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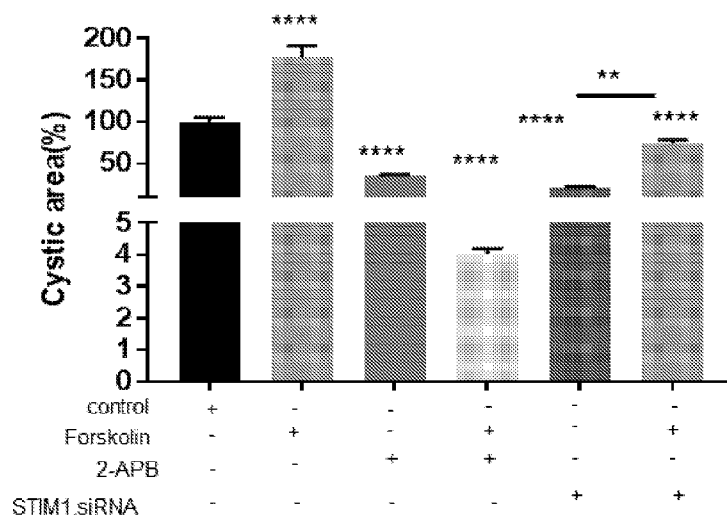
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(54) Title: INHIBITION OF STROMAL INTERACTION MOLECULE 1 (STIM1) AS A CO-TREATMENT FOR ADULT ONSET
POLYCYSTIC KIDNEY DISEASE (ADPKD)

**Fig. 9B**

(57) Abstract: Stromal interaction molecule 1 (STIM1) is identified as a potential therapeutic target for treating diseases or conditions associated with elevated STIM1 levels. Inhibition of HDAC6 in combination with inhibition or knock down of STIM1 was found to synergistically influence cAMP and Ca^{2+} levels in Madin-Darby canine kidney (MDCK) cells, suggesting that inhibition of HDAC6 combined with inhibition or knock down of STIM1 can be used to treat renal diseases, including renal cystic diseases and polycystic renal diseases, such as autosomal dominant polycystic kidney disease (ADPKD), or cancers.



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INHIBITION OF STROMAL INTERACTION MOLECULE 1 (STIM1) AS A CO-TREATMENT FOR ADULT ONSET POLYCYSTIC KIDNEY DISEASE (ADPKD)

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of U.S. Provisional Application No. 62/449,348, filed January 23, 2017, which is incorporated herein by reference in its entirety.

BACKGROUND

10 Autosomal dominant polycystic kidney disease (ADPKD), also referred to as autosomal dominant PKD or adult-onset PKD, is one of the most prevalent, potentially lethal, monogenic human disorders, i.e., a disorder caused by a defect or defects in a single gene. ADPKD is associated with large interfamilial and intrafamilial variability, which can be explained, in part, by its genetic heterogeneity and modifier genes. ADPKD also is the most common of the inherited cystic kidney
15 diseases, a group of disorders having related, but distinct pathogenesis, and which are characterized by the development of renal cysts and various extrarenal manifestations. In the case of ADPKD, these manifestations include cysts in other organs, such as the liver, seminal vesicles, pancreas, and arachnoid membrane, as well as other
20 abnormalities, such as intracranial aneurysms and dolichoectasias, aortic root dilatation aneurysms, mitral valve prolapse, and abdominal wall hernias. More than 50% of patients afflicted with ADPKD eventually develop end stage kidney disease and require dialysis or kidney transplantation. ADPKD is estimated to affect at least 1 in every 1000 individuals worldwide.

25

SUMMARY

 The presently disclosed subject matter, in part, identifies stromal interaction molecule 1 (STIM1) as a potential therapeutic target for treating diseases or conditions associated with elevated STIM1 levels. More particularly, in some
30 aspects, the presently disclosed subject matter demonstrates that inhibition of HDAC6 in combination with inhibition or knock down of STIM1 synergistically influences cAMP and Ca²⁺ levels in Madin-Darby canine kidney (MDCK) cells. This observation suggests that inhibition of HDAC6 combined with inhibition or knock down of STIM1 can be used to treat renal diseases, including renal cystic diseases and

polycystic renal diseases, such as autosomal dominant polycystic kidney disease (ADPKD). Accordingly, in some aspects, the presently disclosed subject matter provides a method of treating ADPKD in a subject in need of treatment thereof, the method comprising administering to the subject a therapeutically effective amount of an HDAC6 inhibitor in combination with a STIM1 inhibitor. In other aspects, inhibition of HDAC6 combined with inhibition or knock down of STIM1 can be used to treat cancer.

Certain aspects of the presently disclosed subject matter having been stated hereinabove, which are addressed in whole or in part by the presently disclosed subject matter, other aspects will become evident as the description proceeds when taken in connection with the accompanying Examples and Figures as best described herein below.

BRIEF DESCRIPTION OF THE FIGURES

Having thus described the presently disclosed subject matter in general terms, reference will now be made to the accompanying Figures, which are not necessarily drawn to scale, and wherein:

FIG. 1A and FIG. 1B show STIM1 expression in PN18/PH2 cells. FIG. 1A is a Western blot showing expression of STIM1 in PN and PH cells treated with tubacin. FIG. 1B shows fold changes relative to PN for STIM1 in PN18 and PH2 cells (total lysate; n=6);

FIG. 2A and FIG. 2B show STIM1 expression in PN18/PH2 cells treated with tubacin. FIG. 2A is a Western blot showing expression of STIM1 in PN and PH cells, as also shown in FIG. 1A. FIG. 2B shows fold changes relative to PN for STIM1 in PN18 and PH2 cells treated with tubacin (total lysate; n=6);

FIG. 3A and FIG. 3B show STIM1 knockdown in PN cells using STIM1 siRNA. FIG. 3A is a representative Western blot showing expression of STIM1 in PN cells transfected with various concentrations of STIM1 siRNA or scrambled RNA for 72 hours. FIG. 3B is representative graphs showing Western blotting analyses revealing the downregulation of STIM1 expression in PN cells at 72 h after STIM1 siRNA (1 nM) transfection. Columns represent averages $\pm$ standard errors of the STIM1 expression. Data were analyzed by nonparametric *t* test. Experiment was repeated 4 times. ****P* < 0.001;

FIG. 4A and FIG. 4B show a.Cyclase 6 (Ac6) expression in PN/STIM1

siRNA cells treated with tubacin. FIG. 4A is a Western blot (left panel) and fold changes relative to PN for STIM1 expression (right panel). FIG. 4B shows fold changes relative to PN for a.cyclase 6;

FIG. 5 shows cAMP levels in siRNA-STIM1-PN cells treated with tubacin/forskolin/IBMX/W7. 3 nM siRNA-STIM1(Origene) was used in this experiment to knockdown STIM1. Each set of data is from 3-6 individual wells. PN18 confluent cells were treated with tubacin (10 μ M) for 16h and then treated with forskolin (100 μ M) or 3-isobutyl-1-methylxanthine (IBMX) (100 μ M) for 30 min before harvesting the cells for assay. Cyclic AMP levels were measured with a direct cAMP enzyme immunoassay kit based on the manufacturer's protocol. Results are expressed as pmole/mL. Columns represent averages $\pm$ SEs. *P < 0.05, **** P < 0.0001. Statistical analysis was performed using a 2-tailed Student t-test. *Compared with PN cells unless indicated with bars;

FIG. 6 shows cAMP levels in siRNA-STIM1-PN cells treated with tubacin/W7 (*N*-(6-Aminoethyl)-5-chloro-1-naphthalenesulfonamide hydrochloride). Each set of data is from 3-6 individual wells. PN18 confluent cells were treated with tubacin (10 μ M) or W7 (50 μ M) for 16h. Cyclic AMP levels were measured with a direct cAMP enzyme immunoassay kit based on the manufacturer's protocol. Results are expressed as pmole/mL. Columns represent averages $\pm$ SEs. *P < 0.05, ** P < 0.001, *** P < 0.0005. Statistical analysis was performed using a 2-tailed Student t-test;

FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E, and FIG. 7F show protein expression of STIM1 in PN and PH cells, Pkd1 mice.

FIG. 8A, FIG. 8B, and FIG. 8C show the effect of STIM siRNA on PN18 cyst size;

FIG. 9A and FIG. 9B show that Forskolin significantly increased the PN18 cyst, whereas 2-APB, Forskolin+2-APB, STIM1 siRNA, Forskolin +STIM1 siRNA, Tubacin, Forskolin+Tubacin significantly inhibited the cyst growth;

FIG. 10A, FIG. 10B, FIG. 10C, FIG. 10D, FIG. 10E, and FIG. 10F demonstrate intracellular Ca^{2+} and ATP-induced release in STIM1 siRNA-PN/PH cells treated with tubacin;

FIG. 11A, FIG. 11B, FIG. 11C, FIG. 11D, FIG. 11E, and FIG. 11F show intracellular Ca^{2+} (F340/F380) levels obtained by ratiometric Fura-2 AM analysis of PN/PH cells treated with Tubacin (10 μ M) for 16h;

FIG. 12A and FIG. 12B show cAMP levels in PN/PH cells treated with STIM1 siRNA and W7;

FIG. 13 shows cAMP levels in PN cells treated with 2APB/Forskolin/IBMX;

FIG. 14A, FIG. 14B, FIG. 14C, FIG. 14D, FIG. 14E, and FIG. 14F show the effect of STIM1 siRNA on ac.αTubulin expression in PN cells; and

FIG. 15A, FIG. 15B, FIG. 15C, FIG. 15D, FIG. 15E, and FIG. 15F show IP3R type I/II/III expression in PN cells treated with STIM1siRNA, 2-APB or Tubacin.

The patent or application file contains at least one drawing executed in color.
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DETAILED DESCRIPTION

The presently disclosed subject matter now will be described more fully hereinafter with reference to the accompanying Figures, in which some, but not all embodiments of the presently disclosed subject matter are shown. Like numbers refer to like elements throughout. The presently disclosed subject matter may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Indeed, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in the foregoing descriptions and the associated Figures. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims.

I. STROMAL INTERACTON MOLECULE 1 (STIM1)

STIM1 is a transmembrane protein with its Ca^{2+} -sensing domain facing the endoplasmic reticulum (ER). When STIM1 senses ER Ca^{2+} , it is translocated to the plasma membrane, where it activates store operated Ca^{2+} entry (SOCE). STIM1 may be a therapeutic target in diseases with dysregulated calcium flux. Dysregulation of calcium flux has been reported in cancers, autoimmune diseases, and other diseases.

Levels of STIM1 may be elevated in subjects with autosomal dominant polycystic kidney disease (ADPKD). In some embodiments, reducing the levels of STIM1 in subjects may treat (ADPKD).

5 II. HISTONE DEACETYLASE 6 (HDAC6)

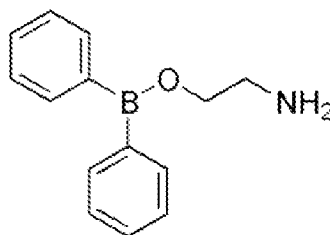
HDAC6, a member of the HDAC family, is predominantly localized to the cytoplasm and has a unique substrate specificity for the deacetylation of tubulin. Many important biological processes are regulated by HDAC6, including transcription, cell migration and proliferation, cell signaling, immune responses, and
10 protein degradation. Increased HDAC6 expression and activity are involved in a number of diseases, including cancer. HDAC6 activity is elevated in ADPKD cells, and HDAC6 may play a role in cyst formation in ADPKD.

III. STIM1 INHIBITORS, STIM1 siRNA, and HDAC6 inhibitors

15 Inhibitors and siRNA may be used to reduce levels or activity of STIM1 in subjects in need thereof. In some embodiments, the one or more agents capable of inhibiting or knocking down STIM1 comprises an siRNA. In some embodiments, the siRNA comprises siRNA STIM 1. In other embodiments, the one or more agents capable of inhibiting or knocking down STIM1 comprises an STIM1 inhibitor.

20 Inhibitors and siRNA may be used to reduce levels or activity of STIM1 in subjects in need thereof. In some embodiments, the one or more agents capable of inhibiting or knocking down STIM1 comprises an siRNA. In some embodiments, the siRNA comprises siRNA STIM 1. In other embodiments, the one or more agents capable of inhibiting or knocking down STIM1 comprises an STIM1 inhibitor.

25 In particular embodiments, the one or more agents capable of inhibiting or knocking down STIM1 comprises 2-aminoethoxydiphenyl borate (2-APB):



2-APB inhibits both IP₃ receptors and TRP channels and is used in research to manipulate intracellular release of calcium ions (Ca²⁺). 2-APB is a powerful modifier
30 of store-operated calcium channels (SOC) function and causes STIM1 clustering in a

cell and prevents STIM1 from moving toward the plasma membrane. In some embodiments, 2-APB is at least as effective, or in other embodiments, is more effective, than siRNA in inhibiting or knocking down STIM1.

One of ordinary skill in the art upon review of the presently disclosed subject matter would recognize that other STIM1 inhibitors, either alone or in combination with 2-APB, are suitable for use with the presently disclosed methods. Commercially available STIM1 inhibitors include ML-9 (1-(5-Chloronaphthalenesulfonyl)homopiperazine hydrochloride) and SKF-96365 (1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl]imidazole, 1-[β-(3-(4-Methoxyphenyl)propoxy)-4-methoxyphenethyl]-1H-imidazole hydrochloride), and derivatives thereof.

In some embodiments, the presently disclosed subject matter demonstrates that histone deacetylase 6 (HDAC6) inhibition (HDAC6i) and STIM1 play a synergistic role in influencing both cyclic adenosine monophosphate (cAMP) and Ca^{2+} levels. STIM1 knockdown has never been shown to be synergistic with an HDAC6 inhibitor, such as tubacin, in reducing cAMP levels and, accordingly, the therapeutic regime provided herein has not been proposed before.

More particularly, in some embodiments, the presently disclosed subject matter demonstrates that histone deacetylase inhibition (HDACi) causes a dramatic drop in resting Ca^{2+} and in thapsigargin-induced release of Ca^{2+} from the ER. The presently disclosed subject matter further shows that HDACi increases the release of Ca^{2+} induced by ATP, mostly likely caused by sustained high levels of STIM1.

In particular embodiments, it was found that an HDAC6 inhibitor can increase the steady state levels of STIM1. Knocking down STIM1 dramatically reduces the ATP-dependent release of Ca^{2+} into the intracellular compartment and the thapsigargin-induced release of Ca^{2+} from the ER. Both of these maneuvers synergize to cause a dramatic, e.g., almost 90%, reduction of resting cAMP levels in the PN cells.

In some embodiments, the HDAC6 inhibitor is selected from the group consisting of tubacin (N-(4-((2R,4R,6S)-4-((4,5-diphenyl-1,3-oxazol-2-yl)sulfanyl)methyl)-6-[4-(hydroxymethyl)phenyl]-1,3-dioxan-2-yl)phenyl)-N'-hydroxyoctanediamide), tubastatin (N-Hydroxy-4-(2-methyl-1,2,3,4-tetrahydropyrido[4,3-b]indol-5-ylmethyl)benzamide hydrochloride; N-Hydroxy-4-((2-methyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzamide hydrochloride), and derivatives thereof.

Representative HDAC6 inhibitors are disclosed in WO2015/061684, to Gradilone and Larusso, published April 30, 2015, which is incorporated herein by reference in its entirety.

5 IV. METHODS OF TREATMENT

The disclosed STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions may be used in methods of treatment for diseases. The disclosed STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions may be useful for treating disease of the kidneys in a subject. The methods of treatment may
10 comprise administering a therapeutically effective amount of the STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors disclosed herein.

a. STIM1 ASSOCIATED DISEASES

Diseases in which STIM1 is thought to be involved include, but are not limited to kidney disease, polycystic disease, autosomal dominant polycystic kidney disease,
15 Alzheimer's disease, cancers and cancer-related diseases, including melanoma, glioblastoma, colorectal cancer, neuroblastoma, breast cancer, non-small cell lung cancer, hepatocellular carcinoma, prostate cancer, and renal cell cancer; immune-related diseases, including severe immunodeficiency, Kaposi sarcoma, thalassemia, systemic lupus erythematosus, cerebral vasospasm, Sjögren's syndrome, and
20 multiple sclerosis, and other diseases, such as hypertension, obesity, diabetes, acute lung injury, and sepsis.

In some embodiments, STIM1 levels may be increased in subjects with kidney disease, polycystic disease, autosomal dominant polycystic kidney disease, Alzheimer's disease, cancers and cancer-related diseases, including melanoma,
25 glioblastoma, colorectal cancer, neuroblastoma, breast cancer, non-small cell lung cancer, hepatocellular carcinoma, prostate cancer, and renal cell cancer; immune-related diseases, including severe immunodeficiency, Kaposi sarcoma, thalassemia, systemic lupus erythematosus, cerebral vasospasm, Sjögren's syndrome, and multiple sclerosis, and other diseases, such as hypertension, obesity, diabetes, acute
30 lung injury, and sepsis.

Accordingly, in some embodiments, the presently disclosed subject matter provides a method for treating a disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1), the method comprising administering to a subject in need of treatment thereof a histone deacetylase 6 (HDAC6) inhibitor in

combination with one or more agents capable of inhibiting or knocking down STIM1.

In some embodiments, the inhibitor composition comprises one or more HDAC6 inhibitors and one or more agents capable of inhibiting or knocking down STIM1.

