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(54) Title: COMPOSITIONS AND METHODS RELATED TO CHOLIC ACID-7-SULFATE AS A TREATMENT FOR DIABETES

(57) Abstract: Provided are methods of treating diabetes and/or obesity in a subject in need thereof, and methods of increasing the amount of cholic acid-7-sulfate (CA7S) in a subject. Further provided herein are methods of administering CA7S to a subject. Also provided are compositions and kits comprising cholic acid-7-sulfate, or a salt thereof for use in the treatment of diabetes and/or obesity.

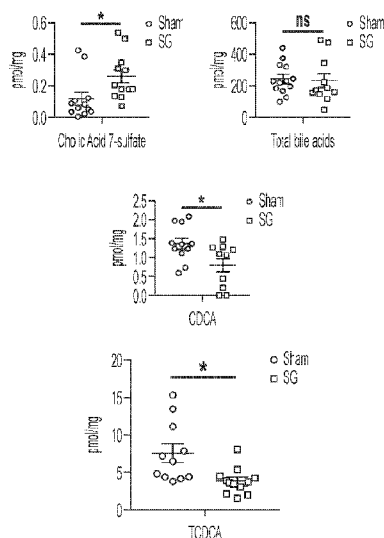


FIG. 3

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COMPOSITIONS AND METHODS RELATED TO CHOLIC ACID-7-SULFATE AS A TREATMENT FOR DIABETES

RELATED APPLICATIONS

[001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application, U.S.S.N. 62/722,010, filed August 23, 2018, which is incorporated herein by reference.

FIELD OF THE INVENTION

[002] The present disclosure relates to the treatment of obesity and diabetes.

BACKGROUND

[003] Obesity and type 2 diabetes (T2D) are medical pandemics. Bariatric surgery, in the form of Roux-en-Y gastric bypass or sleeve gastrectomy (SG), is currently the most effective and lasting treatment for obesity and related comorbidities (Batterham, R. L., *et al.* *Diabetes Care* **2016** 39, 893–901; Gloy, V. L. *et al.*, *BMJ* **2013**, 347, f5934–f5934). While maximal weight-loss occurs at 1 year, remarkably, many patients see resolution of their T2D within hours to days of surgery (Abbasi, J. *JAMA*, **2017**, 317, 571–574). For a majority of patients, remission is durable and lasts for years after surgery.

[004] Two changes consistently observed following bariatric surgery are increased levels of Glucagon-like peptide-1 (GLP-1), a circulating incretin hormone, and changes in the systemic repertoire of bile acids (BAs) (Kaska, L., *et al.* *J. World J. Gastroenterol.* **2016**, 22, 8698–8719). BAs are cholesterol-derived metabolites that play crucial roles in host metabolism by acting as detergents that aid in the absorption of lipids and vitamins, and as ligands for host receptors (Fiorucci, S., *et al.*, *Trends Mol Med.* **2015**, 21, 702–714). BAs have been implicated in post-SG therapeutic benefits due to their ability to mediate farnesoid X receptor (FXR) signaling (Ryan, K. K. *et al.* *Nature* **2014**, 509, 183–188). However, the causal role of BAs in eliciting beneficial metabolic changes post-surgery remains unclear. Thus far, research efforts have focused on overall changes in the total BA pool or in levels of BAs conjugated to amino acids (Patti, M.-E. *et al.* *Obesity (Silver Spring)* **2009**, 17, 1671–1677). Individual BAs, however, have different binding affinities for nuclear hormone receptors (NhrRs) and GPCRs, and thus unique abilities to modulate glucose homeostasis, lipid accumulation, and energy expenditure (Patti, M.-E. *et al.* *Obesity (Silver Spring)* **2009**, 17, 1671–1677; Sayin, S. I. *et al.* *Cell Metab.*, **2013**, 17, 225–235).

[005] Diabetes mellitus is a disease that characterized by the lack of insulin production (e.g. type 1 diabetes) by the pancreas or a lack of insulin sensitivity (e.g. type II diabetes). Patients with diabetes mellitus are diagnosed by a glucose tolerance test. The plasma glucose levels are elevated in patients diagnosed with diabetes compared with healthy patients. Diabetes can result in a number of long term complications including diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes. Current treatments, such as insulin injections, manage the symptoms but do not prevent the long term complications of the disease and require constant monitoring of blood glucose levels. New treatments for diabetes are needed to improve the quality of life and prevent future complications of the disease.

SUMMARY OF THE INVENTION

[006] The compositions and methods described herein are related, in part, to the discovery of cholic acid-7-sulfate as a treatment for diabetes and obesity.

[007] In one aspect, provided herein is a method for treating or preventing diabetes, the method comprising: administering to a subject in need thereof an agent that increases the level of cholic acid-7-sulfate in the subject.

[008] In another aspect, provided herein is a method for treating or preventing obesity, the method comprising: administering to a subject in need thereof an agent that increases the level of cholic acid-7-sulfate in the subject. In another aspect, provided herein are methods for treating or preventing diabetes and/or obesity, comprising administering to a subject in need thereof an effective amount of cholic acid-7-sulfate. In certain aspects, the agent that increases the level of cholic acid-7-sulfate in a subject is cholic acid-7-sulfate.

[009] Compositions and kits comprising cholic acid-7-sulfate and/or compositions, and methods of treatment using cholic acid-7-sulfate or compositions thereof are provided herein.

[0010] The details of certain embodiments of the invention are set forth in the Detailed Description of Certain Embodiments, as described below. Other features, objects, and advantages of the invention will be apparent from the Definitions, Examples, and Claims.

[0011] In one embodiment, the agent is cholic acid-7-sulfate.

[0012] In one embodiment, the agent is a TGR5 agonist.

[0013] In one embodiment, the TGR5 agonist induces GLP-1 secretion from a target cell. In some embodiments, the activity of TGR5 is increased by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to a control. In some embodiments, the

secretion of GLP1 is increased by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to a control.

[0014] In one embodiment, the agent is selected from the group consisting of a small molecule, an antibody, a peptide, a genome editing system, an antisense oligonucleotide, and an RNAi.

[0015] In some embodiments, the agent is a vector that encodes the agent. In one embodiment, the vector is non-integrative or integrative. In one embodiment, the vector is a viral vector.

[0016] In one embodiment, the agent is formulated with a pharmaceutical composition.

[0017] In one embodiment, the pharmaceutical composition is formulated to restrict delivery of an agent to the gastrointestinal tract of the subject.

[0018] In one embodiment, the diabetes is type I, type II, neonatal, or maturity onset diabetes in the young.

[0019] In one embodiment, the administering reduces glucose levels in the serum of a subject.

[0020] In one embodiment, wherein the subject is a mammal.

[0021] In one embodiment, the mammal is a human.

[0022] In one embodiment, the target cell is an enteroendocrine cell, an epithelial cell, an L-cell, or a neuron.

[0023] In one aspect, described herein is a composition comprising an agent that increases the level of cholic acid-7-sulfate in a subject.

[0024] In one embodiment, wherein the agent is cholic acid-7-sulfate.

[0025] In one embodiment, the composition is formulated for treating or preventing diabetes.

[0026] In one embodiment, the composition further comprises a pharmaceutically acceptable carrier or excipient.

[0027] In one embodiment, the carrier or excipient restricts delivery of the composition to the gastrointestinal tract.

[0028] In one aspect, described herein is a method for treating or preventing diabetes, the method comprising: administering to a subject in need thereof a genetically engineered microorganism or population thereof, that expresses an agent that increases the level of cholic acid-7-sulfate.

[0029] In one embodiment, the genetically engineered microorganism is a bacterium.

[0030] In one aspect, described herein is a method for treating or preventing diabetes, the method comprising: administering to a subject in need thereof a genetically engineered microorganism or population thereof, that secretes cholic acid-7-sulfate.

[0031] In one embodiment, the genetically engineered microorganism is a bacterium.

DEFINITIONS

[0032] For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims, are provided below. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed technology, because the scope of the technology is limited only by the claims. 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 to which this technology belongs. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

[0033] An “obesity-related condition” as used herein, includes, but is not limited to, obesity, undesired weight gain (e.g., from medication-induced weight gain, from cessation of smoking) and an over-eating disorder (e.g., binge eating, bulimia, compulsive eating, or a lack of appetite control each of which can optionally lead to undesired weight gain or obesity). “Obesity” and “obese” as used herein, refers to class I obesity, class II obesity, class III obesity, and pre-obesity (e.g., being “over-weight”) as defined by the World Health Organization.

[0034] As used herein the term “an inflammatory disease” refers to any disease that affects the immune system. The term “inflammatory disease” may also refer to a dysregulated inflammatory reaction that causes an exaggerated response by macrophages, granulocytes, and/or T-lymphocytes leading to abnormal tissue damage and/or cell death. An inflammatory disease can be either an acute or chronic inflammatory condition and can result from infections or non-infectious causes. The inflammatory disease can cause at least one symptom of the disease. These symptoms can include but are not limited to, diarrhea, vomiting, nausea, upset stomach, pain, swollen joints, malaise, fever, weight loss, weight gain, bleeding, any change in the consistency or frequency of a bowel movement or stool, or any other symptom associated with an inflammatory disease in a subject. In some embodiments, the inflammatory disease is an autoimmune disease.

[0035] Inflammatory diseases include, without limitation, atherosclerosis, arteriosclerosis, autoimmune disorders, multiple sclerosis, systemic lupus erythematosus, polymyalgia rheumatica (PMR), gouty arthritis, degenerative arthritis, tendonitis, bursitis, food intolerance, psoriasis, cystic fibrosis, diverticulitis, arthroseitis, rheumatoid arthritis, inflammatory arthritis, Sjogren's syndrome, giant cell arteritis, progressive systemic sclerosis (scleroderma), ankylosing spondylitis, polymyositis, dermatomyositis, pemphigus, pemphigoid, diabetes (e.g., Type I), myasthenia gravis, Hashimoto's thyroiditis, gastroesophageal reflux disease (GERD), Graves' disease, Goodpasture's disease, mixed connective tissue disease, sclerosing cholangitis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, pernicious anemia, inflammatory dermatoses, usual interstitial pneumonitis (UIP), asbestosis, silicosis, bronchiectasis, berylliosis, talcosis, pneumoconiosis, sarcoidosis, desquamative interstitial pneumonia, lymphoid interstitial pneumonia, giant cell interstitial pneumonia, cellular interstitial pneumonia, extrinsic allergic alveolitis, Wegener's granulomatosis and related forms of angiitis (temporal arteritis and polyarteritis nodosa), inflammatory dermatoses, hepatitis, delayed-type hypersensitivity reactions (e.g., poison ivy dermatitis), pneumonia, respiratory tract inflammation, Adult Respiratory Distress Syndrome (ARDS), encephalitis, immediate hypersensitivity reactions, asthma, hayfever, allergies, acute anaphylaxis, rheumatic fever, glomerulonephritis, pyelonephritis, cellulitis, cystitis, chronic cholecystitis, ischemia (ischemic injury), reperfusion injury, allograft rejection, host-versus-graft rejection, appendicitis, arteritis, blepharitis, bronchiolitis, bronchitis, cervicitis, cholangitis, chorioamnionitis, conjunctivitis, dacryoadenitis, dermatomyositis, endocarditis, endometritis, enteritis, enterocolitis, epicondylitis, epididymitis, fasciitis, fibrositis, gastritis, gastroenteritis, gingivitis, ileitis, iritis, laryngitis, myelitis, myocarditis, nephritis, omphalitis, oophoritis, orchitis, osteitis, otitis, pancreatitis, parotitis, pericarditis, pharyngitis, pleuritis, phlebitis, pneumonitis, proctitis, prostatitis, rhinitis, salpingitis, sinusitis, stomatitis, synovitis, testitis, tonsillitis, urethritis, urocystitis, uveitis, vaginitis, vasculitis, vulvitis, vulvovaginitis, angitis, chronic bronchitis, osteomyelitis, optic neuritis, temporal arteritis, transverse myelitis, necrotizing fasciitis, and necrotizing enterocolitis.

[0036] An "autoimmune disease" refers to a disease arising from an inappropriate immune response of the body of a subject against substances and tissues normally present in the body. In other words, the immune system mistakes some part of the body as a pathogen and attacks its own cells. This may be restricted to certain organs (e.g., in autoimmune thyroiditis) or involve a particular tissue in different places (e.g., Goodpasture's disease which may affect the basement membrane in both the lung and kidney). The treatment of autoimmune diseases

is typically with immunosuppression, e.g., medications which decrease the immune response. Exemplary autoimmune diseases include, but are not limited to, celiac disease, glomerulonephritis, Goodpasture's syndrome, necrotizing vasculitis, lymphadenitis, peri-arteritis nodosa, systemic lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, psoriasis, ulcerative colitis, systemic sclerosis, dermatomyositis/polymyositis, anti-phospholipid antibody syndrome, scleroderma, pemphigus vulgaris, ANCA-associated vasculitis (e.g., Wegener's granulomatosis, microscopic polyangiitis), uveitis, Sjogren's syndrome, Crohn's disease, Reiter's syndrome, ankylosing spondylitis, Lyme disease, Guillain-Barré syndrome, Hashimoto's thyroiditis, and cardiomyopathy.

[0037] Diabetes refers to a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger).

[0038] Generally, diabetes is characterized and diagnosed by high blood glucose levels in a subject's serum (e.g. hyperglycemia). The diagnosis can be carried out by a physician with a glucose challenge test and/or a glucose tolerance test. For an oral glucose tolerance test in humans, a blood sugar level less than about 140 mg/dL (7.8 mmol/L) is normal. A reading of more than about 200 mg/dL (11.1 mmol/L) after two hours indicates that the subject has diabetes. A reading between about 140 and about 199 mg/dL (7.8 mmol/L and 11.0 mmol/L) can indicate prediabetes.

[0039] There are several types of diabetes. Type I diabetes results from the body's failure to produce insulin, and presently requires the person to inject insulin or wear an insulin pump. Type 2 diabetes results from insulin resistance a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. Gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. Other forms of diabetes include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes, e.g., mature onset diabetes of the young (e.g., MODY 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). Pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of diabetes.

[0040] All forms of diabetes increase the risk of long-term complications. These typically develop after many years, but may be the first symptom in those who have otherwise not received a diagnosis before that time. The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and macrovascular diseases such as ischemic heart disease (angina, myocardial infarction), stroke, and peripheral vascular disease. Diabetes also causes microvascular complications, e.g., damage to the small blood vessels. Diabetic retinopathy, which affects blood vessel formation in the retina of the eye, can lead to visual symptoms, reduced vision, and potentially blindness. Diabetic nephropathy, the impact of diabetes on the kidneys, can lead to scarring changes in the kidney tissue, loss of small or progressively larger amounts of protein in the urine, and eventually chronic kidney disease requiring dialysis. Diabetic neuropathy is the impact of diabetes on the nervous system, most commonly causing numbness, tingling and pain in the feet and also increasing the risk of skin damage due to altered sensation. Together with vascular disease in the legs, neuropathy contributes to the risk of diabetes-related foot problems, e.g., diabetic foot ulcers, that can be difficult to treat and occasionally require amputation.

[0041] As used herein, the terms “treat,” “treatment,” “treating,” or “amelioration” refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down, or stop the progression or severity of a condition associated with diabetes, e.g., type II diabetes. The term “treating” includes reducing or alleviating at least one adverse effect or symptom of diabetes. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a disease is reduced or halted. That is, “treatment” includes not just the improvement of symptoms or markers, but also a cessation of, or at least slowing of, progress or worsening of symptoms compared to what would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, remission (whether partial or total), and/or decreased mortality, whether detectable or undetectable. The term “treatment” of a disease also includes providing relief from the symptoms or side-effects of the disease (including palliative treatment).

[0042] As used herein, the term “prevent” or “preventing” refers to the prevention of at least one symptom associated with diabetes, or complete prevention of diabetes, or the lessening of the severity of diabetes (e.g., preventing the progression of diabetes or complications) in a

subject, and/or delaying one or more symptoms of a diabetes, and/or delaying the onset of diabetes and/or symptoms.

[0043] As used herein, the term “administering,” refers to the placement of a therapeutic (e.g., an agent that increases cholic acid-7 sulfate levels) or pharmaceutical composition as disclosed herein into a subject by a method or route which results in at least partial delivery of the agent to the subject. Pharmaceutical compositions comprising agents as disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[0044] As used herein, a “subject” means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include, for example, chimpanzees, cynomolgus monkeys, spider monkeys, and macaques, e.g., Rhesus. Rodents include, for example, mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include, for example, cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich, and fish, e.g., trout, catfish and salmon. In some embodiments, the subject is a mammal, e.g., a primate, e.g., a human. The terms, “individual,” “patient” and “subject” are used interchangeably herein.

[0045] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used as subjects that represent animal models of disease e.g., diabetic or obesity model. A subject can be male or female. A subject can be at any stage of development.

[0046] A subject can be one who has been previously diagnosed with or identified as suffering from or having a disease or disorder in need of treatment (e.g., diabetes) or one or more complications related to such a disease or disorder, and optionally, have already undergone treatment for the disease or disorder or the one or more complications related to the disease or disorder. Alternatively, a subject can also be one who has not been previously diagnosed as having such disease or disorder or related complications. For example, a subject can be one who exhibits one or more risk factors for the disease or disorder or one or more complications related to the disease or disorder or a subject who does not exhibit risk factors.

