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(54) Title: NICOTINE AND NICOTINIC AGONIST COMPOSITIONS FOR THE PREVENTION AND TREATMENT OF PANCREATITIS AND METHODS OF USING SAME

(57) Abstract: The present disclosure provides compositions and methods for treating and preventing pancreatitis. Particularly, the disclosure provides methods for treating and preventing pancreatitis using a nicotinic acetylcholine receptor agonist.



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**NICOTINE AND NICOTINIC AGONIST COMPOSITIONS FOR THE  
PREVENTION AND TREATMENT OF PANCREATITIS AND METHODS OF  
USING SAME**

**RELATED APPLICATIONS**

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/332,326 filed April 19, 2022, incorporated herein by reference in its entirety for all purposes.

**GOVERNMENT FUNDING**

[0002] This invention was made with Government support under Federal Grant Nos. DK064213, DK098796 and DK109368 awarded by the NIH. The Federal Government has certain rights to this invention.

**FIELD**

[0003] The present disclosure provides compositions and methods for treating and preventing pancreatitis. Particularly, the disclosure provides methods for treating and preventing pancreatitis using a nicotinic acetylcholine receptor agonist.

**BACKGROUND**

[0004] Pancreatitis is a severe, painful, and debilitating disease for which there is no specific treatment. Over 200,000 patients are hospitalized in the United States each year with pancreatitis and severe acute pancreatitis is associated with a ~20% mortality rate. Smoking has been shown to be a risk factor for both acute and chronic pancreatitis in people based primarily on published correlative epidemiological data. Tobacco smoke contains nicotine plus approximately 4000 other chemicals, but there is scant evidence concerning which component of tobacco is responsible for increasing the risk of developing pancreatitis in smokers. As of yet, there are not any effective treatments for pancreatitis.

**SUMMARY**

[0005] The present disclosure is based, in part, on the findings that agonists of nicotinic acetylcholine receptors (e.g., the  $\alpha 7$  nicotinic cholinergic receptor [ $\alpha 7$ nAChR]), act on one or more types of splenocytes to protect the pancreas against acute pancreatitis. Embodiments of the present disclosure provide methods of treating or preventing pancreatitis in a subject. In some embodiments, the pancreatitis is acute pancreatitis.

[0006] In some embodiments, the methods include administering an effective amount of a nicotinic acetylcholine receptor agonist to the subject. In some embodiments, the nicotinic

acetylcholine receptor agonist is a  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR) agonist. In some embodiments, the nicotinic acetylcholine receptor agonist includes one or more of: nicotine, GTS-21, a quinuclidine derivative, a substituted quinuclidine, varenicline, galantamine, 3-(1-methylpyrrolidin-2-yl)pyridine, carbachol, succinylcholine, epibatidine, lobeline, encenicline, or a pharmaceutically acceptable salt or derivative thereof. In some embodiments, the nicotinic acetylcholine receptor agonist is GTS-21 or nicotine.

[0007] In some embodiments, the methods decrease pancreatic edema, plasma amylase levels, pancreatic myeloperoxidase (MPO) concentrations, or a combination thereof.

[0008] In some embodiments, the subject is at high risk of pancreatitis. In some embodiments, the subject had or is having a procedure which increases the risk of pancreatitis. In some embodiments, the subject had or is having endoscopic retrograde cholangiopancreatography (ERCP). In some embodiments, the nicotinic acetylcholine receptor agonist is administered prior to, concomitant with, or after the procedure (e.g., ERCP).

[0009] In some embodiments, the nicotinic acetylcholine receptor agonist is administered after onset of pancreatic damage or pancreatitis.

[0010] In some embodiments, the methods further include administration of a second therapy. In some embodiments, the second therapy includes administration of a TRPVI antagonist, a TRPV4 antagonist, phosphate, or a combination thereof.

[0011] In some embodiments, the methods comprise administering an effective amount of a population of modified splenocytes, wherein the modified splenocytes were treated with a nicotinic acetylcholine receptor agonist prior to administration. In some embodiments, the modified splenocytes are autologous or allogeneic, or a combination thereof.

[0012] In some embodiments, the nicotinic acetylcholine receptor agonist is a  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR) agonist. In some embodiments, the nicotinic acetylcholine receptor agonist includes one or more of: nicotine, GTS-21, a quinuclidine derivative, a substituted quinuclidine, varenicline, galantamine, 3-(1-methylpyrrolidin-2-yl)pyridine, carbachol, succinylcholine, epibatidine, lobeline, encenicline, or a pharmaceutically acceptable salt or derivative thereof. In some embodiments, the nicotinic acetylcholine receptor agonist is GTS-21 or nicotine.

[0013] In some embodiments, the subject is at high risk of pancreatitis. In some embodiments, the subject had or is having a procedure which increases the risk of pancreatitis. In some embodiments, the subject had or is having endoscopic retrograde

cholangiopancreatography (ERCP). In some embodiments, the modified splenocytes are administered prior to, concomitant with, or after the procedure (e.g., ERCP).

[0014] In some embodiments, the modified splenocytes are administered after onset of pancreatic damage or pancreatitis.

[0015] Embodiments of the present disclosure provide compositions comprising a nicotinic acetylcholine receptor agonist; and one or more of a TRPV1 antagonist, a TRPV4 antagonist, phosphate, or a combination thereof. In some embodiments, the nicotinic acetylcholine receptor agonist is a  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR) agonist. In some embodiments, the nicotinic acetylcholine receptor agonist includes one or more of: nicotine, GTS-21, a quinuclidine derivative, a substituted quinuclidine, varenicline, galantamine, 3-(1-methylpyrrolidin-2-yl)pyridine, carbachol, succinylcholine, epibatidine, lobeline, encenicline, or a pharmaceutically acceptable salt or derivative thereof. In some embodiments, the nicotinic acetylcholine receptor agonist is GTS-21 or nicotine.

[0016] Another aspect of the present disclosure provides a compound comprising, consisting of, or consisting essentially of nicotine and/or nicotinic agonist(s) for the prevention and/or treatment of pancreatitis in a subject.

[0017] In some embodiments, the compound is selected from the group consisting of quinuclidine carbamates, quinuclidine amides, quinuclidine ethers, (1) 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino[2,3-h][3]benzazepine, 4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]-benzazepin-6-ol, 3-(1-methylpyrrolidin-2-yl)pyridine, 2-[(aminocarbonyl)oxy]-N,N,N-trimethylethanaminium, 2,2'-[(1,4-dioxobutane-1,4-diyl)bis(oxy)]bis(N,N,N-trimethylethanaminium), 2-(6-chloropyridin-3-yl)-7-azabicyclo[2.2.1]heptane, GTS-21, any salts, esters, and derivatives thereof, and any combinations thereof. In some embodiments, the compound comprises GTS-21.

[0018] Another aspect of the present disclosure provides a pharmaceutical composition comprising a nicotine and/or a nicotinic agonist(s) compound as provided herein and a pharmaceutically acceptable carrier, diluent, and/or excipient.

[0019] Another aspect of the present disclosure provides a method for preventing and/or reducing the severity of, and/or treating pancreatitis in a subject, the method comprising, consisting of, or consisting essentially of administering to the subject a therapeutically effective amount of a nicotine or a nicotinic agonist compound, a pharmaceutical composition thereof, as provided herein such that the pancreatitis is treated.

[0020] Another aspect of the present disclosure provides a method for preventing and/or treating and/or reducing the severity of pancreatitis associated with conditions that increase risk of acute pancreatitis in a subject comprising, consisting of, or consisting essentially of administering to the subject a therapeutically effective amount of a nicotine or a nicotinic agonist compound, a pharmaceutical composition thereof, as provided herein such that the pancreatitis is treated.

[0021] Other aspects and embodiments of the disclosure will be apparent in light of the following detailed description and accompanying figures.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

[0022] The accompanying Figures and Examples are provided by way of illustration and not by way of limitation. The foregoing aspects and other features of the disclosure are explained in the following description, taken in connection with the accompanying example figures (also "FIG.") relating to one or more embodiments, in which:

[0023] FIGS. 1A-1E show nicotine dose-dependently inhibits endoscopic retrograde cholangiopancreatography (ERCP)-induced pancreatitis (pancreatic edema, serum amylase, pancreatic MPO levels, and histopathology) in accordance with one embodiment of the present disclosure. The results are normalized to percent of the mean responses to pressure alone. FIGS. 1A-1D are graphs showing the effects of nicotine on pressure-induced pancreatic edema, plasma amylase, pancreatic MPO, and pancreatic histology score, respectively. FIG. 1E is images of the effects of nicotine on pressure-induced pancreatic histology. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; \*\*\*\*P<0.0001

[0024] FIGS. 2A-2E show the  $\alpha 7$ nAChR agonist, GTS-21, inhibits ERCP pressure-induced pancreatic edema, serum amylase, pancreatic MPO levels, and histopathology and this effect is blocked by the nicotinic antagonist, mecamylamine. The results are normalized to percent of the mean responses to pressure alone. FIGS. 2A-2D are graphs showing the effects of GTS-21 and mecamylamine on pressure-induced pancreatic edema, plasma amylase, pancreatic MPO, and pancreatic histology score, respectively. FIG. 2E is images showing the effects of GTS-21 and mecamylamine on pressure-induced pancreatic histology. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; \*\*\*\*P<0.0001

[0025] FIGS. 3A-3E show the  $\alpha 7$ nAChR agonist, GTS-21, inhibits ERCP pressure-induced pancreatic edema, serum amylase, pancreatic MPO levels, and histopathology and this effect is blocked by prior splenectomy. The results are normalized to percent of the mean responses to pressure alone. FIGS. 3A-3D are graphs showing the effects of GTS-21 and

splenectomy on pressure-induced pancreatic edema, plasma amylase, pancreatic MPO, and pancreatic histology score, respectively. FIG. 3E is images of the effects of GTS-21 and splenectomy on pressure-induced pancreatic histology. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$

[0026] FIGS. 4A-4F show splenocytes from GTS-21-treated mice inhibit ERCP-induced pancreatitis but splenocytes from control (vehicle)-treated mice. The results are normalized to percent of the mean responses to pressure alone. FIG. 4A is flow-cytometric analysis demonstrating the depletion of T cells from a preparation of total mouse splenocytes by the use of magnetic beads coupled to the T cell-specific antigen, CD3e. T cells from the spleens of both vehicle-treated (Control) and GTS-21-treated mice were depleted by about 94%. FIGS. 4B-4E are graphs showing the effects of various splenocyte preparations on pressure-induced pancreatic edema, plasma amylase, pancreatic MPO, and pancreatic histology score, respectively. FIG. 4F is images of various splenocyte preparations on pressure-induced pancreatic histology. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ .

[0027] FIG. 5 shows nicotine dose-dependently inhibits ERCP pressure-induced pancreatic inflammation, including pancreatic edema, serum amylase, pancreatic MPO levels, and histopathology. \*\* $P < 0.01$  vs. Pressure -/Nicotine 0; \*\*\* $P < 0.001$  vs. Pressure -/Nicotine 0; \*\*\*\* $P < 0.0001$  vs. Pressure -/Nicotine 0; # $P < 0.05$  vs. Pressure +/Nicotine 0; ## $P < 0.01$  vs. Pressure +/Nicotine 0; ### $P < 0.001$  vs. Pressure +/Nicotine 0; #### $P < 0.0001$  vs. Pressure +/Nicotine 0.

[0028] FIG. 6 is flow-cytometric analysis demonstrating the depletion of T cells from a preparation of total mouse splenocytes by the use of magnetic beads coupled to the T cell-specific antigen, CD3. T cells from the spleens of both vehicle-treated (Ctrl) and GTS-21-treated mice were depleted by about 94%. The splenocytes depicted in the upper (vehicle-treated controls) and lower (GTS-21-treated) right hand panels, dramatically depleted of T cells, were used to obtain the data shown in FIG. 3.

[0029] FIG. 7 is graphs showing splenocytes depleted of T cells do not protect against pressure-induced acute pancreatitis in response to GTS-21 administration.

[0030] FIGS. 8A-8E show ERCP pressure-induced acute pancreatitis can be inhibited after damage to the pancreas has begun by administration of the nicotinic agonist, GTS-21. The results are normalized to percent of the mean responses to pressure alone. FIGS. 8A-8D show the effects of GTS-21 administered at various intervals after ERCP pressure-induced increases in pancreatic edema, plasma amylase, pancreatic MPO, and pancreatic histology score, respectively. FIG. 8E is images of the effects of GTS-21 administered at various

intervals after ERCP pressure-induced pancreatic histological damage. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; \*\*\*\*P<0.0001

[0031] FIG. 9 is data showing prior splenectomy reduces the protective effects of GTS-21 administered 2 h after ERCP-induced damage to the pancreas has begun. Effects of prior splenectomy on the protective effects of GTS-21 administered 2 h after ERCP pressure-induced increases in pancreatic edema, plasma amylase, and pancreatic MPO. \*\*P<0.01; \*\*\*P<0.001; \*\*\*\*P<0.0001

[0032] FIGS. 10A-10E show splenectomy alone has no effect on inflammation but blocks the protective effects of GTS-21 on caerulein hyperstimulation-induced acute pancreatitis. FIGS. 10A-10D show the effects of prior splenectomy on the protective effects of GTS-21 on ERCP pressure-induced increases in pancreatic edema, plasma amylase, pancreatic MPO, and pancreatic histology score, respectively. FIG. 10E is images of the effects prior splenectomy on the protective effects of GTS-21 on caerulein hyperstimulation-induced acute pancreatitis. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; \*\*\*\*P<0.0001.

[0033] FIG. 11 is a schematic of nicotinic protection against ERCP-induced acute pancreatitis showing that stimulation of  $\alpha 7$  nicotinic cholinergic receptors may protect against ERCP-induced acute pancreatitis.

#### DETAILED DESCRIPTION

[0034] Embodiments of present disclosure relate to the treatment and prevention of pancreatitis. In particular, the present disclosure provides compositions and methods for treating and preventing pancreatitis using nicotinic acetylcholine receptor agonists. For example, as disclosed herein, pretreatment with nicotine dose-dependently inhibited acute pancreatitis caused by infusion of endoscopic retrograde cholangiopancreatography (ERCP) contrast solution into the main pancreatic duct in mice. GTS-21, a specific partial agonist of  $\alpha 7$ nAChRs, also elicited nicotinic protection against ERCP-induced acute pancreatitis, when administered intraperitoneally (ip) at 1, 2, 4, and 12 hours after ERCP surgery. Significant inhibition of most inflammatory indices was observed when GTS-21 was given 2-12 hours after ERCP. Splenectomy performed 7 days prior to ERCP abolished the protective effect of GTS-21, suggesting that a splenocyte mediates the nicotinic protective effect. T cell-depleted splenocytes prepared from GTS-21-treated mice did not protect against ERCP-induced pancreatitis whereas crude splenocytes from GTS-21-treated mice did protect the pancreas.

[0035] Section headings as used in this section and the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

## 1. Definitions

[0036] To facilitate an understanding of the present technology, a number of terms and phrases are defined below. Additional definitions are set forth throughout the detailed description.

[0037] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” 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. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[0038] 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. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise-Indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure.

[0039] As used herein, 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). 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  $\pm 10\%$  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” may be apparent from the context, such as rounding off; for example, “about 1” may also mean from 0.5 to 1.4.

[0040] “Subject” and “patient” as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (e.g., a monkey, such as a cynomolgus or rhesus monkey, chimpanzee, etc.) and a human). In some embodiments, the subject may be a human or a non-human. In one embodiment, the subject is a human.

[0041] As used herein, “treat,” “treating,” and the like means a slowing, stopping, or reversing of progression of a disease or disorder. The term also includes a reversing of the progression of such a disease or disorder to a point of eliminating or greatly reducing the disease. As such, “treating” means an application or administration of the nicotine and/or a nicotinic acetylcholine receptor agonist described herein to a subject, where the subject has a disease or a symptom of a disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease or symptoms of the disease. In some embodiments, the methods disclosed herein cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect pancreatitis or symptoms of pancreatitis.

[0042] As used herein, the term “preventing” refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delay onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delay onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delay progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition. As such, the disclosed methods may partially or completely delay onset of pancreatitis; partially or completely delay onset of one or more symptoms, features, or clinical manifestations of pancreatitis; partially or completely delay onset of one or more symptoms, features, or manifestations of pancreatitis; partially or completely delay progression of pancreatitis; and/or decrease the risk of developing pathology associated with pancreatitis.