5

i. KIDNEY DISEASE

In some embodiments, the disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1) is a polycystic disease. In particular embodiments, the polycystic disease comprises a renal cystic disease. In yet more particular embodiments, the renal cystic disease comprises a polycystic kidney disease. In even yet more particular embodiments, the polycystic kidney disease is selected from the group consisting of an autosomal dominant polycystic kidney disease (ADPKD) and an autosomal recessive kidney disease (ARPKD). In certain embodiments, the polycystic kidney disease comprises an autosomal dominant polycystic kidney disease (ADPKD).

Kidney disease, also known as nephropathy or renal disease, is damage to or disease of a kidney. Nephritis is inflammatory kidney disease. Nephrosis is noninflammatory kidney disease. Kidney disease may cause impaired kidney function. Kidney disease may cause kidney failure. Autosomal dominant polycystic kidney disease is characterized by the progressive enlargement of multiple renal cysts. renal function, resulting in renal failure in 50% of patients. Cysts may develop in every nephron segment through a combination of aberrant epithelial cell proliferation and abnormal fluid secretion. ADPKD may be associated with several abnormalities, including alterations in growth factor receptor distribution and activation, altered extracellular matrix, and mispolarization of essential membrane proteins. Cyst fluid is produced by a cAMP-dependent mechanism similar to that found in secretory epithelia. Misregulation of Ca^{2+} may be associated with cyst formation in ADPKD.

The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may be used to treat autosomal dominant kidney disease. Treating polycystic kidney disease may include, but is not limited to improving renal function, maintaining renal function, preventing renal failure, treating renal failure, reducing the number of renal cysts, and/or reducing the number of renal cysts. The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may be used to prevent autosomal dominant polycystic kidney disease. The STIM1

inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may be used to treat renal cysts. The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may be used to reduce the number of renal cysts. The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may be used to reduce the size of renal cysts. The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may be used to reduce the number of renal cysts and the size of renal cysts. The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may improve renal function. The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may be used to treat renal failure. The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may be used to prevent renal failure. The STIM1 inhibitors, STIM1 siRNA, HDAC6 inhibitors, and compositions disclosed herein may reduce the need for kidney transplants in subjects with ADPKD.

In some embodiments, one or more STIM1 inhibitor may be used to treat autosomal dominant polycystic kidney disease. In some embodiments, one or more STIM1 siRNA may be used to treat autosomal polycystic kidney disease. In some embodiments, one or more HDAC6 inhibitors may be used to treat autosomal polycystic kidney disease. In some embodiments, one or more STIM1 inhibitors and one or more HDAC6 inhibitors may be used to treat autosomal dominant polycystic

In some embodiments, the presently disclosed subject matter provides a method for inhibiting stromal interaction molecule 1 (STIM1) as a co-treatment for ADPKD.

In some embodiments, an inhibitor composition to treat autosomal dominant polycystic kidney disease comprises one or more HDAC6 inhibitors and one or more agents capable of inhibiting or knocking down STIM1.

b. MODES OF ADMINISTRATION

Methods of treatment may include any number of modes of administering a disclosed inhibitors, siRNA, and compositions. Modes of administration may include tablets, pills, dragees, hard and soft gel capsules, granules, pellets, aqueous, lipid, oily or other solutions, emulsions such as oil-in-water emulsions, liposomes, aqueous or oily suspensions, syrups, elixirs, solid emulsions, solid dispersions or dispersible powders. For the preparation of pharmaceutical compositions for oral administration, the agent may be admixed with commonly known and used adjuvants and excipients

such as for example, gum arabic, talcum, starch, sugars (such as, e.g., mannitolose, methyl cellulose, lactose), gelatin, surface-active agents, magnesium stearate, aqueous or non-aqueous solvents, paraffin derivatives, cross-linking agents, dispersants, emulsifiers, lubricants, conserving agents, flavoring agents (e.g., ethereal oils),
5 solubility enhancers (e.g., benzyl benzoate or benzyl alcohol) or bioavailability enhancers (e.g. Gelucire®). In the pharmaceutical composition, the agent may also be dispersed in a microparticle, e.g. a nanoparticulate composition.

For parenteral administration, the agent can be dissolved or suspended in a physiologically acceptable diluent, such as, e.g., water, buffer, oils with or without
10 solubilizers, surface-active agents, dispersants or emulsifiers. As oils for example and without limitation, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil and sesame oil may be used. More generally, for parenteral administration, the agent can be in the form of an aqueous, lipid, oily or other kind of solution or suspension or even administered in the form of liposomes or nano-suspensions.

15 c. COMBINATION THERAPIES

The term “combination” is used in its broadest sense and means that a subject is administered at least two agents. More particularly, the term “in combination” refers to the concomitant administration of two (or more) active agents for the treatment of a, e.g., single disease state. As used herein, the active agents may be
20 combined and administered in a single dosage form, may be administered as separate dosage forms at the same time, or may be administered as separate dosage forms that are administered alternately or sequentially on the same or separate days. In one embodiment of the presently disclosed subject matter, the active agents are combined and administered in a single dosage form. In another embodiment, the active agents
25 are administered in separate dosage forms (e.g., wherein it is desirable to vary the amount of one but not the other). The single dosage form may include additional active agents for the treatment of the disease state.

Further, the presently disclosed compositions can be administered alone or in combination with adjuvants that enhance stability of the agents, facilitate
30 administration of pharmaceutical compositions containing them in certain embodiments, provide increased dissolution or dispersion, increase activity, provide adjuvant therapy, and the like, including other active ingredients. Advantageously, such combination therapies utilize lower dosages of the conventional therapeutics,

thus avoiding possible toxicity and adverse side effects incurred when those agents are used as monotherapies.

The timing of administration of the inhibitors can be varied so long as the beneficial effects of the combination of these agents are achieved. Accordingly, the phrase “in combination with” refers to the administration of at least two inhibitors, and optionally additional agents either simultaneously, sequentially, or a combination thereof. Therefore, a subject administered a combination of at least two inhibitors, and optionally additional agents can receive at least two inhibitors, and optionally additional agents at the same time (i.e., simultaneously) or at different times (i.e., sequentially, in either order, on the same day or on different days), so long as the effect of the combination of all agents is achieved in the subject.

When administered sequentially, the agents can be administered within 1, 5, 10, 30, 60, 120, 180, 240 minutes or longer of one another. In other embodiments, agents administered sequentially, can be administered within 1, 2, 3, 4, 5, 10, 15, 20 or more days of one another. Where the agents are administered simultaneously, they can be administered to the subject as separate pharmaceutical compositions, each comprising either at least one inhibitor, and optionally additional agents, or they can be administered to a subject as a single pharmaceutical composition comprising all agents.

When administered in combination, the effective concentration of each of the agents to elicit a particular biological response may be less than the effective concentration of each agent when administered alone, thereby allowing a reduction in the dose of one or more of the agents relative to the dose that would be needed if the agent was administered as a single agent. The effects of multiple agents may, but need not be, additive or synergistic. The agents may be administered multiple times.

In some embodiments, when administered in combination, the two or more agents can have a synergistic effect. As used herein, the terms “synergy,” “synergistic,” “synergistically” and derivations thereof, such as in a “synergistic effect” or a “synergistic combination” or a “synergistic composition” refer to circumstances under which the biological activity of a combination of an agent and at least one additional therapeutic agent is greater than the sum of the biological activities of the respective agents when administered individually.

Synergy can be expressed in terms of a “Synergy Index (SI),” which generally can be determined by the method described by F. C. Kull et al. Applied Microbiology 9, 538 (1961), from the ratio determined by:

$$Q_a Q_A + Q_b Q_B = \text{Synergy Index (SI)}$$

5 wherein:

Q_A is the concentration of a component A, acting alone, which produced an end point in relation to component A;

Q_a is the concentration of component A, in a mixture, which produced an end point;

10 Q_B is the concentration of a component B, acting alone, which produced an end point in relation to component B; and

Q_b is the concentration of component B, in a mixture, which produced an end point.

Generally, when the sum of Q_a/Q_A and Q_b/Q_B is greater than one, antagonism
 15 is indicated. When the sum is equal to one, additivity is indicated. When the sum is less than one, synergism is demonstrated. The lower the SI, the greater the synergy shown by that particular mixture. Thus, a “synergistic combination” has an activity higher than what can be expected based on the observed activities of the individual components when used alone. Further, a “synergistically effective amount” of a
 20 component refers to the amount of the component necessary to elicit a synergistic effect in, for example, another therapeutic agent present in the composition.

V. PHARMACEUTICAL COMPOSITIONS

The disclosed inhibitors and siRNA may be incorporated into pharmaceutical
 25 compositions suitable for administration to a subject (such as a patient, which may be a human or non-human).

The pharmaceutical compositions may include a “therapeutically effective amount” or a “prophylactically effective amount” of the agent. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time
 30 necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the composition may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the composition to elicit a desired response in the individual. A “prophylactically effective amount” refers to an amount effective, at

dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

5 For example, a therapeutically effective amount of the disclosed compositions may be, but is not limited to, about 1 mg/kg to about 1000 mg/kg, about 5 mg/kg to about 950 mg/kg, about 10 mg/kg to about 900 mg/kg, about 15 mg/kg to about 850 mg/kg, about 20 mg/kg to about 800 mg/kg, about 25 mg/kg to about 750 mg/kg, about 30 mg/kg to about 700 mg/kg, about 35 mg/kg to about 650 mg/kg, about 40
10 mg/kg to about 600 mg/kg, about 45 mg/kg to about 550 mg/kg, about 50 mg/kg to about 500 mg/kg, about 55 mg/kg to about 450 mg/kg, about 60 mg/kg to about 400 mg/kg, about 65 mg/kg to about 350 mg/kg, about 70 mg/kg to about 300 mg/kg, about 75 mg/kg to about 250 mg/kg, about 80 mg/kg to about 200 mg/kg, about 85 mg/kg to about 150 mg/kg, and about 90 mg/kg to about 100 mg/kg.

15 The pharmaceutical compositions may include pharmaceutically acceptable carriers. The term "pharmaceutically acceptable carrier," as used herein, means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as, but not limited to, lactose,
20 glucose and sucrose; starches such as, but not limited to, corn starch and potato starch; cellulose and its derivatives such as, but not limited to, sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as, but not limited to, cocoa butter and suppository waxes; oils such as, but not limited to, peanut oil, cottonseed oil, safflower oil, sesame oil, olive
25 oil, corn oil and soybean oil; glycols; such as propylene glycol; esters such as, but not limited to, ethyl oleate and ethyl laurate; agar; buffering agents such as, but not limited to, magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as, but not limited to, sodium
30 lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

Thus, the compounds and their physiologically acceptable salts and solvates

may be formulated for administration by, for example, solid dosing, eyedrop, in a topical oil-based formulation, injection, inhalation (either through the mouth or the nose), implants, or oral, buccal, parenteral, or rectal administration. Techniques and formulations may generally be found in "Remington's Pharmaceutical Sciences",
5 (Meade Publishing Co., Easton, Pa.). Therapeutic compositions must typically be sterile and stable under the conditions of manufacture and storage.

The route by which the disclosed compositions are administered and the form of the composition will dictate the type of carrier to be used. The composition may be in a variety of forms, suitable, for example, for systemic administration (e.g., oral,
10 rectal, nasal, sublingual, buccal, implants, or parenteral) or topical administration (e.g., dermal, pulmonary, nasal, aural, ocular, liposome delivery systems, transdermal, or iontophoresis).

Carriers for systemic administration typically include at least one of diluents, lubricants, binders, disintegrants, colorants, flavors, sweeteners, antioxidants,
15 preservatives, glidants, solvents, suspending agents, wetting agents, surfactants, combinations thereof, and others. All carriers are optional in the compositions.

Suitable diluents include sugars such as glucose, lactose, dextrose, and sucrose; diols such as propylene glycol; calcium carbonate; sodium carbonate; sugar alcohols, such as glycerin; mannitol; and sorbitol. The amount of diluent(s) in a
20 systemic or topical composition is typically about 50 to about 90%.

Suitable lubricants include silica, talc, stearic acid and its magnesium salts and calcium salts, calcium sulfate; and liquid lubricants such as polyethylene glycol and vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma. The amount of lubricant(s) in a systemic or topical composition is
25 typically about 5 to about 10%.

Suitable binders include polyvinyl pyrrolidone; magnesium aluminum silicate; starches such as corn starch and potato starch; gelatin; tragacanth; and cellulose and its derivatives, such as sodium carboxymethylcellulose, ethyl cellulose, methylcellulose, microcrystalline cellulose, and sodium carboxymethylcellulose. The
30 amount of binder(s) in a systemic composition is typically about 5 to about 50%.

Suitable disintegrants include agar, alginic acid and the sodium salt thereof, effervescent mixtures, croscarmellose, crospovidone, sodium carboxymethyl starch, sodium starch glycolate, clays, and ion exchange resins. The amount of disintegrant(s) in a systemic or topical composition is typically about 0.1 to about

10%.

Suitable colorants include a colorant such as an FD&C dye. When used, the amount of colorant in a systemic or topical composition is typically about 0.005 to about 0.1%.

5 Suitable flavors include menthol, peppermint, and fruit flavors. The amount of flavor(s), when used, in a systemic or topical composition is typically about 0.1 to about 1.0%.

10 Suitable sweeteners include aspartame and saccharin. The amount of sweetener(s) in a systemic or topical composition is typically about 0.001 to about 1%.

Suitable antioxidants include butylated hydroxyanisole ("BHA"), butylated hydroxytoluene ("BHT"), and vitamin E. The amount of antioxidant(s) in a systemic or topical composition is typically about 0.1 to about 5%.

15 Suitable preservatives include benzalkonium chloride, methyl paraben and sodium benzoate. The amount of preservative(s) in a systemic or topical composition is typically about 0.01 to about 5%.

Suitable glidants include silicon dioxide. The amount of glidant(s) in a systemic or topical composition is typically about 1 to about 5%.

20 Suitable solvents include water, isotonic saline, ethyl oleate, glycerine, hydroxylated castor oils, alcohols such as ethanol, and phosphate buffer solutions. The amount of solvent(s) in a systemic or topical composition is typically from about 0 to about 100%.

25 Suitable suspending agents include AVICEL RC-591 (from FMC Corporation of Philadelphia, PA) and sodium alginate. The amount of suspending agent(s) in a systemic or topical composition is typically about 1 to about 8%.

30 Suitable surfactants include lecithin, Polysorbate 80, and sodium lauryl sulfate, and the TWEENS from Atlas Powder Company of Wilmington, Delaware. Suitable surfactants include those disclosed in the C.T.F.A. Cosmetic Ingredient Handbook, 1992, pp.587-592; Remington's Pharmaceutical Sciences, 15th Ed. 1975, pp. 335-337; and McCutcheon's Volume 1, Emulsifiers & Detergents, 1994, North American Edition, pp. 236-239. The amount of surfactant(s) in the systemic or topical composition is typically about 0.1% to about 5%.

Although the amounts of components in the systemic compositions may vary depending on the type of systemic composition prepared, in general, systemic

compositions include 0.01% to 50% of active and 50% to 99.99% of one or more carriers. Compositions for parenteral administration typically include 0.1% to 10% of actives and 90% to 99.9% of a carrier including a diluent and a solvent.

Compositions for oral administration can have various dosage forms. The oral dosage form may be a vaccination. The oral dosage form may be solid forms including tablets, capsules, granules, and bulk powders. These oral dosage forms include a safe and effective amount, usually at least about 5%, and more particularly from about 25% to about 50% of actives. The oral dosage compositions include about 50% to about 95% of carriers, and more particularly, from about 50% to about 75%.

Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed. Tablets typically include an active component, and a carrier comprising ingredients selected from diluents, lubricants, binders, disintegrants, colorants, flavors, sweeteners, glidants, and combinations thereof. Specific diluents include calcium carbonate, sodium carbonate, mannitol, lactose and cellulose. Specific binders include starch, gelatin, and sucrose. Specific disintegrants include alginic acid and croscarmellose. Specific lubricants include magnesium stearate, stearic acid, and talc. Specific colorants are the FD&C dyes, which can be added for appearance. Chewable tablets preferably contain sweeteners such as aspartame and saccharin, or flavors such as menthol, peppermint, fruit flavors, or a combination thereof.

Capsules (including implants, time release and sustained release formulations) typically include an active compound, and a carrier including one or more diluents disclosed above in a capsule comprising gelatin. Granules typically comprise a disclosed compound, and preferably glidants such as silicon dioxide to improve flow characteristics. Implants can be of the biodegradable or the non-biodegradable type.

The selection of ingredients in the carrier for oral compositions depends on secondary considerations like taste, cost, and shelf stability, which are not critical for the purposes of this invention.

Solid compositions may be coated by conventional methods, typically with pH or time-dependent coatings, such that a disclosed compound is released in the gastrointestinal tract in the vicinity of the desired application, or at various points and times to extend the desired action. The coatings typically include one or more components selected from the group consisting of cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methyl cellulose phthalate, ethyl cellulose,

EUDRAGIT coatings (available from Rohm & Haas G.M.B.H. of Darmstadt, Germany), waxes and shellac.

Compositions for oral administration can have liquid forms. For example, suitable liquid forms include aqueous solutions, emulsions, suspensions, solutions
5 reconstituted from non-effervescent granules, suspensions reconstituted from non-effervescent granules, effervescent preparations reconstituted from effervescent granules, elixirs, tinctures, syrups, and the like. Liquid compositions, which may be administered orally, may include a disclosed immunogenic proteins, compositions, and vaccines and a carrier, namely, a carrier selected from diluents, colorants, flavors,
10 sweeteners, preservatives, solvents, suspending agents, and surfactants. Peroral liquid compositions preferably include one or more ingredients selected from colorants, flavors, and sweeteners.