[0047] As used herein, an “agent” refers to e.g., a molecule, protein, peptide, antibody, or nucleic acid, that inhibits activity of a polypeptide or polynucleotide, or binds to, partially or totally blocks stimulation, decreases, prevents, delays activation, inactivates, desensitizes, or down-regulates the activity of the polypeptide or the polynucleotide. Agents that increase

cholic acid-7-sulfate, e.g., increase secretion or act as an agonist of TGR5 receptors, e.g., improve stability, degradation, dissociation, or localization, secretion, metabolism, partially or totally enhance stimulation, activity or enzymatic activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity of a polypeptide or polynucleotide. An agent can act directly or indirectly.

[0048] The term “agent” as used herein means any compound or substance such as, but not limited to, a small molecule, nucleic acid, polypeptide, peptide, drug, ion, lipid, polymer, etc. An “agent” can be any chemical (e.g., an acid or sulfate), entity or moiety, including without limitation synthetic and naturally-occurring proteinaceous and non-proteinaceous entities. In some embodiments, an agent is nucleic acid, nucleic acid analogues, proteins, antibodies, peptides, aptamers, oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNazymes, glycoproteins, siRNAs, mRNAs, lipoproteins, aptamers, and modifications and combinations thereof, etc. In certain embodiments, agents are small molecule having a chemical moiety. For example, chemical moieties included unsubstituted or substituted alkyl, aromatic, or heterocyclcyl moieties including macrolides, leptomycins, and related natural products or analogues thereof. Compounds can be known to have a desired activity and/or property, or can be selected from a library of diverse compounds.

[0049] The agent can be a molecule from one or more chemical classes, e.g., organic molecules, which may include organometallic molecules, inorganic molecules, genetic sequences, etc. Agents may also be fusion proteins from one or more proteins, chimeric proteins (for example domain switching or homologous recombination of functionally significant regions of related or different molecules), synthetic proteins or other protein variations including substitutions, deletions, insertion, and other variants.

[0050] As used herein, the term “small molecule” refers to a chemical agent which can include, but is not limited to, a peptide, a peptidomimetic, an amino acid, an amino acid analog, a polynucleotide, a polynucleotide analog, an aptamer, a nucleotide, a nucleotide analog, an organic or inorganic compound (e.g., including heterorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

[0051] As used herein, the term “salt” refers to any and all salts, and encompasses pharmaceutically acceptable salts.

[0052] The term “pharmaceutically acceptable salt” refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1-19, incorporated herein by reference.

Pharmaceutically acceptable salts of cholic acid-7-sulfate include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids, such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid or by using other methods known in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium, and $N^+(C_{1-4} \text{ alkyl})_4^-$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate.

[0053] The term “RNAi” as used herein refers to interfering RNA or RNA interference. RNAi refers to a means of selective post-transcriptional gene silencing by destruction of specific mRNA by molecules that bind and inhibit the processing of mRNA, for example inhibit mRNA translation or result in mRNA degradation. As used herein, the term “RNAi” refers to any type of interfering RNA, including but are not limited to, siRNA, shRNA,

endogenous microRNA and artificial microRNA. For instance, it includes sequences previously identified as siRNA, regardless of the mechanism of down-stream processing of the RNA.

[0054] As used herein, the term “antibody” refers to a polypeptide that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence and which specifically binds a given antigen. An antibody reagent can comprise an antibody or a polypeptide comprising an antigen-binding domain of an antibody. In some embodiments of any of the aspects, an antibody reagent can comprise a monoclonal antibody or a polypeptide comprising an antigen-binding domain of a monoclonal antibody. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term “antibody reagent” encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab')₂, Fd fragments, Fv fragments, scFv, CDRs, and domain antibody (dAb) fragments (see, e.g. de Wildt *et al.*, Eur J. Immunol. 1996; 26(3):629-39; which is incorporated by reference herein in its entirety) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, or IgM (as well as subtypes and combinations thereof). Antibodies can be from any source, including mouse, rabbit, pig, rat, and primate (human and non-human primate) and primatized antibodies. Antibodies also include broadly neutralizing antibodies, midibodies, nanobodies, humanized antibodies, chimeric antibodies, and the like.

[0055] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and includes any chain or chains of two or more amino acids. Thus, as used herein, terms including, but not limited to “peptide”, “dipeptide”, “tripeptide”, “protein”, “enzyme”, “amino acid chain”, and “contiguous amino acid sequence” are all encompassed within the definition of a “polypeptide”, and the term “polypeptide” can be used instead of, or interchangeably with, any of these terms. The term further includes polypeptides that have undergone one or more post-translational modification(s), including for example, but not limited to, glycosylation, acetylation, phosphorylation, amidation, derivatization, proteolytic cleavage, post-translation processing, or modification by inclusion of one or more non-naturally occurring amino acids. Conventional nomenclature exists in the art for polynucleotide and polypeptide structures. For example, one-letter and three-letter abbreviations are widely employed to describe amino acids: Alanine (A; Ala), Arginine (R; Arg), Asparagine (N; Asn), Aspartic Acid (D; Asp),

Cysteine (C; Cys), Glutamine (Q; Gln), Glutamic Acid (E; Glu), Glycine (G; Gly), Histidine (H; His), Isoleucine (I; Ile), Leucine (L; Leu), Methionine (M; Met), Phenylalanine (F; Phe), Proline (P; Pro), Serine (S; Ser), Threonine (T; Thr), Tryptophan (W; Trp), Tyrosine (Y; Tyr), Valine (V; Val), and Lysine (K; Lys). Amino acid residues described herein are preferred to be in the “L” isomeric form. However, residues in the “D” isomeric form may be substituted for any L-amino acid residue provided the desired properties of the polypeptide are retained.

[0056] Methods and compositions described herein require that the levels and/or activity cholic acid-7-sulfate, TGR5, and/or GLP-1 are increased. TGR5 as described herein is specifically targeted to increase secretion of GLP-1 from a target cell.

[0057] In some embodiments, the methods increase the activity of G-protein coupled bile acid receptor 1 (GPBAR1, i.e., TGR5).

[0058] As used herein, an “control” refers to an untreated, otherwise identical cell or population (e.g., a subject who was not administered an agent described herein, or was administered by only a subset of agents described herein, as compared to a non-control cell).

[0059] As used herein the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the method or composition, yet open to the inclusion of unspecified elements, whether essential or not.

[0060] The singular terms “a”, “an”, and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, “e.g.” is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation “e.g.” is synonymous with the term “for example”.

BRIEF DESCRIPTION OF THE DRAWINGS

[0061] Figure 1 shows that mice are a suitable model for bariatric surgery-induced amelioration of diabetic phenotypes. Figure 1A shows the glucose levels from sham and sleeve gastrectomy (SG) mice following a glucose tolerance test. Figure 1B shows sham and SG mice glucose levels following insulin tolerance tests. High fat diet (HFD) mice post-sleeve show improved glucose tolerance and insulin sensitivity.

[0062] Figure 2 shows that bile acid profiling reveals significant changes in individual bile acids including cholic acid-7-sulfate in mice post-sleeve. Figure 2 shows that mice 6 weeks

post-sleeve have higher levels of cholic acid-7-sulfate in their cecum compared to sham-operated mice.

[0063] Figure 3 shows that sleeve mice livers also showed increased cholic acid-7-sulfate, chenodeoxycholic acid (CDCA), and taurochenodeoxycholic acid (TCDCA).

[0064] Figure 4 shows that cholic acid-7-sulfate is a TGR5 agonist and induces GLP-1 secretion *in vitro*. Figure 4A shows that sleeve mice show increase in GLP-1 in systemic circulation. Figure 4B shows that cholic acid-7-sulfate induces GLP-1 secretion *in vitro* better than the known GLP-1 inducer taurodeoxy cholic acid (TDCA), while cholic acid had no effect. Figure 4C shows that cholic acid-7-sulfate extracted from cecum of mice also has activity in inducing GLP-1 secretion *in vitro*. Figure 4D shows that cholic acid-7-sulfate activates TGR5 in L-cells, dose response curve shows an EC₅₀ of 0.013 μ M.

[0065] Figure 5 shows that acute cholic acid-7-sulfate treatment induces GLP-1 and reduces serum glucose levels *in vivo*. Figure 5A-B shows that cholic acid-7-sulfate is stable in a wide range of pHs, and has no toxicity in intestinal Caco cells *in vitro*. Figure 5C-D shows that treatment of HFD-fed mice with cholic acid-7-sulfate *in vivo* reduced blood glucose levels and induced GLP-1 levels within 15 min. of treatment. Figure 5E shows that dosing with 1 mg cholic acid-7-sulfate resulted in ~2500 μ M cholic acid-7-sulfate in the cecum, similar to the amounts we saw in sleeve-operated mice. Figure 5F-G shows that ectopic introduction of cholic acid-7-sulfate allowed only minor amounts to leak into systemic circulation and in the portal vein, and did not significantly affect other bile acids in the cecum, blood, or the portal vein. Figure 5H shows that feces from human patients pre- and post-sleeve gastrectomy also have an increase in cholic acid-7-sulfate.

[0066] Figure 6 shows that portal vein bile acids induce synthesis of cholic acid-7-sulfate via SULT2A1 enzyme. Figure 6A shows that livers from mice exhibit an increase in SULT2A1 enzyme isoform 1, previously shown to sulfate bile acids. Figure 6B shows that the portal vein has a different repertoire of bile acids compared to circulating blood. Figure 6C shows that the bile acid pool in the portal vein of sleeve-operated mice significantly induced SULT2A1 compared to the portal vein bile acid pool in sham-operated mice. Figure 6D-E show that there was no difference in induction of SULT2A1 between the pools of bile acids mimicking those observed in the antibiotic-treated sleeve- and sham-operated mouse portal veins. Figure 6D also shows that lithocholic acid (LCA), TDCA, cholic acid (CA), and CDCA were absent in the antibiotic-treated mouse portal veins. Figure 6F shows that LCA induced SULT2A1 in HepG2, while others did not in all concentrations tested. Figure 6G

shows the relative expression of SULT2A of siRNA treated groups. Figure 6H shows the relative expression of Pxr in the liver of Sham and SG mice.

[0067] Figure 7 shows that total bile acids and other bile acids did not differ significantly in cecum of mice operated with sleeve or sham surgery.

[0068] Figure 8 shows that total bile acids and other bile acids did not differ significantly in liver of mice operated with sleeve or sham surgery.

[0069] Figure 9 shows that cholic acid-7-sulfate-mediated induction of GLP-1 requires TGR5. Figure 9A shows that knockdown of TGR5 abolished GLP-1 secretion. Figure 9B shows that cholic acid-7-sulfate increases calcium levels in L-cells *in vitro*. Figure 9C shows that cholic acid-7-sulfate induces TGR5 activation in HEK293T cells.

[0070] Figure 10 shows that ectopic introduction of cholic acid-7-sulfate allowed only minor amounts to leak into systemic circulation and in the portal vein, and did not significantly affect other bile acids in the cecum, blood, or the portal vein.

[0071] Figure 11 shows that ectopic introduction of cholic acid-7-sulfate allowed only minor amounts to leak into systemic circulation and in the portal vein, and did not significantly affect other bile acids in the cecum, blood, or the portal vein.

[0072] Figure 12 shows that ectopic introduction of cholic acid-7-sulfate allowed only minor amounts to leak into systemic circulation and in the portal vein, and did not significantly affect other bile acids in the cecum, blood, or the portal vein.

[0073] Figure 13 shows that human fecal samples post-sleeve have a reduction in levels of secondary bile acids LCA, isolithocholic acid (iso-LCA), and ursodeoxy cholic acid (UDCA), similar to what we observed in mice post-sleeve. Other bile acids and total bile acids were not significantly affected, except for CA levels.

[0074] Figure 14 shows that the portal vein had a very different repertoire of bile acids compared to circulating blood.

[0075] Figure 15 shows that there is no cholic acid-7-sulfate in the liver and approximately 200-fold lower levels of cholic acid-7-sulfate in the cecum in antibiotic-treated mice compared to HFD-fed conventional mice.

[0076] Figure 16 shows TCDCA levels of sham and SG mice.

[0077] Figure 17 shows cholic acid-7-sulfate (CA7S), a bile acid metabolite increased in mice and humans following sleeve gastrectomy, is a TGR5 agonist and induces GLP-1 secretion *in vitro*.

[0078] Figure 18 shows acute CA7S administration induces GLP-1 and reduces serum glucose levels *in vivo*.

[0079] Figure 19 shows CA7S gavage induces GLP-1 and improves glucose tolerance *in vivo* via GLP1 receptor.

[0080] Figure 20 shows diet induced obese (DIO) mice show improved insulin sensitivity and a reduction in percent body weight post-SG.

[0081] Figure 21 shows CA7S activates TGR5, induces GLP-1 secretion, and reduces systemic glucose levels.

[0082] Figure 22 shows GLP-1R shRNA knockdown efficiency and stability of CA7S.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0083] The compositions and methods described herein are related, in part, to the discovery that cholic acid-7 sulfate is increased in subjects following bariatric surgery and ameliorates the symptoms of diabetes. Cholic acid-7 sulfate is a TGR5 agonist and induces GLP-1 secretion *in vivo* and *in vitro*.

Methods of Treatment and Uses

[0084] As generally described herein, provided is a method of treating or preventing diabetes, the method comprising administering to a subject in need thereof an agent that increases the level of cholic acid-7-sulfate in the subject. In one embodiment, the agent is cholic acid-7-sulfate. In one embodiment, the agent is a TGR5 agonist. In one embodiment, the TGR5 agonist is cholic acid-7-sulfate.

[0085] In one embodiment, the TGR5 agonist induces GLP-1 secretion from a target cell. In some embodiments, the activity of TGR5 is increased by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to a control. In some embodiments, the secretion of GLP1 is increased by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to a control.

[0086] In one embodiment, the agent is selected from the group consisting of a small molecule, an antibody, a peptide, a genome editing system, an antisense oligonucleotide, and an RNAi.

[0087] In some embodiments, the agent is a vector that encodes the agent. In one embodiment, the vector is non-integrative or integrative. In one embodiment, the vector is a viral vector.

[0088] In one embodiment, the agent is formulated with a pharmaceutical composition.

[0089] In one embodiment, the pharmaceutical composition is formulated to restrict delivery of an agent to the gastrointestinal tract of the subject.

[0090] In one embodiment, the diabetes is type I, type II, neonatal, or maturity onset diabetes in the young.

[0091] In one embodiment, the administering reduces glucose levels in the serum of a subject.

[0092] In one embodiment, wherein the subject is a mammal.

[0093] In one embodiment, the mammal is a human. In certain embodiments, the human is an adult human.

[0094] In one embodiment, the target cell is an enteroendocrine cell, an epithelial cell, an L-cell, or a neuron. In certain embodiments, the target cell is an immune cell, leukocyte, muscle cell, or an adipocyte.

[0095] In one aspect, described herein is a composition comprising an agent that increases the level of cholic acid-7-sulfate in a subject.

[0096] In one embodiment, wherein the agent is cholic acid-7-sulfate.

[0097] In one embodiment, the composition is formulated for treating or preventing diabetes.

[0098] In one embodiment, the composition further comprises a pharmaceutically acceptable carrier or excipient.

[0099] In one embodiment, the carrier or excipient restricts delivery of the composition to the gastrointestinal tract.

[00100] In one aspect, described herein is a method for treating or preventing diabetes, the method comprising administering to a subject in need thereof a genetically engineered microorganism or population thereof, that expresses an agent that increases the level of cholic acid-7-sulfate.

[00101] In one embodiment, the genetically engineered microorganism is a bacterium.

[00102] In one aspect, described herein is a method for treating or preventing diabetes, the method comprising administering to a subject in need thereof a genetically engineered microorganism or population thereof, that secretes cholic acid-7-sulfate.

[00103] In one embodiment, the genetically engineered microorganism is a bacterium.

[00104] The present disclosure contemplates using cholic acid-7-sulfate for the treatment of diabetes and/or obesity.

[00105] Thus, as generally described herein, provided is a method of treating diabetes and/or obesity comprising administering an effective amount of cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof, to a subject in need thereof. Such a method can be conducted *in vivo* (*i.e.*, by administration to a subject). Treating, as used herein, encompasses therapeutic treatment and prophylactic treatment.

[00106] In some embodiments, the diabetes is type I diabetes, type II diabetes, neonatal diabetes, maturity onset diabetes in the young, or gestational diabetes.

[00107] In some embodiments, the diabetes is type II diabetes.

[00108] Diabetes can cause many complications. Acute complications (*e.g.*, hypoglycemia, ketoacidosis, or nonketotic hyperosmolar coma) may occur if the disease is not adequately controlled. Serious long-term complications (*i.e.*, chronic side effects) include cardiovascular disease (doubled risk), inflammatory diseases, chronic renal failure, retinal damage (which can lead to blindness), nerve damage (of several kinds), and microvascular damage, which may cause impotence and poor wound healing. Poor healing of wounds, particularly of the feet, can lead to gangrene, and possibly to amputation.