[0043] An “effective amount” refers to an amount sufficient to elicit a desired biological response (e.g., treating a condition). As will be appreciated by those skilled in the art, the effective amount may vary depending on such factors as the desired biological endpoint, the pharmacokinetics, the condition being treated, the mode of administration, and the age and health of the subject. An effective amount encompasses therapeutic and prophylactic treatment. For example, a “therapeutically effective amount” is an amount sufficient to provide a therapeutic benefit in the treatment of a condition, or to delay or minimize one or more

symptoms associated with the condition. In some embodiments, a therapeutically effective amount is an amount sufficient to provide a therapeutic benefit in the treatment of a condition or to minimize one or more symptoms associated with the condition. A therapeutically effective amount means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the condition. The term “therapeutically effective amount” can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of the condition, or enhances the therapeutic efficacy of another therapeutic agent.

[0044] As used herein, the terms “providing”, “administering,” “introducing,” are used interchangeably herein and refer to the placement into a cell, tissue, organism, or subject by a method or route which results in at least partial localization to a desired site. The administration can be by any appropriate route which results in delivery to a desired location in the cell, tissue, organism, or subject.

[0045] The term “contacting” as used herein refers to bring or put in contact, to be in or come into contact. The term “contact” as used herein refers to a state or condition of touching or of immediate or local proximity. Contacting a nicotinic acetylcholine receptor agonist of the disclosed methods to a target destination, such as, but not limited to, an organ, tissue, cell, or tumor, may occur by any means of administration known to the skilled artisan.

[0046] Unless otherwise defined herein, scientific, and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear; in the event, however of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0047] 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 disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

## **2. Nicotinic acetylcholine receptor agonist**

[0048] The present disclosure is based, in part, on the findings that nicotinic acetylcholine receptor agonists act on one or more types of splenocytes to protect the pancreas against acute pancreatitis. As used herein, the term “nicotinic acetylcholine receptor agonist” refers to a

compound that mimics the action of acetylcholine (ACh) at a nicotinic acetylcholine receptor (nAChR). Suitable agonist compounds include small molecules, proteinaceous molecules such as peptides, polypeptides and proteins and genetic molecules such as RNA, DNA and mimetics and chemical analogs thereof as well as cellular agents. In particular embodiments, the nicotinic acetylcholine receptor agonist is a small molecule.

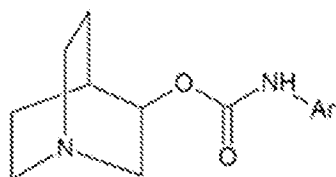
[0049] Types of nicotinic acetylcholine receptors (nAChRs) can be classified based on their site of expression, for example muscle type (subfamily I-III, based on protein sequence) or neuronal type (subfamily IV). Neuronal nAChRs are transmembrane proteins that form pentameric structures assembled from a family of subunits composed of  $\alpha 2$ – $\alpha 10$  and  $\beta 2$ – $\beta 4$ . Muscle nAChRs have both embryonic and adult forms, differing in the inclusion of a gamma or epsilon subunit. For example, embryonic forms are composed of  $\alpha_1$ ,  $\beta_1$ ,  $\gamma$ , and  $\delta$  subunits in a 2:1:1:1 ratio ( $(\alpha_1)_2\beta_1\gamma\delta$ ) whereas the adult form is composed of  $\alpha_1$ ,  $\beta_1$ ,  $\delta$ , and  $\epsilon$  subunits in a 2:1:1:1 ratio ( $(\alpha_1)_2\beta_1\delta\epsilon$ ). Herein, nicotinic acetylcholine receptor agonist comprises any compound which agonizes any one or more of the various classes or families of known nAChRs fully or partially. As such, the invention is not limited by the type of nicotinic acetylcholine receptor or by the extent of the effect (e.g., full or partial inhibition).

[0050] In some embodiments, the nicotinic acetylcholine receptor agonist acts on an  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR), thus the nicotinic acetylcholine receptor agonist is an  $\alpha 7$  nicotinic acetylcholine receptor agonist. The terms “ $\alpha 7$  nicotinic acetylcholine receptor agonist” and “ $\alpha 7$  nicotinic agonist” refer to compounds that bind and stimulate the  $\alpha 7$  nicotinic acetylcholine receptor (nAChR). The agonist effect of a compound may be determined using routine methods routine, for example, by measuring electrophysiologically or radioisotopically the ion flux or change in intracellular calcium concentration. The  $\alpha 7$  nicotinic acetylcholine receptor agonist may be a full agonist or a partial agonist. A partial agonist is a compound that stimulates the  $\alpha 7$  receptor, but whose maximal response is less than that of natural ligands (e.g., acetylcholine) when measured under the same conditions. A full agonist is a compound whose maximal response is the same or greater than that of natural ligands when measured under the same conditions. In some embodiments, the  $\alpha 7$  nicotinic acetylcholine receptor agonist selectively binds to an  $\alpha 7$  nicotinic acetylcholine receptor relative to other nicotinic acetylcholine receptors. In some embodiments, the  $\alpha 7$  nicotinic acetylcholine receptor agonist also binds to other nicotinic acetylcholine receptors. Extensive studies have identified and evaluated  $\alpha 7$  nicotinic acetylcholine receptor modulators. Exemplary  $\alpha 7$  nicotinic acetylcholine receptor agonists are reviewed in Papke RL and Horenstein NA, Pharmacol Rev.

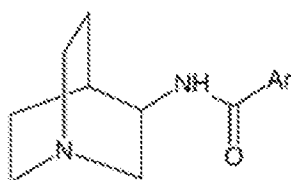
2021 Jul;73(3):1118-1149, Yang T, et al., Acta Pharm Sin B. 2017 Nov;7(6):611-622, Zhou YQ, et al., Front Mol Neurosci. 2022 Sep 30;15:970040, and Toyohara J and Hashimoto K, Open Med Chem J. 2010 May 27;4:37-56, each incorporated herein by reference in their entirety.

[0051] Exemplary nicotinic acetylcholine receptor agonists include, but are not limited to, nicotine, choline, cytisine, 2-(3-pyridyl)azaadamantanes, diazabicyclic compounds, pyridylazabicyclic compounds, cinnamamides of 3-aminoquinuclidine, arylcarbamates of 3-quinuclidinol, aromatic amides of 3-aminoquinuclidine, spiroquinuclidines, pyrazolo pyrimidine derivatives, and benzylideneanabaseines. For example, acetylcholine receptor agonists include, but are not limited to, diroximel fumarate, carbamoylcholine, epibatidine, succinylcholine (also known as suxamethonium), levamisole, varenicline, carenicline, ethadone, dimethylphenylpiperazinium (DMPP), rivanicline, bradanicline, anabesine, anabaseine and analogs thereof (e.g., 3-(2,4)-dimethoxybenzylidene anabaseine (DMXB-A or GTS-21), 3-(4)-dimethylaminobenzylidene anabaseine (DMAB), and 3-(4)-dimethylaminocinnamylidene (DMAC)), and epiboxidine.

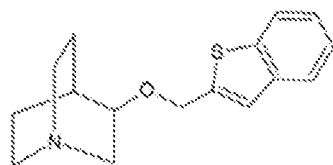
[0052] In some embodiments, the nicotinic acetylcholine receptor agonist includes, but is not limited to: (a) a quinuclidine derivative or a substituted quinuclidine, and any salts, esters, and derivatives thereof, such as quinuclidine carbamate e.g.,



, and any salts, esters, and derivatives thereof, quinuclidine amides e.g.,

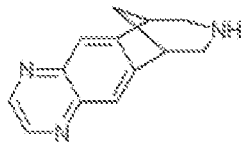


, and any salts, esters, and derivatives thereof, quinuclidine ethers e.g.,



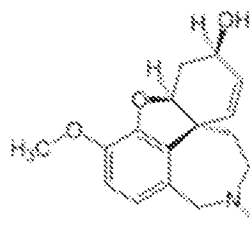
, and any salts, esters, and derivatives thereof;

(b) 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino[2,3-h][3]benzazepine, also known as varenicline, having the structure



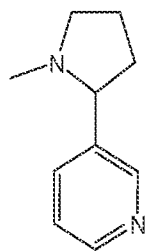
, and any esters, salts, and derivatives thereof;

(c) 4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]-benzazepin-6-ol, also known as galanthamine or galantamine, having the structure



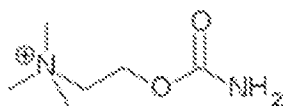
, and any salts, esters, and derivatives thereof;

(d) 3-(1-methylpyrrolidin-2-yl)pyridine having the structure



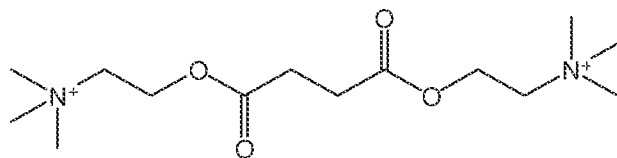
, and any salts, esters, and derivatives thereof;

(e) 2-[(aminocarbonyl)oxy]-N,N,N-trimethylethanaminium, also known as carbachol, having the structure



, and any salts, esters, and derivatives thereof;

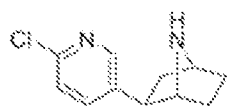
(f) 2,2'-[(1,4-dioxobutane-1,4-diyl)bis(oxy)]bis(N,N,N-trimethylethanaminium), also known as succinylcholine, having the structure



, and any salts, esters, and derivatives

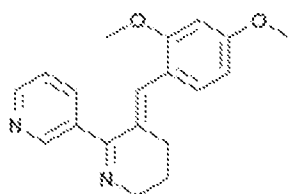
thereof;

(g) 2-(6-chloropyridin-3-yl)-7-azabicyclo[2.2.1]heptane, also known as epibatidine, having the structure



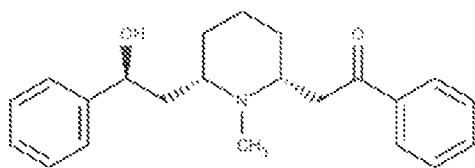
, and any salts, esters, and derivatives thereof; and

(h) 3-(2,4-dimethoxybenzylidene)anabaseine (also known as GTS-21 or DMBX-a) having the structure



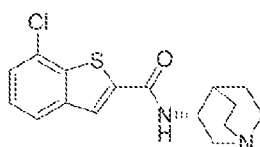
, and any salts, esters, and derivatives thereof;

(i) 2-[(2R,6S)-6-[(2S)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethan-1-one, also known as lobeline, having the structure



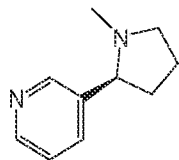
, and any salts, esters, and derivatives thereof; and

(j) N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-7-chloro-1-benzothiophene-2-carboxamide, also known as encenicline, having the structure



, and any salts, esters, and derivatives thereof.

[0053] In some embodiments, the nicotinic acetylcholine receptor agonist is nicotine, also known as 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine or a compound comprising nicotine. Nicotine has the general structure



[0054] In some embodiments, the nicotinic acetylcholine receptor agonist comprises one or more of: nicotine, GTS-21, a quinuclidine derivative, a substituted quinuclidine, varenicline, galantamine, 3-(1-methylpyrrolidin-2-yl)pyridine, carbachol, succinylcholine, epibatidine, lobeline, encenicline, or a pharmaceutically acceptable salt or derivative thereof, or a pharmaceutically acceptable salt or derivative thereof. In select embodiments, the nicotinic acetylcholine receptor agonist comprises GTS-21.

[0055] In select embodiments, the nicotinic acetylcholine receptor agonist comprises nicotine. In some embodiments, the nicotinic acetylcholine receptor agonist is not nicotine or a compound comprising nicotine.

[0056] In some embodiments, the nicotinic acetylcholine receptor agonist comprises quinuclidine carbamates, quinuclidine amides, quinuclidine ethers, 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino[2,3-h][3]benzazepine, 4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]-benzazepin-6-ol, 3-(1-methylpyrrolidin-2-yl)pyridine, 2-[(aminocarbonyl)oxy]-N,N,N-trimethylethanaminium, 2-(6-chloropyridin-3-yl)-7-azabicyclo[2.2.1]heptane, GTS-21, any salts, esters, and derivatives thereof, and any combinations thereof.

[0057] The nicotinic acetylcholine receptor agonist may be utilized as a composition which further comprises an excipient or pharmaceutically acceptable carrier. The choice of excipients or pharmaceutically acceptable carriers will depend on factors including, but not limited to, the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form.

[0058] Excipients and carriers may include any and all solvents, dispersion media, antibacterial and antifungal agents, isotonic and absorption delaying agents. Some examples of materials which can serve as excipients and/or carriers are sugars including, but not limited to, lactose, glucose and sucrose; starches including, but not limited to, corn starch and potato starch; cellulose and its derivatives including, but not limited to, sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients including, but not limited to, cocoa butter and suppository waxes; oils including, but not limited to, peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols; including propylene glycol; esters including, but not limited to, ethyl oleate and ethyl laurate; agar; buffering agents including, 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 including, but not limited to, sodium lauryl sulfate and magnesium stearate, as well

as coloring agents, releasing agents, preservatives, and antioxidants. Techniques and formulations may be found, for example, in Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company, 1995).

[0059] The phrase “pharmaceutically acceptable,” as used in connection with compositions and/or cells of the present disclosure, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a subject (e.g., a mammal, a human). Preferably, as used herein, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans. “Acceptable” means that the carrier is compatible with the active ingredient of the composition (e.g., the nucleic acids, vectors, cells, or therapeutic antibodies) and does not negatively affect the subject to which the composition(s) are administered. Any of the pharmaceutical compositions and/or cells to be used in the present methods can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formations or aqueous solutions.

[0060] Pharmaceutically acceptable carriers, including buffers, are well known in the art, and may comprise phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; amino acids; hydrophobic polymers; monosaccharides; disaccharides; and other carbohydrates; metal complexes; and/or non-ionic surfactants.

[0061] The compositions may be formulated for any appropriate manner of administration, and thus administered, including for example, oral, nasal, intraocular, intravenous, intravaginal, epicutaneous, sublingual, intracranial, intradermal, intraperitoneal, subcutaneous, intramuscular administration, or via inhalation. Techniques and formulations may generally be found in “Remington's Pharmaceutical Sciences,” (Meade Publishing Co., Easton, Pa.). Therapeutic or pharmaceutical compositions must typically be sterile and stable under the conditions of manufacture and storage.

### **3. Methods of Treatment**

[0062] Embodiments of the present disclosure include methods for treating and/or preventing pancreatitis. In some embodiments, the methods comprise administering an effective amount of a nicotinic acetylcholine receptor agonist, as described above, to a subject in need thereof.

[0063] Embodiments of the present disclosure include methods for treating and/or preventing pancreatitis with adoptive cell transfer. Adoptive cell transfer or ACT refers to the infusion of cells of various lineages into a subject to treat disease. In some embodiments, the methods for treating and/or preventing pancreatitis comprise administering an effective amount of a population of modified splenocytes, wherein the modified splenocytes were treated with a nicotinic acetylcholine receptor agonist, as described above, prior to administration. Splenocytes are a cell population obtained from the spleen of a subject. Splenocytes usually comprise T cells, B cells as well as antigen presenting cells. Protocols and techniques for isolation and treatment of splenocytes are known in the art.

[0064] The modified splenocytes may be autologous and/or allogeneic (e.g., the population may comprise only autologous cells, only allogeneic cells, or combinations of both autologous and allogeneic cells). As used herein, allogeneic cells are those isolated from one individual (the donor) and infused into another (the recipient or host); whereas autologous cells refer to those cells that are isolated and infused back into the same individual (recipient or host). In some embodiments, the modified splenocytes are syngeneic with respect to a subject; sufficiently identical to the subject so as to prevent an immune rejection upon transplantation. Alternatively, an established cell line may be made from allogeneic or autologous sources. The cell line can be stored, expanded, and modified until needed.

[0065] The pancreatitis may be any form of pancreatitis, acute pancreatitis, chronic pancreatitis, and hereditary pancreatitis. In some embodiments, the pancreatitis is acute pancreatitis. In certain embodiments, acute pancreatitis can be mild to moderate to severe disease. In certain embodiments, acute pancreatitis includes disease post ERCP (endoscopic retrograde cholangiopancreatography).