Other compositions useful for attaining systemic delivery of the subject compounds include sublingual, buccal and nasal dosage forms. Such compositions
15 typically include one or more of soluble filler substances such as diluents including sucrose, sorbitol and mannitol; and binders such as acacia, microcrystalline cellulose, carboxymethyl cellulose, and hydroxypropyl methylcellulose. Such compositions may further include lubricants, colorants, flavors, sweeteners, antioxidants, and glidants.

20 The disclosed compositions may be topically administered. Topical compositions that can be applied locally to the skin may be in any form including solids, solutions, oils, creams, ointments, gels, lotions, shampoos, leave-on and rinse-out hair conditioners, milks, cleansers, moisturizers, sprays, skin patches, and the like. The carrier of the topical composition preferably aids penetration of the compounds
25 into the skin. The carrier may further include one or more optional components. Transdermal administration may be used to facilitate delivery.

The amount of the carrier employed in conjunction with a disclosed compound is sufficient to provide a practical quantity of composition for administration per unit dose of the medicament. Techniques and compositions for making dosage forms
30 useful in the methods of this invention are described in the following references: Modern Pharmaceutics, Chapters 9 and 10, Banker & Rhodes, eds. (1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms, 2nd Ed., (1976).

A carrier may include a single ingredient or a combination of two or more

ingredients. In the topical compositions, the carrier includes a topical carrier. Suitable topical carriers include one or more ingredients selected from phosphate buffered saline, isotonic water, deionized water, monofunctional alcohols, symmetrical alcohols, aloe vera gel, allantoin, glycerin, vitamin A and E oils, mineral oil, propylene glycol, PPG-2 myristyl propionate, dimethyl isosorbide, castor oil, combinations thereof, and the like. More particularly, carriers for skin applications include propylene glycol, dimethyl isosorbide, and water, and even more particularly, phosphate buffered saline, isotonic water, deionized water, monofunctional alcohols, and symmetrical alcohols.

10 The carrier of a topical composition may further include one or more ingredients selected from emollients, propellants, solvents, humectants, thickeners, powders, fragrances, pigments, and preservatives, all of which are optional.

 Suitable emollients include stearyl alcohol, glyceryl monoricinoleate, glyceryl monostearate, propane-1,2-diol, butane-1,3-diol, mink oil, cetyl alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, isocetyl stearate, oleyl alcohol, isopropyl laurate, hexyl laurate, decyl oleate, octadecan-2-ol, isocetyl alcohol, cetyl palmitate, di-n-butyl sebacate, isopropyl myristate, isopropyl palmitate, isopropyl stearate, butyl stearate, polyethylene glycol, triethylene glycol, lanolin, sesame oil, coconut oil, arachis oil, castor oil, acetylated lanolin alcohols, petroleum, mineral oil, butyl myristate, isostearic acid, palmitic acid, isopropyl linoleate, lauryl lactate, myristyl lactate, decyl oleate, myristyl myristate, and combinations thereof. Specific emollients for skin include stearyl alcohol and polydimethylsiloxane. The amount of emollient(s) in a skin-based topical composition is typically about 5% to about 95%.

 Suitable propellants include propane, butane, isobutane, dimethyl ether, carbon dioxide, nitrous oxide, and combinations thereof. The amount of propellant(s) in a topical composition is typically about 0% to about 95%.

 Suitable solvents include water, ethyl alcohol, methylene chloride, isopropanol, castor oil, ethylene glycol monoethyl ether, diethylene glycol monobutyl ether, diethylene glycol monoethyl ether, dimethylsulfoxide, dimethyl formamide, tetrahydrofuran, and combinations thereof. Specific solvents include ethyl alcohol and homotopic alcohols. The amount of solvent(s) in a topical composition is typically about 0% to about 95%.

 Suitable humectants include glycerin, sorbitol, sodium 2-pyrrolidone-5-carboxylate, soluble collagen, dibutyl phthalate, gelatin, and combinations thereof.

Specific humectants include glycerin. The amount of humectant(s) in a topical composition is typically 0% to 95%.

The amount of thickener(s) in a topical composition is typically about 0% to about 95%.

5 Suitable powders include beta-cyclodextrins, hydroxypropyl cyclodextrins, chalk, talc, fullers earth, kaolin, starch, gums, colloidal silicon dioxide, sodium polyacrylate, tetra alkyl ammonium smectites, trialkyl aryl ammonium smectites, chemically-modified magnesium aluminum silicate, organically-modified Montmorillonite clay, hydrated aluminum silicate, fumed silica, carboxyvinyl
10 polymer, sodium carboxymethyl cellulose, ethylene glycol monostearate, and combinations thereof. The amount of powder(s) in a topical composition is typically 0% to 95%.

The amount of fragrance in a topical composition is typically about 0% to about 0.5%, particularly, about 0.001% to about 0.1%.

15 Suitable pH adjusting additives include HCl or NaOH in amounts sufficient to adjust the pH of a topical pharmaceutical composition.

The presently disclosed pharmaceutical compositions can be manufactured in a manner known in the art, *e.g.* by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or
20 lyophilizing processes.

In some embodiments, the presently disclosed pharmaceutical compositions can be administered by rechargeable or biodegradable devices. For example, a variety of slow-release polymeric devices have been developed and tested *in vivo* for the controlled delivery of drugs, including proteinacious biopharmaceuticals. Suitable
25 examples of sustained release preparations include semipermeable polymer matrices in the form of shaped articles, *e.g.*, films or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. Patent No. 3,773,919; EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., Biopolymers 22:547, 1983), poly (2-hydroxyethyl-methacrylate) (Langer et al. (1981)
30 *J. Biomed. Mater. Res.* 15:167; Langer (1982), *Chem. Tech.* 12:98), ethylene vinyl acetate (Langer et al. (1981) *J. Biomed. Mater. Res.* 15:167), or poly-D-(-)-3-hydroxybutyric acid (EP 133,988A). Sustained release compositions also include liposomally entrapped compositions comprising at least one inhibitor which can be prepared by methods known in the art (Epstein et al. (1985) *Proc. Natl. Acad. Sci.*

U.S.A. 82:3688; Hwang et al. (1980) *Proc. Natl. Acad. Sci. U.S.A.* 77:4030; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324A). Ordinarily, the liposomes are of the small (about 200-800 angstroms) unilamellar type in which the lipid content is greater than about 30 mol % cholesterol, the selected proportion being adjusted for the optimal therapy. Such materials can comprise an implant, for example, for sustained release of the presently disclosed compositions, which, in some embodiments, can be implanted at a particular, pre-determined target site.

Pharmaceutical compositions for parenteral administration include aqueous solutions of compositions comprising, for example, at least two inhibitors. For injection, the presently disclosed pharmaceutical compositions can be formulated in aqueous solutions, for example, in some embodiments, in physiologically compatible buffers, such as Hank's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of compositions include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility of the compositions comprising, for example, at least one two inhibitors to allow for the preparation of highly concentrated solutions.

For nasal or transmucosal administration generally, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Additional ingredients can be added to compositions for topical administration, as long as such ingredients are pharmaceutically acceptable and not deleterious to the epithelial cells or their function. Further, such additional ingredients should not adversely affect the epithelial penetration efficiency of the composition, and should not cause deterioration in the stability of the composition. For example, fragrances, opacifiers, antioxidants, gelling agents, stabilizers, surfactants, emollients, coloring agents, preservatives, buffering agents, and the like can be present. The pH of the presently disclosed topical composition can be adjusted to a physiologically acceptable range of from about 6.0 to about 9.0 by adding buffering agents thereto such that the composition is physiologically compatible with a subject's skin.

Regardless of the route of administration selected, the presently disclosed

compositions are formulated into pharmaceutically acceptable dosage forms, such as described herein or by other conventional methods known to those of skill in the art.

In general, the “effective amount” or “therapeutically effective amount” of an active agent or drug delivery device refers to the amount necessary to elicit the
5 desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of an agent or device may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the composition of the encapsulating matrix, the target tissue, and the like.

Generally, the presently disclosed compositions (e.g., comprising at least two
10 inhibitors) can be administered to a subject for therapy by any suitable route of administration, including orally, nasally, transmucosally, ocularly, rectally, intravaginally, parenterally, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articular, intra-sternal, intra-synovial, intra-hepatic, intralesional, intracranial, intraperitoneal,
15 intranasal, or intraocular injections, intracisternally, topically, as by powders, ointments or drops (including eyedrops), including buccally and sublingually, transdermally, through an inhalation spray, or other modes of delivery known in the art. The phrases “systemic administration”, “administered systemically”, “peripheral administration” and “administered peripherally” as used herein mean the
20 administration of compositions, e.g., comprising at least two inhibitors, such that it enters the patient’s system and, thus, are subject to metabolism and other like processes, for example, subcutaneous administration.

The phrases “parenteral administration” and “administered parenterally” as used herein mean modes of administration other than enteral and topical
25 administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intarterial, intrathecal, intracapsular, intraorbital, intraocular, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

30 In another aspect, the presently disclosed subject matter provides a pharmaceutical composition including at least two inhibitors, and optionally additional agents, alone or in combination with one or more additional therapeutic agents in admixture with a pharmaceutically acceptable excipient.

More particularly, the presently disclosed subject matter provides a

pharmaceutical composition comprising at least two inhibitors, and optionally additional agents, and a pharmaceutically acceptable carrier. In therapeutic and/or diagnostic applications, the compounds of the disclosure can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington: The Science and Practice of Pharmacy (20th ed.) Lippincott, Williams and Wilkins (2000).

Use of pharmaceutically acceptable inert carriers to formulate the compounds herein disclosed for the practice of the disclosure into dosages suitable for systemic administration is within the scope of the disclosure. With proper choice of carrier and suitable manufacturing practice, the compositions of the present disclosure, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the disclosure to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject (e.g., patient) to be treated.

For nasal or inhalation delivery, the agents of the disclosure also may be formulated by methods known to those of skill in the art, and may include, for example, but not limited to, examples of solubilizing, diluting, or dispersing substances, such as saline; preservatives, such as benzyl alcohol; absorption promoters; and fluorocarbons.

Pharmaceutical compositions suitable for use in the present disclosure include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. Generally, the compounds according to the disclosure are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from 0.01 to 1000 mg, from 0.5 to 100 mg, from 1 to 50 mg per day, and from 5 to 40 mg per day are examples of dosages that may be used. A non-limiting dosage is 10 to 30 mg per day. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the subject to be treated, the body weight of the subject to be treated, and the preference and experience of the attending physician.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral
5 administration may be in the form of tablets, dragees, capsules, or solutions.

In some embodiments the pharmaceutical composition may comprise at least one pharmaceutically acceptable carrier, a therapeutically effective amount of a histone deacetylase 6 (HDAC6) inhibitor, and one or more agents capable of inhibiting or knocking down STIM1. In some embodiments, the one or more agents
10 capable of inhibiting or knocking down STIM1 in the pharmaceutical composition is an siRNA. In some embodiments, the one or more agents capable of inhibiting or knocking down STIM1 in the pharmaceutical composition is selected from ML-9 (1-(5-Chloronaphthalenesulfonyl) homopiperazine hydrochloride), SKF-96365 (1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl]imidazole, 1-[β-(3-(4-
15 Methoxyphenyl)propoxy)-4-methoxyphenethyl]-1H-imidazole hydrochloride), and derivatives thereof. In some embodiments, the HDAC6 inhibitor in the pharmaceutical composition is selected from: tubacin, tubastatin, and derivatives thereof.

20 VI. KITS

The inhibitors, siRNA, and compositions disclosed herein may be included in kits comprising the inhibitors, siRNA, and/or compositions; and information, instructions, or both that use of the kit will provide treatment for medical conditions in mammals (particularly humans). The kit may include an additional pharmaceutical
25 composition for use in combination therapy. The kit may include buffers, reagents, or other components to facilitate the mode of administration. The kit may include materials to facilitate oral administration. The kit may include materials to facilitate nasal mucousal administration. The kit may include materials that facilitate sublingual administration. The information and instructions may be in the form of
30 words, pictures, or both, and the like. In addition or in the alternative, the kit may include the medicament, a composition, or both; and information, instructions, or both, regarding methods of application of medicament, or of composition, preferably with the benefit of treating or preventing medical conditions in mammals (e.g., humans).

VII. DEFINITIONS

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case
5 of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials,
10 methods, and examples disclosed herein are illustrative only and not intended to be limiting.

The modifier “about” used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (for example, it includes at least the degree of error associated with the measurement of the particular quantity).
15 The modifier “about” should also be considered as disclosing the range defined by the absolute values of the two endpoints. For example, the expression “from about 2 to about 4” also discloses the range “from 2 to 4.” The term “about” may refer to plus or minus 10% of the indicated number. For example, “about 10%” may indicate a range of 9% to 11%, and “about 1” may mean from 0.9-1.1. Other meanings of “about”
20 may be apparent from the context, such as rounding off, so, for example “about 1” may also mean from 0.5 to 1.4. Further, the term “about” when used in connection with one or more numbers or numerical ranges, should be understood to refer to all such numbers, including all numbers in a range and modifies that range by extending the boundaries above and below the numerical values set forth. The recitation of
25 numerical ranges by endpoints includes all numbers, e.g., whole integers, including fractions thereof, subsumed within that range (for example, the recitation of 1 to 5 includes 1, 2, 3, 4, and 5, as well as fractions thereof, e.g., 1.5, 2.25, 3.75, 4.1, and the like) and any range within that range. For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing amounts, sizes,
30 dimensions, proportions, shapes, formulations, parameters, percentages, parameters, quantities, characteristics, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about” even though the term “about” may not expressly appear with the value, amount or range. Accordingly, unless indicated to the contrary, the numerical parameters set

forth in the following specification and attached claims are not and need not be exact, but may be approximate and/or larger or smaller as desired, reflecting tolerances, conversion factors, rounding off, measurement error and the like, and other factors known to those of skill in the art depending on the desired properties sought to be
5 obtained by the presently disclosed subject matter. For example, the term “about,” when referring to a value can be meant to encompass variations of, in some embodiments, $\pm 100\%$ in some embodiments $\pm 50\%$, in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some embodiments $\pm 0.1\%$ from the specified
10 amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

The terms “administration” or “administering” as used herein may include the process in which the compositions disclosed herein, alone or in combination with other compounds or compositions, are delivered to a subject. The disclosed
15 compositions may be administered in various routes including, but not limited to, oral, mucosal, mucosal nasal, parenteral (including intravenous, intra-arterial, and other appropriate parenteral routes), intrathecally, intramuscularly, subcutaneously, colonically, rectally, and nasally, transcutaneously, among others. The dosing of the compositions described herein to obtain a therapeutic or prophylactic effect may be
20 determined by the circumstances of the subject, as known in the art. The dosing of a subject herein may be accomplished through individual or unit doses of the compositions described herein or by a combined or prepackaged or pre-formulated dose of the compositions described herein.

Administration may depend upon the amount of the compositions described
25 herein administered, the number of doses, and duration of treatment. For example, multiple doses of the compositions described herein may be administered. The frequency of administration of the compositions described herein may vary depending on any of a variety of factors. The duration of administration of the compositions described herein, e.g., the period of time over which the compositions described
30 herein are administered, may vary, depending on any of a variety of factors, including subject response, etc.

The amount of the disclosed compositions administered may vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, idiosyncratic responses of the individual, the dosimetry, and the

like. Detectably effective amounts of the disclosed compositions of the present disclosure may also vary.

As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the content clearly dictates otherwise.

5 Thus, for example, reference to “a subject” includes a plurality of subjects, unless the context clearly is to the contrary (e.g., a plurality of subjects), and so forth. As used in this specification and the appended claims, the term “or” is generally employed in its sense including “and/or” unless the context clearly dictates otherwise.

The terms “comprise(s)”, “include(s)”, “having”, “has”, “can”, “contain(s)”,
10 and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. Likewise, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items. The present disclosure
15 also contemplates other embodiments “comprising”, “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

The term “parenterally,” as used herein, refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous
20 and intraarticular injection and infusion.

A “pharmaceutically acceptable excipient,” “pharmaceutically acceptable diluent,” “pharmaceutically acceptable carrier,” or “pharmaceutically acceptable adjuvant” means an excipient, diluent, carrier, and/or adjuvant that are useful in preparing a pharmaceutical composition that are generally safe, non-toxic and neither
25 biologically nor otherwise undesirable, and includes an excipient, diluent, carrier, and adjuvant that are acceptable for veterinary use and/or human pharmaceutical use. “A pharmaceutically acceptable excipient, diluent, carrier and/or adjuvant” as used herein includes one or more such excipients, diluents, carriers, and adjuvants.

As used herein, the terms “subject” and “patient” are used interchangeably
30 herein. The subject treated by the presently disclosed methods, uses, inhibitors, and compositions comprising those inhibitors in their many embodiments is desirably a human subject, although it is to be understood that the methods described herein are effective with respect to all vertebrate species, which are intended to be included in the term “subject.” Accordingly, a “subject” can include a human subject for medical

purposes, such as for the treatment of an existing condition or disease or the prophylactic treatment for preventing the onset of a condition or disease, or an animal subject for medical, veterinary purposes, or developmental purposes. Suitable animal subjects include mammals including, but not limited to, primates, e.g., humans, 5 monkeys, apes, and the like; bovines, e.g., cattle, oxen, and the like; ovines, e.g., sheep and the like; caprines, e.g., goats and the like; porcines, e.g., pigs, hogs, and the like; equines, e.g., horses, donkeys, zebras, and the like; felines, including wild and domestic cats; canines, including dogs; lagomorphs, including rabbits, hares, and the like; and rodents, including mice, rats, and the like. An animal may be a transgenic 10 animal. In some embodiments, the subject is a human including, but not limited to, fetal, neonatal, infant, juvenile, and adult subjects. Further, a “subject” can include a patient afflicted with or suspected of being afflicted with a condition or disease. In some embodiments, the subject is a human, rat, mouse, cat, dog, horse, sheep, cow, monkey, avian, or amphibian. In some embodiments, the subject is a human.