[00109] In some embodiments, the diabetes caused by obesity. In one aspect, provided herein is a method of treating obesity in a subject. The term “obesity” refers to excess fat in the body. Obesity can be determined by any measure accepted and utilized by those of skill in the art. Currently, an accepted measure of obesity is body mass index (BMI), which is a measure of body weight in kilograms relative to the square of height in meters. Generally, for an adult over age 20, a BMI between about 18.5 and 24.9 is considered normal, a BMI between about 25.0 and 29.9 is considered overweight, a BMI at or above about 30.0 is considered obese, and a BMI at or above about 40 is considered morbidly obese. (See, *e.g.*, Gallagher et al. (2000) *Am J Clin Nutr* 72:694-701.) These BMI ranges are based on the effect of body weight on increased risk for disease. Some common conditions related to high BMI and obesity include cardiovascular disease, high blood pressure (*i.e.*, hypertension), osteoarthritis, cancer, and diabetes. Although BMI correlates with body fat, the relation between BMI and actual body fat differs with age and gender. For example, women are more likely to have a higher percent of body fat than men for the same BMI. Furthermore, the BMI threshold that separates normal, overweight, and obese can vary, *e.g.*, with age, gender, ethnicity, fitness, and body type, amongst other factors. In some embodiments, a subject with obesity can be a subject with a body mass index of at least about 25 kg/m² prior to administration of a treatment as described herein. In some embodiments, a subject with obesity can be a subject with a body mass index of at least about 30 kg/m² prior to administration of a treatment, compound, or agent as described herein.

[00110] In one aspect, provided herein is a method of treating an inflammatory disease in a subject. As used herein, the term “inflammation” or “inflamed” or “inflammatory” refers to activation or recruitment of the immune system or immune cells (*e.g.*, T cells, B cells, macrophages). A tissue that has inflammation can become reddened, white, swollen, hot,

painful, exhibit a loss of function, or have a film or mucus. Methods of identifying inflammation are well known in the art. Inflammation generally occurs following injury or infection by a microorganism.

[00111] In certain embodiments, the inflammatory disease is Crohn's disease. In certain embodiments, the inflammatory disease is ulcerative colitis. In certain embodiments, the inflammatory disease is pancreatitis. In certain embodiments, the inflammatory disease is hepatitis. In certain embodiments, the inflammatory disease is appendicitis. In certain embodiments, the inflammatory disease is gastritis. In certain embodiments, the inflammatory disease is diverticulitis. In certain embodiments, the inflammatory disease is celiac disease. In certain embodiments, the inflammatory disease is food intolerance. In certain embodiments, the inflammatory disease is enteritis. In certain embodiments, the inflammatory disease is ulcer. In certain embodiments, the inflammatory disease is gastroesophageal reflux disease (GERD). In certain embodiments, the inflammatory disease is psoriatic arthritis. In certain embodiments, the inflammatory disease is psoriasis. In certain embodiments, the inflammatory disease is rheumatoid arthritis.

[00112] In some embodiments, the inflammatory disease is an intestinal inflammatory disease. In certain embodiments, the inflammatory disease is associated with inflammation of the gastrointestinal tract. In certain embodiments, the inflammatory disease is selected from the group consisting of: Crohn's disease, inflammatory bowel disease, ulcerative colitis, pancreatitis, hepatitis, appendicitis, gastritis, diverticulitis, celiac disease, food intolerance, enteritis, ulcer, and gastroesophageal reflux disease (GERD), psoriatic arthritis, psoriasis, and rheumatoid arthritis.

[00113] In certain embodiments, the inflammatory disease is an inflammatory bowel disease. In certain embodiments, the inflammatory bowel disease is Crohn's disease. In certain embodiments, the inflammatory bowel disease is ulcerative colitis.

[00114] In certain embodiments, the inflammatory disease is an autoimmune disease. In certain embodiments, the autoimmune disease is celiac disease.

[00115] In certain embodiments, the effective amount is a therapeutically effective amount. For example, in certain embodiments, the method slows the progression of diabetes and/or obesity in the subject. In certain embodiments, the method improves the condition of the subject suffering from diabetes and/or obesity.

[00116] In certain embodiments, the effective amount is a prophylactically effective amount. For example, in certain embodiments, the method prevents or reduces the likelihood of obesity and/or diabetes, e.g., in certain embodiments, the method comprises administering

CA7S to a subject in need thereof in an amount sufficient to prevent or reduce the likelihood of obesity and/or diabetes. In certain embodiments, the subject is at risk of obesity and/or diabetes.

[00117] As generally described herein, further provided is a method of increasing the amount of cholic acid-7-sulfate in a subject comprising administering an effective amount of cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof, to a subject in need thereof. Such a method can be conducted *in vivo* (i.e., by administration to a subject).

[00118] As generally described herein, further provided is a method of increasing the activity of TGR5 comprising administering an effective amount of cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof, to a subject in need thereof. Such a method can be conducted *in vivo* (i.e., by administration to a subject).

[00119] As generally described herein, further provided is a method of increasing GLP-1 secretion in a subject comprising administering an effective amount of cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof, to a subject in need thereof. Such a method can be conducted *in vivo* (i.e., by administration to a subject).

Pharmaceutical Compositions and Administration

[00120] The present disclosure provides pharmaceutical compositions comprising cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

[00121] Pharmaceutically acceptable excipients include any and all solvents, diluents, or other liquid vehicles, dispersions, suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. General considerations in formulation and/or manufacture of pharmaceutical compositions agents can be found, for example, in *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), and *Remington: The Science and Practice of Pharmacy*, 21st Edition (Lippincott Williams & Wilkins, 2005).

[00122] Pharmaceutical compositions described herein can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the steps of bringing the cholic acid-7-sulfate into association with a carrier and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00123] Pharmaceutical compositions can be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of the pharmaceutical composition comprising a predetermined amount of cholic acid-7-sulfate. The amount of cholic acid-7-sulfate is generally equal to the dosage of cholic acid-7-sulfate which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00124] Relative amounts of cholic acid-7-sulfate, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) cholic acid-7-sulfate.

[00125] Pharmaceutically acceptable excipients used in the manufacture of provided pharmaceutical compositions include inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and perfuming agents may also be present in the composition.

[00126] Liquid dosage forms for oral and parenteral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to cholic acid-7-sulfate, the liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents, and emulsifiers, and mixtures thereof. Besides inert diluents, the oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents. In certain embodiments for parenteral administration, the conjugates are mixed with solubilizing agents, and mixtures thereof.

[00127] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

[00128] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the cholic acid-7-sulfate is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

[00129] Dosage forms for topical and/or transdermal administration of cholic acid-7-sulfate may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants and/or patches. Generally, the cholic acid-7-sulfate is admixed under sterile conditions with a pharmaceutically acceptable carrier and/or any needed preservatives and/or buffers as can be required.

[00130] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with ordinary experimentation.

[00131] Cholic acid-7-sulfate may be formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily amount of CA7S will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disease, disorder, or condition being treated and the severity of the disorder; the specific composition employed; the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the

treatment; drugs used in combination or coincidental with CA7S; and like factors well known in the medical arts.

[00132] Cholic acid-7-sulfate and compositions provided herein can be administered by any route, including enteral (e.g., oral), parenteral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, bucal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent, the therapeutic regimen, and/or the condition of the subject. Oral administration is the preferred mode of administration. However, in certain embodiments, the subject may not be in a condition to tolerate oral administration, and thus intravenous, intramuscular, and/or rectal administration are also preferred alternative modes of administration.

[00133] An effective amount of cholic acid-7-sulfate may be included in a single dose (e.g., single oral dose) or multiple doses (e.g., multiple oral doses). In certain embodiments, when multiple doses are administered to a subject or applied to a tissue or cell, any two doses of the multiple doses include different or substantially the same amounts of CA7S. In certain embodiments, when multiple doses are administered to a subject or applied to a tissue or cell, the frequency of administering the multiple doses to the subject or applying the multiple doses to the tissue or cell is three doses a day, two doses a day, one dose a day, one dose every other day, one dose every third day, one dose every week, one dose every two weeks, one dose every three weeks, or one dose every four weeks. In certain embodiments, a dose (e.g., a single dose, or any dose of multiple doses) described herein includes independently between 0.1 μ g and 1 μ g, between 0.001 mg and 0.01 mg, between 0.01 mg and 0.1 mg, between 0.1 mg and 1 mg, between 1 mg and 3 mg, between 3 mg and 10 mg, between 10 mg and 30 mg, between 30 mg and 100 mg, between 100 mg and 300 mg, between 300 mg and 1,000 mg, or between 1 g and 10 g, inclusive, of CA7S.

[00134] It will be also appreciated that cholic acid-7-sulfate or a composition comprising CA7S, as described herein, can be administered in combination with one or more additional therapeutically active agents. CA7S or a composition comprising CA7S can be administered concurrently with, prior to, or subsequent to, one or more additional therapeutically active agents. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. It will further be appreciated that the additional therapeutically active agent utilized in this combination can be administered together in a single composition

or administered separately in different compositions. The particular combination to employ in a regimen will take into account compatibility of CA7S with the additional therapeutically active agent and/or the desired therapeutic effect to be achieved. In general, it is expected that additional therapeutically active agents utilized in combination be utilized at levels that do not exceed the levels at which they are utilized individually. In certain embodiments, the levels utilized in combination will be lower than those utilized individually.

[00135] In certain embodiments, the additional therapeutic agent is an anti-diabetic agent. In certain embodiments, the anti-diabetic agent is selected from the group consisting of insulin, an insulin analog, nateglinide, repaglinide, metformin, thiazolidinediones, glitazones such as troglitazone, pioglitazone and rosiglitazone, glisoxepid, glyburide, glibenclamide, acetohexamide, chloropropamide, glibornuride, tolbutamide, tolazamide, glipizide, carbutamide, gliquidone, glyhexamide, phenbutamide, tolcyclamide, glimepiride and gliclazide.

[00136] In certain embodiments, the additional pharmaceutical agent is a dipeptidyl peptidase 4 (DPP-4) inhibitor (e.g., sitagliptin, linagliptin, alogliptin, saxagliptin, vildagliptin).

[00137] Also provided herein are uses for cholic acid-7-sulfate in treating or preventing disease (e.g., diabetes, obesity) in a subject in need thereof. In certain embodiments, provided herein is cholic acid-7-sulfate, or a composition comprising CA7S, for use in treating or preventing diabetes in a subject. In certain embodiments, provided herein is cholic acid-7-sulfate, or a composition comprising CA7S, for use in treating or preventing obesity in a subject.

[00138] Also encompassed by this disclosure are kits (e.g., pharmaceutical packs). The kits provided may comprise a pharmaceutical composition, as described herein, or CA7S and a container (e.g., a vial, ampule, bottle, syringe, and/or dispenser package, or other suitable container). In certain embodiments, provided kits may optionally further include a second container comprising a pharmaceutical excipient for dilution or suspension of a pharmaceutical composition or CA7S. In certain embodiments, the pharmaceutical composition or CA7S provided in the container and the second container are combined to form one unit dosage form.

EXAMPLES

[00139] In order that the invention described herein may be more fully understood, the following examples are set forth. The synthetic and biological examples described in this application are offered to illustrate the compounds (e.g., cholic acid-7-sulfate),

pharmaceutical compositions, uses, and methods provided herein and are not to be construed in any way as limiting their scope.

General Procedures

[00140] Animals. Diet induced obese (DIO), male, C57Bl/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME) at 11-16 weeks of age. They were housed under standard conditions in a climate-controlled environment with 12 hour light and dark cycles and reared on a high fat diet (HFD, 60% Kcal fat; RD12492; Research Diets, NJ). They were allowed to acclimate for at least 1 week prior to undergoing any procedures. All animals were cared for according to guidelines set forth by the American Association for Laboratory Animal Science. All procedures were approved by the Institutional Animal Care and Use Committee.

[00141] Sleeve gastrectomy (SG) and sham procedures. 11-week-old DIO mice were purchased and housed as described above. Mice were weight-matched into two groups and either underwent SG or sham operation. SG was performed through a 1.5 cm midline laparotomy under isoflurane anesthesia. The stomach was gently dissected free from its surrounding attachments, the vessels between the spleen and stomach (short gastric vessels) were divided, and a tubular stomach was created by removing 80% of the glandular and 100% of the non-glandular stomach with a linear-cutting surgical stapler. Sham operation consisted of a similar laparotomy, stomach dissection, ligation of short gastric vessels, and manipulation of the stomach along the staple line equivalent. Mice were then individually housed thereafter to allow for monitoring of food intake, weight, and behavior. SG and Sham mice were maintained on Recovery Gel Diet (Clear H₂O, Westbrook, ME) from 1 day prior through 6 days after surgery and then were restarted on HFD on the morning of post-operative day (POD) 7. Mice were sacrificed 5-7 weeks post-surgery.

[00142] Functional glucose testing. After a 4 hour fast (8 am to noon), intraperitoneal glucose tolerance testing (IPGTT) and insulin tolerance testing (ITT) were performed at post-operative week 4 and 5, respectively. During IPGTT, mice received 2 mg/g of intraperitoneal D-Glucose (Sigma-Aldrich, St. Louis, MO) and serum glucose levels were measured from the tail vein at 15, 30, 60, and 120 min with a OneTouch Glucometer (Life technologies, San Diego, CA). ITT was performed by intraperitoneal instillation of 0.6u/kg of regular human insulin (Eli Lilly and Company, Indianapolis, IN) and measurement of serum glucose levels at 15, 30, and 60 min. Baseline glucose was measured for each set prior to medication administration.

[00143] Body weight and food intake measurements. Mice were individually housed and weighed daily for the first post-operative week and then twice weekly until sacrifice. Food intake was measured twice weekly and daily food intake was calculated by averaging the grams eaten per day over the preceding days. Note that food intake measurements were started on POD 10 as animals were transitioned from Gel Diet to high fat diet on the morning of POD 7.

[00144] Bile acid analysis. Bile acid analyses were performed using a previously reported method (Yao, L. *et al.* *eLife* **2018**, 7, 675).

[00145] Reagents. Stock solutions of all bile acids were prepared by dissolving the compounds in molecular biology grade DMSO (VWR International, Radnor, PA). These solutions were used to establish standard curves. CA7S was purchased from (Caymen Chemicals, Ann Arbor, MI. Cat. No. 9002532). Glyocholic acid (GCA) (Sigma) was used as the internal standard for measurements in mouse tissues. HPLC grade solvents were used for preparing and running UPLC-MS samples.

[00146] Extraction. Cecal, liver, and human fecal samples (approximately 50 mg each) and mouse portal veins were pre-weighed in lysis tubes containing ceramic beads (Precellys lysing kit tough micro-organism lysing VK05 tubes for cecal, fecal samples, and portal veins; tissue homogenizing CKMix tubes for liver samples; Bertin technologies, Montigny-le-Bretonneux, France). 400 µL of methanol containing 10 µM internal standard (GCA) was added and the tubes were homogenized in a MagNA Lyser (6000 speed for 90 s*2, 7000 speed for 60 s). 50 µl of mouse serum was collected in Eppendorf tubes, followed by addition of 50 µL of methanol containing 10 µM internal standard (GCA). After vortexing for 1 min, the sample was cooled to -20 °C for 20 min. All methanol-extracted tissue samples were centrifuged at 4 °C for 30 min at 15,000 rpm. The supernatant was diluted 1:1 in 50% methanol/water and centrifuged again at 4 °C for 30 min at 15000 rpm. The supernatant was transferred into mass spec vials and injected into the UPLC-MS.

[00147] UPLC-MS. Samples were injected onto a Phenomenex 1.7 µm, C18 100 Å, 100 × 21 mm LC column at room temperature and eluted using a 30 min gradient of 75% A to 100% B (A = water + 0.05% formic acid; B = acetone + 0.05% formic acid) at a flow rate of 0.350 mL/min. Samples were analyzed using an Agilent Technologies 1290 Infinity II UPLC system coupled online to an Agilent Technologies 6120 Quadrupole LC/MS spectrometer in negative electrospray mode with a scan range of 350–550 m/z (MSD settings: fragmentor - 250, gain - 3.00, threshold - 150, Step size - 0.10, speed (u/sec) - 743). Capillary voltage was 4500 V, drying gas temperature was 300°C, and drying gas flow was 3 L/min. Analytes were

identified according to their mass and retention time. For quantification of the analytes, standard curves were obtained using known bile acids, and then each analyte was quantified based on the standard curve and normalized based on the internal standard. The limit of detection for CA7S is 0.05 picomol/ μ L. Note that CA7S and cholic acid-3-sulfate can be distinguished based on retention time using this UPLC-MS method.

[00148] Purification of CA7S. Extracted cecal contents from 11 SG mice (same shown in Figure 1) were pooled to provide sufficient material for purification. Pooled extract was purified via MS-guided HPLC of m/z 487 using a Luna RP C18 semi-preparative column and water and acetonitrile with 0.1% formic acid as an additive.

[00149] NMR Spectroscopy. CA7S and purified m/z 487 (<1 mg) were dissolved in 250 μ L DMSO- d_6 . Nuclear magnetic resonance (NMR) spectra were acquired on a Varian INOVA 500 MHz and are referenced internally according to residual solvent signals (DMSO to 2.50, HOD to 3.33).

[00150] Cell culture. NCI-H716 cells and Caco-2 cells were obtained from American Type Culture Collection (Manassas, VA). HEK-293T cells were a kind gift from the Blacklow lab (BCMP, HMS). Caco-2 and HEK-293T cells were maintained in Minimum Essential Medium (MEM) with GlutaMAX and Earle's Salts (Gibco, Life Technologies, UK). NCI-H716 cells were maintained in RPMI 1640 with L-glutamine (GenClone, San Diego, CA). All cell culture media were supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μ g/ml streptomycin (GenClone). Cells were grown in FBS- and antibiotic-supplemented 'complete' media at 37 °C in an atmosphere of 5% CO₂.

