arg
    1070                1075                1080

Asn Asn  Leu Thr Ala Pro Val  Ser Arg Thr Ile Phe  His Asp Ile
    1085                1090                1095

Thr Arg  Leu His Leu Asp Asp  Gly Leu Ile Asn Cys  Ala Lys Tyr
    1100                1105                1110

Phe Ile  Asn Tyr Phe Phe Tyr  Lys Phe Gly Leu Glu  Thr Cys Phe
    1115                1120                1125

Leu Met  Ser Val Asn Val Ile  Gly Gln Arg Met Asp  Phe Tyr Ala
    1130                1135                1140

Met Ile  His Ala Cys Trp Leu  Ile Ala Val Leu Tyr  Arg Arg Arg
    1145                1150                1155

Arg Lys  Ala Ile Ala Glu Ile  Trp Pro Lys Tyr Cys  Cys Phe Leu
    1160                1165                1170

Ala Cys  Ile Ile Thr Phe Gln  Tyr Phe Ile Cys Ile  Gly Ile Pro
    1175                1180                1185

Pro Ala  Pro Cys Arg Asp Tyr  Pro Trp Arg Phe Lys  Gly Ala Ser
    1190                1195                1200

Phe Asn  Asp Asn Ile Ile Lys  Trp Leu Tyr Phe Pro  Asp Phe Ile
    1205                1210                1215

Val Arg  Pro Asn Pro Val Phe  Leu Val Tyr Asp Phe  Met Leu Leu
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Leu Cys  Ala Ser Leu Gln Arg  Gln Ile Phe Glu Asp  Glu Asn Lys
    1235                1240                1245

Ala Ala  Val Arg Ile Met Ala  Gly Asp Asn Val Glu  Ile Cys Met
    1250                1255                1260

Asn Leu  Asp Ala Ala Ser Phe  Ser Gln His Asn Pro  Val Pro Asp
    1265                1270                1275

Phe Ile  His Cys Arg Ser Tyr  Leu Asp Met Ser Lys  Val Ile Ile
    1280                1285                1290

Phe Ser  Tyr Leu Phe Trp Phe  Val Leu Thr Ile Ile  Phe Ile Thr
    1295                1300                1305

Gly Thr  Thr Arg Ile Ser Ile  Phe Cys Met Gly Tyr  Leu Val Ala
    1310                1315                1320

Cys Phe  Tyr Phe Leu Leu Phe  Gly Gly Asp Leu Leu  Leu Lys Pro
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Ile Lys  Ser Ile Leu Arg Tyr  Trp Asp Trp Leu Ile  Ala Tyr Asn
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Val Phe  Val Ile Thr Met Lys  Asn Ile Leu Ser Ile  Gly Ala Cys
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Gly Tyr  Ile Gly Thr Leu Val  His Asn Ser Cys Trp  Leu Ile Gln
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Ala Phe  Ser Leu Ala Cys Thr  Val Lys Gly Tyr Gln  Met Pro Ala
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Ala Asn  Ser Pro Cys Thr Leu  Pro Ser Gly Glu Ala  Gly Ile Ile
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Trp Asp  Ser Ile Cys Phe Ala  Phe Leu Leu Leu Gln  Arg Arg Val
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Phe Met  Ser Tyr Tyr Phe Leu  His Val Val Ala Asp  Ile Lys Ala
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Ser Gln  Ile Leu Ala Ser Arg  Gly Ala Glu Leu Phe  Gln Ala Thr
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Ile Val  Lys Ala Val Lys Ala  Arg Ile Glu Glu Glu  Lys Lys Ser
    1460                1465                1470

Met Asp  Gln Leu Lys Arg Gln  Met Asp Arg Ile Lys  Ala Arg Gln
    1475                1480                1485

Gln Lys  Tyr Lys Lys Gly Lys  Glu Arg Met Leu Ser  Leu Thr Gln
    1490                1495                1500