15 The “therapeutically effective amount” for purposes herein may be determined by such considerations as are known in the art. A therapeutically effective amount of a compound may include the amount necessary to provide a therapeutically effective result *in vivo*. The amount of the compound or composition must be effective to achieve a response, including but not limited to a total prevention of (e.g., protection 20 against) of a condition, improved survival rate or more rapid recovery, improvement or elimination of symptoms associated with the condition (such as cancer), or other indicators as are selected as appropriate measures by those skilled in the art. As used herein, a suitable single dose size includes a dose that is capable of preventing or alleviating (reducing or eliminating) a symptom in a subject when administered one or 25 more times over a suitable time period. The “therapeutically effective amount” of a compound or composition as described herein may depend on the route of administration, type of subject being treated, and the physical characteristics of the subject. These factors and their relationship to dose are well known to one of skill in the medicinal art, unless otherwise indicated.

30 As used herein, “treat”, “treatment”, “treating”, and the like refer to acting upon a condition with an agent to affect the condition by improving or altering it. The condition includes, but is not limited to conditions in which STIM1 is involved in the pathophysiology, such as kidney disease. The aforementioned terms cover one or more treatments of a condition in a subject (e.g., a mammal, typically a human or

non-human animal of veterinary interest), and include: (a) reducing the risk of occurrence of the condition in a subject determined to be predisposed to the condition but not yet diagnosed, (b) impeding the development of the condition, and/or (c) relieving the condition, e.g., causing regression of the condition and/or relieving one or more condition symptoms.

For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

The presently disclosed compositions and methods will be better understood by reference to the following examples, which are intended as an illustration of and not a limitation upon the scope of the invention.

15

EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The synthetic descriptions and specific examples that follow are only intended for the purposes of illustration, and are not to be construed as limiting in any manner to make compounds of the disclosure by other methods.

25

EXAMPLE 1

Inhibition of STIM1 as a Co-Treatment for Adult Onset Polycystic Kidney Disease

Referring now to FIG. 1A and FIG. 1B, STIM1 expression in PN18/ PH2 cells treated with tubacin was measured. These data indicate that protein expression of STIM1 is significantly increased in PN cells compared with PH cells. Further, these data show that the HDAC6 inhibitor, tubacin, has no effect on STIM 1 expression.

Referring now to FIG. 2A and FIG. 2B, STIM1 expression in PN18/ PH2 cells treated with tubacin was again measured. Consistent with the data shown in FIG. 1A

and FIG. 1B, the HDAC6 inhibitor, tubacin, has no effect on STIM1 expression.

FIG. 3A and FIG. 3B show STIM1 knockdown in PN cells using STIM1 siRNA. PN cells were cultured as mentioned in culture methods. Mouse STIM1 siRNA or Scramble siRNA was transfected according to the manufacturer's instructions. FIG. 3A is a representative Western blot showing expression of STIM1 in PN cells transfected with various concentrations of STIM1 siRNA or scrambled RNA for 72h. FIG. 3B is representative graphs showing Western blotting analyses revealing the downregulation of STIM1 expression in PN cells at 72 h after STIM1 siRNA (1nM) transfection. These data show that protein expression of STIM1 is significantly decreased in PN cells compared with PH cells after treatment with siRNA STIM 1.

FIG. 4A and FIG. 4B show a cyclase 6 expression in PN/STIM1siRNA cells treated with tubacin. These data indicate that protein expression of Ad. Cyclase 6 is significantly decreased in PN cells after treatment with STIM1 siRNA or in combination with tubacin compared with control PN cells.

Referring now to FIG. 5, are cAMP levels in siRNA-STIM1-PN cells treated with Tubacin/Forskolin/IBMX/W7. 3nM siRNA-STIM1(Origene) was used in this experiment to knockdown STIM1. Each set of data is from 3-6 individual wells. PN18 confluent cells were treated with tubacin (10 μ M) for 16h and then treated with Forskolin (100 μ M) or IBMX (100 μ M) for 30 min before harvesting the cells for assay. Cyclic AMP levels were measured with a direct cAMP Enzyme immunoassay Kit based on the manufacturer's protocol. Results are expressed as pmole/mL. Columns represent averages $\pm$ SEs. *P < 0.05, **** P < 0.0001. Statistical analysis was performed using a 2-tailed Student t-test. *Compared with PN cells unless indicated with bars; and

FIG. 6 shows cAMP levels in siRNA-STIM1-PN cells treated with Tubacin/W7. Each set of data is from 3-6 individual wells. PN18 confluent cells were treated with tubacin (10 μ M) or W7 (50 μ M) for 16h. Cyclic AMP levels were measured with a direct cAMP. Enzyme immunoassay Kit based on the manufacturer's protocol. Results are expressed as pmole/ml. Columns represent averages $\pm$ SEs. *P < 0.05, ** P < 0.001, *** P < 0.0005. Statistical analysis was performed using a 2-tailed Student t-test.

EXAMPLE 2

Protein Expression of STIM1 in PN and PH Cells, Pkd1 Mice

FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E, and FIG. 7F show protein
 5 expression of STIM1 in PN and PH cells, Pkd1 mice. In this example, Pkd1 null (PN)
 and heterozygous (PH) cells were grown in 10-cm culture dishes at permissive
 conditions (33 °C) with γ -interferon in culture media. Cells were then transferred to
 non-permissive conditions at 37 °C, in γ -interferon free culture media, and evaluated
 at full confluency. At day five, cells were harvested for Western blot assay. FIG. 7A
 10 is a Western blot showing expression of STIM1 in PN and PH cells. In FIG. 7B, the
 columns represent averages $\pm$ standard errors of the STIM1 expression in PN and PH
 cells. **** $P < 0.0001$. Data were analyzed by nonparametric t-test. The experiment
 was repeated 6 times. These data indicate that protein expression of STIM1 is
 significantly increased in PN cells compared to PH cells. Protein expression of
 15 STIM1 also is significantly increased in induced Pkd1 mice.

EXAMPLE 3

Effect of STIM siRNA on PN18 Cyst Size

Referring now to FIG. 8A, FIG. 8B, and FIG. 8C, cysts were treated with
 20 Control (opti-MEM), transfection reagent control, siRNA control, STIM1 siRNA
 3nM on day 11. Pictures were taken on Day 15 and Day 18. Columns represent mean
 $\pm$ SEM (n=6-10). Average cyst from control group was considered 100%, and the rest
 of the cysts were compared with this cyst. *** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.
 STIM1 siRNA: SR419122, Origene; Matrigel matrix: 354230, Corning. These data
 25 indicate that STIM1 siRNA decreased the cyst size.

5

EXAMPLE 4

Inhibition of Cyst Growth with 2-APB, Forskolin+2-APB, STIM1 siRNA,Forskolin +STIM1 siRNA, Tubacin, Forskolin+Tubacin

30 Referring to FIG. 9A and FIG. 9B, Forskolin significantly increased the PN18
 cyst. In contrast, 2-APB, Forskolin+2-APB, STIM1 siRNA, Forskolin +STIM1
 siRNA, Tubacin, Forskolin+Tubacin significantly inhibited the cyst growth.

EXAMPLE 5

Intracellular Ca^{2+} and ATP-induced Release in STIM1 siRNA-PN/PH
Cells Treated with Tubacin

FIG. 10A, FIG. 10B, and FIG. 10C demonstrate intracellular Ca^{2+} and ATP-induced release in STIM1siRNA-PN/PH cells treated with tubacin. FIG. 10A shows representative traces of intracellular Ca^{2+} release in response to 100 μM ATP in STIM1 siRNA-PN/PH cells treated with tubacin. FIG. 10B is a graph summarizing the average amplitude of Ca^{2+} release in response to ATP. FIG. 10C shows that PN cells have a higher resting Ca^{2+} than PH cells. Amplitude was measured as standard deviation of signal base to peak $\Delta f/f$. Asterisk indicates significance between the two groups (student's t-test, * $P < 0.05$, **** $P < 0.0001$, $n=4$).

EXAMPLE 6

Intracellular Ca^{2+} (F340/F380) Levels of PN/PH Cells Treated with Tubacin

FIG. 11A, FIG. 11B, FIG. 11C, FIG. 11D, FIG. 11E, and FIG. 11F show intracellular Ca^{2+} (F340/F380) levels obtained by ratiometric Fura-2 AM analysis of PN/PH cells treated with Tubacin (10 μM) for 16h. FIG. 11A shows representative traces of intracellular Ca^{2+} release in response to Thapsigargin (4 μM) in PN/PH cells treated with tubacin. FIG. 11B is a graph summarizing resting calcium levels; and FIG. 11C is a graph summarizing the average amplitude of Ca^{2+} release in response to thapsigargin. Amplitude was measured as standard deviation of signal base to peak $\Delta f/f$. Asterisk indicates significance between the two groups (student's t-test, ** $P < 0.01$, **** $P < 0.0001$, $n=4$).

EXAMPLE 7

cAMP Levels in PN/PH Cells Treated with STIM1 siRNA and W7

Referring now to FIG. 12A and FIG. 12B, cAMP levels in PN/PH cells treated with STIM1 siRNA and W7 are shown. Pkd1 null (PN) cells were grown in 10-cm culture dishes at permissive conditions (33 °C) with γ -interferon in culture media. Cells were then transferred to non-permissive conditions at 37 °C, γ -interferon free culture media. In the case of PN/S cells, mouse STIM1 siRNA (3 nM) was transfected using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions and evaluated at full confluency. At day five confluent cells were treated with Forskolin (100 μM) or IBMX (100 μM) for 30 min before harvesting the cells

for assay. Cyclic AMP levels were measured with a direct cAMP Enzyme immunoassay Kit based on the manufacturer's protocol. Results are expressed as pmole/mL. Columns represent averages $\pm$ SEs. *P < 0.05. Statistical analysis was performed using a 2-tailed Student t-test. n=3-6. These data indicate that STIM1 knockdown using siRNA STIM1 decreased the cAMP levels in PN cells, but no significant effect was observed in forskolin- or IBMX-induced cAMP levels.

EXAMPLE 8

cAMP Levels in PN Cells Treated with 2-APB/Forskolin/IBMX

FIG. 13 shows cAMP levels in PN cells treated with 2-APB/Forskolin/IBMX. PN18 confluent cells were treated with 2-APB (10 μ M) for 16 and then treated with Forskolin (100 μ M) or IBMX (100 μ M) for 30 min. before harvesting the cells for assay. Cyclic AMP levels were measured with a direct cAMP Enzyme immunoassay Kit based on the manufacturer's protocol. Results are expressed as pmole/ml. Columns represent averages $\pm$ SEs. *P < 0.05, **P < 0.01, ****P < 0.0001. Statistical analysis was performed using a 2-tailed Student's t-test. n=3. Reagents: 2-APB from Sigma (# D9754); cAMP kit from Sigma (# CA200); Forskolin : Cayman (11018); IBMX: Sigma (I5879) were used in this Example. These data indicate that PN cells exposed to 2-APB (10 μ M) increased the cAMP levels. Further, it did not show any effect on forskolin and IBMX induced cAMP levels compared to respective control cells.

EXAMPLE 9

Effect of STIM1 siRNA on ac. α Tubulin Expression in PN Cells

FIG. 14A and FIG. 14B show the effect of STIM1 siRNA on ac. α Tubulin expression in PN cells. Pkd1 null (PN) cells were grown in 10-cm culture dishes at permissive conditions (33 °C) with γ -interferon in culture media. Cells were then transferred to non-permissive conditions at 37 °C, γ -interferon free culture media. In the case of PN/S cells, mouse STIM1 siRNA (3 nM) was transfected using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions and evaluated at full confluency. At day five, cells were harvested for WB experiments. FIG. 14A is a Western blot showing expression of ac. α Tubulin in PN and PN/S cells. In FIG. 14B, columns represent averages $\pm$ standard errors of the

ac.aTubulin expression. Experiment was repeated for four times at least. $*P < 0.05$.
Data were analyzed by nonparametric t test. Experiment was repeated 6 times.
Ac.aTubulin -55; b.Actin -42.

These data indicate that protein expression of Adenylyl Cyclase 6 is
5 significantly decreased in PN cells after treatment with STIM1 siRNA compared with
control PN cells. Protein expression of Adenylyl Cyclase 3 is significantly increased
in PN cells treated with STIM1 siRNA compared with PN cells. Without being bound
to any one particular theory, one possible mechanism is by altering intracellular
calcium.

10

EXAMPLE 10

IP3R type I/II/III Expression in PN Cells Treated with STIM1 siRNA, 2-APB or Tubacin

FIG. 15A, FIG. 15B, FIG. 15C, FIG. 15D, FIG. 15E, and FIG. 15F show IP3R
15 type I/II/III expression in PN cells treated with STIM1 siRNA, 2-APB or Tubacin.
These data demonstrate that the total protein expression of IP3 receptors is
significantly downregulated in PN cells treated with 2-APB and Tubacin compared
with the control PN cells. The binding of IP3 receptors to PC2 is increased in cells
treated with 2-APB and Tubacin.

20

REFERENCES

All publications, patent applications, patents, and other references mentioned
in the specification are indicative of the level of those skilled in the art to which the
presently disclosed subject matter pertains. All publications, patent applications,
25 patents, and other references (e.g., websites, databases, etc.) mentioned in the
specification are herein incorporated by reference in their entirety to the same extent
as if each individual publication, patent application, patent, and other reference was
specifically and individually indicated to be incorporated by reference. It will be
understood that, although a number of patent applications, patents, and other
30 references are referred to herein, such reference does not constitute an admission that
any of these documents forms part of the common general knowledge in the art. In
case of a conflict between the specification and any of the incorporated references, the
specification (including any amendments thereof, which may be based on an
incorporated reference), shall control. Standard art-accepted meanings of terms are

used herein unless indicated otherwise. Standard abbreviations for various terms are used herein.

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5 migrates from the Ca^{2+} store to the plasma membrane, *Nature*, 437, 902-905 (2005);
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is determined by the depletion of subplasma membrane endoplasmic reticulum Ca^{2+}
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Integrators of Signalling Pathways in Development, Differentiation, and Disease, *J.
Cell. Mol.*, 14(7), 1890-1903 (2010).

15 International PCT patent application publication no. WO2015/061684 A1 for
Treatment of Polycystic Diseases with an HDAC6 Inhibitor, to Gradilone and
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For reasons of completeness, various aspects of the present disclosure are set
20 out in the following numbered clauses:

Clause 1. A method for treating a disease or disorder associated with an
elevated level of stromal interaction molecule 1 (STIM1), the method comprising
administering to a subject in need of treatment thereof one or more agents capable of
25 inhibiting or knocking down STIM1.

Clause 2. The method of clause 1, wherein the one or more agents
capable of inhibiting or knocking down STIM1 comprises an siRNA.

Clause 3. The method of clause 1, wherein the one or more agents
capable of inhibiting or knocking down STIM1 is 2-ABP.

30 Clause 4. The method of clause 1, wherein the disease or disorder
associated with an elevated level of stromal interaction molecule 1 (STIM1) is a
polycystic disease.

Clause 5. The method of clause 4, wherein the polycystic disease is a
renal cystic disease.

Clause 6. The method of clause 5, wherein the renal cystic disease is a polycystic kidney disease.

Clause 7. The method of clause 6, wherein the polycystic kidney disease is selected from autosomal dominant polycystic kidney disease (ADPKD) and
5 autosomal recessive kidney disease (ARPKD).

Clause 8. The method of clause 7, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

Clause 9. The method of clause 1, wherein the disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1) is
10 selected from: Alzheimer's disease; cancers and cancer-related diseases, including melanoma, glioblastoma, colorectal cancer, neuroblastoma, breast cancer, non-small cell lung cancer, hepatocellular carcinoma, prostate cancer, and renal cell cancer; immune-related diseases, including severe immunodeficiency, Kaposi sarcoma, thalassemia, systemic lupus erythematosus, cerebral vasospasm, Sjögren's syndrome,
15 multiple sclerosis, hypertension, obesity, diabetes, acute lung injury, and sepsis.

Clause 10. A method for treating a disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1), the method comprising administering to a subject in need of treatment thereof a histone deacetylase 6 (HDAC6) inhibitor in combination with one or more agents capable of inhibiting or
20 knocking down STIM1.

Clause 11. The method of clause 10, wherein the one or more agents capable of inhibiting or knocking down STIM1 is an siRNA.

Clause 12. The method of clause 10, wherein the one or more agents capable of inhibiting or knocking down STIM1 is 2-ABP.

Clause 13. The method of clause 10, wherein the HDAC6 inhibitor is
25 selected from: tubacin, tubastatin, and derivatives thereof.

Clause 14. The method of clause 10, wherein the disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1) is a polycystic disease.

Clause 15. The method of clause 14, wherein the polycystic disease is
30 renal cystic disease.

Clause 16. The method of clause 15, wherein the renal cystic disease is polycystic kidney disease.

Clause 17. The method of clause 16, wherein the polycystic kidney disease is selected from autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive kidney disease (ARPKD).

Clause 18. The method of clause 17, wherein the polycystic kidney disease
5 is autosomal dominant polycystic kidney disease (ADPKD).