[0066] All forms of pancreatitis are characterized by inflammation and edema of the pancreas. Moderate and severe pancreatitis is further characterized by pancreatic necrosis and secondary damage to extra-pancreatic organs, with moderate acute pancreatitis patients suffering from transient (<48 hours) organ failure, while severe acute pancreatitis patients suffer from persistent (>48 hours) organ failure. Chronic pancreatitis is a long-term inflammation of the pancreas that alters the normal structure and function of organs. It may be associated with the onset of acute pancreatitis or persistent abdominal pain or digestive defects. Chronic pancreatitis is often diagnosed based on tests of pancreatic structure and function. Serum amylase and lipase may or may not be moderately elevated in the case of chronic pancreatitis, due to uncertain levels of productive cell damage. Increased lipase was the more likely result to be found in both. Elevated amylase and lipase enzymes are almost

always found in acute conditions, as well as elevated inflammatory markers of CRP that are generally consistent with the severity of the condition. The method disclosed herein can decrease pancreatic edema, plasma amylase levels, pancreatic myeloperoxidase (MPO) concentrations, or a combination thereof.

[0067] The subject may have a history of pancreatitis or be at high risk of pancreatitis. Subjects with a high risk of pancreatitis, for example, include those individuals suffering from pancreas divisum, a blocked duct, diabetes, obesity, or a history of gallstone, engaging in heavy alcohol use, or taking certain medications (e.g., corticosteroids such as prednisolone, HIV drugs such as didanosine and pentamidine, diuretics, anticonvulsants such as valproic acid, chemotherapeutic agents such as L-asparaginase and azathioprine, estrogens, blood triglyceride increasing drugs, statin drugs such as cholesterol lowering statin drugs, antihyperglycemic drugs such as metformin and statin drugs such as vildagliptin, sitagliptin, and linagliptin (linagliptin), tetracycline, sulfanilamide, azathioprine, mercaptopurine, pentamidine, trimethoprim-sulfamethoxazole, and salicylates).

[0068] In some embodiments, the subject had or is having a procedure which increases the risk of pancreatitis. For example, procedures which require an injection of or use of a contrast agent. In some embodiments, the procedure is endoscopic retrograde cholangiopancreatography (ERCP). The nicotinic acetylcholine receptor agonist may be administered prior to, concomitant with, or after the procedure or ERCP.

[0069] The nicotinic acetylcholine receptor agonist or the modified splenocytes may be administered any time prior to or after onset of pancreatic damage. In some embodiments, the nicotinic acetylcholine receptor agonist or the modified splenocytes is administered prior to the onset of pancreatic damage. For example, to a subject at high risk or a subject having or planning to have a procedure which increases the risk of pancreatitis. In some embodiments, the nicotinic acetylcholine receptor agonist or the modified splenocytes is administered after the onset of pancreatic damage (e.g., as caused from a procedure) or pancreatitis.

[0070] A wide range of second therapies may be used with the disclosed methods. The second therapy may be administration of a therapeutic agent or may be a second therapy not connected to administration of another agent (e.g., fluids, diet management). In some embodiments, the second therapy may be a therapeutic agent (e.g., analgesics, antibiotics, TRPV1 antagonist, TRPV4 antagonist, phosphate).

[0071] In select embodiments, the methods further comprise administering one or more of a TRPV1 antagonist and or a TRPV4 antagonist. Transient receptor potential vanilloid 1 (TRPV1) is an ion channel present on sensory neurons which is activated by heat, protons,

capsaicin and a variety of endogenous lipids termed endovanilloids. TRPV1 antagonists include, but are not limited to, capsaicin related compounds (e.g., oleoylvanillamine (olvanil), phenylacetylvanil), thiourea derivatives (e.g., capsazepine, JYL1421), urea derivatives (e.g., piperazinyl urea, A-425619, SB-705498 and SB-452533 (GSK) and ABT-102 (Abbott), cinnamides, carboxamides, imidazole derivatives, diarylethers and amines. TRPV4, another member of the vanilloid subfamily in the transient receptor potential (TRP) superfamily of ion channels, is activated by a wide range of stimuli including temperature, pH and osmolarity. TRPV4 antagonists include, but are not limited to, spirocarbamates, sulfonamides, rosmarinic acid and its derivatives, 2-phenyl-pyrrole carboxamide and indole carboxamide derivatives, ruthenium red, RN-1734, HC-067047, and RN-9893.

[0072] In select embodiments, the methods further comprise administering phosphate. See, for example, Farooq, A., et al., *Am J Physiol Gastrointest Liver Physiol.* 2022 Jun 1;322(6):G561-G570, incorporated herein by reference. Phosphate may be provided as a supplement to a food or drink, formulated with the nicotinic acetylcholine receptor agonists for administration by any method listed above, or in a separate dosage unit (e.g., for intravenous administration). The phosphate may be inorganic (salts of phosphoric acid) or organic phosphate (esters of phosphoric acid salt).

[0073] The second therapy may be administered at the same time as the disclosed methods, either in the same composition or in a separate composition administered at substantially the same time. When administered separately, the second therapy may be administered in the same or different manner as the nicotinic acetylcholine receptor agonists. The second therapy may be administered in any manner, e.g., oral, nasal, intraocular, intravenous, intravaginal, epicutaneous, sublingual, intracranial, intradermal, intraperitoneal, subcutaneous, intramuscular administration, or via inhalation. In some embodiments, the second therapy may precede or follow the disclosed methods by time intervals ranging from hours to days.

[0074] As such, also provided herein are compositions comprising: a nicotinic acetylcholine receptor agonist; and one or more of a TRPV1 antagonist, a TRPV4 antagonist, phosphate, or a combination thereof.

[0075] When utilized as a method of treatment, the effective amount may depend on the particular agonist or composition selected but also with the route of administration, the level of risk of the patient, the nature and symptoms of the disease, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner.

[0076] The determination of effective dosage levels, that is the dosage levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods, for example, human clinical trials, in vivo studies, and in vitro studies. For example, useful dosages of a nicotinic acetylcholine receptor agonist, or composition thereof, can be determined by comparing their in vitro activity, and in vivo activity in animal models.

[0077] Dosage amount and interval may be adjusted individually to provide plasma levels of the active agent which are sufficient to maintain the modulating effects, or minimal effective concentration (MEC). The MEC will vary for each a nicotinic acetylcholine receptor agonist but can be estimated from in vivo and/or in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, bioassays can be used to determine plasma concentrations. Dosage intervals can also be determined using MEC value. Nicotinic acetylcholine receptor agonist, the second therapy, or compositions thereof should be administered using a regimen, which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the nicotinic acetylcholine receptor agonist or the second therapy may not be related to plasma concentration.

[0078] It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity or organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate, precluding toxicity. The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the symptoms to be treated and the route of administration. Further, the dose, and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be also used in veterinary medicine for non-human subjects.

[0079] A nicotinic acetylcholine receptor agonist or a composition thereof can be evaluated for efficacy and toxicity using known methods. For example, the toxicology of a particular nicotinic acetylcholine receptor agonist may be established by determining in vitro toxicity towards a cell line, such as a mammalian, and preferably human, cell line. The results of such studies are often predictive of toxicity in animals, such as mammals, or more specifically, humans. Alternatively, the toxicity of a particular nicotinic acetylcholine receptor agonist in an animal model, such as mice, rats, rabbits, dogs, or monkeys, may be determined using known methods. The efficacy of a particular nicotinic acetylcholine receptor agonist may be

established using several recognized methods, such as in vitro methods, animal models, or human clinical trials. When selecting a model to determine efficacy, the skilled artisan can be guided by the state of the art to choose an appropriate model, dose, route of administration and/or regime.

#### **4. Examples**

[0080] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the present disclosure described herein are readily applicable and appreciable, and may be made using suitable equivalents without departing from the scope of the present disclosure or the aspects and embodiments disclosed herein. Having now described the present disclosure in detail, the same will be more clearly understood by reference to the following examples, which are merely intended only to illustrate some aspects and embodiments of the disclosure, and should not be viewed as limiting to the scope of the disclosure. The disclosures of all journal references, U.S. patents, and publications referred to herein are hereby incorporated by reference in their entireties.

[0081] The present disclosure has multiple aspects, illustrated by the following non-limiting examples.

##### **Example 1**

##### **Nicotinic Stimulation of Splenic T Cells is Protective in ERCP-Induced Acute Pancreatitis in Mice**

[0082] Acute pancreatitis develops in 3.5% of all patients after endoscopic retrograde cholangiopancreatography (ERCP) and up to 40-50% of high-risk ERCPs. The role of intra-pancreatic duct pressure on acute pancreatitis in the mouse was studied previously by infusing buffered isotonic saline into the main pancreatic duct after tying off the common bile duct to prevent backflow into the gallbladder and liver. Increasing intra-pancreatic duct pressure from 7-11 mm Hg to 25-33 mm Hg using this method induced acute pancreatitis via activation of the mechanoreceptor, Piezo1, and selective knockout of Piezo1 in pancreatic acinar cells prevented acute pancreatitis caused by elevated pressure. To more closely mimic human ERCP conditions herein, the radiocontrast solution used in ERCP was infused at the same volume and rate of infusion used previously. This procedure caused reproducible increases in several indices of acute pancreatitis: increased pancreatic edema, elevated plasma amylase levels, increased pancreatic myeloperoxidase (MPO) concentrations (a marker of neutrophil influx into the pancreas), and histopathology (FIG. 2).

[0083] When the mice were pretreated 30 min before ERCP surgery with intraperitoneal injections of nicotine hydrogen tartrate there was a significant nicotine dose-dependent inhibition of ERCP-induced pancreatic edema, plasma amylase elevation, pancreatic MPO concentrations, and histopathology (FIGS. 1A-1D). This was consistent with the mechanism of the cholinergic anti-inflammatory reflex. The effects of nicotine in the cholinergic anti-inflammatory reflex are mediated by  $\alpha 7$ nAChRs, and it was also found that the inflammatory effects of ERCP were inhibited by a specific  $\alpha 7$ nAChR agonist drug, GTS-21 (FIG. 2). To confirm that the effects of GTS-21 were specific to nicotinic receptors and not some other nonspecific effect, the nicotinic receptor antagonist drug, mecamylamine, blocked the protective effects of GTS-21 (FIG. 2).

[0084] The role of the spleen in these effects was evaluated. Prior splenectomy alone had no effect on ERCP-induced acute pancreatitis, but prior splenectomy blocked the protective effects of GTS-21 on ERCP-induced pancreatic inflammation (FIG. 3). Splenocytes prepared from mice treated 24 hours previously with GTS-21 exhibited significantly less ERCP-induced inflammation than splenocytes prepared from control mice treated with the GTS-21 vehicle (FIG. 4), suggesting that a cell type (or types) that is stimulated by  $\alpha 7$ nAChRs mediates the splenic protection of the pancreas in this model. Thus, adoptive transfer of splenocytes from GTS-21-treated mice could prevent ERCP-induced acute pancreatitis in intact, normal mice. T cells were specifically deleted from a suspension of crude splenocytes using immunomagnetic depletion of CD3 $\epsilon$ -expressing T cells. T cell depletion was confirmed by flow cytometric analysis (FIG. 4A). When injected intravenously into intact mice, splenocytes depleted of T cells from GTS-21-treated mice did not inhibit acute pancreatic inflammation (FIGS. 4B-4F).

[0085] The results described above have come from pretreating the mice with nicotine or GTS-21 30 min prior to initiating pancreatic damage by ERCP or prior to harvesting splenocytes/T cells. To determine if nicotinic stimulation can reverse pancreatic damage after it has begun, GTS-21 was administered at either 1, 2, 4, or 12 hours after performing the ERCP procedure. When given after initiating pancreatic damage, GTS-21 was able to inhibit inflammation significantly and time-dependently (FIG. 8).

[0086] To determine if this post-treatment nicotinic protection was mediated by splenocytes, the same experiment was repeated using splenectomized mice. Just as in the pretreatment study, splenectomy abolished the protective effects of GTS-21 administered after ERCP surgery (FIG. 9), suggesting that nicotinic  $\alpha 7$ nAChR stimulation can treat as well as prevent acute pancreatitis in this model via a spleen-dependent mechanism.

[0087] Finally, pretreatment with GTS-21 was protective in the caerulein hyperstimulation model of acute pancreatitis and that this effect is blocked by prior splenectomy (FIG. 10).

#### Materials and Methods

[0088] Nicotine hydrogen tartrate, GTS-21 (3-2,4-dimethoxybenzylidene anabaseine; DMXB-A), mecamlamine, and human myeloperoxidase (MPO) were obtained from Sigma-Aldrich (St. Louis, MO).

[0089] *Animals.* Male C57BL/6J mice 6-8 weeks old (Jackson Labs) were used. Mice were housed in a 12:12-h light-dark cycle and given water and chow ad libitum. All studies were approved by the Duke University Animal Care and Use Committee.

[0090] *ERCP Surgery.* Surgery was performed as previously described (See, R. S. Shahid SJD, et al., *Gastroenterology* 146: S297, 2014, Shahid RA, et al., *Cell Mol Gastroenterol Hepatol* 1: 75-86, 2015, Swain SM, et al., *J Clin Invest* 130: 2527-2541, 2020, each incorporated herein by reference), with the following modifications. Mice were anesthetized by intraperitoneal injection of a mixture of 87.5% ketamine/12.5% xylazine, and a midline laparotomy was used to expose the first portion of the duodenum. A puncture wound was made in the antimesenteric surface of the duodenum opposite the ampulla of Vater, and a 30G catheter attached with tubing to an infusion pump was passed through the puncture wound and then into the common bile duct via the ampulla of Vater. The infusion catheter was secured in the distal common bile duct distal to the entrance of the pancreatic duct with a ligature, and the bile duct near the liver was occluded with a bulldog clamp to prevent backflow into the gallbladder and liver. The infusate consisted of ERCP contrast medium pumped into the pancreatic duct using a high performance PHD ultra-syringe I/W programmable pump with pressure transducer APT300 Hg from Harvard Apparatus (Holliston, MA) that was set to a range of 0-200 mm Hg. Intraductal pressure was monitored continuously during injection using the software dedicated to the instrument. Contrast medium was injected at a rate of 80  $\mu$ l/min for 5 min as previously described. Methylene blue (1%) was included in the infusate to allow identification of leakage from the duct lumen. After 10 minutes, the catheter, ligature, and bulldog clamp were removed, and the duodenotomy was closed using a purse-string suture. The laparotomy was closed in two layers, and analgesia was achieved by subcutaneous injection of buprenorphine hydrochloride at a dose of 50 mg/kg. The animals were given free access to food and water upon recovery. The mice were killed 24 hours after surgery by CO<sub>2</sub> asphyxiation and then were weighed. The pancreas was removed and weighed; a portion was frozen at 80°C for later myeloperoxidase (MPO) assay, and a separate portion was fixed

overnight at 4°C in 10% formalin for the histopathologic analysis. Mixed arteriovenous blood was also collected by decapitation for serum amylase measurement.

[0091] *Splenectomy.* Seven days before ERCP surgery, mice were anesthetized with isoflurane, a small incision was made in the left flank, and the splenic vasculature was then ligated and the spleen removed through the incision as previously described (See, Inoue T, et al., J Clin Invest 126: 1939-1952, 2016). Sham-operated controls were treated identically but without splenic artery ligation and spleen removal.

[0092] One group of mice was pretreated 30 min before ERCP surgery ip with either 0.1, 0.5, or 1.0 mg/kg nicotine hydrogen tartrate (equivalent to 32.5, 162.5, and 325 µg/kg of pure nicotine, respectively). In some groups, mice were pretreated 30 min before ERCP surgery ip with 4 mg/kg GTS-21. In another group, mice were administered 1 mg/kg mecamylamine (ip 30 min before GTS-21 and again 12 hours later). Some mice were injected ip with 4 mg/kg GTS-21 or saline (controls) as described 24 h before they were killed and their spleens removed for in vitro splenocyte preparation. Crude splenocytes or T cell-depleted splenocytes (see below) were injected iv into intact recipient mice via the retro-orbital sinus under isoflurane anesthesia 24 hours before ERCP surgery.

[0093] *Splenocytes.* After removal, spleens from mice pretreated 24 hr previously with either 4 mg/kg GTS-21 or vehicle were mashed through a 70 µm filter using a 3 ml syringe plunger with a rubber tip, incubated with DNase-I for 10 min at 37°C, treated to lyse red blood cells, and washed by centrifugation in PBS three times as previously described. These crude splenocytes were either injected iv into untreated recipient mice or used to prepare a suspension of splenocytes lacking T cells. In this preparation, crude splenocytes were incubated with a biotinylated anti-CD3ε antibody (eBioscience; 2.5 µl/ml) for 30 min at 4°C. After washing the cells by centrifugation, the cells were then incubated with 25 µl/ml of MACS streptavidin microbeads (Miltenyi Biotec (Somerville, MA) for 15 min at 4°C. The cells were then washed again by centrifugation and then passed through negative selection LD columns attached to a MACS multistand magnetic separator (Miltenyi Biotec, Somerville, MA) at unit gravity. After washing, the purified mouse splenocytes lacking T cells, or crude splenocytes from mice treated with the GTS-21 vehicle, were resuspended in sterile PBS and then 100 µl of the cell suspensions containing 20 million cells were injected iv into the retro-orbital sinus in intact mice under isoflurane anesthesia. An aliquot of the cells was separately analyzed by flow cytometry to assess the extent of the removal of T cells.