Glu Pro  Gly Glu Gly Gln Asp  Met Gln Lys Leu Ser  Glu Glu Asp
    1505                1510                1515

Asp Glu  Arg Glu Ala Asp Lys  Gln Lys Ala Lys Gly  Lys Lys Lys
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Gln Trp  Trp Arg Pro Trp Val  Asp His Ala Ser Met  Val Arg Ser
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Gly Asp  Tyr Tyr Leu Phe Glu  Thr Asp Ser Glu Glu  Glu Glu Glu
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Glu Glu  Leu Lys Lys Glu Asp  Glu Glu Pro Pro Arg  Arg Ser Ala
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Phe Gln  Phe Val Tyr Gln Ala  Trp Ile Thr Asp Pro  Lys Thr Ala
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Leu Arg  Gln Arg His Lys Glu  Lys Lys Arg Ser Ala  Arg Glu Glu
    1595                1600                1605

Arg Lys  Arg Arg Arg Lys Gly  Ser Lys Glu Gly Pro  Val Glu Trp
    1610                1615                1620

Glu Asp  Arg Glu Asp Glu Pro  Ile Lys Lys Lys Ser  Asp Gly Pro
    1625                1630                1635

Asp Asn  Ile Ile Lys Arg Ile  Phe Asn Ile Leu Lys  Phe Thr Trp
    1640                1645                1650

Val Leu  Phe Leu Ala Thr Val  Asp Ser Phe Thr Thr  Trp Leu Asn
    1655                1660                1665

Ser Ile  Ser Arg Glu His Ile  Asp Ile Ser Thr Val  Leu Arg Ile
    1670                1675                1680

Glu Arg  Cys Met Leu Thr Arg  Glu Ile Lys Lys Gly  Asn Val Pro
    1685                1690                1695

Thr Arg  Glu Ser Ile His Met  Tyr Tyr Gln Asn His  Ile Met Asn
    1700                1705                1710

Leu Ser  Arg Glu Ser Gly Leu  Asp Thr Ile Asp Glu  His Pro Gly
    1715                1720                1725
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Ala Ala  Ser Gly Ala Gln Thr  Ala His Arg Met Asp  Ser Leu Asp
    1730                1735                1740

Ser His  Asp Ser Ile Ser Ser  Glu Pro Thr Gln Cys  Thr Met Leu
    1745                1750                1755

Tyr Ser  Arg Gln Gly Thr Thr  Glu Thr Ile Glu Glu  Val Glu Ala
    1760                1765                1770

Glu Gln  Glu Glu Glu Ala Gly  Ser Thr Ala Pro Glu  Pro Arg Glu
    1775                1780                1785

Ala Lys  Glu Tyr Glu Ala Thr  Gly Tyr Asp Val Gly  Ala Met Gly
    1790                1795                1800

Ala Glu  Glu Ala Ser Leu Thr  Pro Glu Glu Glu Leu  Thr Gln Phe
    1805                1810                1815

Ser Thr  Leu Asp Gly Asp Val  Glu Ala Pro Pro Ser  Tyr Ser Lys
    1820                1825                1830

Ala Val  Ser Phe Glu His Leu  Ser Phe Gly Ser Gln  Asp Asp Ser
    1835                1840                1845

Ala Gly  Lys Asn Arg Met Ala  Val Ser Pro Asp Asp  Ser Arg Thr
    1850                1855                1860

Asp Lys  Leu Gly Ser Ser Ile  Leu Pro Pro Leu Thr  His Glu Leu
    1865                1870                1875

Thr Ala  Ser Glu Leu Leu Leu  Lys Lys Met Phe His  Asp Asp Glu
    1880                1885                1890

Leu Glu  Glu Ser Glu Lys Phe  Tyr Val Gly Gln Pro  Arg Phe Leu
    1895                1900                1905

Leu Leu  Phe Tyr Ala Met Tyr  Asn Thr Leu Val Ala  Arg Ser Glu
    1910                1915                1920