Clause 19. The method of clause 10, wherein the disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1) is selected from: Alzheimer's disease; cancers and cancer-related diseases, including melanoma, glioblastoma, colorectal cancer, neuroblastoma, breast cancer, non-small
10 cell lung cancer, hepatocellular carcinoma, prostate cancer, and renal cell cancer; immune-related diseases, including severe immunodeficiency, Kaposi sarcoma, thalassemia, systemic lupus erythematosus, cerebral vasospasm, Sjögren's syndrome, multiple sclerosis, hypertension, obesity, diabetes, acute lung injury, and sepsis.

Clause 20. A pharmaceutical composition comprising at least one
15 pharmaceutically acceptable carrier, a therapeutically effective amount of a histone deacetylase 6 (HDAC6) inhibitor, and one or more agents capable of inhibiting or knocking down STIM1.

Clause 21. The pharmaceutical composition of clause 20, wherein the one or more agents capable of inhibiting or knocking down STIM1 is an siRNA.

Clause 22. The pharmaceutical composition of clause 20, wherein the one
20 or more agents capable of inhibiting or knocking down STIM1 is 2-ABP.

Clause 23. The pharmaceutical composition of clause 20, wherein the HDAC6 inhibitor is selected from: tubacin, tubastatin, and derivatives thereof.

25 Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.

30

THAT WHICH IS CLAIMED:

1. A method for treating a disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1), the method comprising administering to a subject in need of treatment thereof one or more agents capable of inhibiting or knocking down STIM1.

2. The method of claim 1, wherein the one or more agents capable of inhibiting or knocking down STIM1 comprises an siRNA.

3. The method of claim 1, wherein the one or more agents capable of inhibiting or knocking down STIM1 is 2-ABP.

4. The method of claim 1, wherein the disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1) is a polycystic disease.

5. The method of claim 4, wherein the polycystic disease is a renal cystic disease.

6. The method of claim 5, wherein the renal cystic disease is a polycystic kidney disease.

7. The method of claim 6, wherein the polycystic kidney disease is selected from autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive kidney disease (ARPKD).

8. The method of claim 7, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

9. The method of claim 1, wherein the disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1) is selected from: Alzheimer's disease; cancers and cancer-related diseases, including melanoma, glioblastoma, colorectal cancer, neuroblastoma, breast cancer, non-small cell lung cancer, hepatocellular carcinoma, prostate cancer, and renal cell cancer; immune-

related diseases, including severe immunodeficiency, Kaposi sarcoma, thalassemia, systemic lupus erythematosus, cerebral vasospasm, Sjögren's syndrome, multiple sclerosis, hypertension, obesity, diabetes, acute lung injury, and sepsis.

10. A method for treating a disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1), the method comprising administering to a subject in need of treatment thereof a histone deacetylase 6 (HDAC6) inhibitor in combination with one or more agents capable of inhibiting or knocking down STIM1.

11. The method of claim 10, wherein the one or more agents capable of inhibiting or knocking down STIM1 is an siRNA.

12. The method of claim 10, wherein the one or more agents capable of inhibiting or knocking down STIM1 is 2-ABP.

13. The method of claim 10, wherein the HDAC6 inhibitor is selected from: tubacin, tubastatin, and derivatives thereof.

14. The method of claim 10, wherein the disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1) is a polycystic disease.

15. The method of claim 14, wherein the polycystic disease is renal cystic disease.

16. The method of claim 15, wherein the renal cystic disease is polycystic kidney disease.

17. The method of claim 16, wherein the polycystic kidney disease is selected from autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive kidney disease (ARPKD).

18. The method of claim 17, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD).

19. The method of claim 10, wherein the disease or disorder associated with an elevated level of stromal interaction molecule 1 (STIM1) is selected from: Alzheimer's disease; cancers and cancer-related diseases, including melanoma, glioblastoma, colorectal cancer, neuroblastoma, breast cancer, non-small cell lung cancer, hepatocellular carcinoma, prostate cancer, and renal cell cancer; immune-related diseases, including severe immunodeficiency, Kaposi sarcoma, thalassemia, systemic lupus erythematosus, cerebral vasospasm, Sjögren's syndrome, multiple sclerosis, hypertension, obesity, diabetes, acute lung injury, and sepsis.

20. A pharmaceutical composition comprising at least one pharmaceutically acceptable carrier, a therapeutically effective amount of a histone deacetylase 6 (HDAC6) inhibitor, and one or more agents capable of inhibiting or knocking down STIM1.

21. The pharmaceutical composition of claim 20, wherein the one or more agents capable of inhibiting or knocking down STIM1 is an siRNA.

22. The pharmaceutical composition of claim 20, wherein the one or more agents capable of inhibiting or knocking down STIM1 is 2-ABP.

23. The pharmaceutical composition of claim 20, wherein the HDAC6 inhibitor is selected from: tubacin, tubastatin, and derivatives thereof.