[0094] *Assays.* The serum amylase concentration was measured as previously described elsewhere (See, Shahid RA, et al., Cell Mol Gastroenterol Hepatol 1: 75-86, 2015, incorporated

herein by reference), except that Phadebas amylase test tablets (Magle Life Sciences, Cambridge, MA) were used as substrate instead of Procion Yellow starch.

[0095] Pancreatic tissue activity of MPO, an enzyme produced by neutrophils and used as a marker of inflammation associated with neutrophil infiltration, was measured as previously described elsewhere using the substrate tetramethylbenzidine (See, Shahid RA, et al., Cell Mol Gastroenterol Hepatol 1: 75-86, 2015, incorporated herein by reference). The pancreatic total protein was measured using micro-BCA kits (Thermo Scientific, Rockford, IL).

[0096] Portions of pancreas were fixed overnight in phosphate-buffered 10% formalin. The tissue was then embedded in paraffin, sectioned at 5  $\mu$ m, stained with H&E, and coded for examination by two investigators blinded to the experimental design. The severity of pancreatitis was graded using a modified scoring criterion previously described elsewhere.

[0097] *Statistics.* Results are expressed as mean  $\pm$  standard error of the mean (SEM). To control for day-to-day variations all results were normalized as percent of the mean values obtained in mice treated with elevated intraductal pressure alone. Mean differences among groups were examined by one-way Analysis of Variance followed by the Tukey-Kramer posttest (GraphPad Prism, ver. 8.03; GraphPad Software, San Diego, CA).  $P < 0.05$  was considered statistically significant.

[0098] One skilled in the art will readily appreciate that the present disclosure is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present disclosure described herein is presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the present disclosure. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the present disclosure as defined by the scope of the claims.

[0099] No admission is made that any reference, including any non-patent or patent document cited in this specification, constitutes prior art. In particular, it will be understood that, unless otherwise stated, reference to any document herein does not constitute an admission that any of these documents forms part of the common general knowledge in the art in the United States or in any other country. Any discussion of the references states what their authors assert, and the applicant reserves the right to challenge the accuracy and pertinence of any of the documents cited herein. All references cited herein are fully incorporated by reference, unless explicitly indicated otherwise. The present disclosure shall control in the event there are any disparities between any definitions and/or description found in the cited references.

## CLAIMS

What is claimed is:

1. A method of treating or preventing pancreatitis in a subject in need thereof comprising administering an effective amount of a nicotinic acetylcholine receptor agonist to the subject.
2. The method of claim 1, wherein the nicotinic acetylcholine receptor agonist is a  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR) agonist.
3. The method of claim 1 or 2, wherein the nicotinic acetylcholine receptor agonist comprises one or more of: nicotine, GTS-21, a quinuclidine derivative, a substituted quinuclidine, varenicline, galantamine, 3-(1-methylpyrrolidin-2-yl)pyridine, carbachol, succinylcholine, epibatidine, lobeline, encenicline, or a pharmaceutically acceptable salt or derivative thereof.
4. The method of any of claims 1-3, wherein the nicotinic acetylcholine receptor agonist comprises GTS-21 or nicotine.
5. The method of any of claims 1-4, wherein the methods decrease pancreatic edema, plasma amylase levels, pancreatic myeloperoxidase (MPO) concentrations, or a combination thereof.
6. The method of any of claims 1-5, wherein the subject is at high risk of pancreatitis.
7. The method of any of claims 1-6, wherein the subject had or is having a procedure which increases the risk of pancreatitis.
8. The method of claim 7, wherein the procedure is endoscopic retrograde cholangiopancreatography.
9. The method of claim 7 or 8, wherein the nicotinic acetylcholine receptor agonist is administered prior to, concomitant with, or after the procedure.
10. The method of any of claims 1-9, wherein the nicotinic acetylcholine receptor agonist is administered after onset of pancreatic damage or pancreatitis.
11. The method of any of claims 1-10, further comprising administration of a second therapy.
12. The method of claim 11, wherein the second therapy comprises administration of a TRPV1 antagonist, a TRPV4 antagonist, phosphate, or a combination thereof.
13. The method of any of claims 1-12, wherein the pancreatitis is acute pancreatitis.

14. A method of treating or preventing pancreatitis in a subject in need thereof comprising administering an effective amount of a population of modified splenocytes, wherein the modified splenocytes were treated with a nicotinic acetylcholine receptor agonist prior to administration.
15. The method of claim 14, wherein the nicotinic acetylcholine receptor agonist is an  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR) agonist.
16. The method of claim 14 or 15, wherein the nicotinic acetylcholine receptor agonist comprises one or more of: nicotine, GTS-21, a quinuclidine derivative, a substituted quinuclidine, varenicline, galantamine, 3-(1-methylpyrrolidin-2-yl)pyridine, carbachol, succinylcholine, epibatidine, lobeline, encenicline, or a pharmaceutically acceptable salt or derivative thereof.
17. The method of any of claims 14-16, wherein the subject is at high risk of pancreatitis.
18. The method of claim 17, wherein the subject has or is having an endoscopic retrograde cholangiopancreatography.
19. The method of claim 17 or 18, wherein modified splenocytes are administered prior to, concomitant with, or after the procedure.
20. The method of any of claims 14-19, wherein the modified splenocytes are administered after onset of pancreatic damage or pancreatitis.
21. The method of any of claims 14-20, wherein the modified splenocytes are administered to the subject before, at the same time as, or after a second therapy.
22. The method of any of claims 14-21, wherein the pancreatitis is acute pancreatitis.
23. The method of any of claims 14-22, wherein the modified splenocytes are autologous or allogeneic, or a combination thereof.
24. A composition comprising:
- a) a nicotinic acetylcholine receptor agonist; and
  - b) one or more of a TRPVI antagonist, a TRPV4 antagonist, phosphate, or a combination thereof.
25. The composition of claim 24, wherein the nicotinic acetylcholine receptor agonist is an  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR) agonist.

26. The composition of claim 24 or 25, wherein the nicotinic acetylcholine receptor agonist comprises one or more of: nicotine, GTS-21, a quinuclidine derivative, a substituted quinuclidine, varenicline, galantamine, 3-(1-methylpyrrolidin-2-yl)pyridine, carbachol, succinylcholine, epibatidine, lobeline, encenicline, or a pharmaceutically acceptable salt or derivative thereof.

27. The composition of any of claims 24-26, wherein the nicotinic acetylcholine receptor agonist comprises GTS-21 or nicotine.

28. A composition comprising a nicotinic acetylcholine receptor agonist for use in treating or preventing pancreatitis.

29. The composition of claim 28, wherein the composition further comprises one or more of a TRPVI antagonist, a TRPV4 antagonist, phosphate, or a combination thereof, for use in treating or preventing pancreatitis.

FIG. 1A:

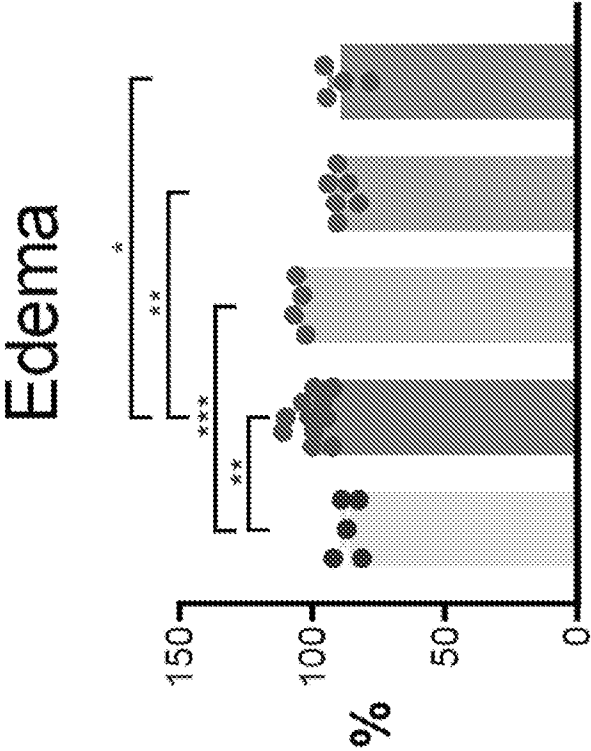
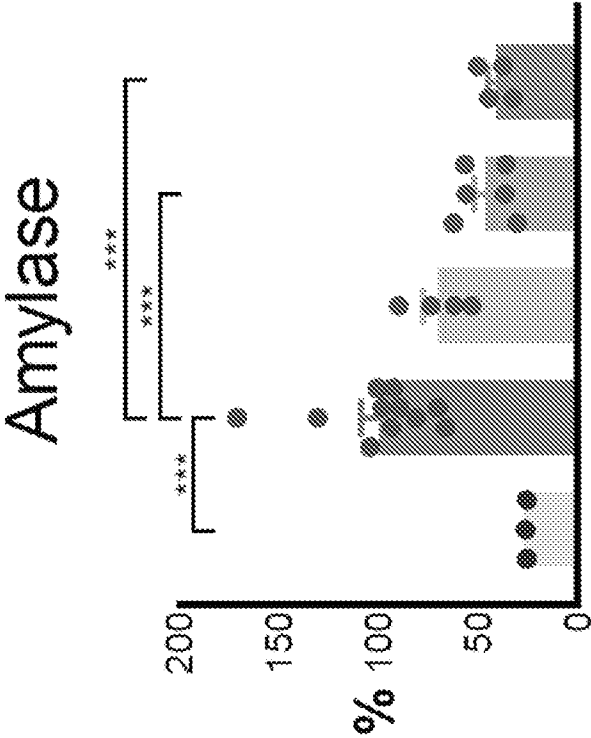


FIG. 1B:



FIGS. 1A-1E

FIG. 1C:

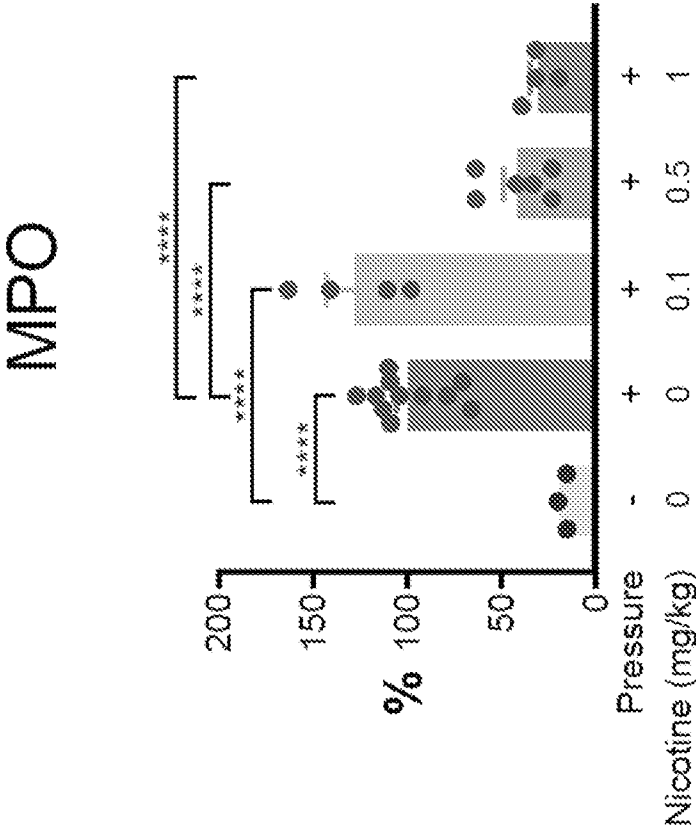
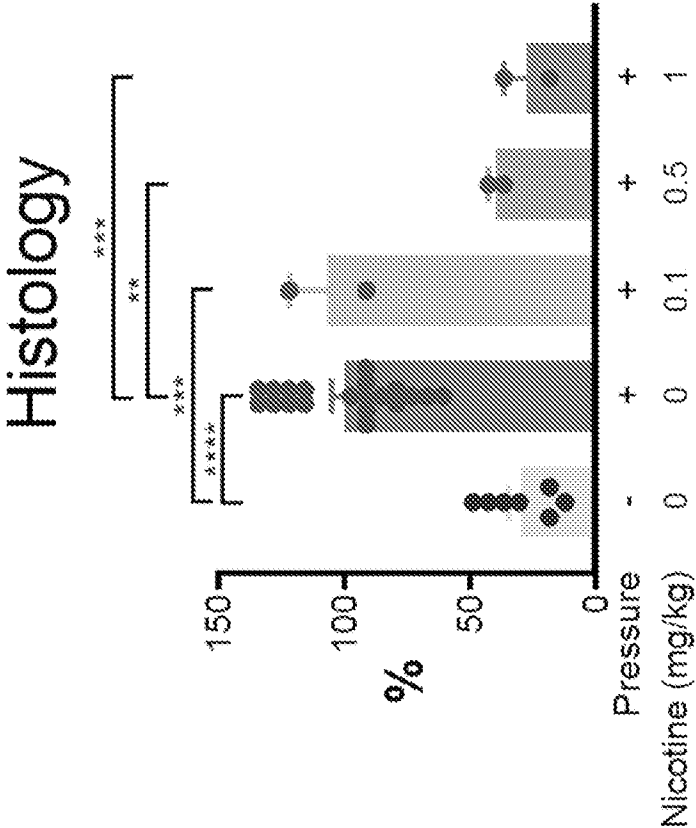
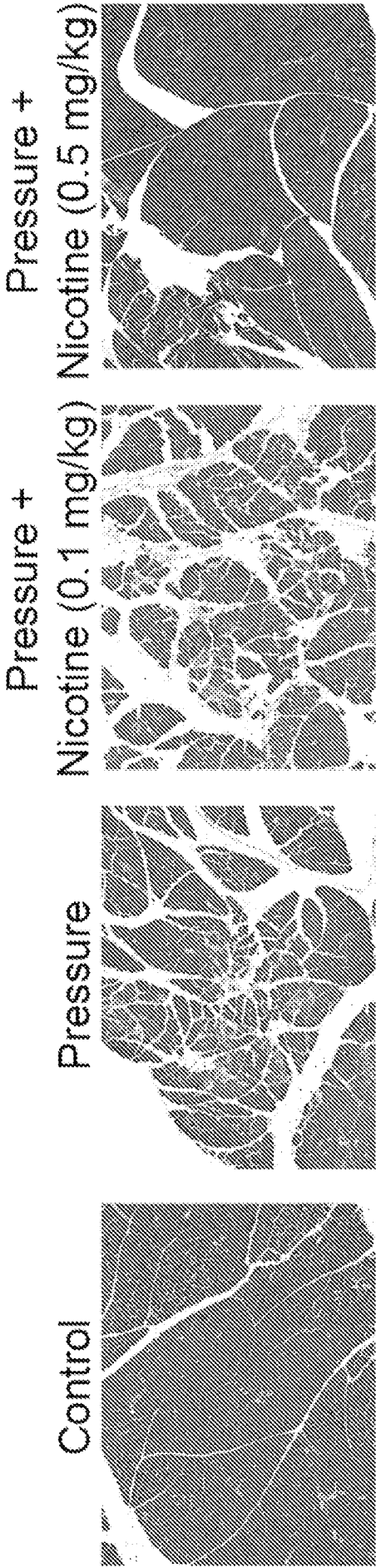


FIG. 1D:



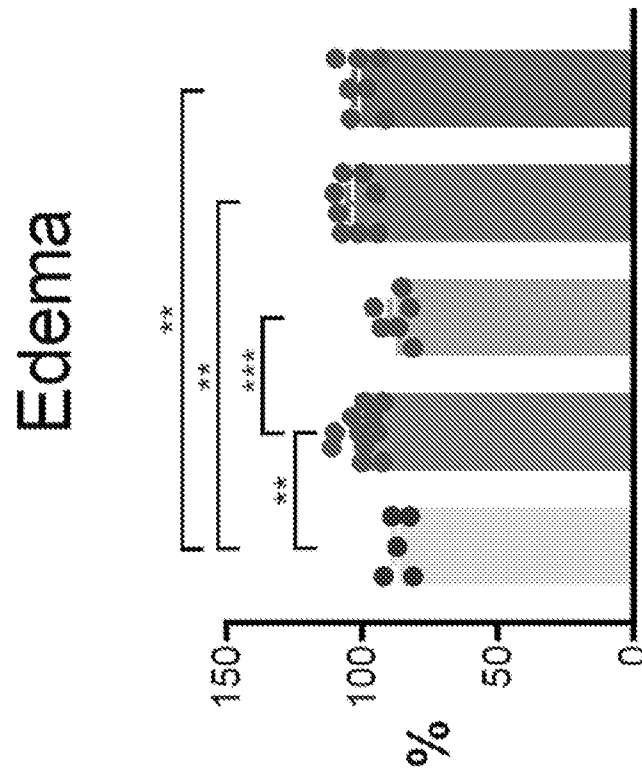
FIGS. 1A-1E (cont'd)