[00151] *In vitro* bile acid treatments. NCI-H716 cells were seeded in cell culture plates coated with Matrigel (Corning, NY. Cat. No. 356234) diluted in Hank's Balanced Salt Solution (HBSS, Gibco) according to manufacturer's instructions. The cells were allowed to grow for 2 days in complete RPMI media. On the day of the treatment, cells were rinsed gently with low serum (0.5% FBS) RPMI 1640 medium without antibiotics. Bile acids cholic acid-7-sulfate (CA7S), cholic acid (CA) (Sigma) and taurodeoxycholic acid (TDCA) (Sigma) were diluted in dimethyl sulfoxide (DMSO, VWR International) and added to cells in the low serum media (0.5% FBS, RPMI 1640) without antibiotics. The concentration of DMSO was kept constant throughout the treatments and used as a negative control. Cells were incubated at 37°C in an atmosphere of 5% CO₂ for 2 hours. After the incubation period, cell culture media was collected in Eppendorf tubes containing 1% trifluoroacetic acid (TFA, Sigma) in sterile purified water (GenClone) to make a final TFA concentration of 0.1% and frozen at -80 °C for further GLP-1 measurements. Cells on cell culture plates were placed on ice and

gently washed with PBS (GenClone). Cells used for GLP-1 measurements were treated with ice-cold cell lysis solution of 1% TFA, 1N hydrochloric acid, 5% formic acid, and 1% NaCl (all from Sigma), scraped off of the Matrigel coating, and collected in lysing tubes with ceramic beads (Precellys lysing kit tough micro-organism lysing VK05 tubes). For calcium measurements, PBS was added to cells, and were collected in lysing tubes containing ceramic beads (Precellys lysing kit tough micro-organism lysing VK05 tubes). Cells were thereafter lysed in a MagNA Lyser and stored at -80 °C for further analysis. Cells used for RNA extraction were treated with TRIzol (Ambion, Life Technologies, Thermo Fisher Scientific, Waltham, MA) and stored at -80 °C for further analysis.

[00152] GLP-1 and Insulin measurements. Total GLP-1 peptide measurements were performed using the GLP-1 EIA Kit (Sigma, Cat. No. RAB0201) and total insulin levels were measured using the Mouse Ins1/Insulin-1 ELISA kit (Sigma, Cat. No. RAB0817) according to manufacturer's instructions. Mouse serum samples, NCI-H716 cell lysates, and cell culture media samples were stored at -80 °C and thawed on ice prior to performance ELISA assay. 20 µl of mouse serum samples were used directly in the GLP-1 ELISA assay, while 50 µl of mouse serum samples were used directly in the Insulin ELISA assay. Cell culture media were centrifuged at 12000 rpm, and the supernatant was directly used in the GLP-1 ELISA assay. Cell lysates were subjected to peptide purification using Sep Pak C18 Classic columns (Waters Corporation, Milford, MA). The column was pretreated with a solution of 0.1% TFA in 80% isopropyl alcohol (EMD Millipore) and equilibrated with 0.1% TFA in water. Cell lysates were loaded onto the column and washed with 0.1% TFA in 80% isopropyl alcohol. The peptides were eluted in 0.1% TFA in water. The eluate was concentrated by drying under vacuum and resuspended in 0.1% TFA in water. Water was used as 'blank' reading for serum GLP-1 ELISA, while 0.1% TFA in water was used as 'blank' for cell culture media and purified cell lysate ELISAs. Excess samples were stored at -80°C for later analyses. Total GLP-1 amounts in the cell culture media (secreted) and cell lysates were calculated using a standard curve provided in the EIA kit. Percentage GLP-1 secretion was calculated as follows: % GLP-1 secretion = total GLP-1 secreted (media) / (total GLP-1 secreted (media) + total GLP-1 in cell lysates) * 100. Relative GLP-1 secretion was calculated compared to DMSO control.

[00153] Plasmids and transient transfections. Human TGR5 was cloned using cDNA from human Caco-2 cells as template and a forward primer with an *EcoRI* restriction-site (5'-CGGAATTCGCACTTGGTCCTTGTGCTCT-3') and a reverse primer with a *XhoI*-site (5'-GTCTCGAGTTAGTTCAAGTCCAGGTCGA-3'). The PCR product was cloned into the

pCDNA 3.1+ plasmid (Promega Corporation, Madison, WI) and transfected at a concentration of 0.4 µg/ml of media. For luciferase reporter assays for TGR5 activation, the pGL4.29[luc2P/CRE/Hygro] plasmid (Promega Corporation), and the pGL4.74[hRluc/CMV] plasmid (Promega Corporation) were used at concentration of 2 µg/ml and 0.05 µg/ml of media respectively. All plasmids were transfected using Opti-MEM (Gibco) and Lipofectamine 2000 (Invitrogen, Life Technologies, Grand Island, NY, USA) according to manufacturer's instructions. Plasmid transfection were performed in antibiotic-free media (MEM for HEK293T and RPMI for Matrigel-attached NCI-H716 cells) with 10% FBS. After overnight incubation, bile acids were added in complete media and incubated overnight. Cells were harvested the next day for luciferase assay. TGR5 siRNA (Santa Cruz Biotechnology, Dallas, TX) and negative siRNA (Ambion) transfection was performed using Opti-MEM and Lipofectamine 2000 according to manufacturer's instructions. After siRNA transfection, cells were incubated in antibiotic- and serum-free media (RPMI for Matrigel-attached NCI-H716 cells) for 24 hours. The next day, the media was replaced by complete media and incubated overnight. Bile acids were added 48 hours post-siRNA transfection in complete media and incubated overnight. Cells were harvested the next day for luciferase assay or RNA extraction.

[00154] Luciferase reporter assay. Luminescence was measured using the Dual-Luciferase Reporter Assay System (Promega Corporation) according to manufacturer's instructions. Cells were washed gently with PBS and lysed in PLB from the kit. Matrigel-attached cells were scraped in PLB. Luminescence was measured using the SpectraMax M5 plate reader (Molecular Devices, San Jose, CA) at the ICCB-Longwood Screening Facility at Harvard Medical School. Luminescence was normalized to *Renilla* luciferase activity and percentage relative luminescence was calculated compared to DMSO control.

[00155] Calcium measurement. CA7S-treated NCI-H716 cells collected in PBS were used to measure intracellular calcium using the Calcium Assay Kit (Fluorometric) (Abcam, UK). Cell lysates were centrifuged at 12000 rpm, and the supernatant was directly used in the calcium assay according to manufacturer's instructions. Fluorescence was measured using the SpectraMax M5 plate reader (Molecular Devices, San Jose, CA) at the ICCB-Longwood Screening Facility at HMS. Percentage relative fluorescence was calculated compared to DMSO control.

[00156] Cell viability assay. Caco-2 cells were treated with CA7S diluted in DMSO in complete MEM media. The concentration of DMSO was kept constant and used as a negative control. Cells were incubated with CA7S overnight at 37 °C in an atmosphere of 5% CO₂.

The next day, cells were treated with 0.25% trypsin in HBSS (GenClone) for 10 min at 37°C. Cell viability was measured in Countess II automated cell counter (Invitrogen). Percentage relative viability was calculated compared to DMSO control.

[00157] pH stability test. Stability of CA7S in physiological pH's was determined using the Waters pH stability test. Briefly, buffers of pH 1 (0.1 M HCl), pH 7.4 (PBS) and pH 9 (a 10 mM solution of ammonium formate adjusted to pH 9 with ammonium hydroxide) (all from Sigma) were prepared. CA7S was incubated in the pH buffers overnight at 37 °C with gentle shaking (50 rpm). The next day, the CA7S solution was diluted in methanol, transferred into mass spec vials and injected into the UPLC-MS.

[00158] RNA extraction and qPCR. Cells frozen in TRIzol (Ambion) were collected in RNase-free Eppendorf tubes and vortexed for 30 seconds. Tissues were collected in Precellys tubes with ceramic beads and TRIzol, followed by homogenization in a MagNA Lyser (Roche, Switzerland). Tubes were kept on ice whenever possible. Chloroform was added (200µl chloroform/1ml TRIzol) and vortexed for 30 seconds. Tubes were centrifuged at 12,000 rpm for 15 min at 4 °C. The clear top layer was transferred to new RNase-free Eppendorf tubes containing 2-propanol and inverted to mix (500µl 2-propanol/1ml TRIzol). Tubes were centrifuged at 12,000 rpm for 10 min at 4 °C. The pellet was washed with 70% EtOH and centrifuged at 14,000rpm for 5 minutes at 4 °C. The RNA pellet was air-dried and resuspended in RNase-free H₂O (GenClone). cDNA synthesis was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Invitrogen, Foster City, CA). qPCR was performed using the Lightcycler 480 SYBR Green I Mater (Roche, Switzerland) in a 384-well format using a LightCycler 480 System (Roche) at the ICCB-Longwood Screening Facility at Harvard Medical School. The $2^{-\Delta\Delta C_t}$ method was used to calculate the relative change in gene expression. Human *TGR5* gene expression were normalized to the human *HPRT1* (*HGPRT*). Mouse *GLP-1R* gene expression was normalized to 18S. Primer sequences were:

human *TGR5*: Forward: 5'-CCTAGGAAGTGCCAGTGCAG-3', Reverse: 5'-CTTGGGTGGTAGGCAATGCT-3'; human *HGPRT*: Forward: 5'-CCTGGCGTCGTGATTAGTGA-3', Reverse: 5'-CGAGCAAGACGTTTCAGTCCT-3'; mouse *GLP-1R*: Forward: 5'-AGGGCTTGATGGTGGCTATC -3', Reverse: 5'-GGACACTTGAGGGGCTTCAT -3'; mouse 18S: Forward: 5'-ATTGGAGCTGGAATTACCGC-3', Reverse: 5'-CGGCTACCACATCCAAGGAA-3'.

[00159] *In vivo* enteral treatment with CA7S. 13-week-old male C57Bl/6J mice were purchased, acclimated, and housed as above. They were weight matched into two groups

($p=0.88$). After an overnight fast (17:00 to 0800), mice received either CA7S or PBS via direct duodenal and rectal administration. The optimal, physiologic dose of CA7S was extrapolated from the average pmol concentration of CA7S found in cecal samples from SG animals (average of 3000 pmol/mg of stool with 500 mg of stool per animal corresponds to 0.75 mg of CA7S per cecum).

[00160] Under isoflurane general anesthesia, 0.25 mg and 0.75 mg of CA7S in PBS (pH 7.2) was delivered by slow infusion (5 min) antegrade into the duodenum and retrograde into the rectum, respectively. The total volume of instillation was 2 mL (0.5 mg CA7S/mL). Control animals received similar volumes of PBS alone. 15 min post infusion, serum glucose was measured via tail vein followed by whole blood collection via cardiac puncture into K+EDTA tubes containing DPPIV inhibitor (Merck Millipore, Billerica, MA), Perfabloc (Sigma), and apoprotinin (Sigma). Organs were harvested for analysis. In order to account for changes in fasting times and hormonal diurnal rhythms, this experiment was carried out on four consecutive days such that only four mice were tested per day.

[00161] *In vivo* CA7S gavage. 16-week-old DIO mice were purchased and housed as described above. Mice were gavaged orally with 100 mg/kg CA7S from 20 mg/mL solution, or equivalent volume of PBS using 20G x 38mm gavage needle. 5 hours after CA7S/PBS administration, whole blood and intestinal segments were collected.

[00162] *In vivo* CA7S and OGTT. Age matched, DIO mice were kept on HFD and their blood glucose levels were monitored until average fasting glucose levels were >160 mg/dL. Animals were fasted for 4 hours on the day of the experiment. Mice were matched into two groups based on fasting glucose levels and received either 100 mg/kg CA7S from a 20 mg/mL solution or an equivalent volume of PBS by oral gavage. Three hours later, an OGTT was performed using an oral gavage of 2 mg/g oral D-glucose (Sigma-Aldrich, St. Louis, MO). Blood glucose levels were measured at baseline and at minutes 15, 30, 60 and 120 with a OneTouch glucometer.

[00163] Lentiviral IP injection. GLP-1R shRNA-containing lentiviral particles (LVP) were purchased from the MISSION TRC library (Sigma-Aldrich, St. Louis, MO). LVPs containing a mixture of three GLP-1R shRNA plasmid clones (TRCN0000004629, TRCN0000004630, and TRCN0000004633) were purchased, stored at -80 °C, and thawed on ice before use. DIO mice were maintained on a HFD until average fasting glucose >160 mg/dL in a BL2 facility. Under sterile conditions, mice were injected intraperitoneally with 0.2 mL of 5×10^5 GLP-1R shRNA LVPs with a 27G needle (Tiscornia, G., *et al.* PNAS **2003**, 100, 1844–1848; Blosser, W. *et al.* PLOS ONE **2014**, 9, e96036). 72 hours after LVP injection, mice underwent

CA7S/PBS gavage followed by OGTT as above. After the OGTT was completed, mice were sacrificed and their tissues were harvested. GLP-1R knock-down efficiency was measured in tissues by qPCR as described above.

[00164] Human stool collection. After obtaining institutional review board approval, we prospectively collected stool specimen from obese human subjects undergoing SG. Pre-operative stool specimens were collected on the day of surgery and post-operative stool specimen were obtained from post-operative day 14 to 99 (mode 15 days; median 36 days). Specimens were snap frozen in liquid nitrogen and stored at -80 °C until bile acid analysis was performed (as above).

Example 1. Glucose and Bile acid profiles post-gastric sleeve surgery mouse model

[00165] The mouse model described herein is used to study the amelioration of diabetic phenotypes post-sleeve surgery. Mice are suitable model for bariatric surgery-induced amelioration of diabetic phenotypes. High fat diet-fed mice post-sleeve show improved glucose tolerance and insulin sensitivity (**Figure 1A and 1B**) consistent with what has been observed before in humans.

[00166] Bile acid profiling was performed and revealed significant changes in individual bile acids in mice post-sleeve. Mice 6 weeks post-sleeve have higher levels of cholic acid-7-sulfate in their cecum compared to sham-operated mice (**Figure 2**). It was confirmed that the molecule in the bile acid was cholic acid-7-sulfate by NMR. Furthermore, mice post-sleeve have lower levels of secondary bile acid LCA and components of the “CDCA pathway” including CDCA, TCDCA, and iso-LCA in their cecum (**Figure 2**).

[00167] The total bile acids and other bile acids did not differ significantly in cecum of mice operated with sleeve or sham surgery (**Figure 7**). Sleeve mice livers showed increased cholic acid-7-sulfate, CDCA, and TCDCA (**Figure 3**). However, total bile acids and other bile acids did not differ significantly in liver of mice operated with sleeve or sham surgery (**Figure 8**).

Example 2. Increased GLP1 and TGR5 activation with cholic acid-7-sulfate

[00168] It was observed that sleeve mice show increase in GLP-1 in systemic circulation (**Figure 4A**). Cholic acid-7-sulfate induces GLP-1 secretion in vitro better than the known GLP-1 inducer TDCA, while cholic acid had no effect (**Figure 4B and Figure 9**).

[00169] To identify a particular target of cholic acid-7-sulfate, it was discovered that cholic acid-7-sulfate-mediated induction of GLP-1 and requires TGR5. This was confirmed when

knockdown of TGR5 abolished GLP-1 secretion (**Figure 4B and Figure 9A**). Therefore, cholic acid-7-sulfate is a TGR5 agonist and induces GLP-1 secretion in vitro.

[00170] To further investigate this mechanism, cholic acid-7-sulfate was extracted from cecum of mice and found to also exhibit activity inducing GLP-1 secretion in vitro (**Figure 4C**). Cholic acid-7-sulfate activates TGR5 in L-cells, dose response curve shows an EC₅₀ of 0.013 μ M (**Figure 4D**). Cholic acid-7-sulfate increased calcium levels in L-cells in vitro (**Figure 9B**). Cholic acid-7-sulfate induces TGR5 activation in HEK293T cells (**Figure 9C**).

[00171] Cholic acid-7-sulfate is stable in a wide range of pHs, and has no toxicity in intestinal Caco cells *in vitro* (**Figure 5A and 5B**). Treatment of HFD-fed mice with cholic acid-7-sulfate in vivo reduced blood glucose levels and induced GLP-1 levels within 15 min. of treatment (**Figure 5C and 5D**). Therefore, acute cholic acid-7-sulfate treatment induces GLP-1 and reduces serum glucose levels in vivo. Dosing with 1 mg cholic acid-7-sulfate resulted in ~2500 μ M cholic acid-7-sulfate in the cecum, similar to the amounts we saw in sleeve-operated mice (**Figure 5E**). Ectopic introduction of cholic acid-7-sulfate allowed only minor amounts to leak into systemic circulation and in the portal vein, and did not significantly affect other bile acids in the cecum, blood, or the portal vein (**Figure 5F and 5G, Figure 10, 11, and 12**). Feces from human patients pre- and post-sleeve gastrectomy also have an increase in cholic acid-7-sulfate (**Figure 5H**).

[00172] Human fecal samples post-sleeve have a reduction in levels of secondary bile acids LCA, iso-LCA, and UDCA, similar to what was observed in mice post-sleeve (**Figure 13**). Other bile acids and total bile acids were not significantly affected, except for calcium levels. (**Figure 13**).