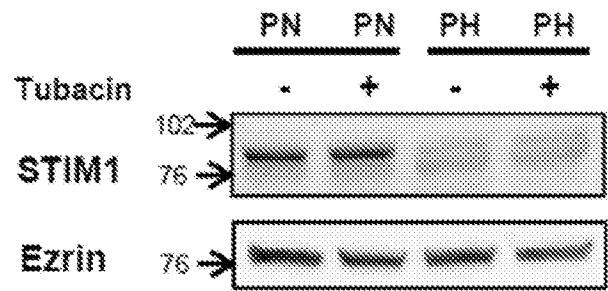


Fig. 1A

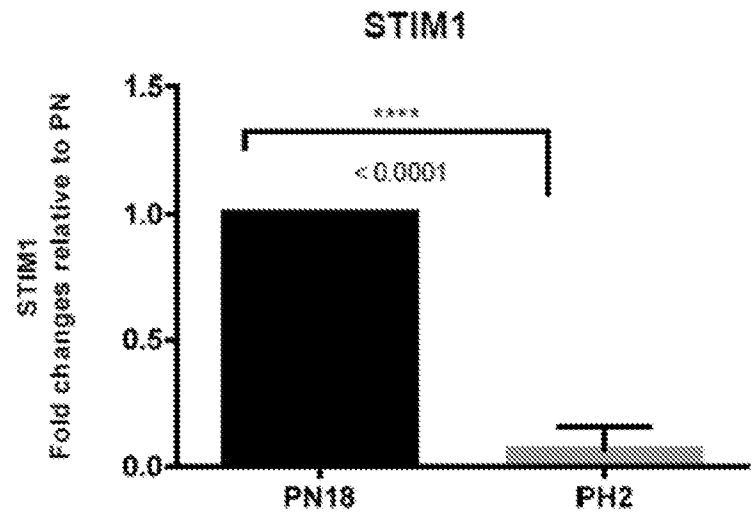


Fig. 1B

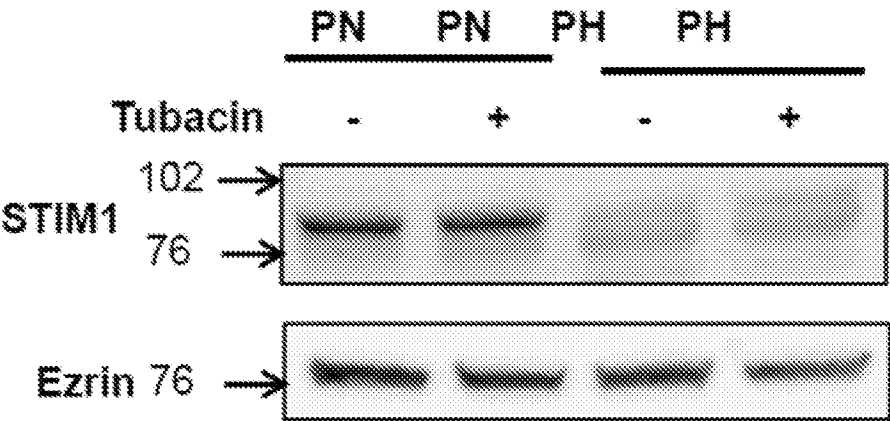


Fig. 2A

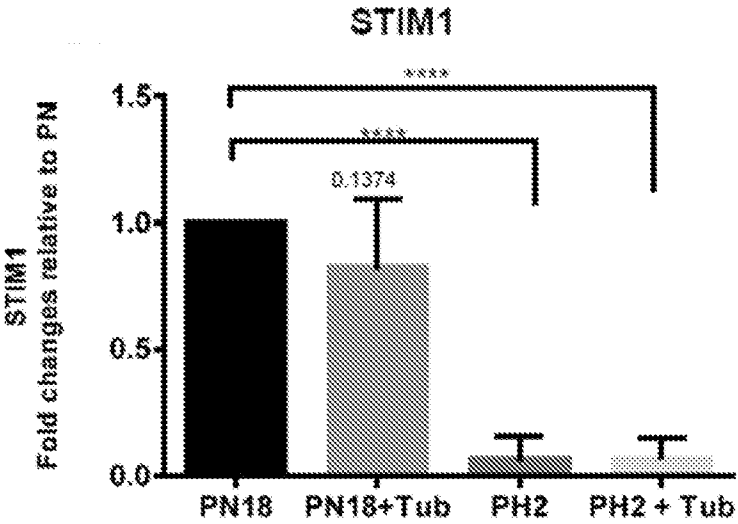


Fig. 2B

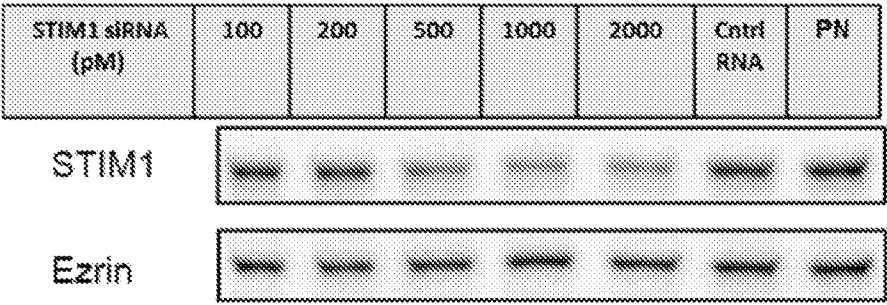


Fig. 3A

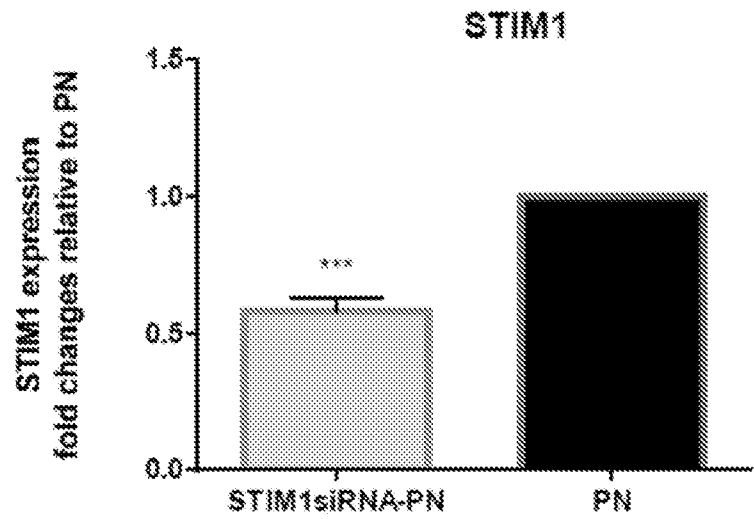


Fig. 3B

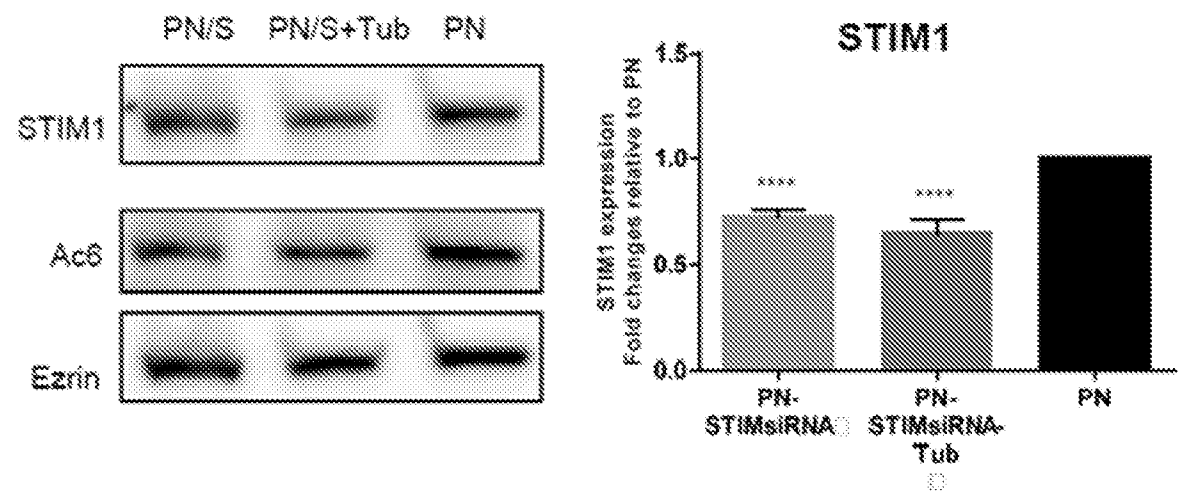


Fig. 4A

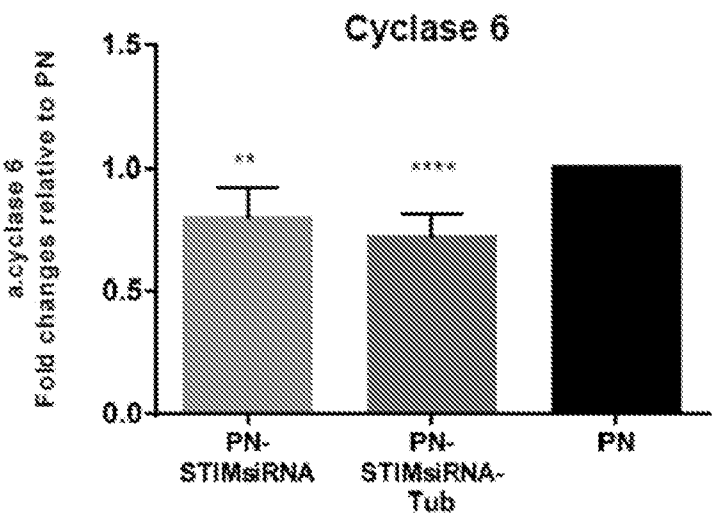


Fig. 4B

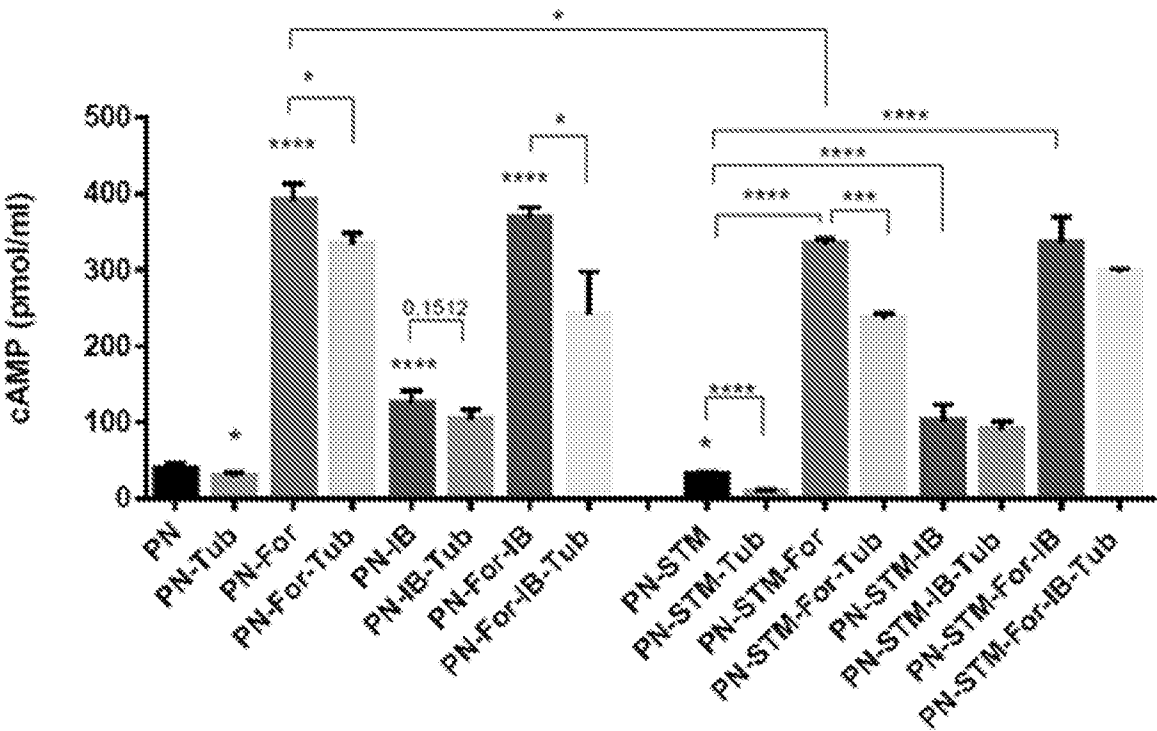


Fig. 5

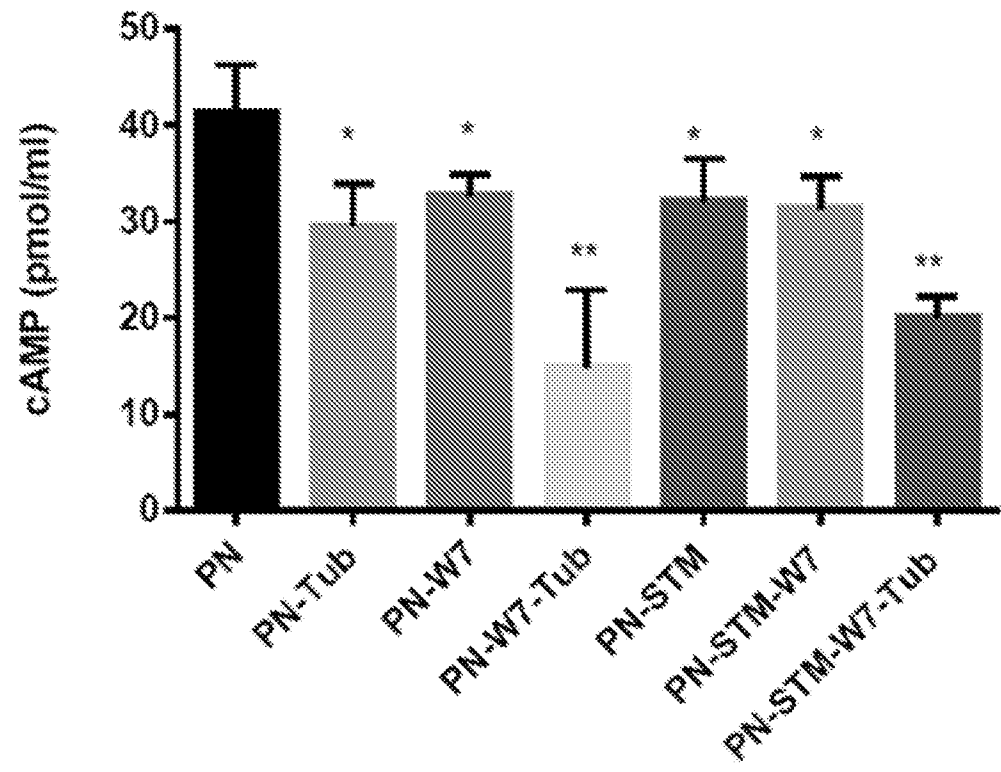


Fig. 6

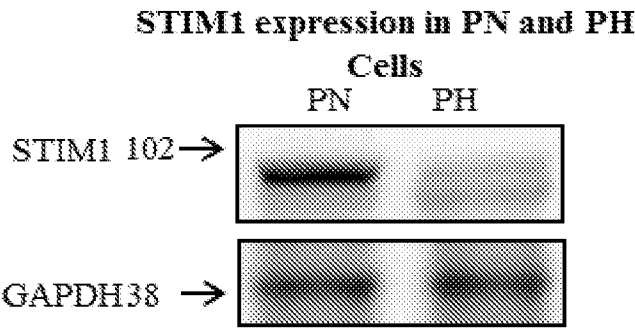


Fig. 7A

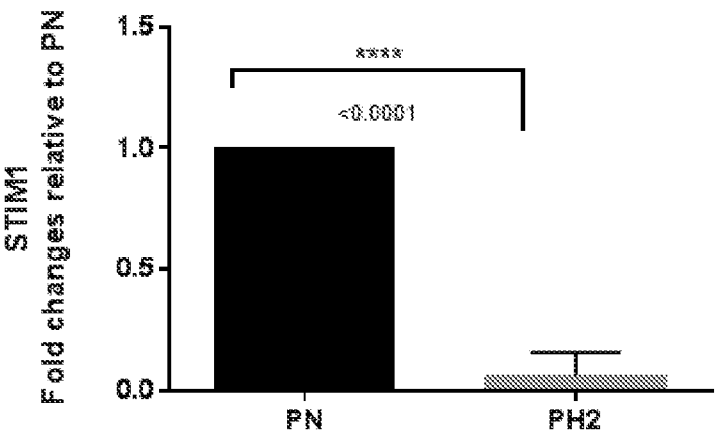


Fig. 7B

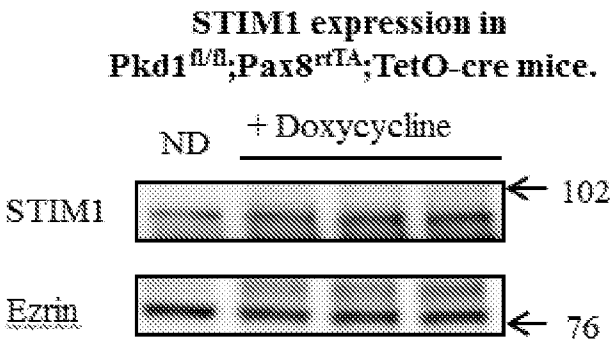


Fig. 7C

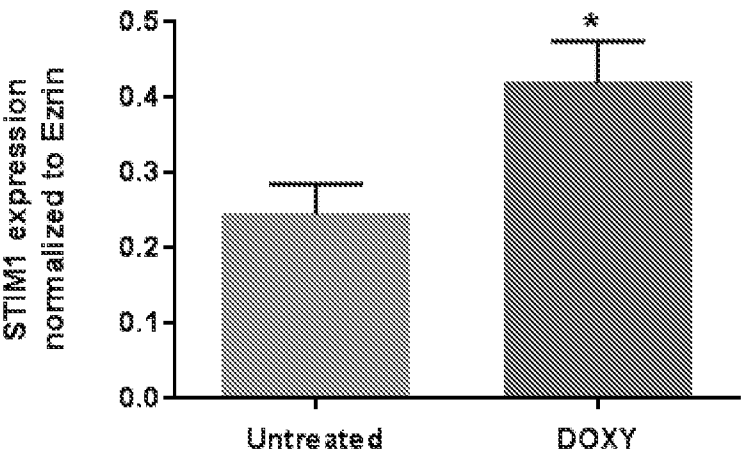


Fig. 7D

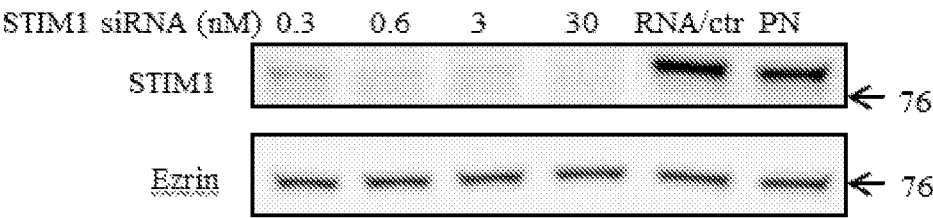


Fig. 7E

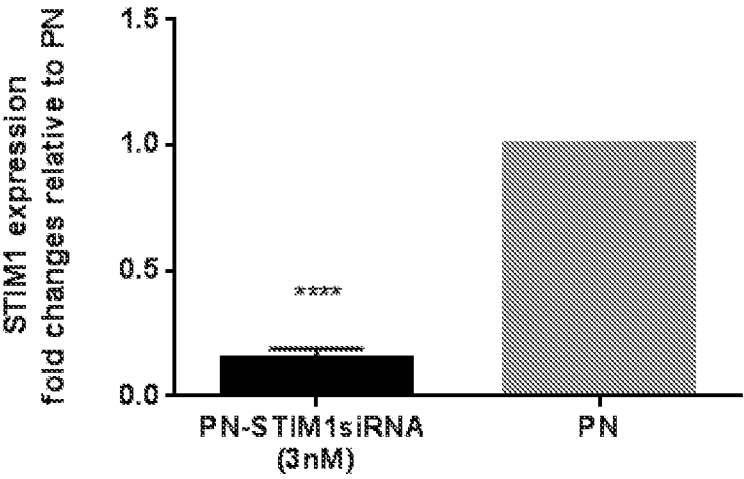


Fig. 7F

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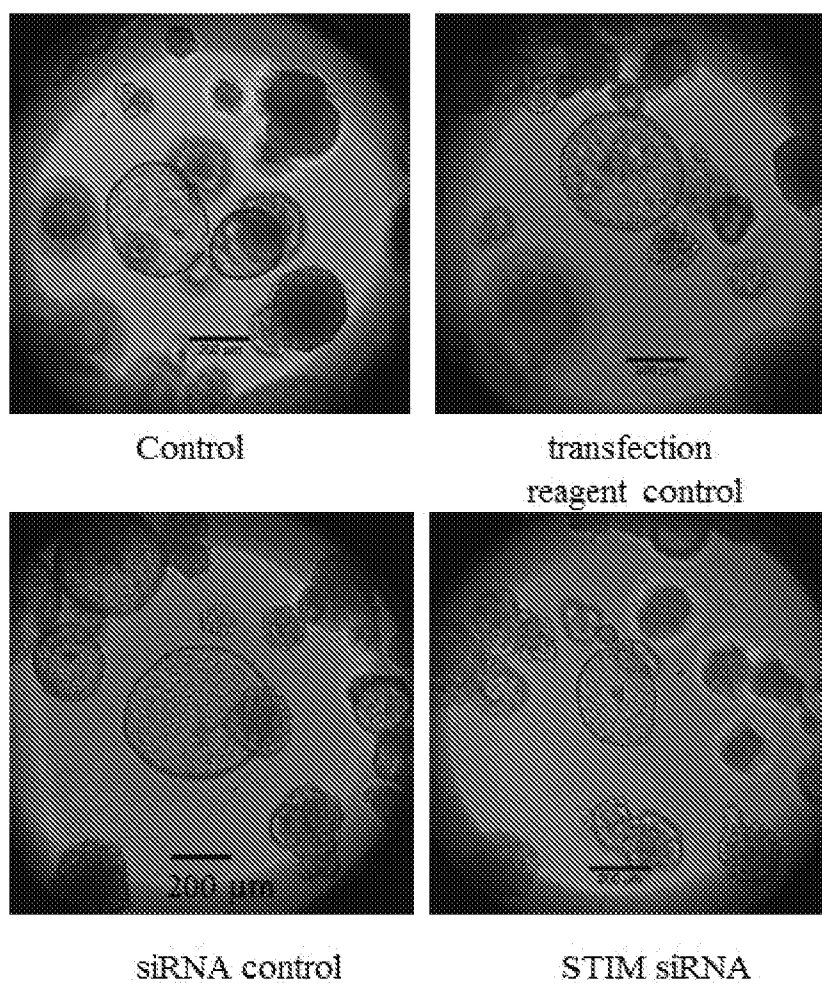


Fig. 8A

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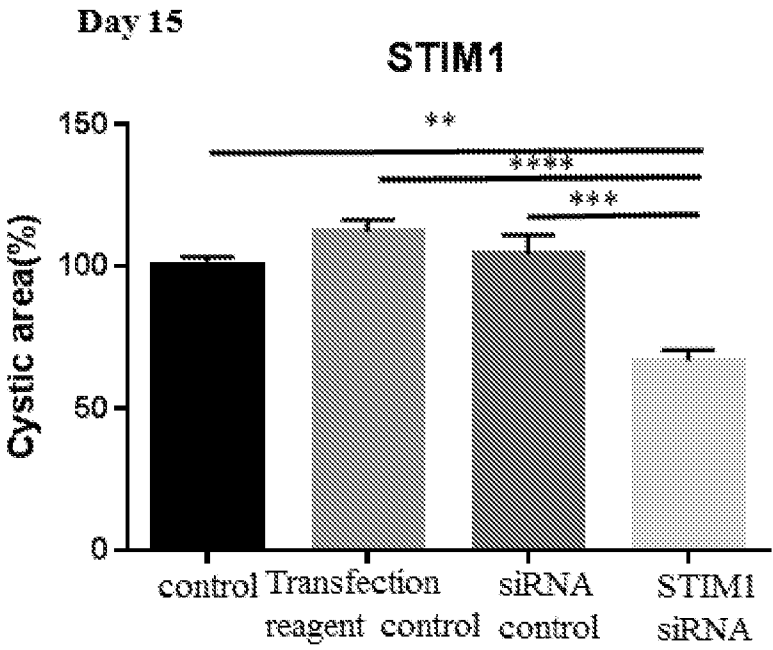


Fig. 8B

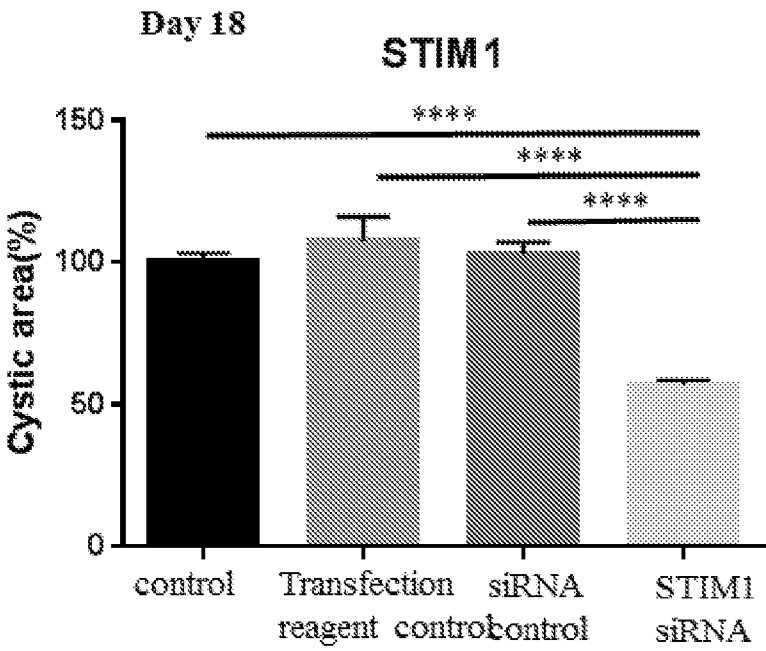
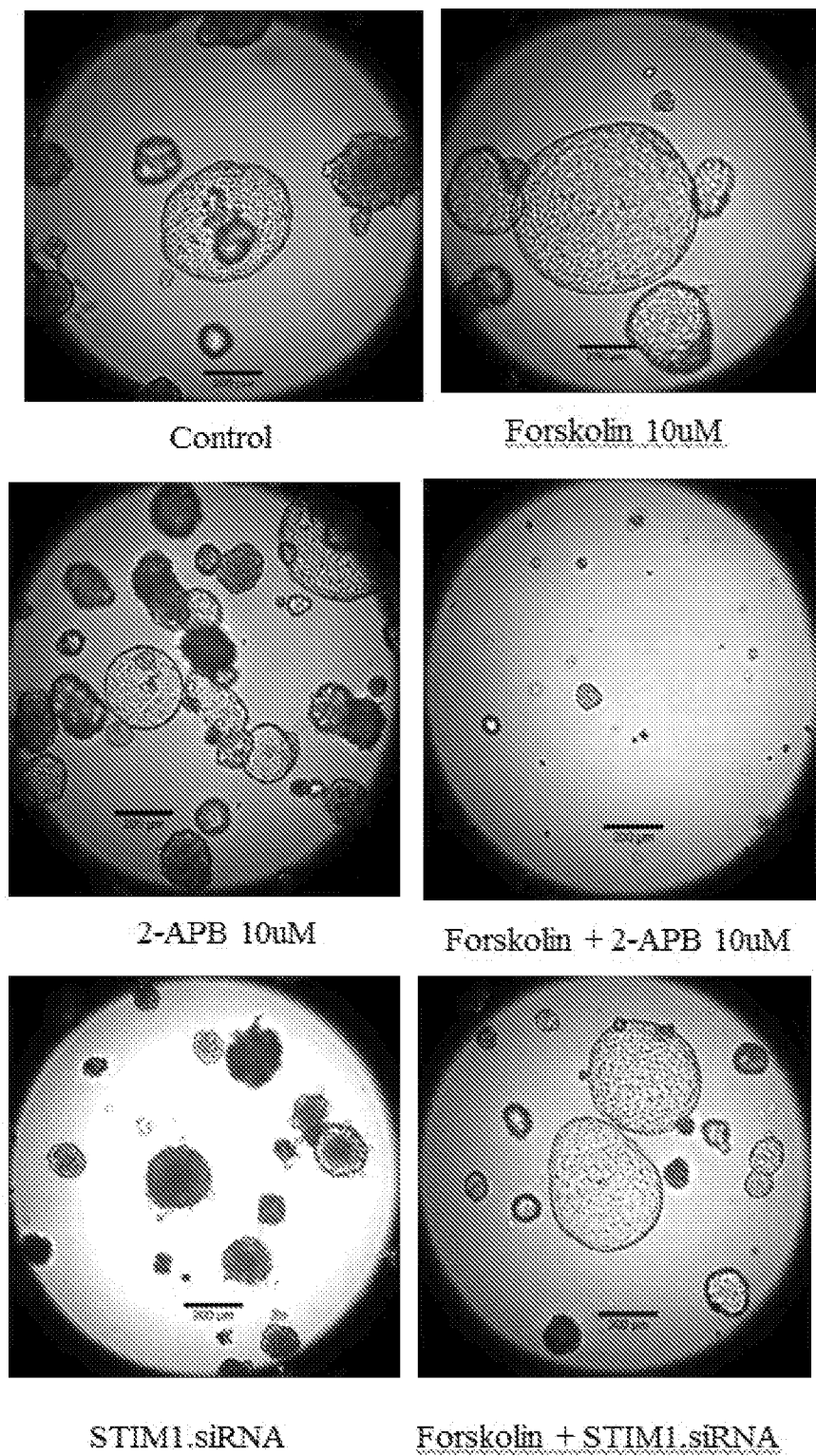