Met Val  Cys Tyr Phe Val Ile  Ile Leu Asn His Met  Val Ser Ala
    1925                1930                1935

Ser Met  Ile Thr Leu Leu Leu  Pro Ile Leu Ile Phe  Leu Trp Ala
    1940                1945                1950

Met Leu  Ser Val Pro Arg Pro  Ser Arg Arg Phe Trp  Met Met Ala
    1955                1960                1965

Ile Val  Tyr Thr Glu Val Ala  Ile Val Val Lys Tyr  Phe Phe Gln
    1970                1975                1980

Phe Gly  Phe Phe Pro Trp Asn  Lys Asn Val Glu Val  Asn Lys Asp
    1985                1990                1995

Lys Pro  Tyr His Pro Pro Asn  Ile Ile Gly Val Glu  Lys Lys Glu
    2000                2005                2010

Gly Tyr  Val Leu Tyr Asp Leu  Ile Gln Leu Leu Ala  Leu Phe Phe
    2015                2020                2025

His Arg  Ser Ile Leu Lys Cys  His Gly Leu Trp Asp  Glu Asp Asp
    2030                2035                2040

Met Thr  Glu Ser Gly Met Ala  Arg Glu Glu Ser Asp  Asp Glu Leu
    2045                2050                2055

Ser Leu  Gly His Gly Arg Arg  Asp Ser Ser Asp Ser  Leu Lys Ser
    2060                2065                2070

Ile Asn  Leu Ala Ala Ser Val  Glu Ser Val His Val  Thr Phe Pro
    2075                2080                2085

Glu Gln  Gln Thr Ala Val Arg  Arg Lys Arg Ser Gly  Ser Ser Ser
    2090                2095                2100

Glu Pro  Ser Gln Arg Ser Ser  Phe Ser Ser Asn Arg  Ser Gln Arg
    2105                2110                2115
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Gly Ser  Thr Ser Thr Arg Asn  Ser Ser Gln Lys Gly  Ser Ser Val
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Leu Ser  Ile Lys Gln Lys Gly  Lys Arg Glu Leu Tyr  Met Glu Lys
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Leu Gln  Glu His Leu Ile Lys  Ala Lys Ala Phe Thr  Ile Lys Lys
    2150                2155                2160

Thr Leu  Glu Ile Tyr Val Pro  Ile Lys Gln Phe Phe  Tyr Asn Leu
    2165                2170                2175

Ile His  Pro Glu Tyr Ser Ala  Val Thr Asp Val Tyr  Val Leu Met
    2180                2185                2190

Phe Leu  Ala Asp Thr Val Asp  Phe Ile Ile Ile Val  Phe Gly Phe
    2195                2200                2205

Trp Ala  Phe Gly Lys His Ser  Ala Ala Ala Asp Ile  Thr Ser Ser
    2210                2215                2220

Leu Ser  Glu Asp Gln Val Pro  Gly Pro Phe Leu Val  Met Val Leu
    2225                2230                2235

Ile Gln  Phe Gly Thr Met Val  Val Asp Arg Ala Leu  Tyr Leu Arg
    2240                2245                2250

Lys Thr  Val Leu Gly Lys Val  Ile Phe Gln Val Ile  Leu Val Phe
    2255                2260                2265

Gly Ile  His Phe Trp Met Phe  Phe Ile Leu Pro Gly  Val Thr Glu
    2270                2275                2280

Arg Lys  Phe Ser Gln Asn Leu  Val Ala Gln Leu Trp  Tyr Phe Val
    2285                2290                2295

Lys Cys  Val Tyr Phe Gly Leu  Ser Ala Tyr Gln Ile  Arg Cys Gly
    2300                2305                2310

Tyr Pro  Thr Arg Val Leu Gly  Asn Phe Leu Thr Lys  Ser Tyr Asn
    2315                2320                2325