Example 3. SULT2A induction and bile acid profile in liver and blood

[00173] Sulfation is a detoxification method to excrete toxic bile acids. Bile acids have been shown to tightly regulate their own synthesis, conjugation, and sulfation. The liver is the major site for synthesis and sulfation of bile acids, therefore bile acids in the hepatic portal vein were analyzed to determine the origin of sulfated cholic acid and a mechanism for the increase in cholic acid-7-sulfate in sleeve mice. The hepatic portal vein is part of the enterohepatic circulation of bile acids. The liver receives 80% of its blood from the hepatic portal vein. The portal vein has a different repertoire of bile acids compared to circulating blood (**Figure 6B & Figure 14**).

[00174] Mice livers show an increase in SULT2A enzyme isoform 1, previously shown to sulfate bile acids (**Figure 6A**).

[00175] To not be bound by a particular theory, it was hypothesized that bile acids in the hepatic portal vein signal in the liver to induce sulfation of cholic acid. Pools of bile acids were tested mimicking those observed in the sleeve- and sham-operated mouse portal veins in inducing SULT2A1 in vitro. Using HepG2 cells, it was observed that the bile acid pool in the portal vein of sleeve-operated mice significantly induced SULT2A1 compared to the portal vein bile acid pool in sham-operated mice (**Figure 6C**).

[00176] Bile acids are modified in the intestine by the microbiome. Therefore, the influence of the microbiome in inducing sulfation of bile acids in the liver was tested. Sleeve gastrectomy was performed and sham surgery on HFD-fed mice treated with antibiotics. Pools of bile acids mimicking those observed in the antibiotic-treated sleeve- and sham-operated mouse portal veins were tested inducing SULT2A1 in HepG2 cells. There was no difference in induction of SULT2A1 between the pools observed (**Figure 6D and 6E**).

[00177] Consistently, it was observed that there was no cholic acid-7-sulfate in the liver and approximately 200-fold lower levels of cholic acid-7-sulfate in the cecum in antibiotic-treated mice (**Figure 14 and Figure 15**) compared to HFD-fed conventional mice. Also, there was no significant difference in cholic acid-7-sulfate levels between antibiotic-treated sleeve- and sham-operated mouse cecum (**Figure 9**). This suggests that a microbiome is required for sulfation of cholic acid. In support of this hypothesis, germ-free animals fed a high fat diet also show 200-fold lower cholic acid-7-sulfate in their cecum (**Figure 9**).

[00178] To test which bile acid(s) may be involved in inducing SULT2A1 enzyme, the bile acids in the portal vein that were significantly different between HFD-fed conventional mice and HFD-fed mice treated with antibiotics were analyzed. It was observed that LCA, TDCA, CA, and CDCA were absent in the antibiotic-treated mouse portal veins (**Figure 6D**).

[00179] Amongst these, LCA induced SULT2A1 in HepG2, while others did not in all concentrations tested (**Figure 6F**). LCA levels were also increased in sleeve mice compared to sham-operated, while the total bile acid levels did not differ significantly, suggesting that LCA is an inducer of SULT2A1 expression (**Figure 6B**). To identify the receptor involved in LCA-mediated induction of SULT2A1 in liver cells, siRNA of known receptors was performed. The PXR receptor was consistently upregulated in mice post-sleeve in the liver (**Figure 6G and 6H**).

Example 4. Identification of cholic acid-7-sulfate

[00180] The individual bile acids in cecal contents of post-gastric sleeve (SG) and sham mice were quantified using UPLC-MS. A significant increase in a monosulfated, trihydroxy BA in

cecal contents of SG mice was observed. Using NMR spectroscopy, the compound was identified as cholic acid-7-sulfate (CA7S) (**Figure 17D and 17E**). This molecule is a sulfated metabolite of cholic acid (CA), which is an abundant primary BA in both mice and humans. Sulfation of BAs predominantly occurs in the liver (Alnouti, Y. *Toxicol. Sci.* **2009**, 108, 225–246). Increased levels of CA7S in the liver of SG mice were also found (**Figure 17F**). CA7S was the only BA detected whose levels were significantly higher in SG mouse livers and cecal contents.

[00181] Bile acids in stool from human patients who had undergone SG were quantified. Fecal CA7S levels were also significantly increased in patients post-SG compared to their pre-surgery levels (**Figure 1G**).

Example 5. CA7S activation of human TGR5 in HEK293T cells

[00182] The activation of human TGR5 was examined in human embryonic kidney cells (HEK293T) by CA7S, CA, or tauro-deoxycholic acid (TDCA) which is a naturally occurring BA and potent TGR5 agonist (Brighton, C. A. *et al.* *Endocrinology* **2015** 156, 3961–3970). It was found that CA7S activated human TGR5 in a dose-dependent manner and to a similar extent as TDCA. CA7S also displayed a lower EC₅₀ (0.17 μ M) than CA (12.22 μ M) (**Figure 17H**).

[00183] CA7S induced GLP-1 secretion in human intestinal L-cells (NCI-H716) to a similar degree as TDCA in a dose-dependent manner, while CA had no effect on GLP-1 secretion (**Figure 17I and 21A**). CA7S extracted directly from cecal contents of SG mice also induced GLP-1 secretion in vitro (**Figure 21B**). Furthermore, siRNA-mediated knockdown of TGR5 abolished both CA7S and TDCA-mediated secretion of GLP-1 (**Figure 17I, 21A, and 21C**). This result indicates that induction of GLP-1 secretion by CA7S requires TGR5. TGR5 agonism also results in elevated intracellular calcium levels (Kuhre, R. E. *et al.* *Journal of Molecular Endocrinology* **2016**, 56, 201–211). Consistent with this previous finding, we observed a dose-dependent increase in calcium levels in NCI-H716 cells treated with CA7S (**Figure 21D**). Taken together, these results demonstrate that CA7S, a naturally occurring BA metabolite, is a potent TGR5 agonist and GLP-1 secretagogue.

Example 6. Evaluation of acute anti-diabetic effects in vivo

[00184] DIO mice were treated with either CA7S or PBS via duodenal and rectal catheters (**Figure 18A**). Administration of 1 mg of CA7S resulted in an average of 2500 pmol/mg wet

mass of CA7S in cecal contents, a concentration similar to observed post-SG levels (**Figure 17E, 18B**, Table 1).

Table 1. Cholic acid-7-sulfate concentration in indicated tissues and blood

Treatment	Tissue/blood	CA7S concentration (mean $\pm$ SEM)
DIO mice; sham surgery	Cecum	1726 $\pm$ 267 pmol/mg
	Liver	0.12 $\pm$ 0.04 pmol/mg
	Portal vein	n.d.
	Systemic blood	n.d.
DIO mice; sleeve gastrectomy	Cecum	2661 $\pm$ 331 pmol/mg
	Liver	0.27 $\pm$ 0.04 pmol/mg
	Portal vein	n.d.
	Systemic blood	n.d.
DIO mice; enteral PBS	Cecum	161.1 $\pm$ 46.4 pmol/mg
	Portal vein	0.07 $\pm$ 0.06 pmol/mg
	Systemic blood	n.d.
DIO mice; enteral CA7S	Cecum	2577 $\pm$ 185 pmol/mg
	Portal vein	6.13 $\pm$ 2.11 pmol/mg
	Systemic blood	0.5 $\pm$ 0.2 pmol/ μ l
DIO mice; PBS gavage	Cecum	947 $\pm$ 349 pmol/mg
	Portal vein	n.d.
	Systemic blood	n.d.
DIO mice; CA7S gavage	Cecum	14345 $\pm$ 1451 pmol/ μ l
	Portal vein	13.2 $\pm$ 7.7 pmol/ μ l
	Systemic blood	n.d.

n.d. not detected, all data are presented as mean $\pm$ SEM.

[00185] CA7S-treated mice displayed increased systemic GLP-1 levels compared to PBS-treated mice within 15 minutes (**Figure 18C**). Moreover, CA7S-treated mice exhibited reduced blood glucose levels and increased insulin levels compared to PBS-treated mice (**Figure 18D, 18E, and 21E**). GLP-1-producing enteroendocrine L-cells are enriched in the distal compared to the proximal gut (Eissele, R. *et al.* Eur. J. Clin. Invest. **1992** 22, 283–291; Harach, T. *et al.* Sci Rep **2012**, 2, 430). It was observed that TGR5 expression was increased in the colon, but not the terminal ileum, of CA7S-treated mice (**Figure 18F**). Without wishing to be bound by any particular theory, these results may suggest that in an acute

setting, distal action of CA7S in the GI tract induces systemic glucose clearance and thus ameliorates hyperglycemia.

Example 7. Antidiabetic effects of CA7S over prolonged periods

[00186] DIO mice were orally gavaged with CA7S at a dose of 100 mg/kg (**Figure 19A**). Analysis of cecal contents 5 hours post-gavage showed an accumulation of 15,000 picomol/mg wet mass of CA7S (mean value, **Figure 19B**), a concentration that is within an order of magnitude of the mean amount measured in post-SG mice. These data indicate that we had administered a physiologically relevant concentration of this metabolite. Systemic levels of GLP-1 were increased in CA7S-gavaged mice compared to PBS-treated mice 5 hours post-treatment (**Figure 19C**). This result is consistent with the findings from enteral administration and demonstrates that oral CA7S treatment can increase circulating GLP-1 for several hours.

The effect of CA7S on glucose tolerance over time was also determined using an oral glucose tolerance test (OGTT). DIO mice were gavaged with CA7S (100 mg/kg) or PBS and then administered an oral glucose bolus 3 hours later. CA7S treatment resulted in an increased rate of blood glucose clearance (**Figure 19D**). Moreover, the total and incremental areas under the glucose versus time curves (AUC and iAUC) were significantly decreased in CA7S-compared to vehicle-treated mice (**Figure 19E**).

Example 8. Dependency of anti-diabetic effects of CA7S on GLP-1

[00187] Lentiviral shRNA-mediated knockdown of GLP-1R was carried out in vivo. DIO mice were injected intraperitoneally with 5×10^5 shRNA lentiviral particles targeting GLP-1R. At day 3 post-injection, expression of GLP-1R in the intestines, heart, liver, and stomach was significantly reduced, and importantly, the expression of GLP-1R was undetectable in the pancreas (**Figure 22A**). Mice were then gavaged with CA7S (100 mg/kg) or PBS and subjected to an OGTT 3 hours post-gavage. There were no significant differences in the glycemic curves or AUCs between CA7S-treated and PBS-treated mice in the absence of GLP-1R, suggesting that in an acute setting, the blood glucose clearing-effects of CA7S are largely dependent on GLP-1 (**Figure 19F and 19G**).

EQUIVALENTS AND SCOPE

[00188] In the claims articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions

that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[00189] Furthermore, the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Where elements are presented as lists, e.g., in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements and/or features, certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements and/or features. For purposes of simplicity, those embodiments have not been specifically set forth *in haec verba* herein. It is also noted that the terms “comprising” and “containing” are intended to be open and permits the inclusion of additional elements or steps. Where ranges are given, endpoints are included. Furthermore, unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub-range within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00190] This application refers to various issued patents, published patent applications, journal articles, and other publications, all of which are incorporated herein by reference. If there is a conflict between any of the incorporated references and the instant specification, the specification shall control. In addition, any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Because such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the invention can be excluded from any claim, for any reason, whether or not related to the existence of prior art.

[00191] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments described herein. The scope of the present embodiments described herein is not intended to be limited to the above Description, but rather is as set forth in the appended claims. Those of ordinary skill in the art will appreciate that various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following claims.

CLAIMS

What is claimed is:

1. A method for treating or preventing diabetes, the method comprising: administering to a subject in need thereof an agent that increases the level of cholic acid-7-sulfate in the subject.
2. The method of any preceding claim, wherein the agent is cholic acid-7-sulfate.
3. The method of any preceding claim, wherein the agent is a TGR5 agonist.
4. The method of any preceding claim, wherein the TGR5 agonist induces GLP-1 secretion from a target cell.
5. The method of any preceding claim, wherein the agent is selected from the group consisting of a small molecule, an antibody, a peptide, a genome editing system, an antisense oligonucleotide, and an RNAi.
6. The method of any preceding claim, wherein the agent is formulated with a pharmaceutical composition.
7. The method of any preceding claim, wherein the pharmaceutical composition is formulated to restrict delivery of an agent to the gastrointestinal tract of the subject.
8. The method of any preceding claim, wherein the diabetes is type I, type II, neonatal, or maturity onset diabetes in the young.
9. The method of any preceding claim, wherein the administering reduces glucose levels in the serum of a subject.
10. The method of any preceding claim, wherein the subject is a mammal.
11. The method of any preceding claim, wherein the mammal is a human.

12. The method of any preceding claim, wherein the target cell is an enteroendocrine cell, an epithelial cell, an L-cell, or a neuron.
13. A composition comprising an agent that increases the level of cholic acid-7-sulfate in a subject.
14. The composition of any preceding claim, wherein the agent is cholic acid-7-sulfate.
15. The composition of any preceding claim, wherein the composition is formulated for treating or preventing diabetes.
16. The composition of any preceding claim, wherein the composition further comprises a pharmaceutically acceptable carrier or excipient.
17. The composition of any preceding claim, wherein the carrier or excipient restricts delivery of the composition to the gastrointestinal tract.
18. A method for treating or preventing diabetes, the method comprising: administering to a subject in need thereof a genetically engineered microorganism or population thereof, that expresses an agent that increases the level of cholic acid-7-sulfate.
19. The method of any preceding claim, wherein the genetically engineered microorganism is a bacterium.
20. A method for treating or preventing diabetes, the method comprising: administering to a subject in need thereof a genetically engineered microorganism or population thereof, that secretes cholic acid-7-sulfate.
21. The method of any preceding claim, wherein the genetically engineered microorganism is a bacterium.

22. The method of any preceding claim, wherein the activity of TGR5 is increased by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to a control.
23. The method of any preceding claim, wherein the secretion of GLP1 is increased by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to a control.
24. The method of any preceding claim, wherein the agent is a vector that encodes the agent.
25. The method of any preceding claim, wherein the vector is non-integrative or integrative.
26. The method of any preceding claim, wherein the vector is a viral vector.
27. A method of treating or preventing diabetes in a subject, the method comprising: administering to a subject in need thereof cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof.
28. A method of treating or preventing obesity in a subject, the method comprising: administering to a subject in need thereof cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof.
29. The method of any one of claims 1 to 12, or 18 to 28, further comprising administering to the subject in need thereof, an additional pharmaceutical agent.
30. The method of claim 29, wherein the additional pharmaceutical agent is an anti-diabetic agent.
31. The method of claim 30, wherein the anti-diabetic agent is selected from the group consisting of insulin, an insulin analog, nateglinide, repaglinide, metformin, thiazolidinediones, glitazones such as troglitazone, pioglitazone and rosiglitazone, glisoxepid, glyburide, glibenclamide, acetohexamide, chloropropamide, glibornuride, tolbutamide, tolazamide,

glipizide, carbutamide, gliquidone, glyhexamide, phenbutamide, tolcyclamide, glimepiride and gliclazide.

32. The method of claim 29, wherein the additional pharmaceutical agent is a dipeptidyl peptidase 4 (DPP-4) inhibitor.

33. The method of claim 32, wherein the DPP-4 inhibitor is sitagliptin, linagliptin, alogliptin, saxagliptin, or vildagliptin.

34. A pharmaceutical composition, comprising cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof.

35. The composition of any one of claims 13 to 17, or 34, further comprising an additional pharmaceutical agent.

36. The composition of claim 35, wherein the additional pharmaceutical agent is an anti-diabetic agent.

37. The composition of claim 36, wherein the anti-diabetic agent is selected from the group consisting of insulin, an insulin analog, nateglinide, repaglinide, metformin, thiazolinediones, glitazones such as troglitazone, pioglitazone and rosiglitazone, glisoxepid, glyburide, glibenclamide, acetohexamide, chloropropamide, glibornuride, tolbutamide, tolazamide, glipizide, carbutamide, gliquidone, glyhexamide, phenbutamide, tolcyclamide, glimepiride and gliclazide.

38. The composition of claim 35, wherein the additional pharmaceutical agent is a dipeptidyl peptidase 4 (DPP-4) inhibitor.

39. The composition of claim 38, wherein the DPP-4 inhibitor is sitagliptin, linagliptin, alogliptin, saxagliptin, or vildagliptin.

40. A method of treating or preventing an inflammatory disease in a subject, the method comprising: administering to a subject in need thereof cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof.

41. The method of claim 40, wherein the inflammatory disease is selected from the group consisting of: Crohn's disease, inflammatory bowel disease, ulcerative colitis, pancreatitis, hepatitis, appendicitis, gastritis, diverticulitis, celiac disease, food intolerance, enteritis, ulcer, and gastroesophageal reflux disease (GERD), psoriatic arthritis, psoriasis, and rheumatoid arthritis.
42. The method of claim 41, wherein the inflammatory disease is an inflammatory bowel disease.
43. The method of claim 42, wherein the inflammatory bowel disease is Crohn's disease.
44. The method of claim 42, wherein the inflammatory bowel disease is ulcerative colitis.
45. The method of claim 41, wherein the inflammatory disease is enteritis.
46. The method of claim 41, wherein the inflammatory disease is colitis.
47. Use of cholic acid-7-sulfate in treating or preventing diabetes in a subject.
48. Use of cholic acid-7-sulfate in treating or preventing obesity in a subject.
49. Use of cholic acid-7-sulfate in treating or preventing an inflammatory disease in a subject.
50. A kit comprising cholic acid-7-sulfate, or a pharmaceutically acceptable salt thereof, or a composition of any one of claims 13 to 17, or 34 to 39; and
instructions for using the compound, or pharmaceutically acceptable salt, or the composition.