Fig. 8C

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***Fig. 9A***

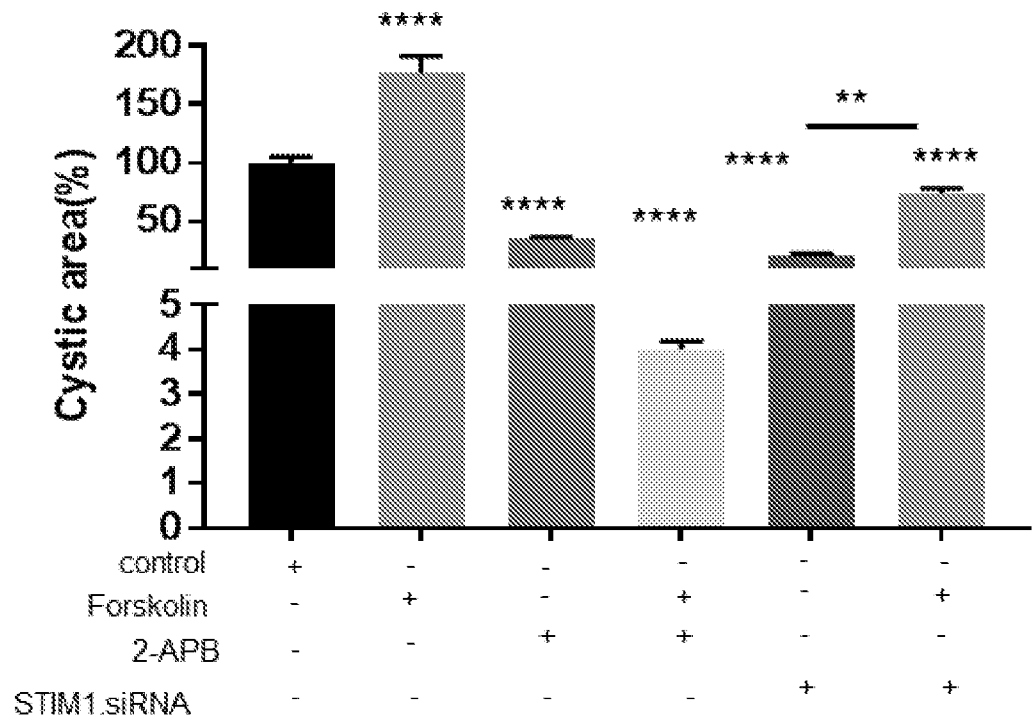


Fig. 9B

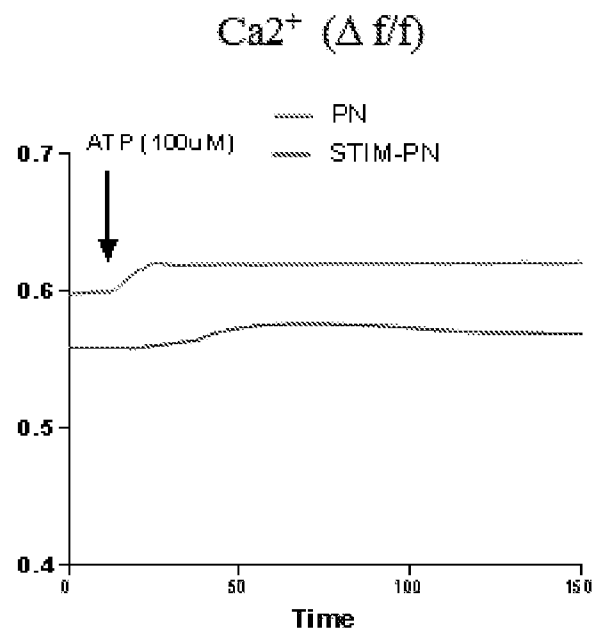


Fig. 10A

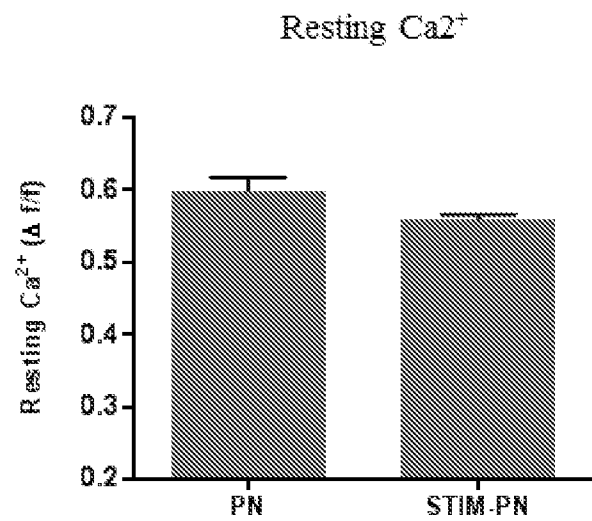


Fig. 10B

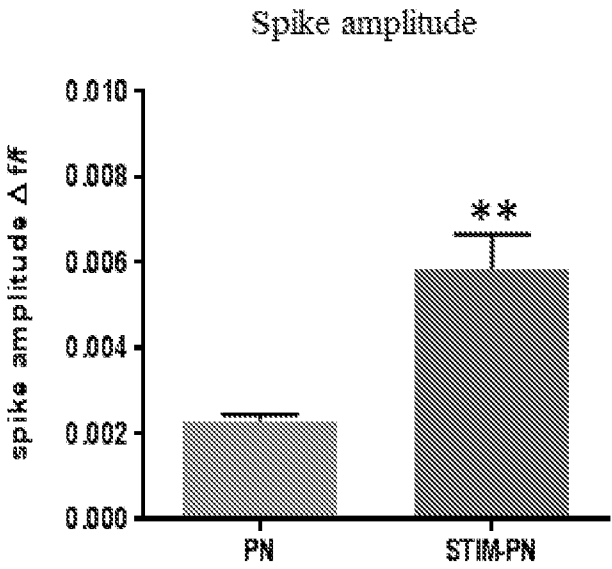


Fig. 10C

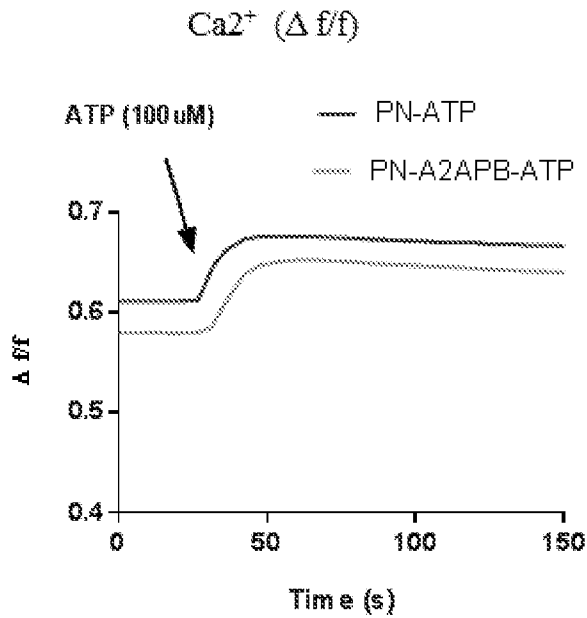


Fig. 10D

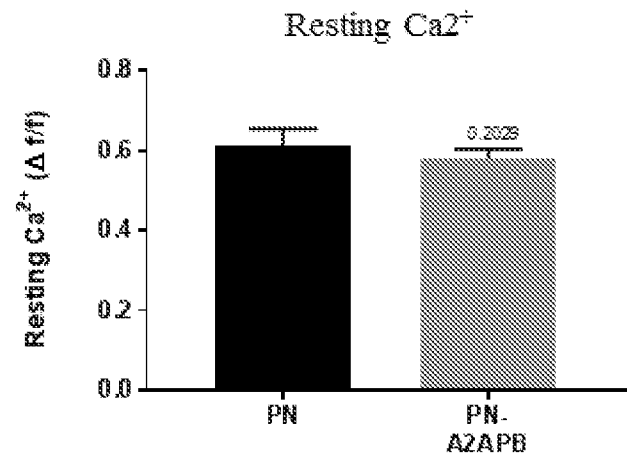


Fig. 10E

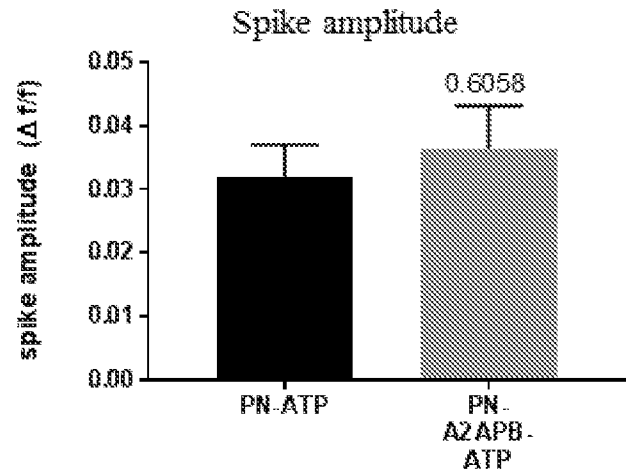


Fig. 10F

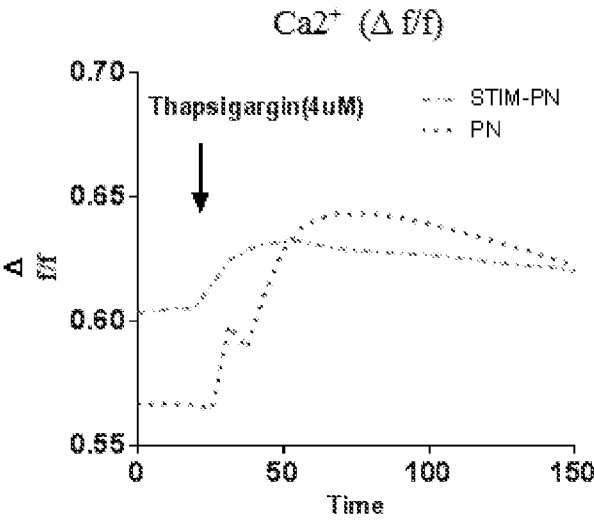


Fig. 11A

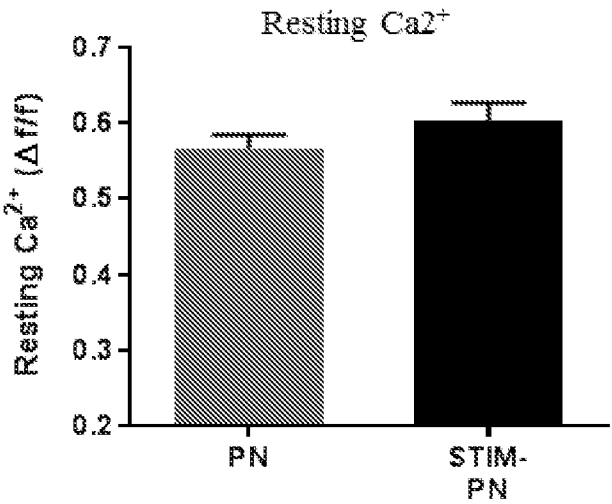


Fig. 11B

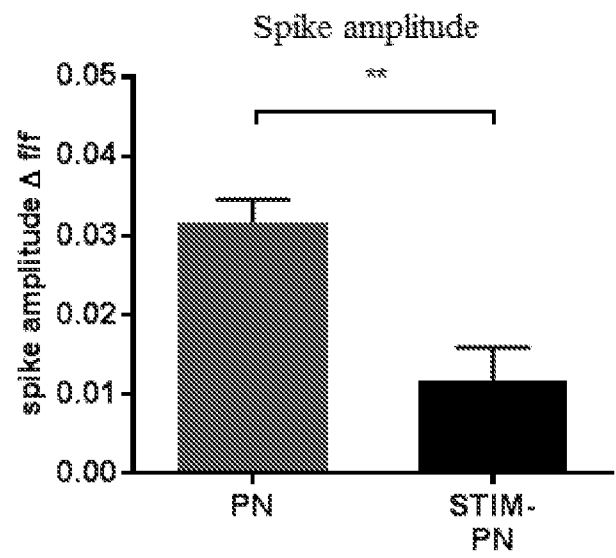


Fig. 11C

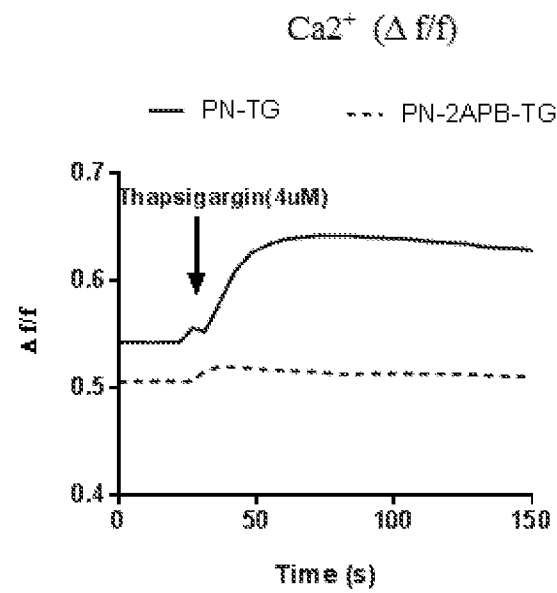


Fig. 11D

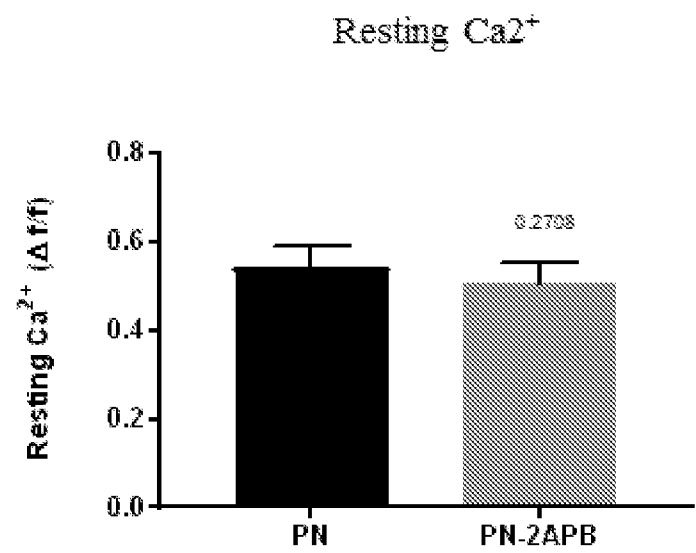


Fig. 11E

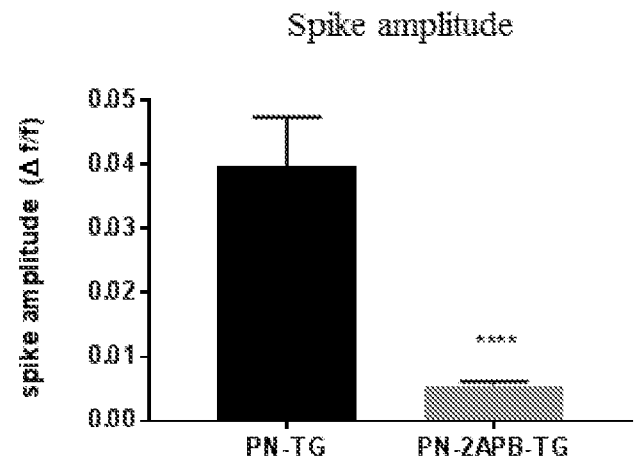


Fig. 11F

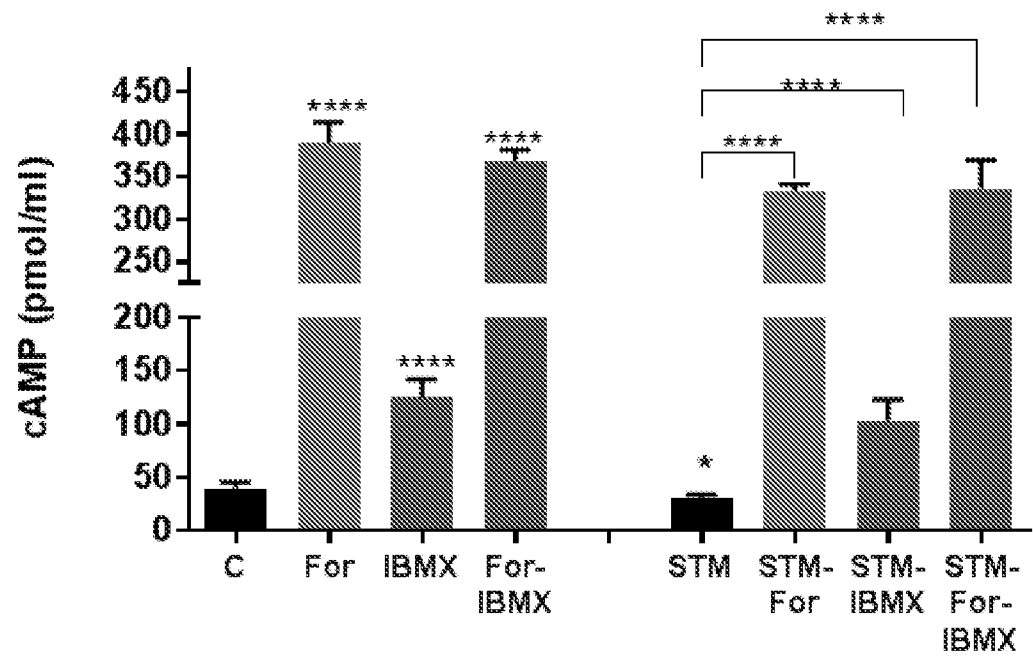


Fig. 12A

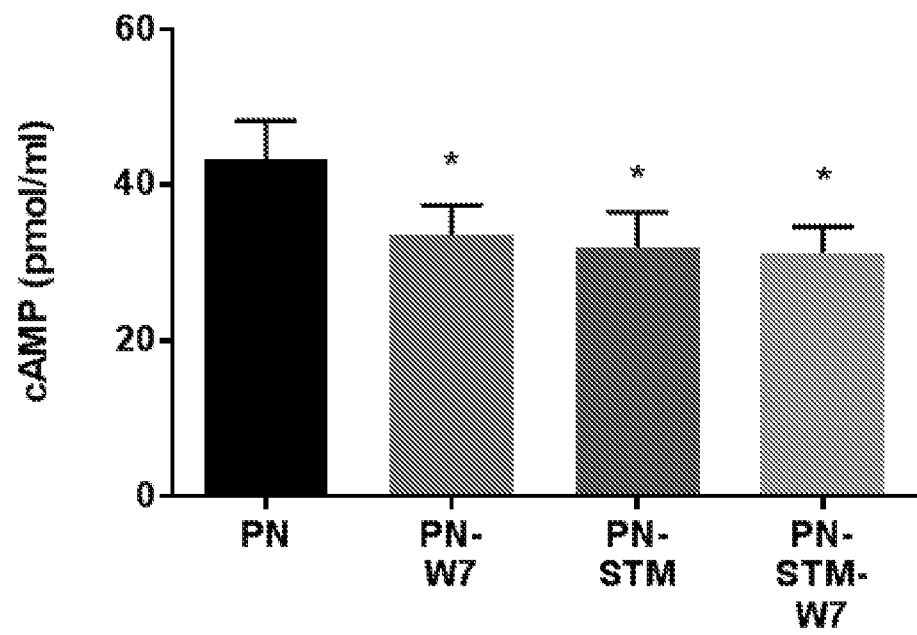


Fig. 12B

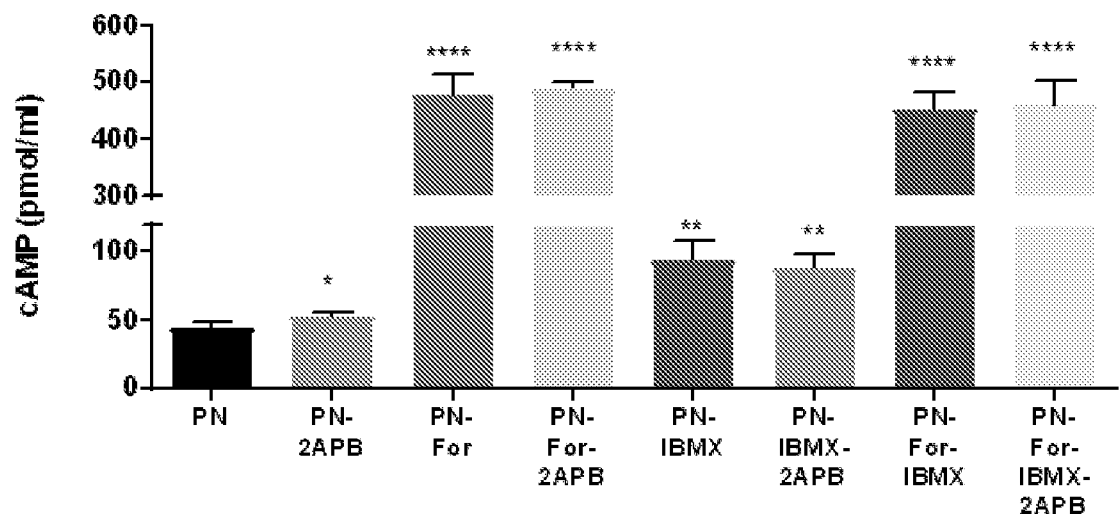


Fig. 13

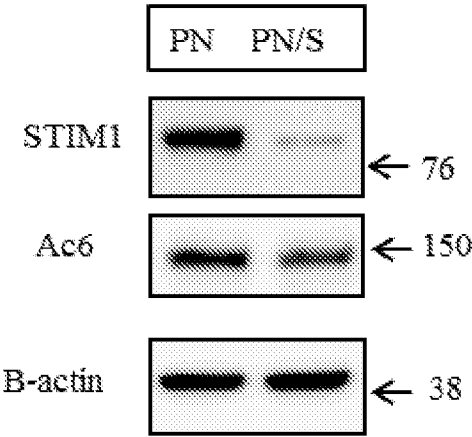


Fig. 14A

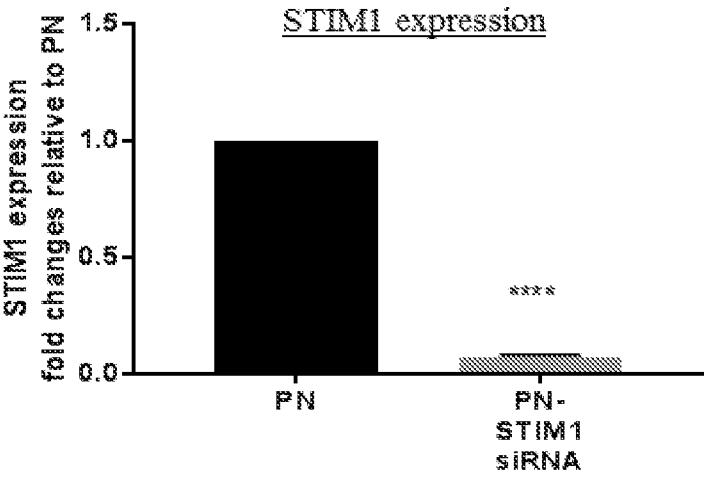


Fig. 14B

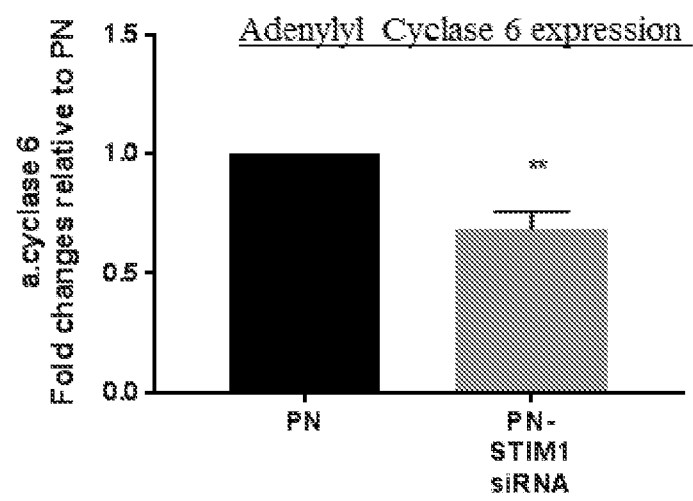


Fig. 14C

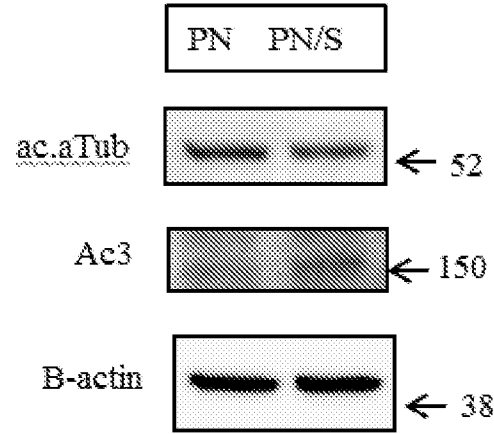


Fig. 14D

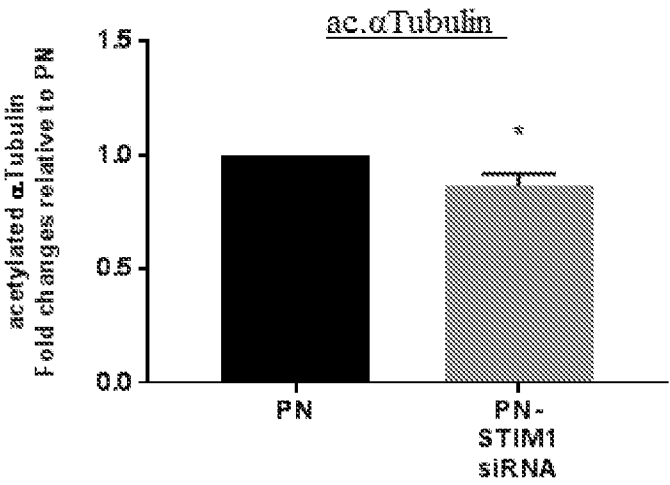


Fig. 14E

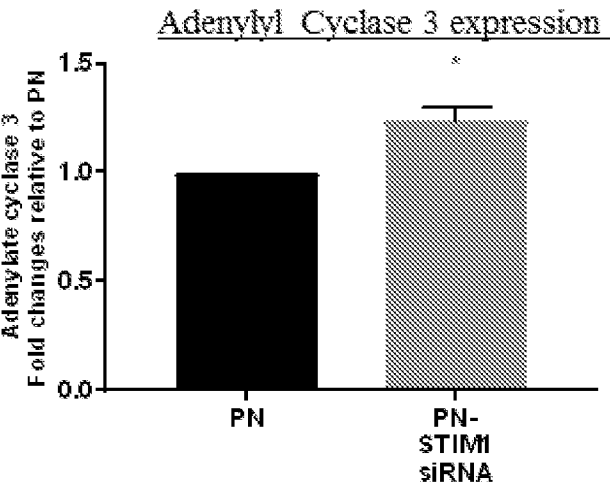


Fig. 14F

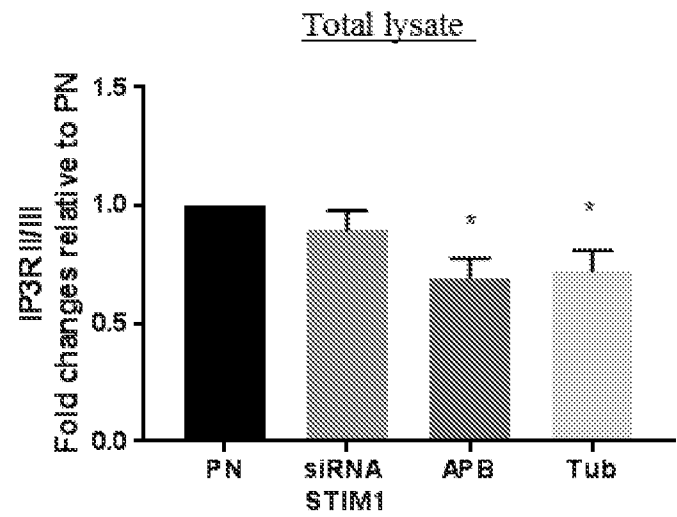


Fig. 15A

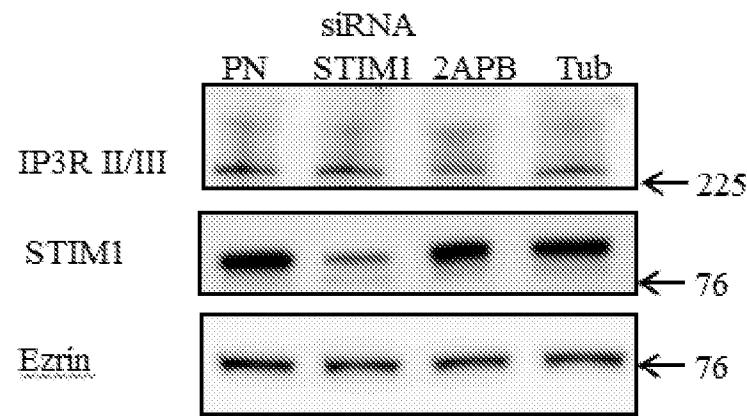


Fig. 15B

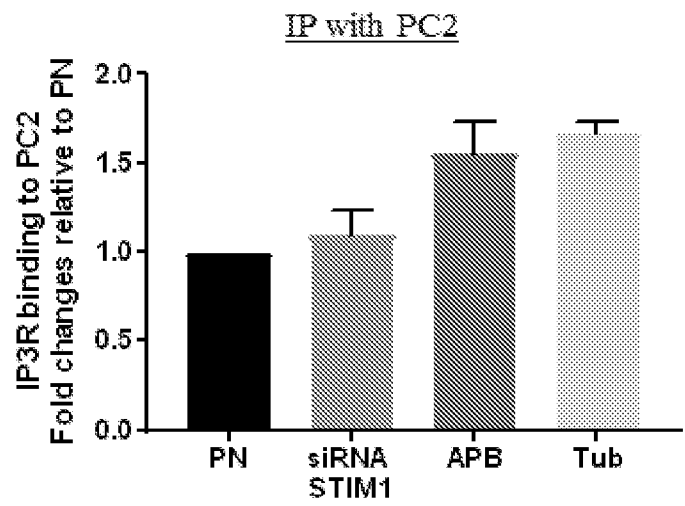


Fig. 15C

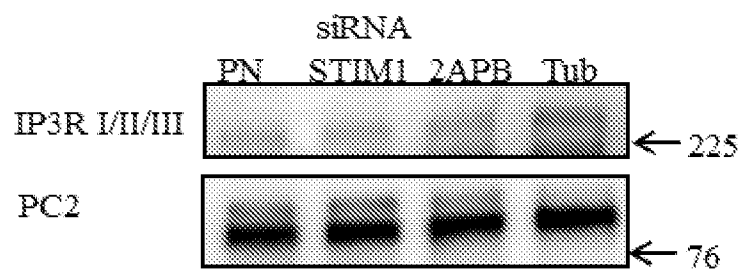


Fig. 15D

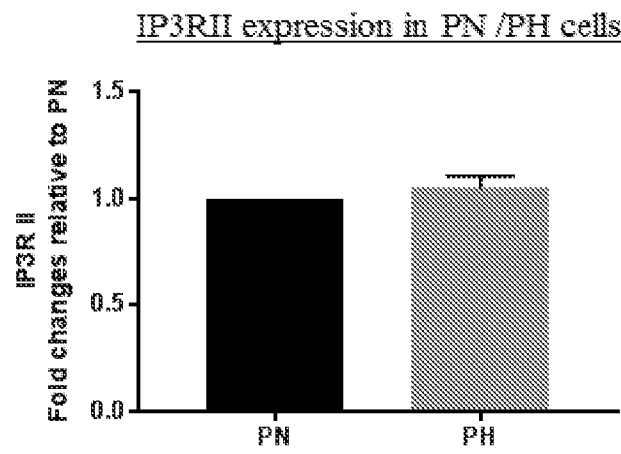


Fig. 15E

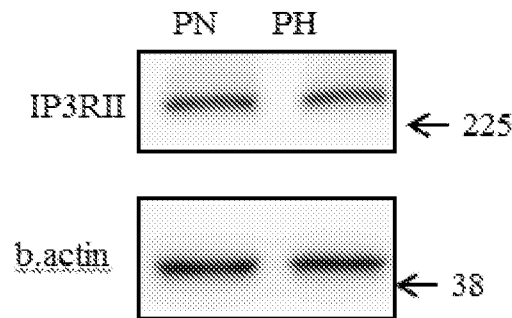


Fig. 15F

A. CLASSIFICATION OF SUBJECT MATTER**A61K 48/00(2006.01)i, A61K 31/422(2006.01)i, A61P 13/12(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 48/00; A61K 31/422; A61P 13/12

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & keywords: HDAC6, tubacin, tubastatin, STIM1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEN, Y. -T. et al., "Microtubule-associated histone deacetylase 6 supports the calcium store sensor STIM1 in mediating malignant cell behaviors", Cancer Research, 2013, Vol. 73, No. 14, pp. 4500-4509 See abstract; and figure 5.	20, 21, 23