Tyr Val  Asn Leu Phe Leu Phe  Gln Gly Phe Arg Leu  Val Pro Phe
    2330                2335                2340

Leu Thr  Glu Leu Arg Ala Val  Met Asp Trp Val Trp  Thr Asp Thr
    2345                2350                2355

Thr Leu  Ser Leu Ser Ser Trp  Ile Cys Val Glu Asp  Ile Tyr Ala
    2360                2365                2370

His Ile  Phe Ile Leu Lys Cys  Trp Arg Glu Ser Glu  Lys Arg Tyr
    2375                2380                2385

Pro Gln  Pro Arg Gly Gln Lys  Lys Lys Lys Val Val  Lys Tyr Gly
    2390                2395                2400

Met Gly  Gly Met Ile Ile Val  Leu Leu Ile Cys Ile  Val Trp Phe
    2405                2410                2415

Pro Leu  Leu Phe Met Ser Leu  Ile Lys Ser Val Ala  Gly Val Ile
    2420                2425                2430

Asn Gln  Pro Leu Asp Val Ser  Val Thr Ile Thr Leu  Gly Gly Tyr
    2435                2440                2445

Gln Pro  Ile Phe Thr Met Ser  Ala Gln Gln Ser Gln  Leu Lys Val
    2450                2455                2460

Met Asp  Gln Gln Ser Phe Asn  Lys Phe Ile Gln Ala  Phe Ser Arg
    2465                2470                2475

Asp Thr  Gly Ala Met Gln Phe  Leu Glu Asn Tyr Glu  Lys Glu Asp
    2480                2485                2490

Ile Thr  Val Ala Glu Leu Glu  Gly Asn Ser Asn Ser  Leu Trp Thr
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    2495                2500                2505

Ile Ser  Pro Pro Ser Lys Gln  Lys Met Ile His Glu  Leu Leu Asp
    2510                2515                2520

Pro Asn  Ser Ser Phe Ser Val  Val Phe Ser Trp Ser  Ile Gln Arg
    2525                2530                2535

Asn Leu  Ser Leu Gly Ala Lys  Ser Glu Ile Ala Thr  Asp Lys Leu
    2540                2545                2550

Ser Phe  Pro Leu Lys Asn Ile  Thr Arg Lys Asn Ile  Ala Lys Met
    2555                2560                2565

Ile Ala  Gly Asn Ser Thr Glu  Ser Ser Lys Thr Pro  Val Thr Ile
    2570                2575                2580

Glu Lys  Ile Tyr Pro Tyr Tyr  Val Lys Ala Pro Ser  Asp Ser Asn
    2585                2590                2595

Ser Lys  Pro Ile Lys Gln Leu  Leu Ser Glu Asn Asn  Phe Met Asp
    2600                2605                2610

Ile Thr  Ile Ile Leu Ser Arg  Asp Asn Thr Thr Lys  Tyr Asn Ser
    2615                2620                2625

Glu Trp  Trp Val Leu Asn Leu  Thr Gly Asn Arg Ile  Tyr Asn Pro
    2630                2635                2640

Asn Ser  Gln Ala Leu Glu Leu  Val Val Phe Asn Asp  Lys Val Ser
    2645                2650                2655

Pro Pro  Ser Leu Gly Phe Leu  Ala Gly Tyr Gly Ile  Met Gly Leu
    2660                2665                2670

Tyr Ala  Ser Val Val Leu Val  Ile Gly Lys Phe Val  Arg Glu Phe
    2675                2680                2685

Phe Ser  Gly Ile Ser His Ser  Ile Met Phe Glu Glu  Leu Pro Asn
    2690                2695                2700

Val Asp  Arg Ile Leu Lys Leu  Cys Thr Asp Ile Phe  Leu Val Arg
    2705                2710                2715

Glu Thr  Gly Glu Leu Glu Leu  Glu Glu Asp Leu Tyr  Ala Lys Leu
    2720                2725                2730