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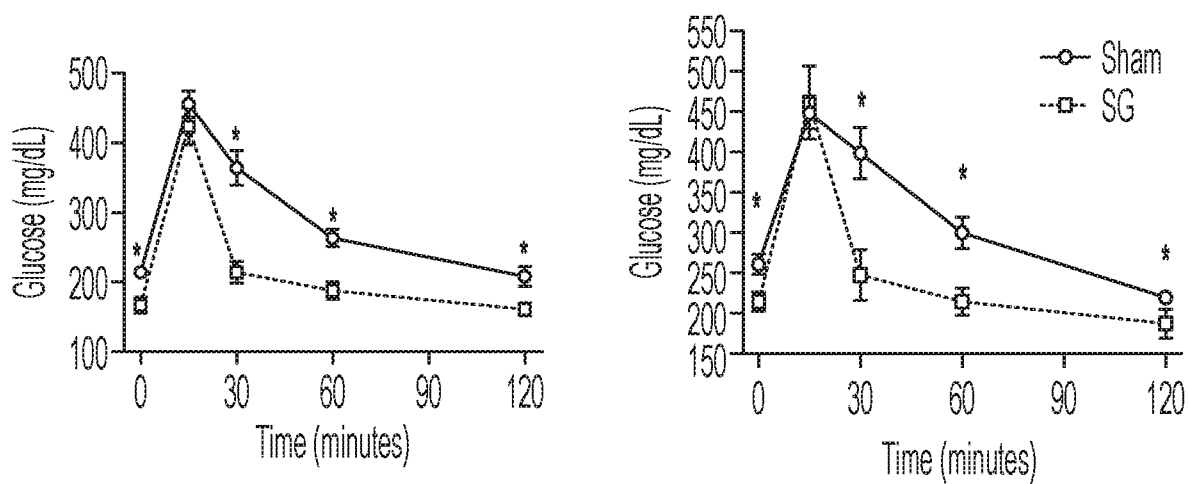


FIG. 1A

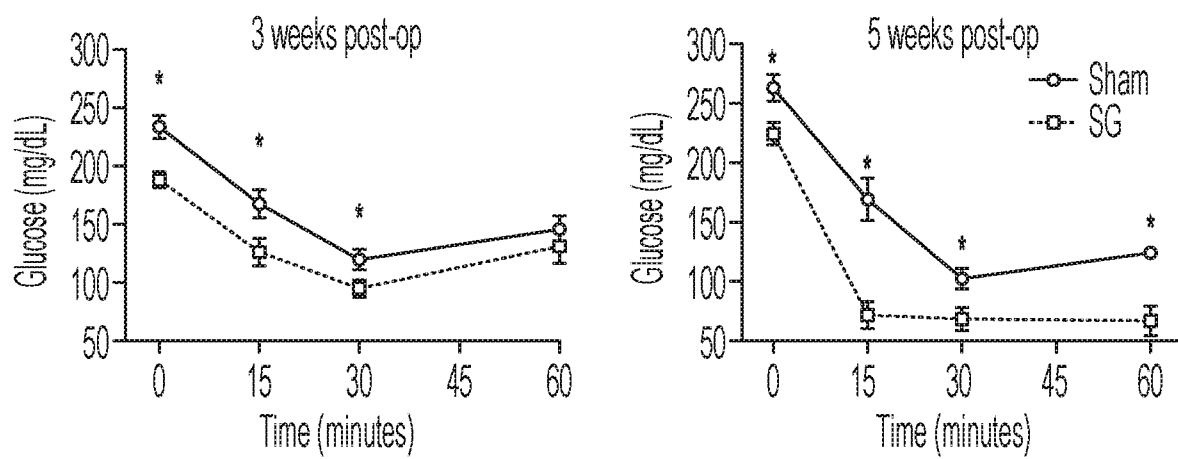


FIG. 1B

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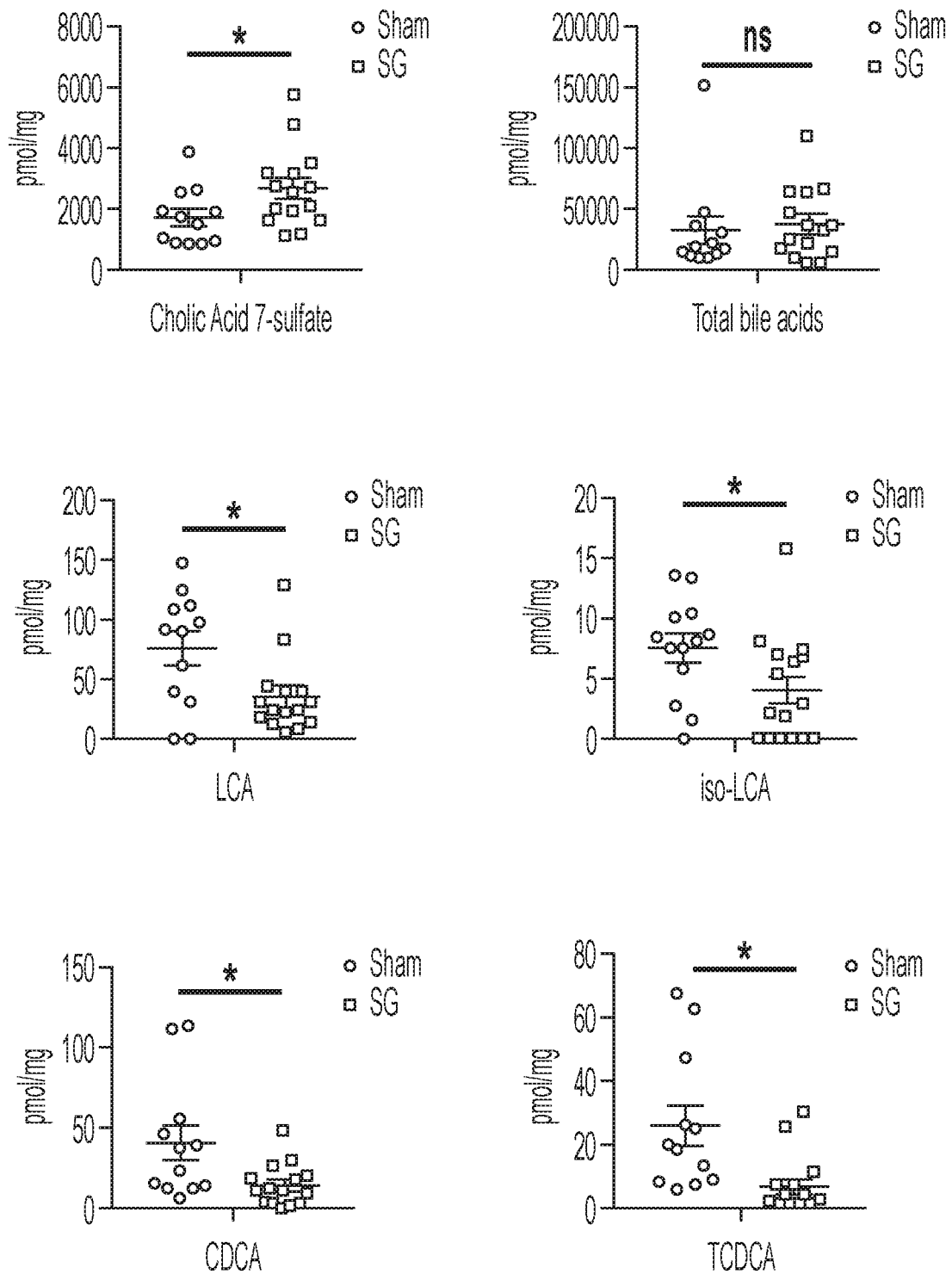


FIG. 2

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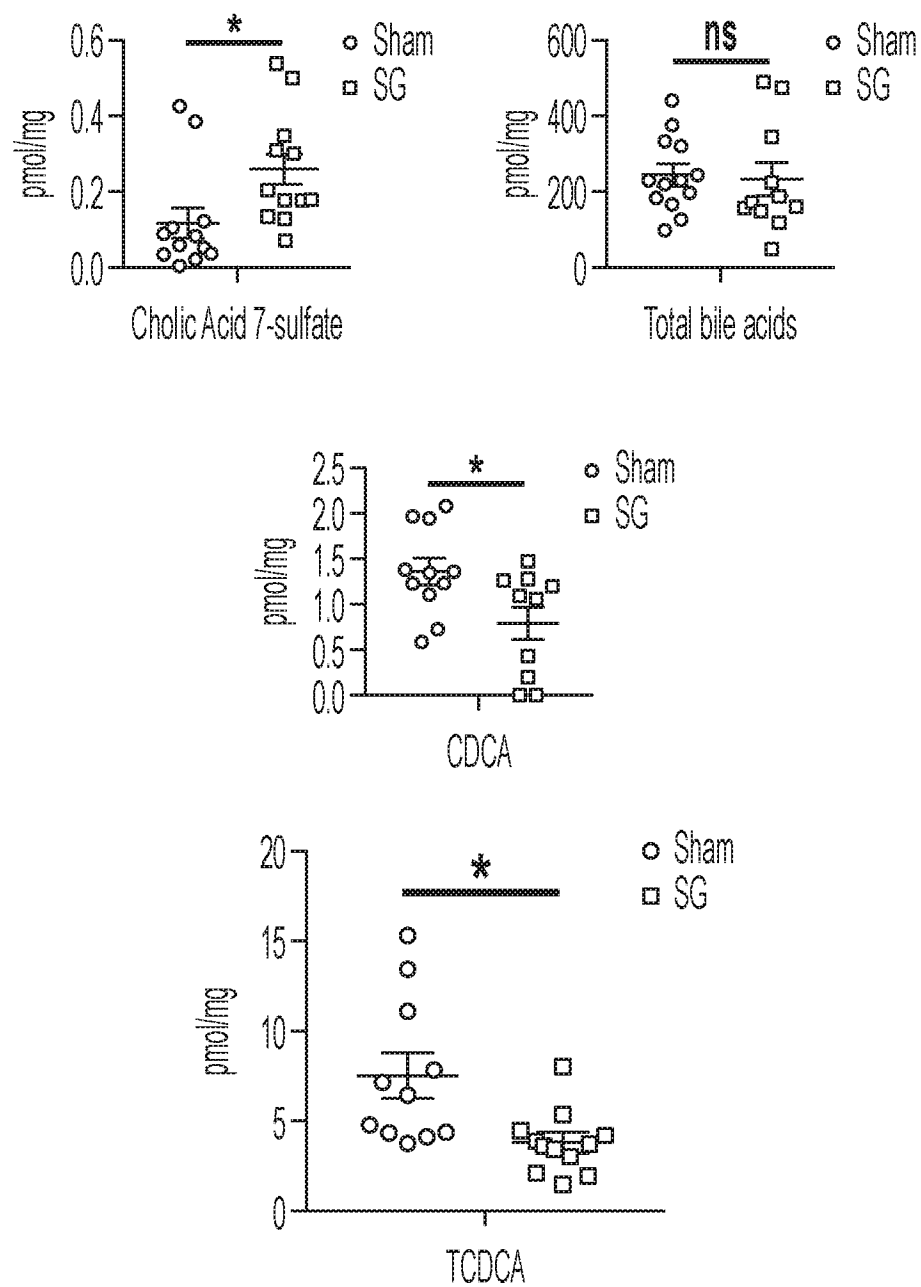


FIG. 3

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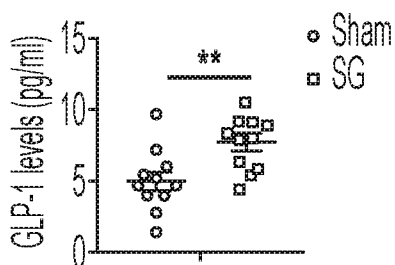


FIG. 4A

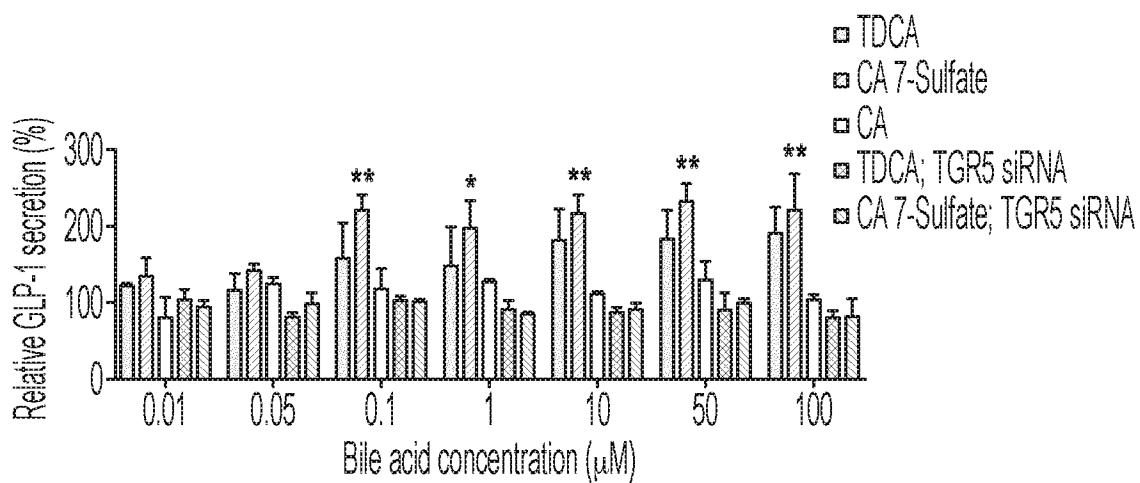


FIG. 4B

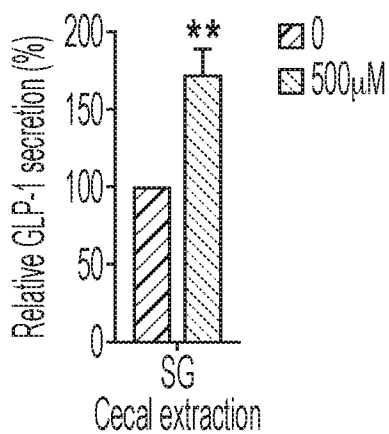


FIG. 4C

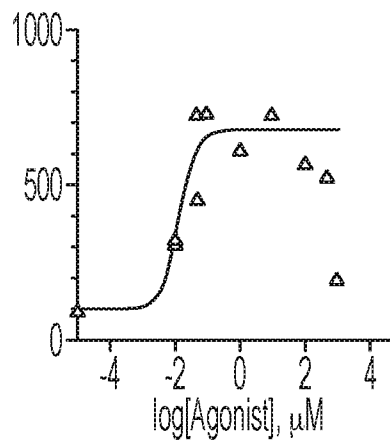


FIG. 4D

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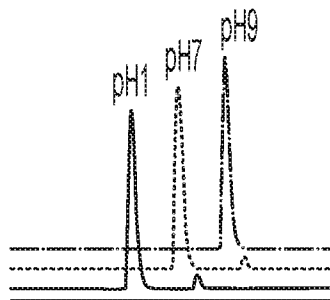