FIG. 1E:



FIGS. 1A-1E (cont'd)

FIG. 2A:



FIGS. 2A-2E

FIG. 2B:

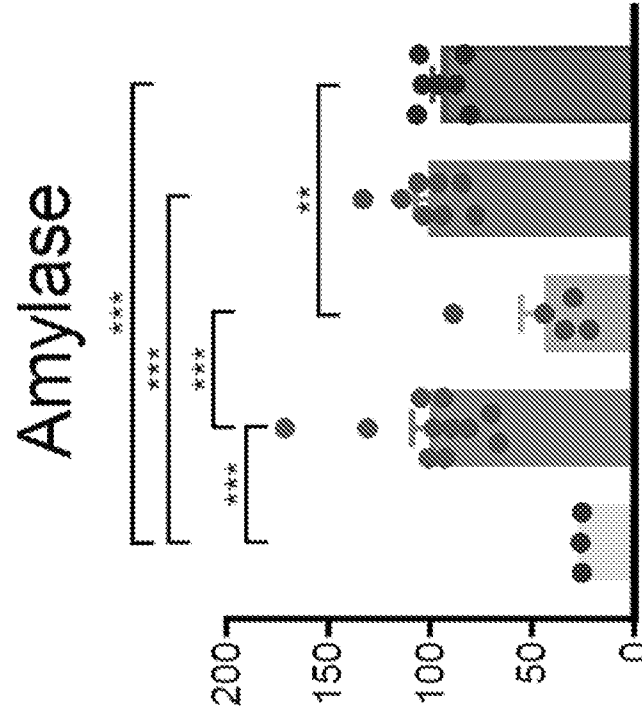


FIG. 2C:

MPO

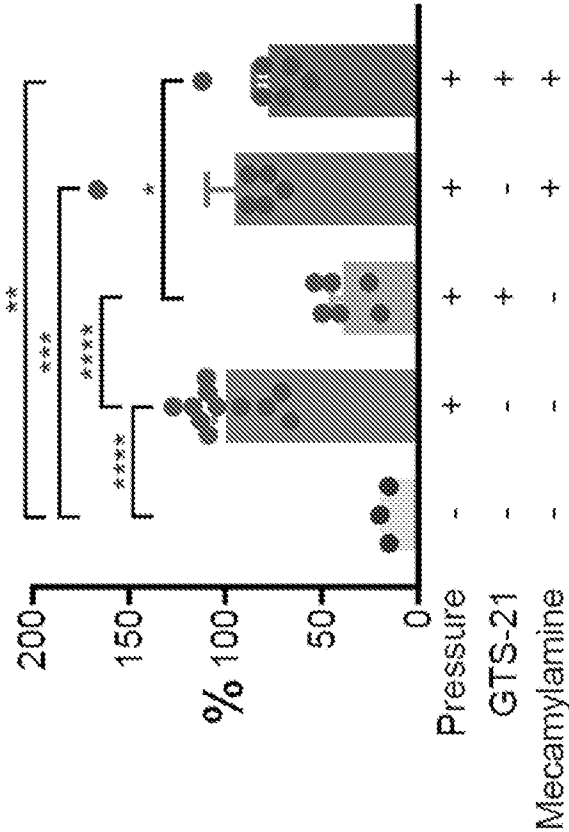
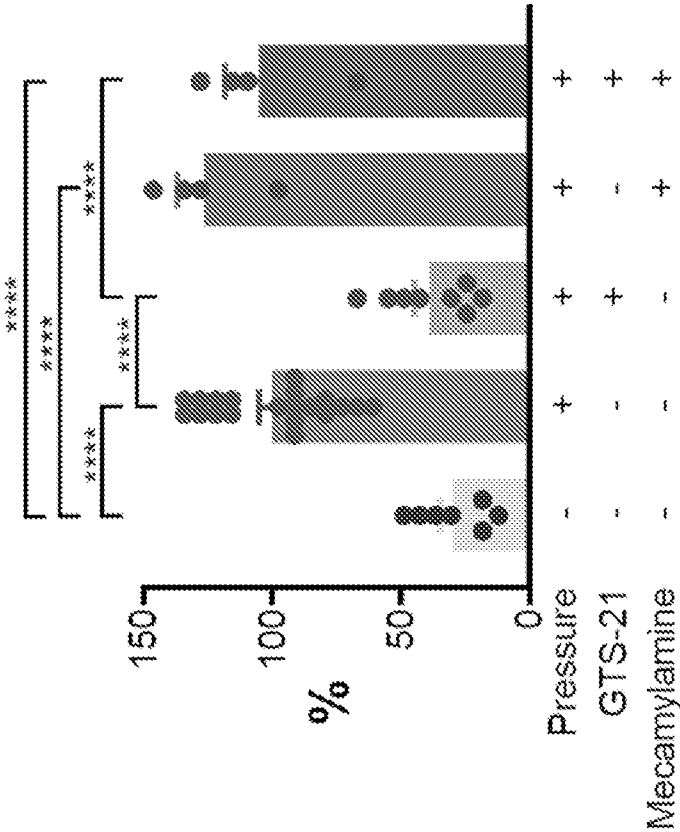


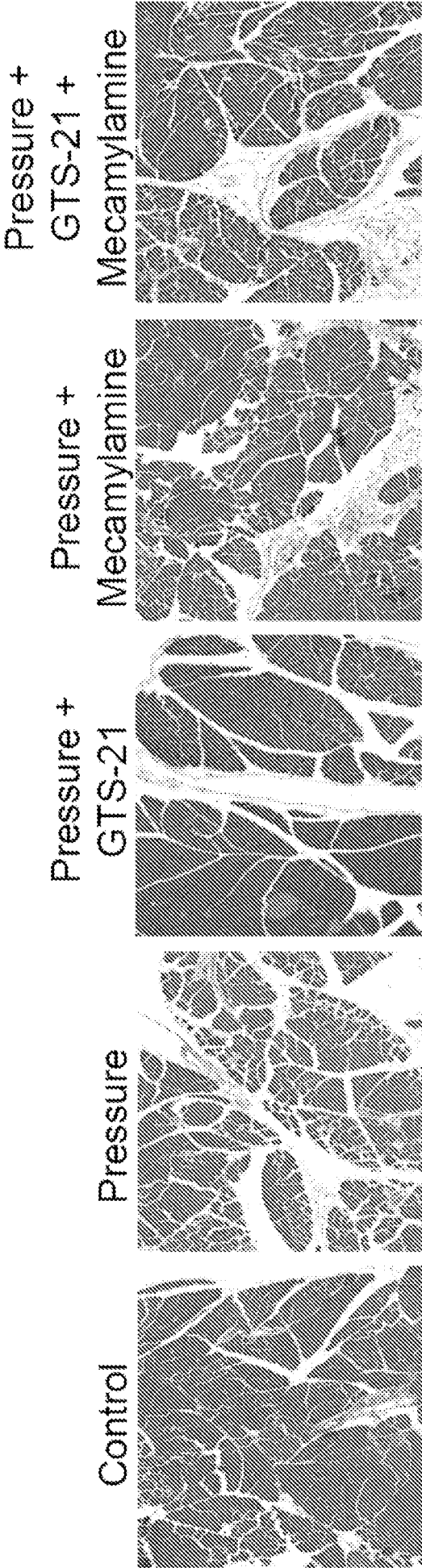
FIG. 2D:

Histology



FIGS. 2A-2E (cont'd)

FIG. 2E:



FIGS. 2A-2E (cont'd)

FIG. 3A:

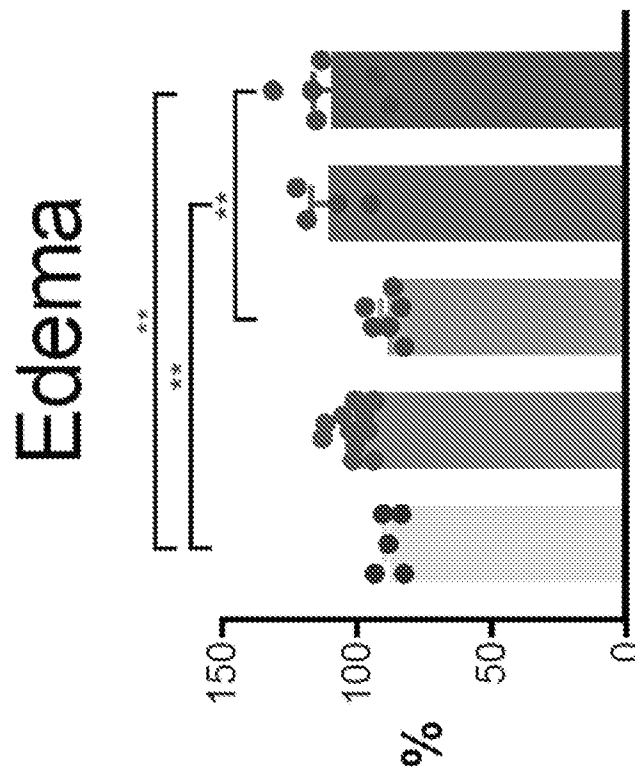
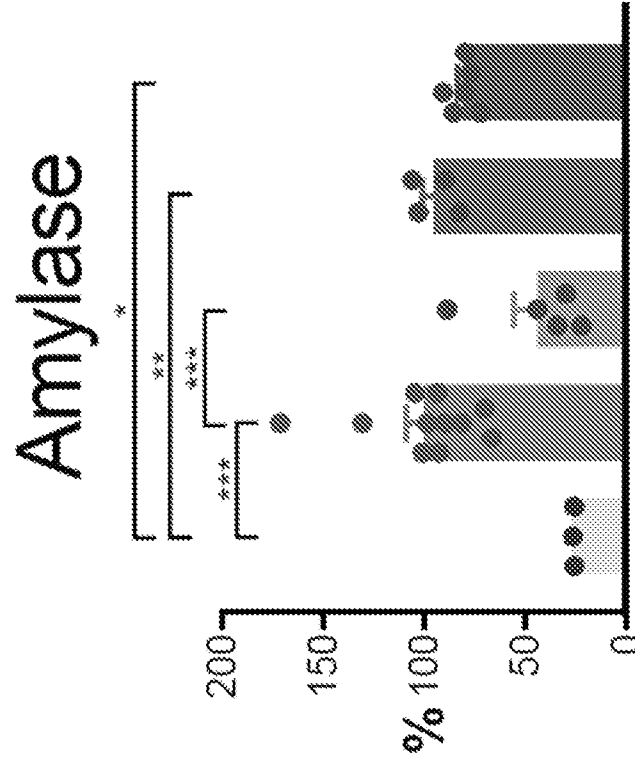


FIG. 3B:



FIGS. 3A-3E

FIG. 3C:

MPO

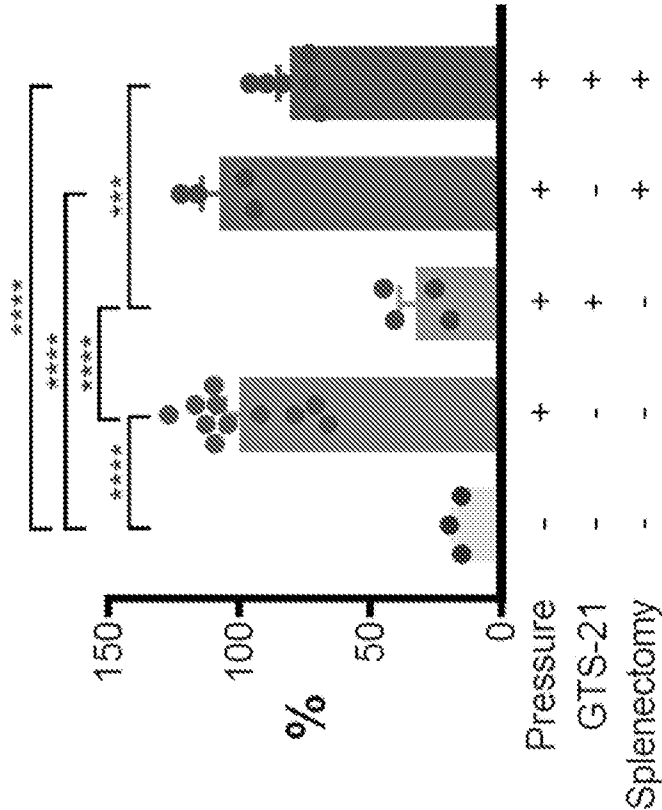
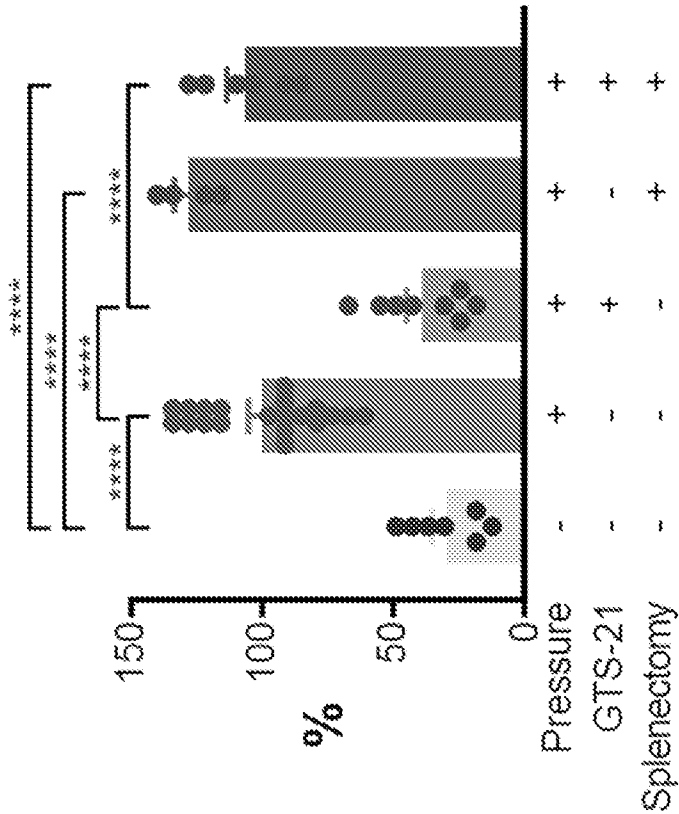


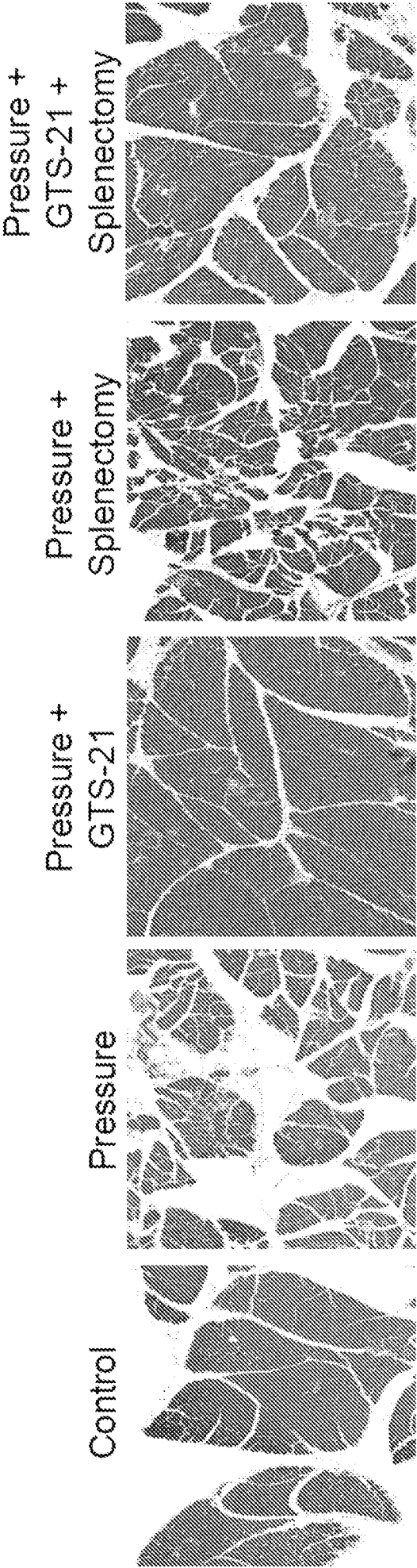
FIG. 3D:

Histology

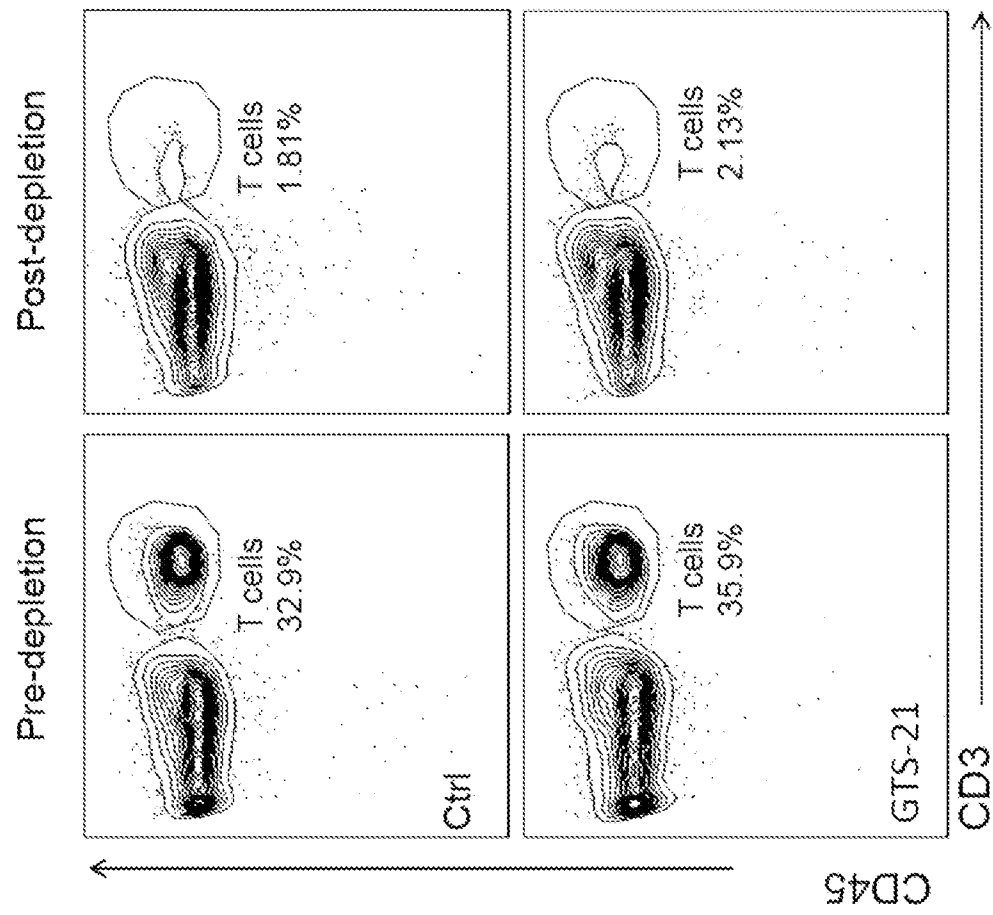


FIGS. 3A-3E (cont'd)

FIG. 3E:



FIGS. 3A-3E (cont'd)



FIGS. 4A-4F

FIG. 4B:

Edema

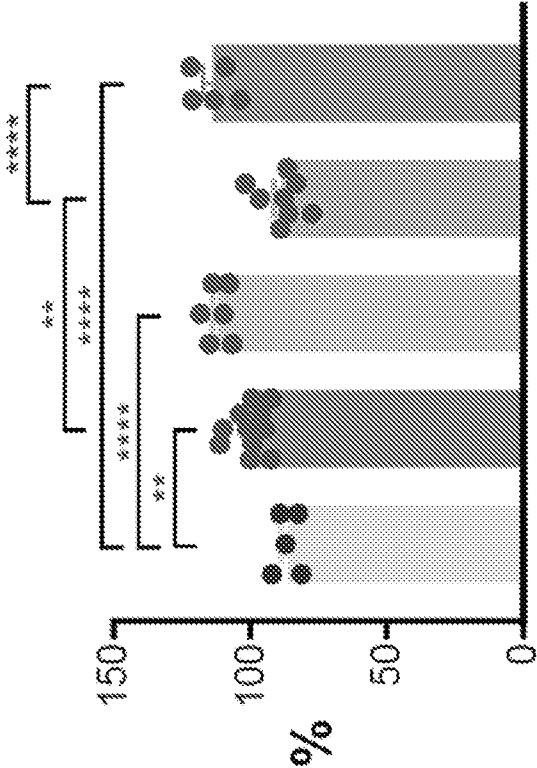
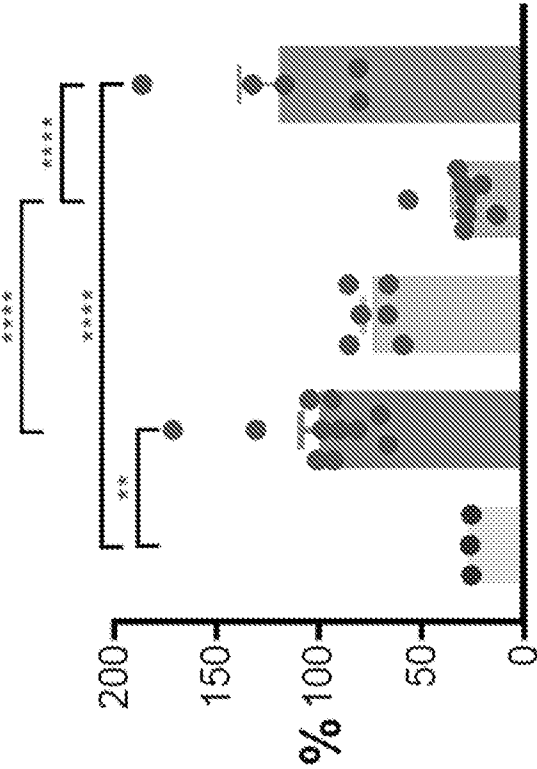


FIG. 4C:

Amylase



FIGS. 4A-4F (cont'd)

FIG. 4D:

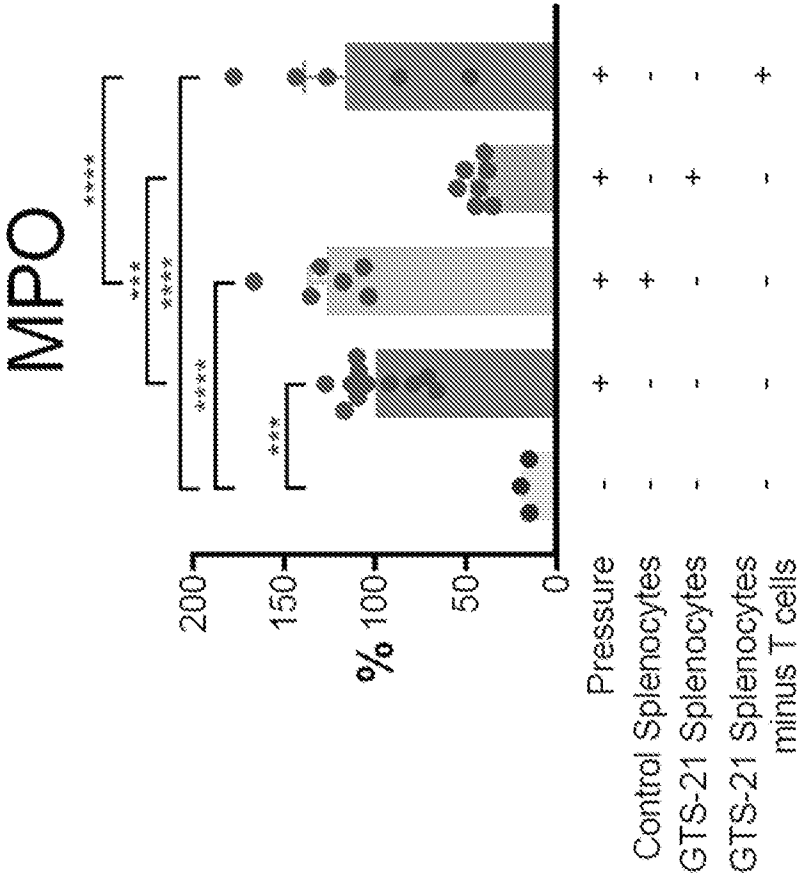
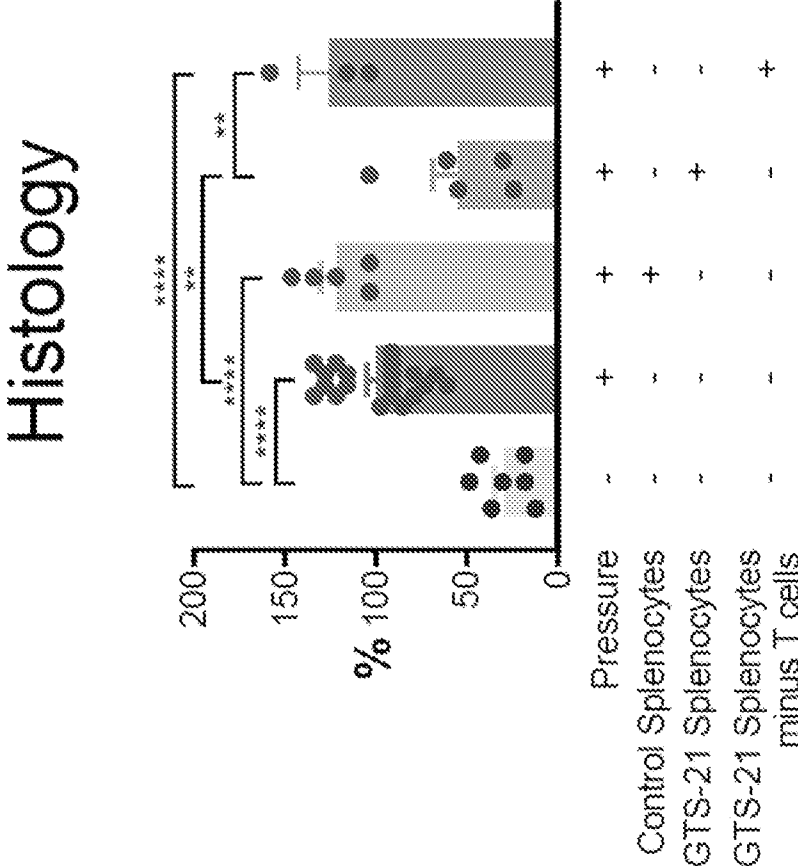
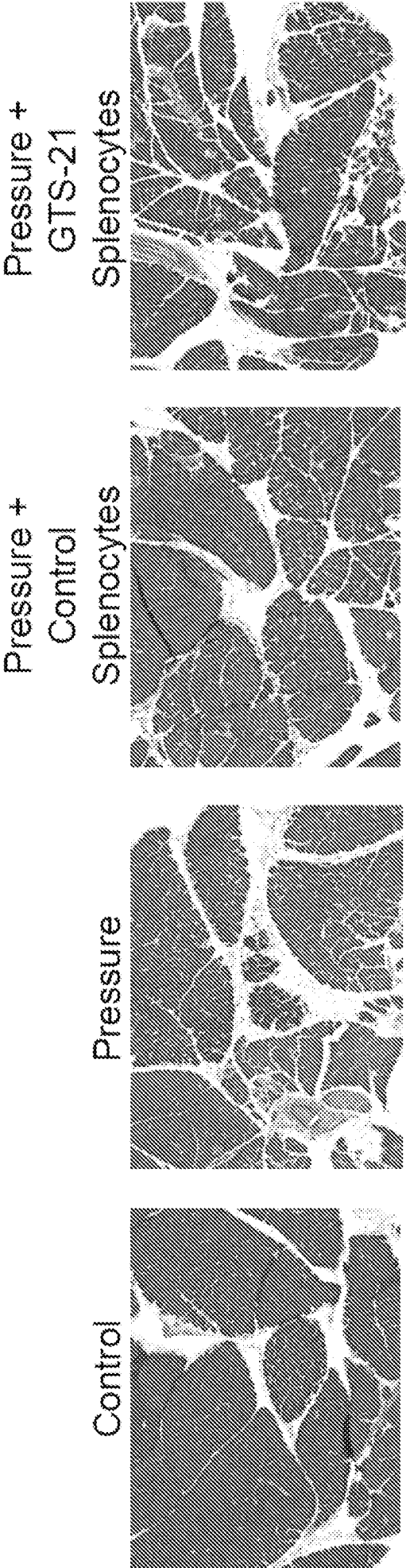


FIG. 4E:



FIGS. 4A-4F (cont'd)

FIG. 4F:



FIGS. 4A-4F (cont'd)

FIG. 5:

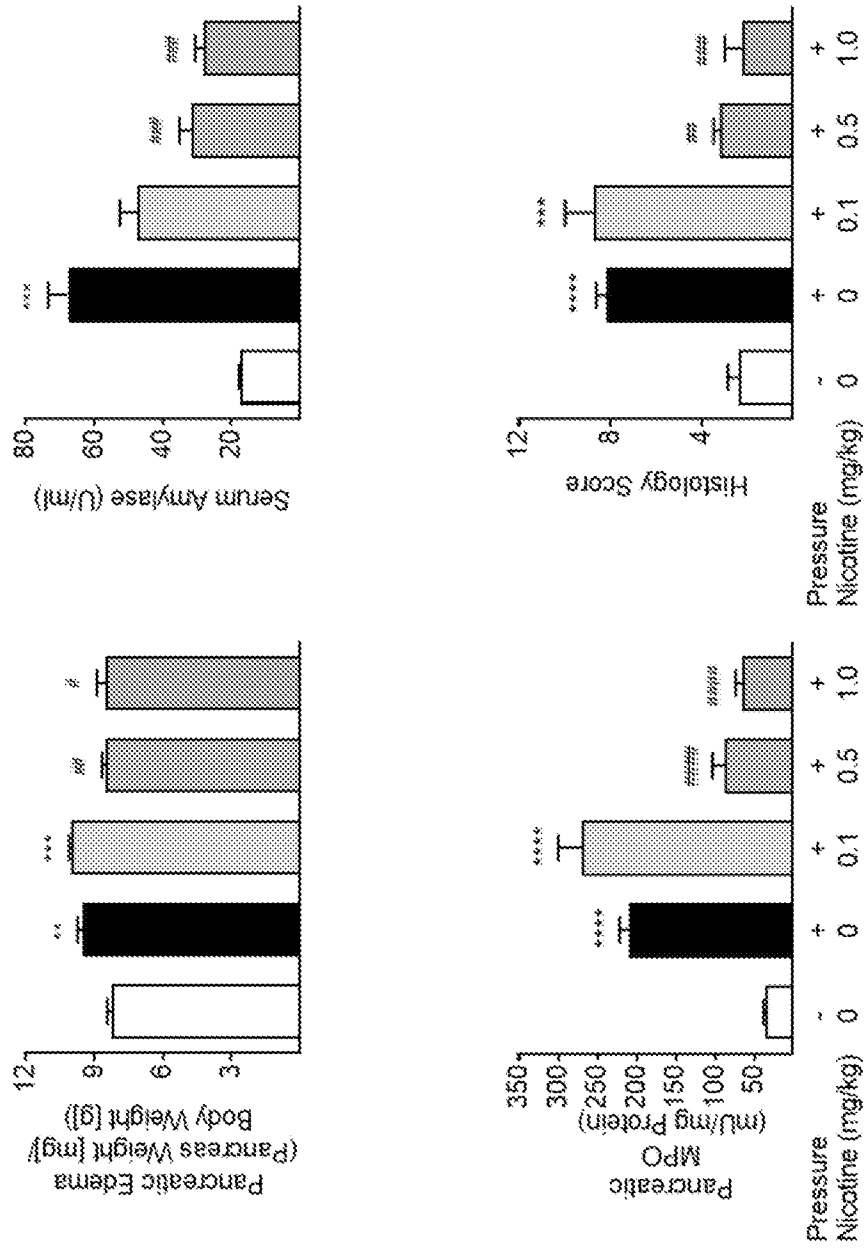


FIG. 6:

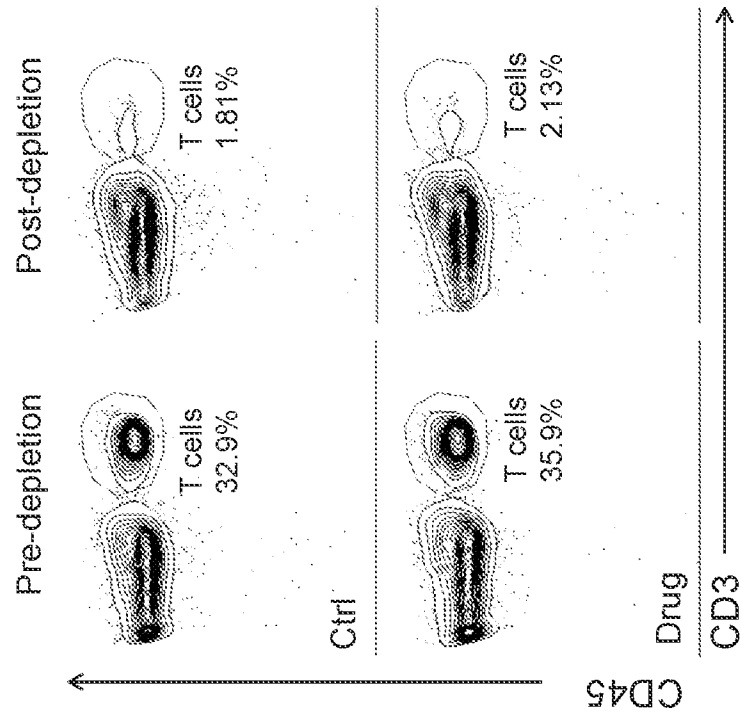


FIG. 7:

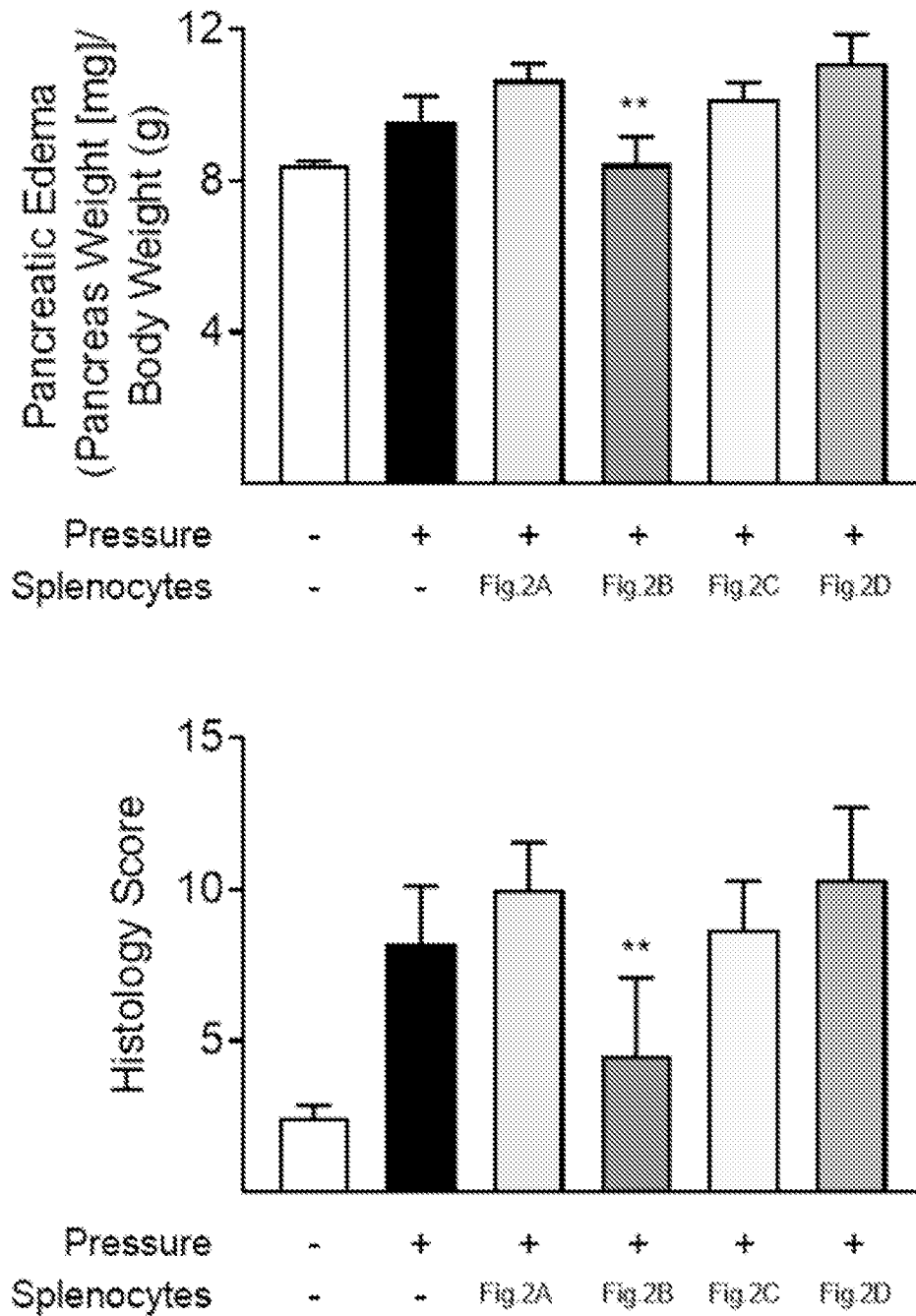


FIG. 8A:

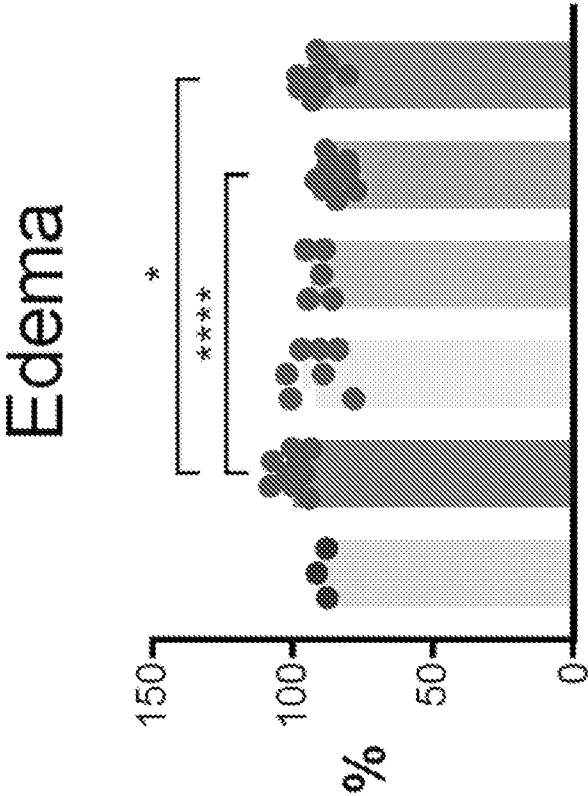
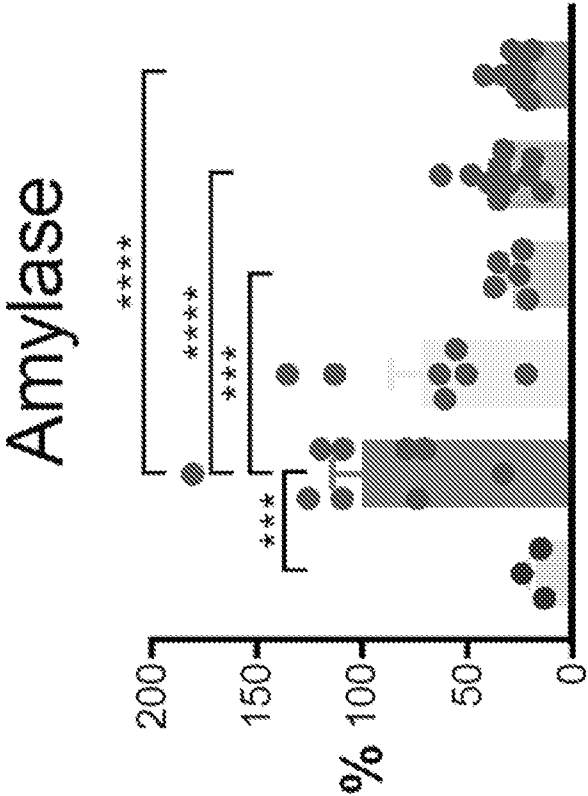


FIG. 8B:



FIGS. 8A-8E

FIG. 8C:

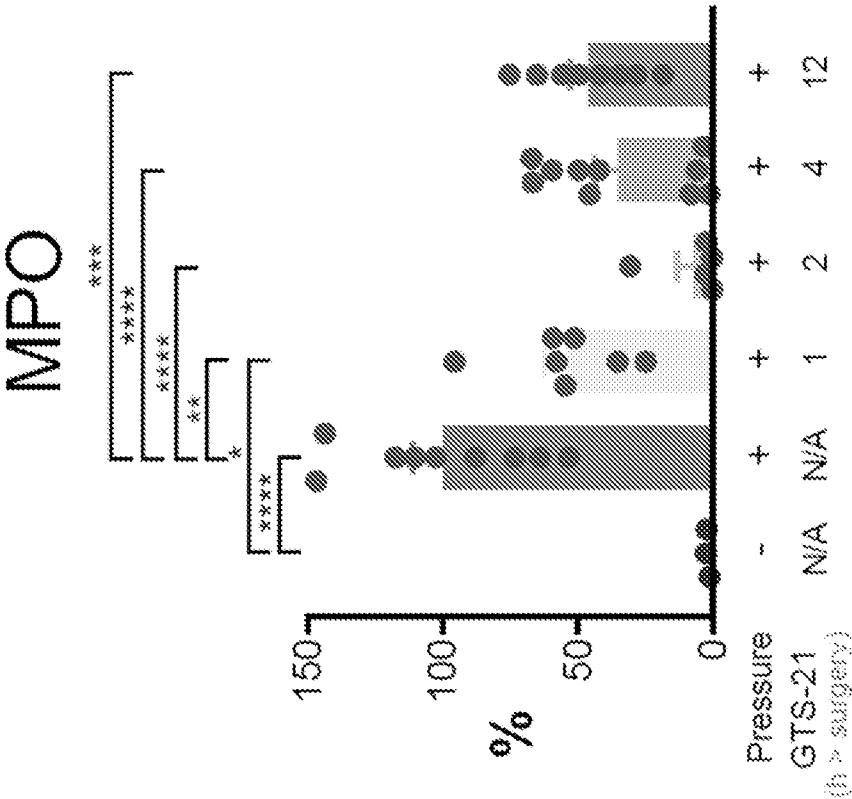
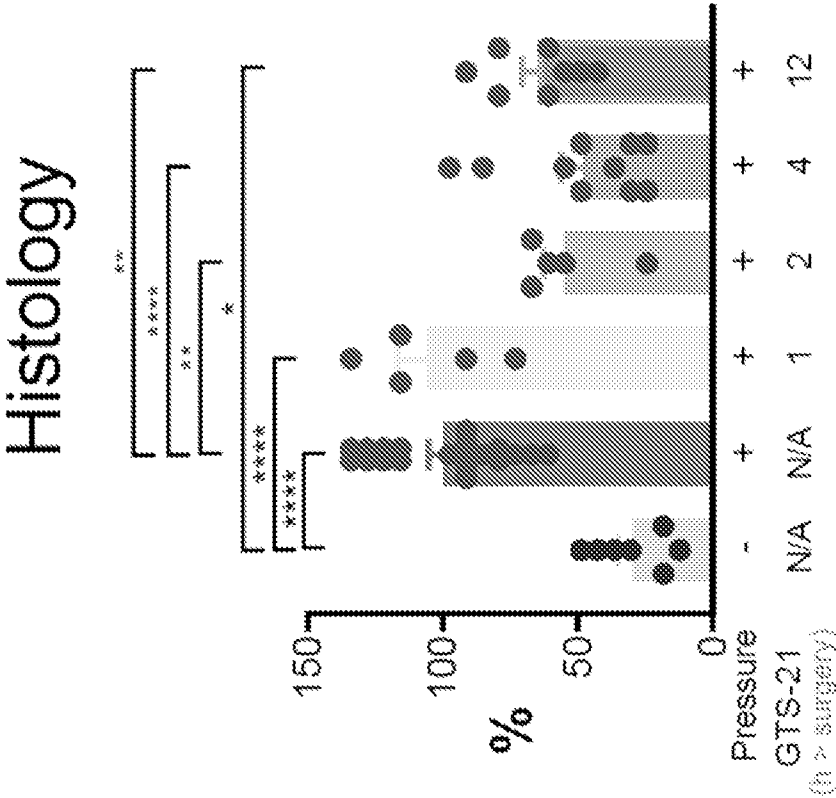
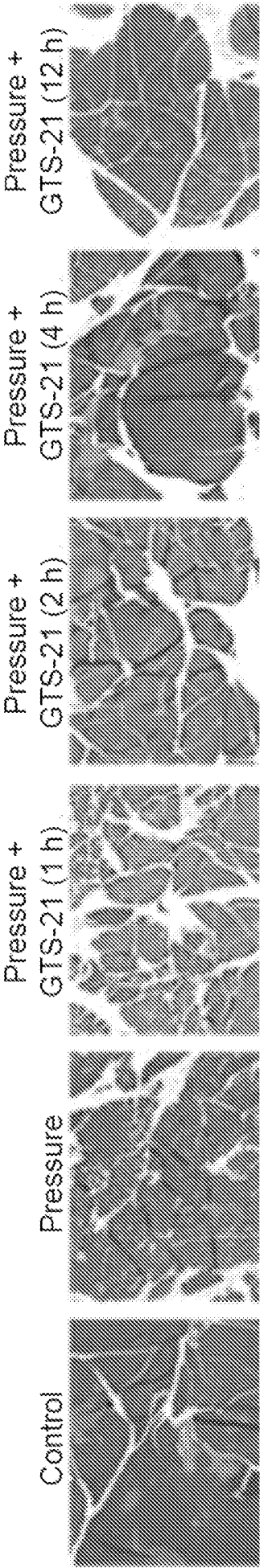


FIG. 8D:



FIGS. 8A-8E (cont'd)

FIG. 8E:



FIGS. 8A-8E (cont'd)

FIG. 9:

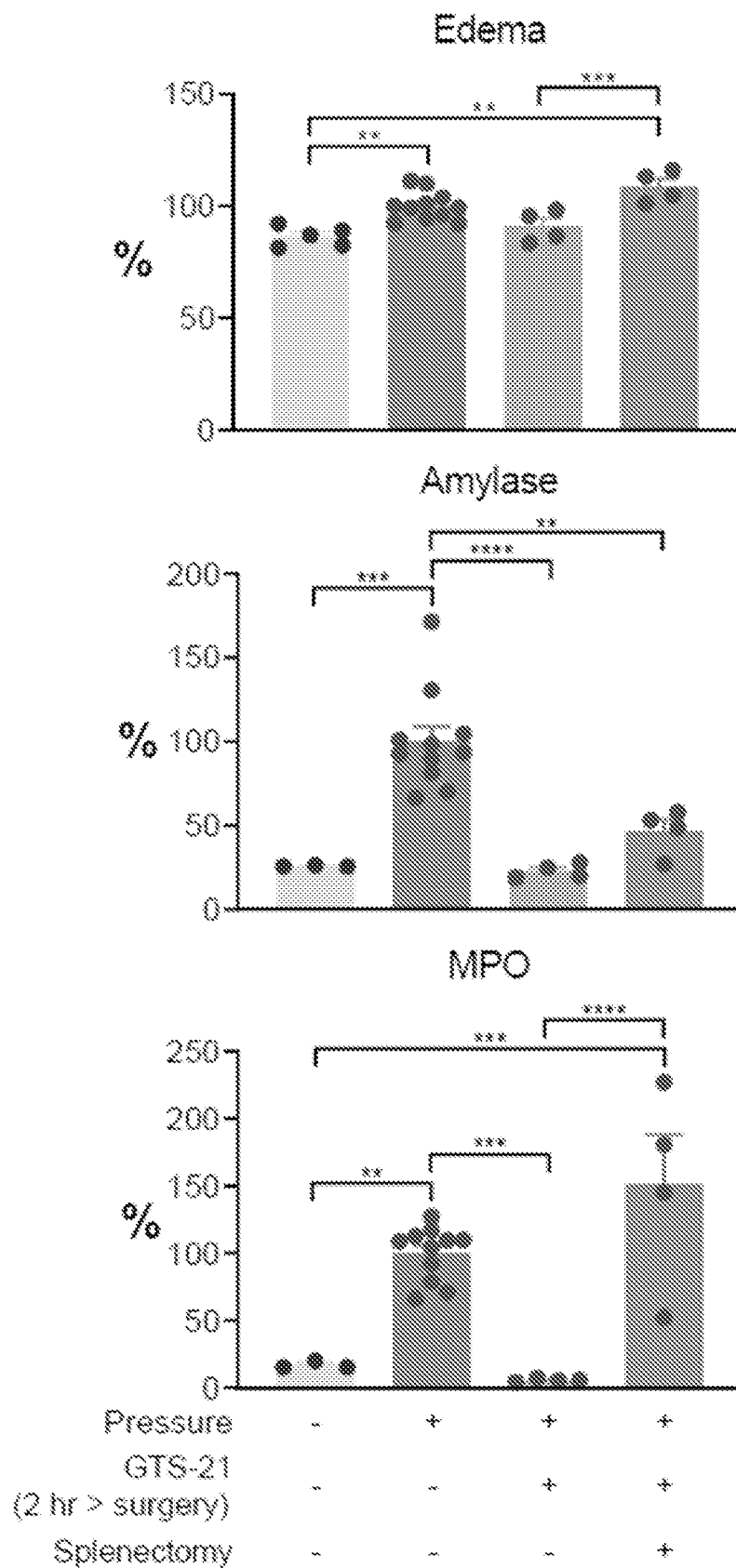


FIG. 10A:

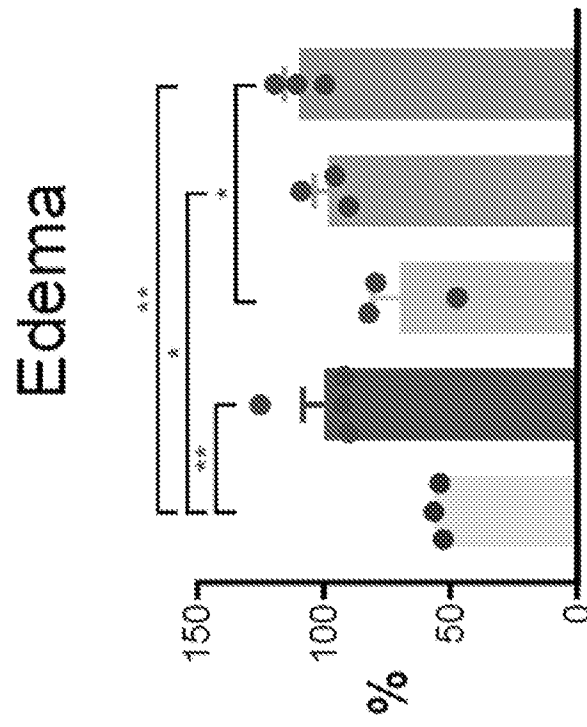
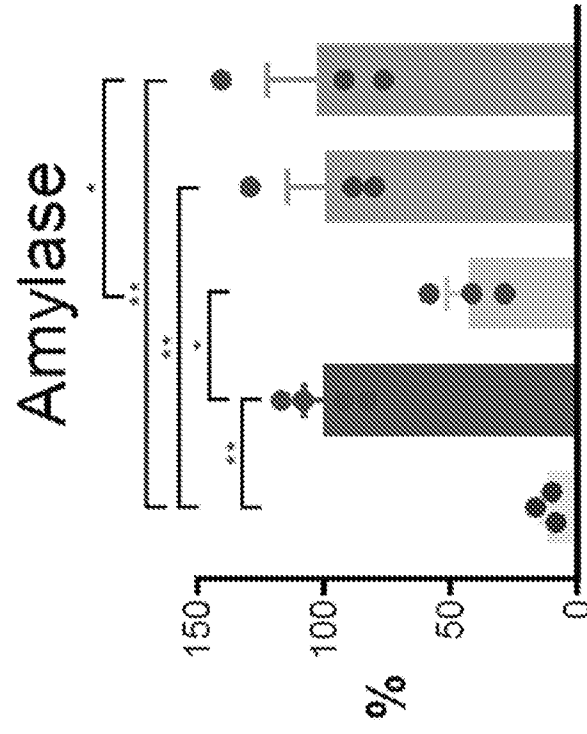


FIG. 10B:



FIGS. 10A-10E

FIG. 10C:

MPO

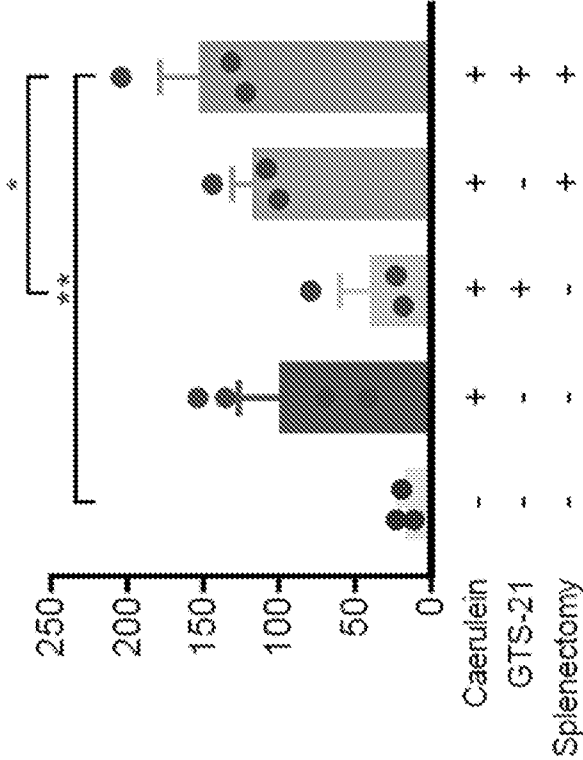
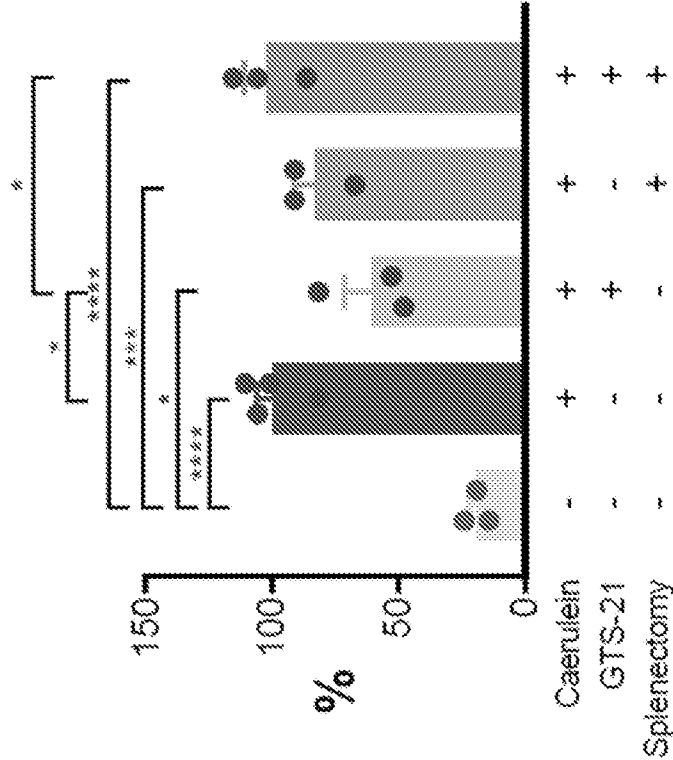


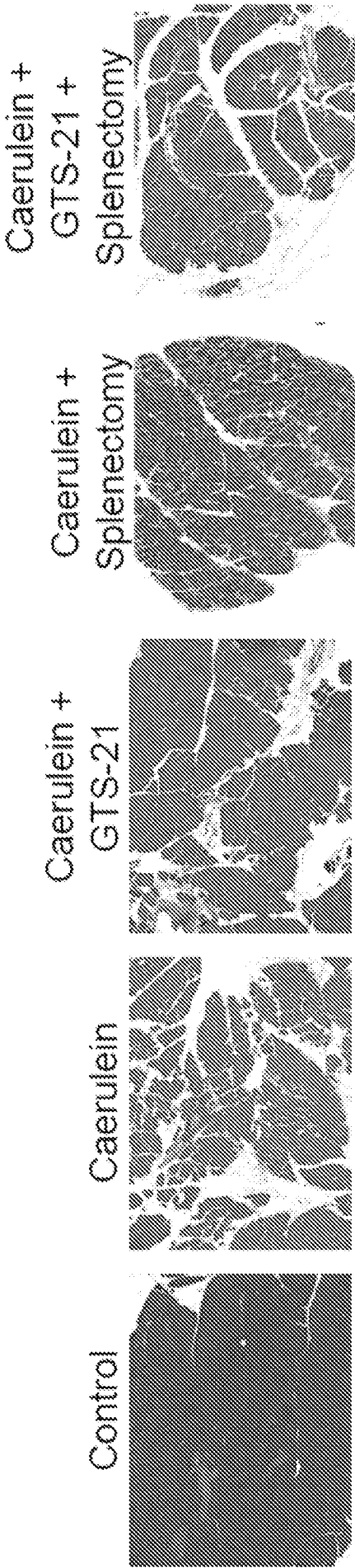
FIG. 10D:

Histology



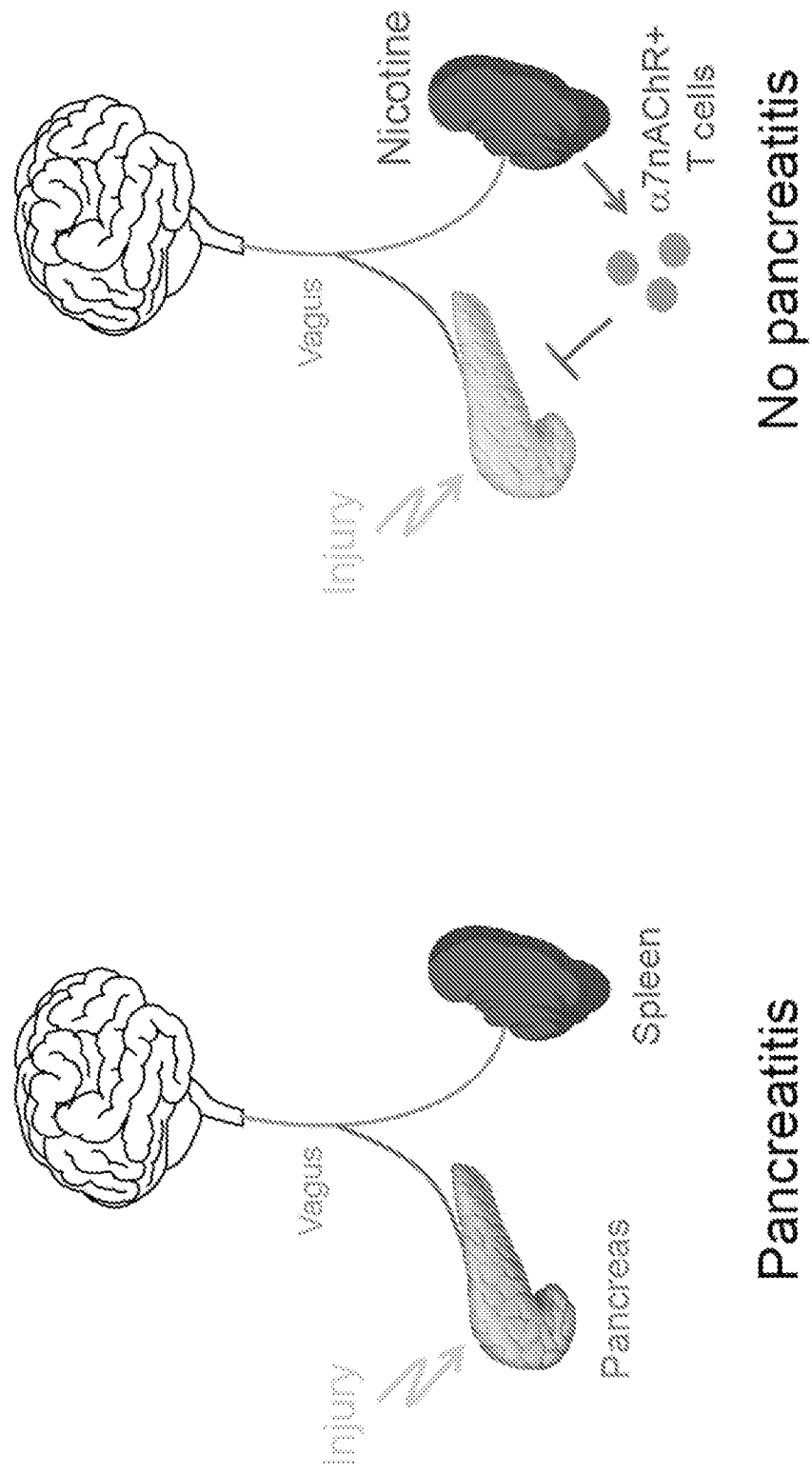
FIGS. 10A-10E (cont'd)

FIG. 10E:



FIGS. 10A-10E (cont'd)

FIG. 11:



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/019117

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - INV. - A61P 1/18; A61K 31/465 (2023.01)

ADD. - A61K 31/09; A61K 31/435; A61P 29/00 (2023.01)

CPC - INV. - A61P 1/18; A61K 31/465 (2023.05)

ADD. - A61K 31/09; A61K 31/435; A61P 29/00 (2023.05)

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)  
See Search History documentDocumentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
See Search History documentElectronic database consulted during the international search (name of database and, where practicable, search terms used)  
See Search History document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                            | Relevant to claim No.     |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| X<br>---  | WO 2021/062347 A1 (DUKE UNIVERSITY) 01 April 2021 (01.04.2021) entire document                                                                                                                                                                                                                                                                                                                                                                                                                                | 1-3, 24-26, 28, 29        |
| Y         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 14-16                     |
| Y         | ~ CN 101289653 A (SHANGHAI CANCER INSTITUTE) 22 October 2008 (22.10.2008) machine translation                                                                                                                                                                                                                                                                                                                                                                                                                 | 14-16                     |
| A         | US 2011/0166148 A1 (TRACEY et al.) 07 July 2011 (07.07.2011) entire document                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1-3, 14-16, 24-26, 28, 29 |
| A         | ~ Wang et al., Stimulation of a7 nicotinic acetylcholine receptor by nicotine increases suppressive capacity of naturally occurring CD4+CD25+ regulatory T cells in mice in vitro, Journal of Pharmacology and Experimental Therapeutics, Vol. 335, No. 3, 01 December 2010 [Retrieved on 06 June 2023]. Retrieved from the Internet: <URL: <a href="https://jpet.aspetjournals.org/content/335/3/553">https://jpet.aspetjournals.org/content/335/3/553</a> >. Pgs. 553-561                                   | 1-3, 14-16, 24-26, 28, 29 |
| A         | ~ CN 101693885 A (XIAMEN UNIVERSITY) 14 April 2010 (14.04.2010) machine translation                                                                                                                                                                                                                                                                                                                                                                                                                           | 1-3, 14-16, 24-26, 28, 29 |
| P, X      | ~ Shahid et al., Nicotinic stimulation of splenic T cells is protective in endoscopic retrograde cholangiopancreatography-induced acute pancreatitis in mice, American Journal of Physiology-Gastrointestinal and Liver Physiology, Vol. 323, No. 5, 20 September 2022 [Retrieved on 06 June 2023]. Retrieved from the Internet: <URL: <a href="https://journals.physiology.org/doi/full/10.1152/ajpgi.00156.2022">https://journals.physiology.org/doi/full/10.1152/ajpgi.00156.2022</a> >. Pgs. G420 - G427. | 1-3, 14-16, 24-26, 28, 29 |

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

|                                                                                                                                                                         |                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents:                                                                                                                                | "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                                              |
| "A" document defining the general state of the art which is not considered to be of particular relevance                                                                | "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                                                                     |
| "D" document cited by the applicant in the international application                                                                                                    | "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 |
| "E" earlier application or patent but published on or after the international filing date                                                                               | "&" document member of the same patent family                                                                                                                                                                                                    |
| "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                                                                  |                                                                                                                                                                                                                                                  |

Date of the actual completion of the international search

06 June 2023

Date of mailing of the international search report

JUL 12 2023

Name and mailing address of the ISA/  
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Taina Matos

Telephone No. PCT Helpdesk: 571-272-4300

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/019117

## 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.:  
because they relate to subject matter not required to be searched by this Authority, namely:
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.: 4-13, 17-23, 27  
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 additional fees, this Authority did not invite payment of 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.