Y		22
Y	GOTO, J. -I. et al., "Two novel 2-aminoethyl diphenylborinate (2-APB) analogues differentially activate and inhibit store-operated Ca ²⁺ entry via STIM proteins", Cell Calcium, 2010, Vol. 47, pp. 1-10 See abstract; and figure 1.	22
X	CHEN, Y. -F. et al., "The store-operated Ca ²⁺ entry-mediated signaling is important for cancer spread", Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 2016, Vol. 1863, pp. 1427-1435 See page 1433, right column, paragraph 1.	20, 21, 23
Y	FELIX, R. et al., "The Orai-1 and STIM-1 complex controls human dendritic cell maturation", PloS One, 2013, Vol. 8, No. 5, e61595, internal pages 1-11 See abstract.	22



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

21 May 2018 (21.05.2018)

Date of mailing of the international search report

23 May 2018 (23.05.2018)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea

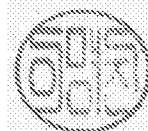


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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/014844

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	CHEN, Y, -C. et al., "Differential Ca²⁺ mobilization and mast cell degranulation by FcϵRI-and GPCR-mediated signaling", Cell Calcium, 2017, Vol. 67, pp. 31-39, [Epub.] 12 August 2017 See abstract; and page 32, left column, paragraph 3, page 36, left column, paragraph 1.	20-23

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US2018/014844**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

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

1. ☒ Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-19 pertain to a method for treating a human body, and thus relate to a subject matter not required to search under PCT Article 17(2)(a)(i) and Rule 39.1(iv).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2018/014844Patent document
cited in search reportPublication
datePatent family
member(s)Publication
date

None