FIG. 5A

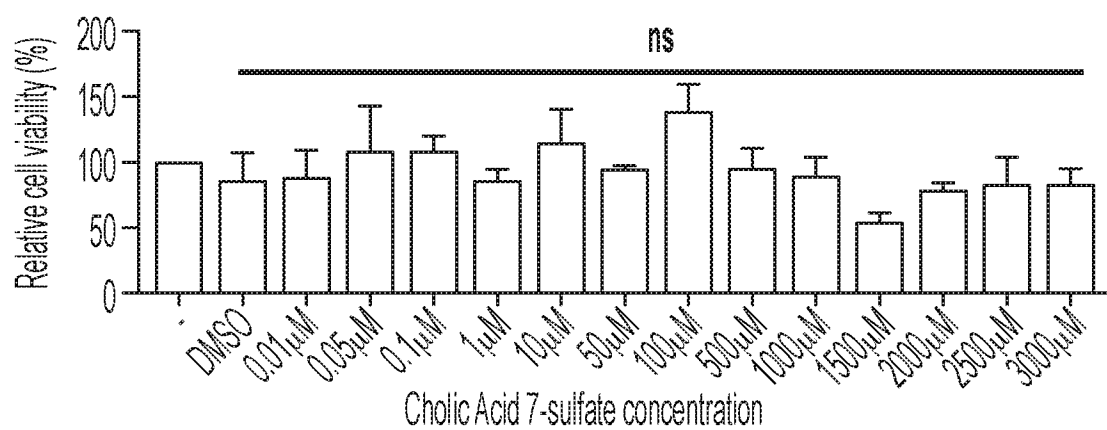


FIG. 5B

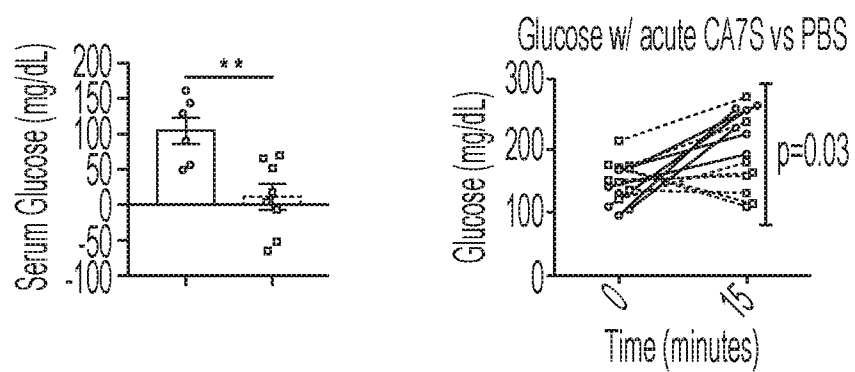


FIG. 5C

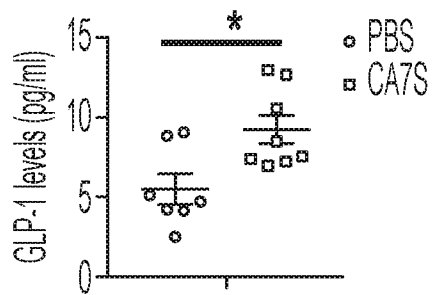


FIG. 5D

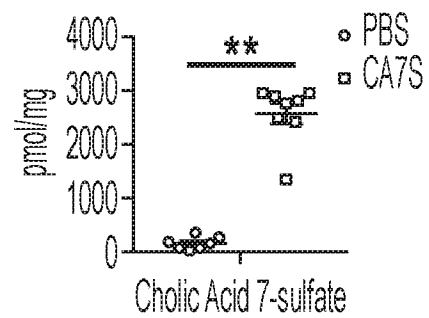


FIG. 5E

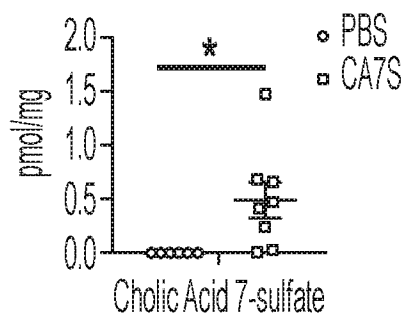


FIG. 5F

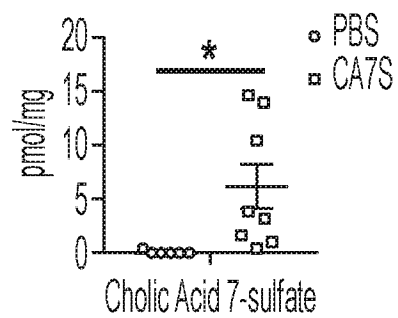


FIG. 5G

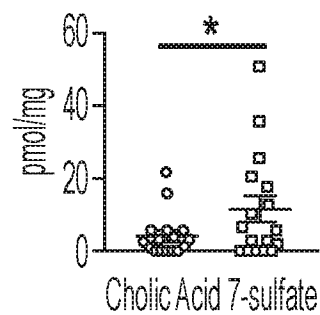


FIG. 5H

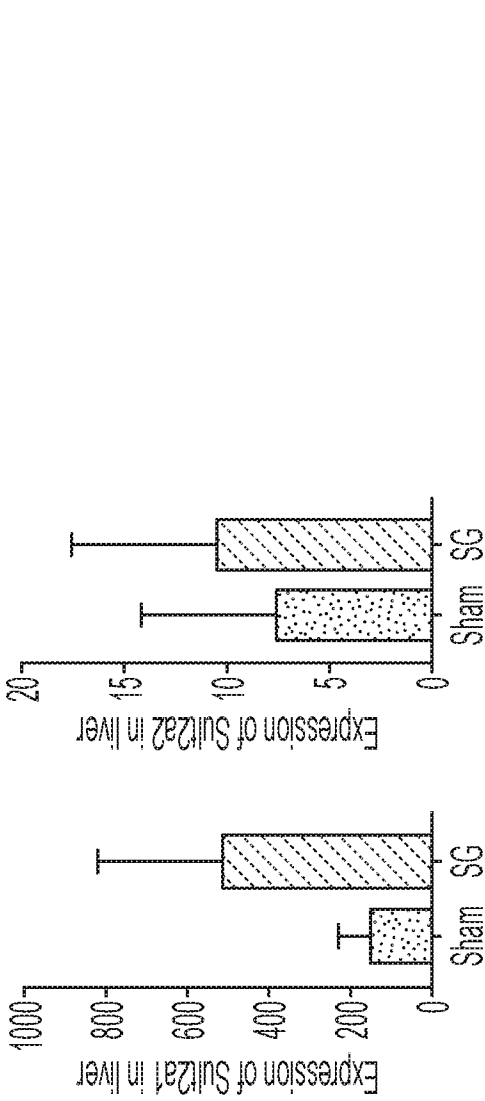


FIG. 6A

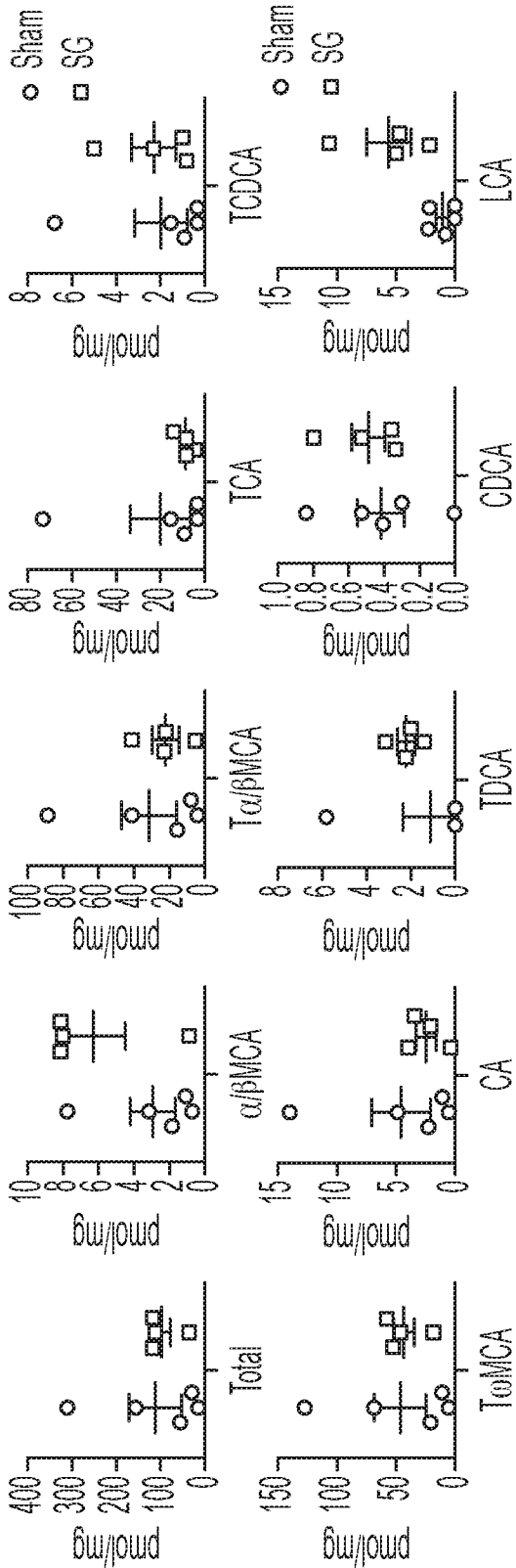
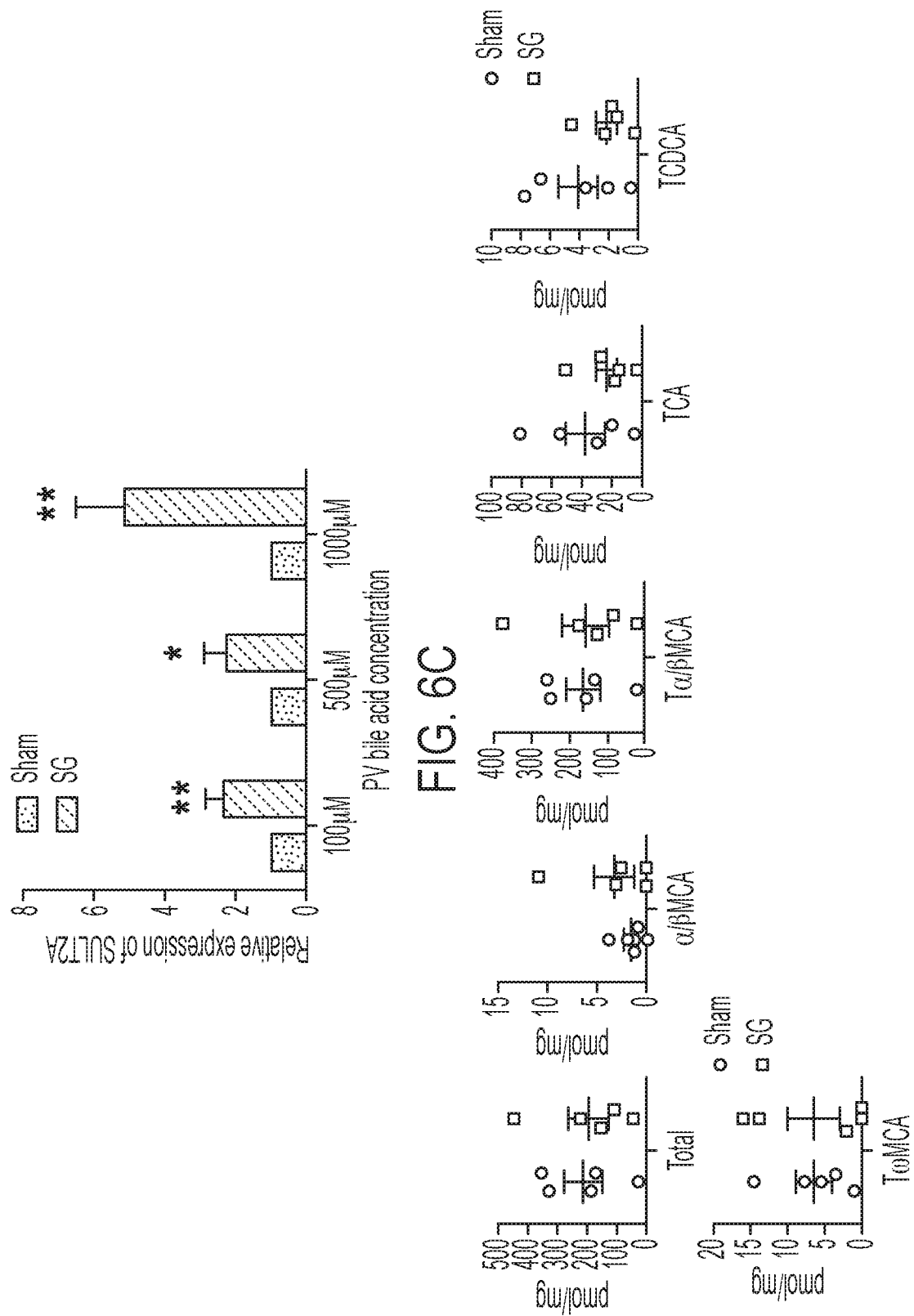


FIG. 6B



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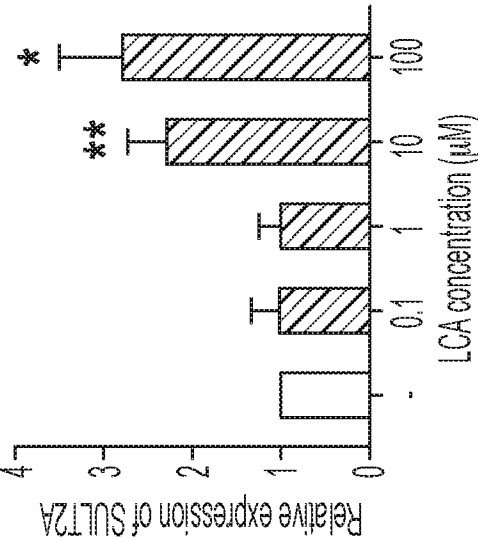


FIG. 6F

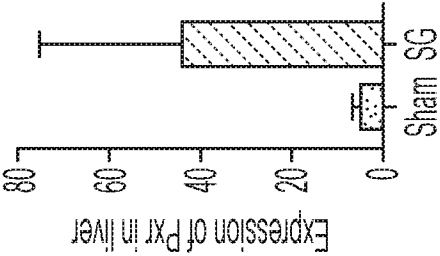


FIG. 6H

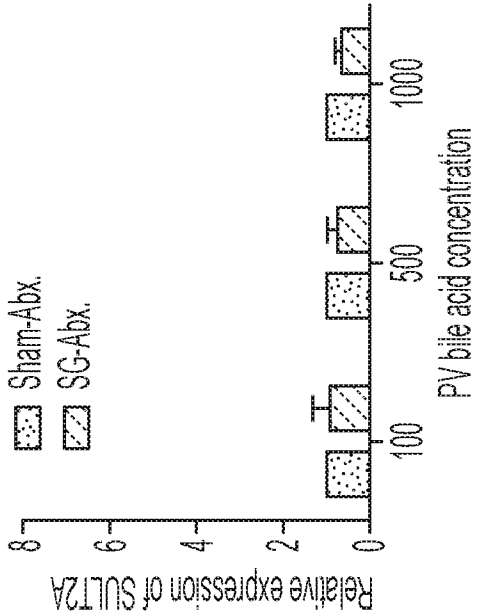


FIG. 6E

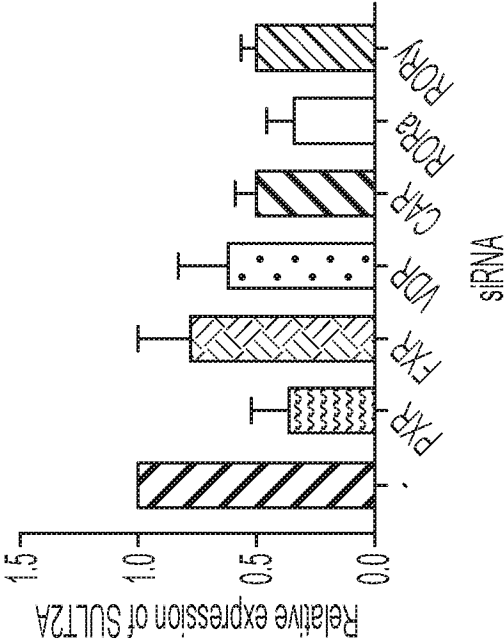


FIG. 6G

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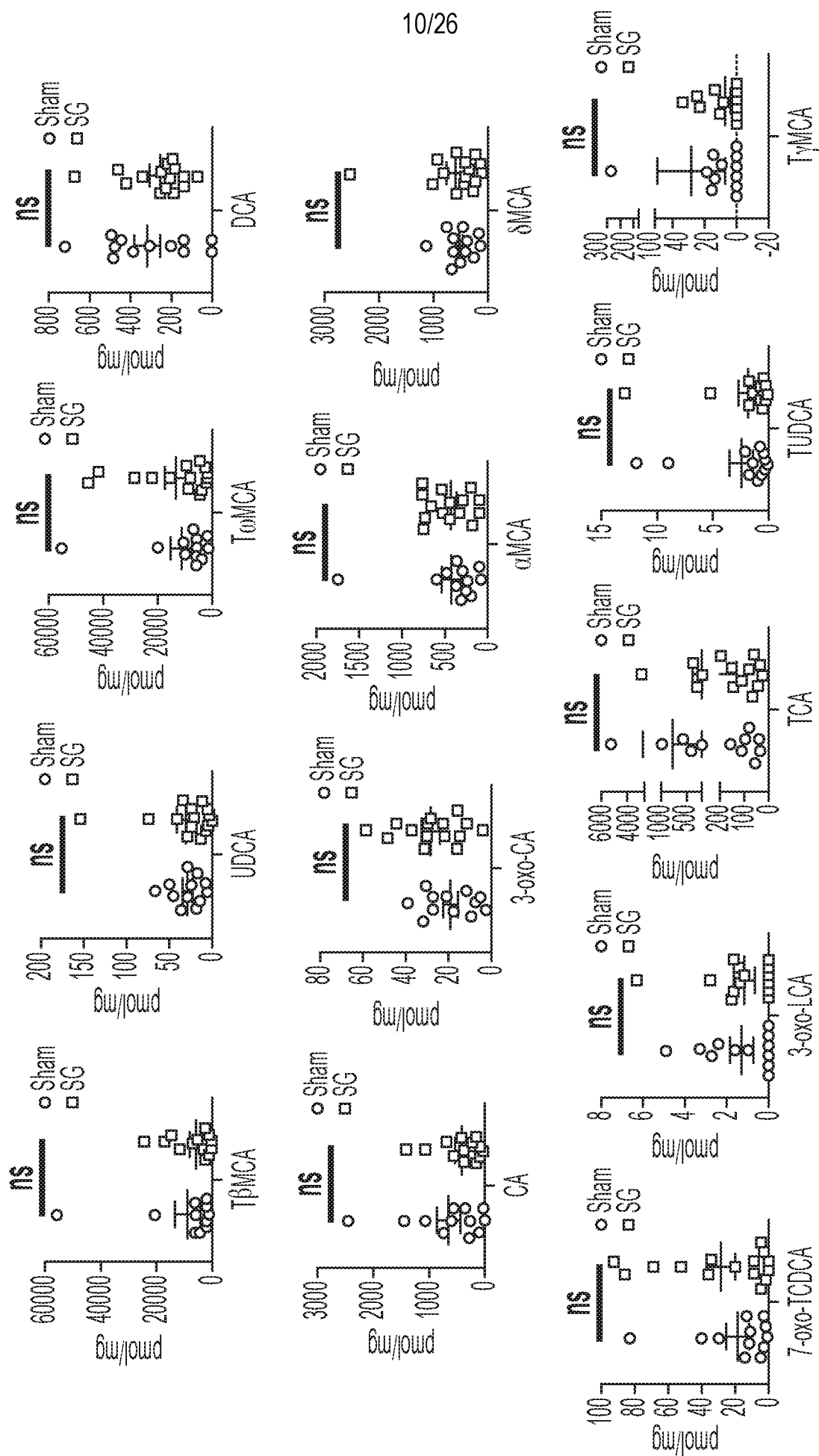