Ile Phe  Leu Tyr Arg Ser Pro  Glu Thr Met Ile Lys  Trp Thr Arg
    2735                2740                2745

Glu Lys  Thr Asn
    2750


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1-45. (canceled)

46. A method of ameliorating pain in a subject, the method comprising administering to the subject a) an antibody that antagonizes the activity of a mechanically activated cation channel polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20, or b) an antisense oligonucleotide or small interfering RNA (siRNA) complementary to at least 15 contiguous nucleotides of SEQ ID NOs 1, 3, 17, or 19 wherein the antisense oligonucleotide or siRNA inhibits production of the mechanically activated cation channel polypeptide.

47. The method of claim **1**, wherein the polypeptide comprises SEQ ID NO:4 or SEQ ID NO:20.

48. The method of claim **1** wherein the polypeptide is expressed in bladder, colon, kidney, lung, or skin.

49. The method of claim **1**, wherein the polypeptide is expressed in a dorsal root ganglion neuron.

50. The method of claim **1**, wherein the subject is a mammal.

51. The method of claim **1**, wherein the subject is a human.

52. The method of claim **1**, wherein the pain is selected from the group consisting of acute mechanical pain, chronic mechanical pain, mechanical hyperalgesia, mechanical allodynia, arthritis, inflammation, dental pain, cancer pain, and labor pain.

53. The method of claim **1**, wherein the antibody is a monoclonal antibody, a humanized antibody or a chimeric antibody.

54. The method of claim **1**, wherein the antisense oligonucleotide or siRNA comprises any one of SEQ ID NOs 5-16.

55. An isolated antisense oligonucleotide or small interfering RNA (siRNA) complementary to at least 15 contiguous nucleotides of SEQ ID NOs:1, 3, 17, or 19 and encodes a mechanically-activated cation channel polypeptide, wherein the antisense oligonucleotide or siRNA inhibits production of the mechanically-activated cation channel polypeptide.

56. The isolated antisense oligonucleotide or siRNA of claim **10**, wherein the antisense oligonucleotide or siRNA comprises any one of SEQ ID NOs:5-16.

57. An expression cassette comprising a promoter operably linked to a polynucleotide comprising the antisense oligonucleotide or siRNA of claim **10**.

58. A vector comprising the expression cassette of claim **12**.

59. A cell comprising the expression cassette or expression vector of claim **13**, wherein the expression cassette or expression vector is heterologous to the cell.

60. An antibody that antagonizes the activity of a mechanically-activated cation channel selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20.

61. The antibody of claim **15**, wherein the antibody is a monoclonal antibody, a humanized antibody or a chimeric antibody.

62. A method of screening for an agent that modulates the activity of a mechanically-activated cation channel, the method comprising:

contacting a mechanically-activated cation channel polypeptide having at least 70% amino acid sequence identity to at least one of SEQ ID NOs:2, 4, 18, or 20 with an agent; and

selecting the agent that modulates the activity of the mechanically-activated cation channel polypeptide.

63. The method of claim **17**, wherein the activity of the mechanically-activated cation channel polypeptide is determined by measuring an electrophysiological change mediated by the polypeptide comprising any of a change in membrane potential, a change in current, an influx of a cation, or a mechanically activated electrophysiological change; and wherein measuring optionally comprises any of:

i) measuring a membrane potential with a membrane potential dye,

ii) measuring an electrophysiological change with a patch-clamp assay, and

iii) measuring a mechanically activated electrophysiological change.

64. The method of claim **17**, wherein the polypeptide is expressed in a cell and the contacting comprises contacting the cell with the agent; wherein the cell optionally:

i) comprises a heterologous expression cassette comprising a promoter operably linked to a polynucleotide encoding the mechanically-activated cation channel polypeptide;

ii) is a eukaryotic cell;
iii) is a neuron; or
iv) is in an animal.

65. The method of claim **17**, wherein the polypeptide comprises SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:18, or SEQ ID NO:20.

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