FIG. 7

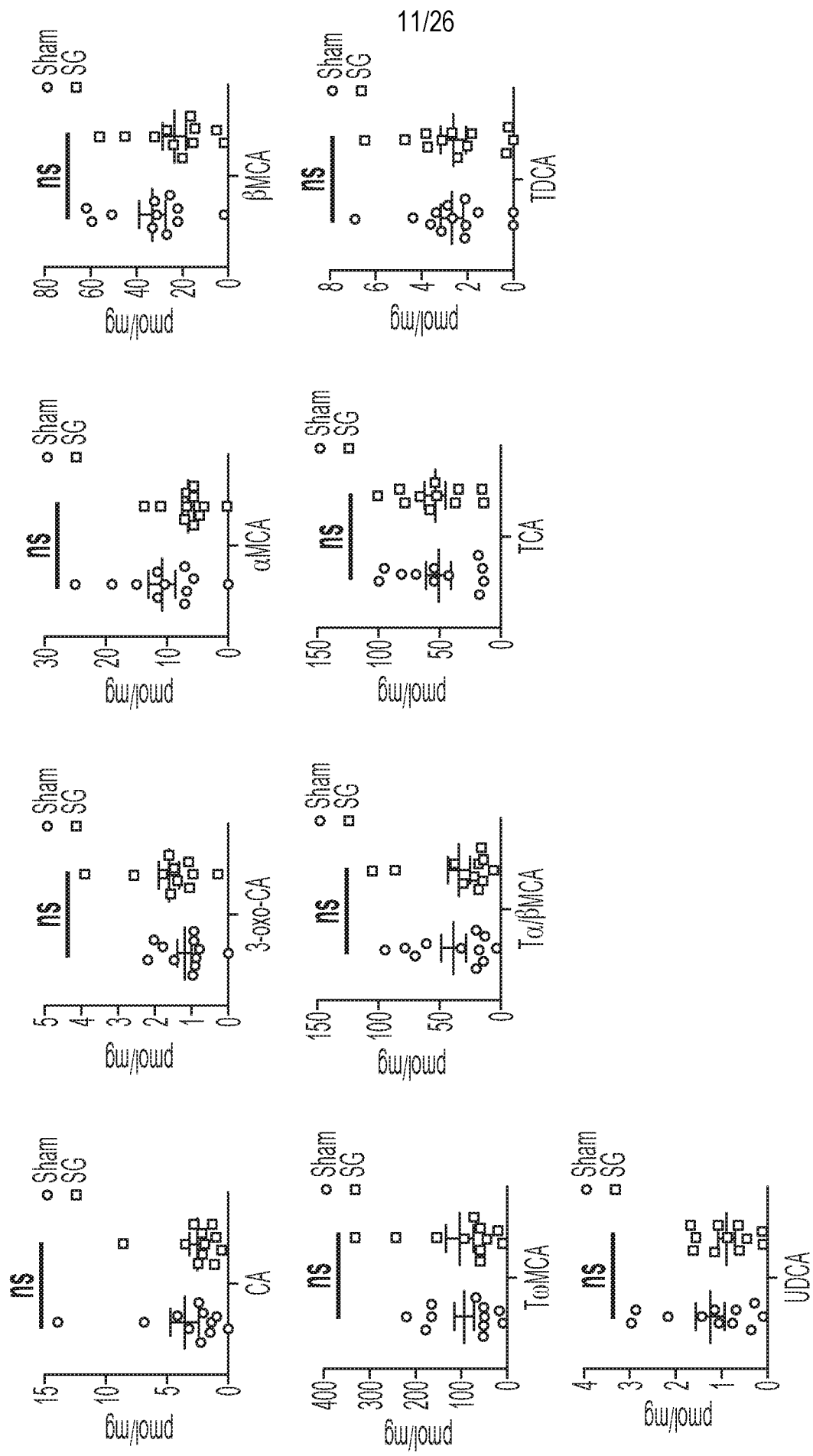


FIG. 8

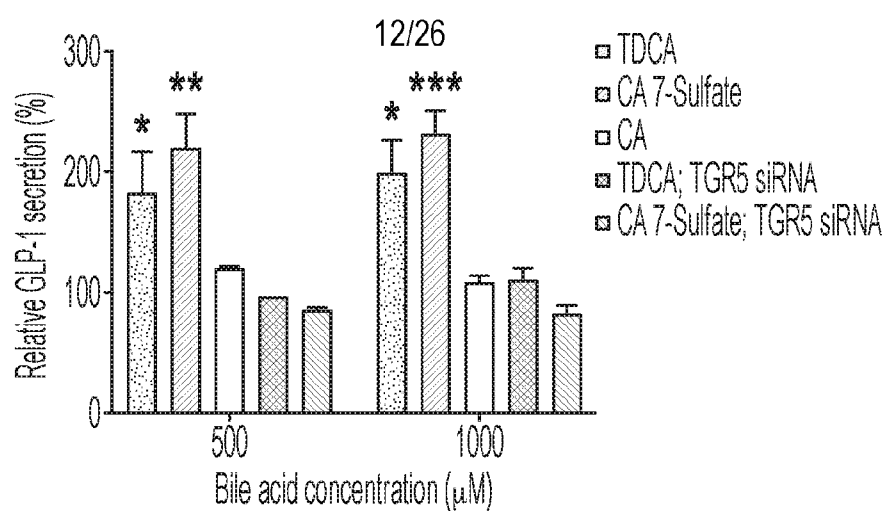


FIG. 9A

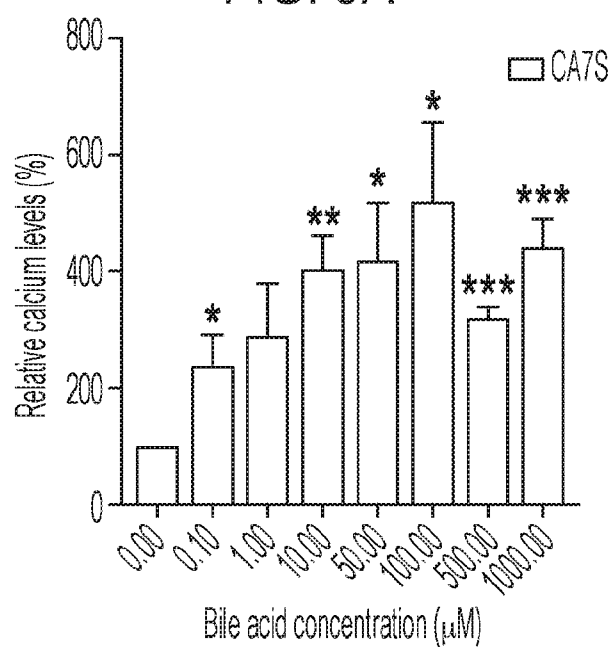


FIG. 9B

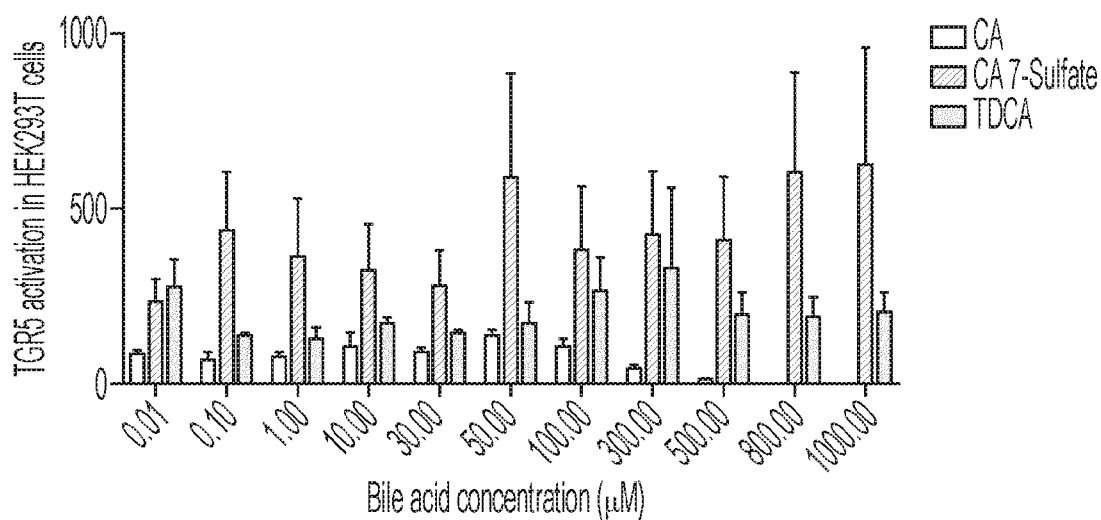


FIG. 9C

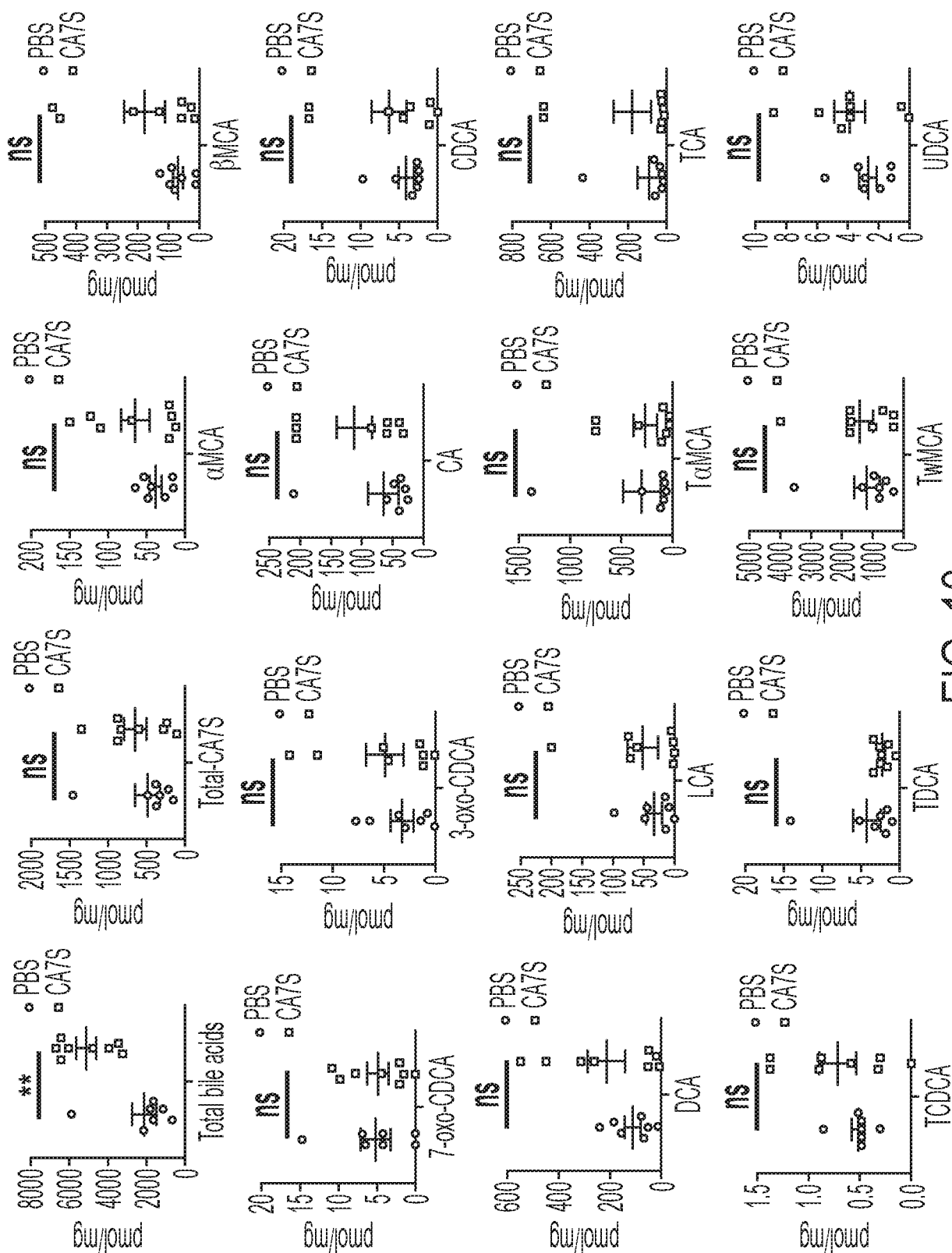


FIG. 10

Figure 2 displays 12 dot plots showing the concentration of various bile acids in the serum of CA7S and PBS groups. The y-axis for all plots is labeled 'pmol/mg' and the x-axis is labeled 'CA7S' and 'PBS'. The bile acids measured are: Total bile acids, Total-CA7S, CA, CDCA, CDCA, DCA, DCA, TCA, TCA, TMCA, TMCA, and TWCA. In all cases, the CA7S group shows significantly higher levels than the PBS group, indicated by 'ns' (not significant) above the bars.

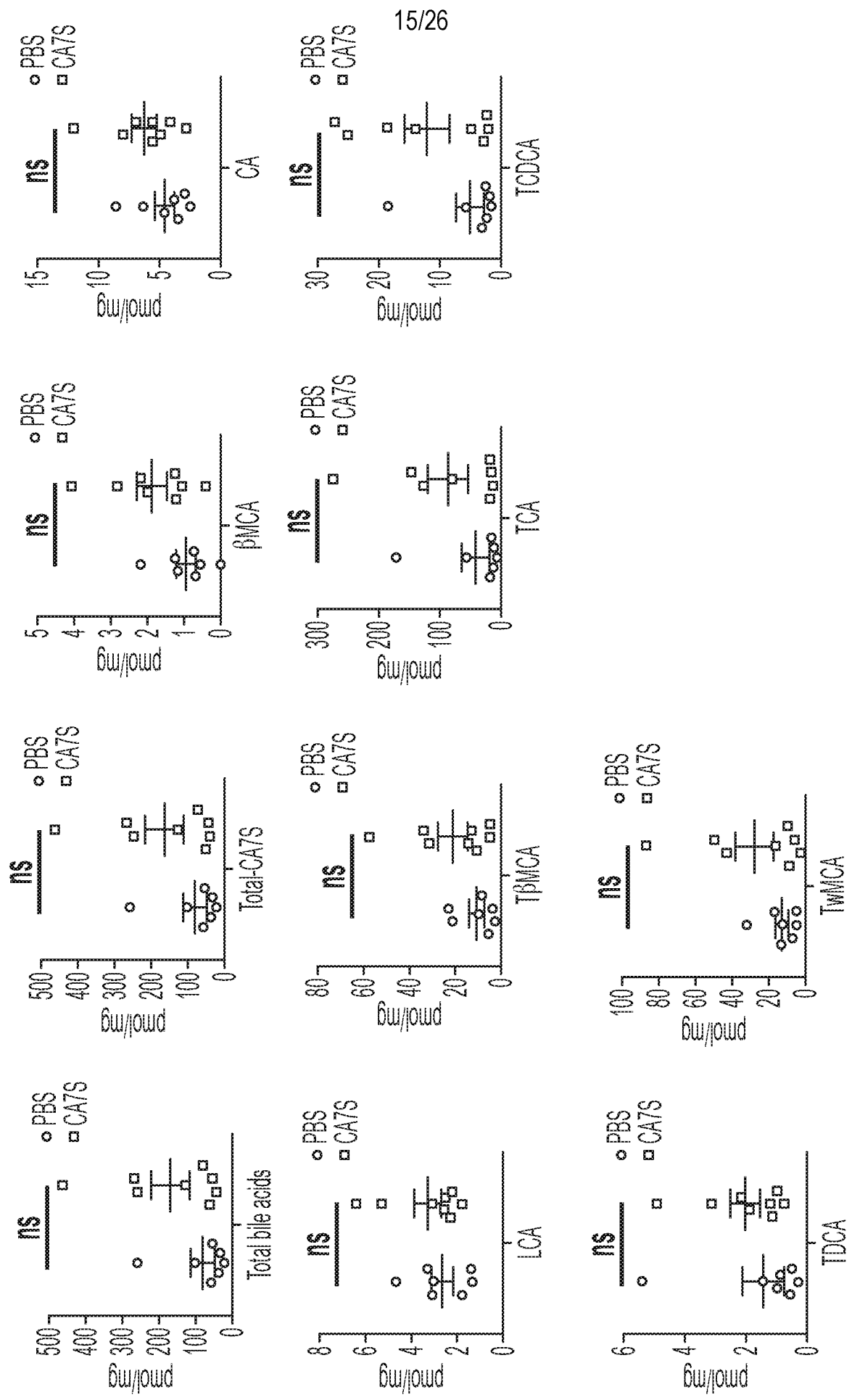


FIG. 12

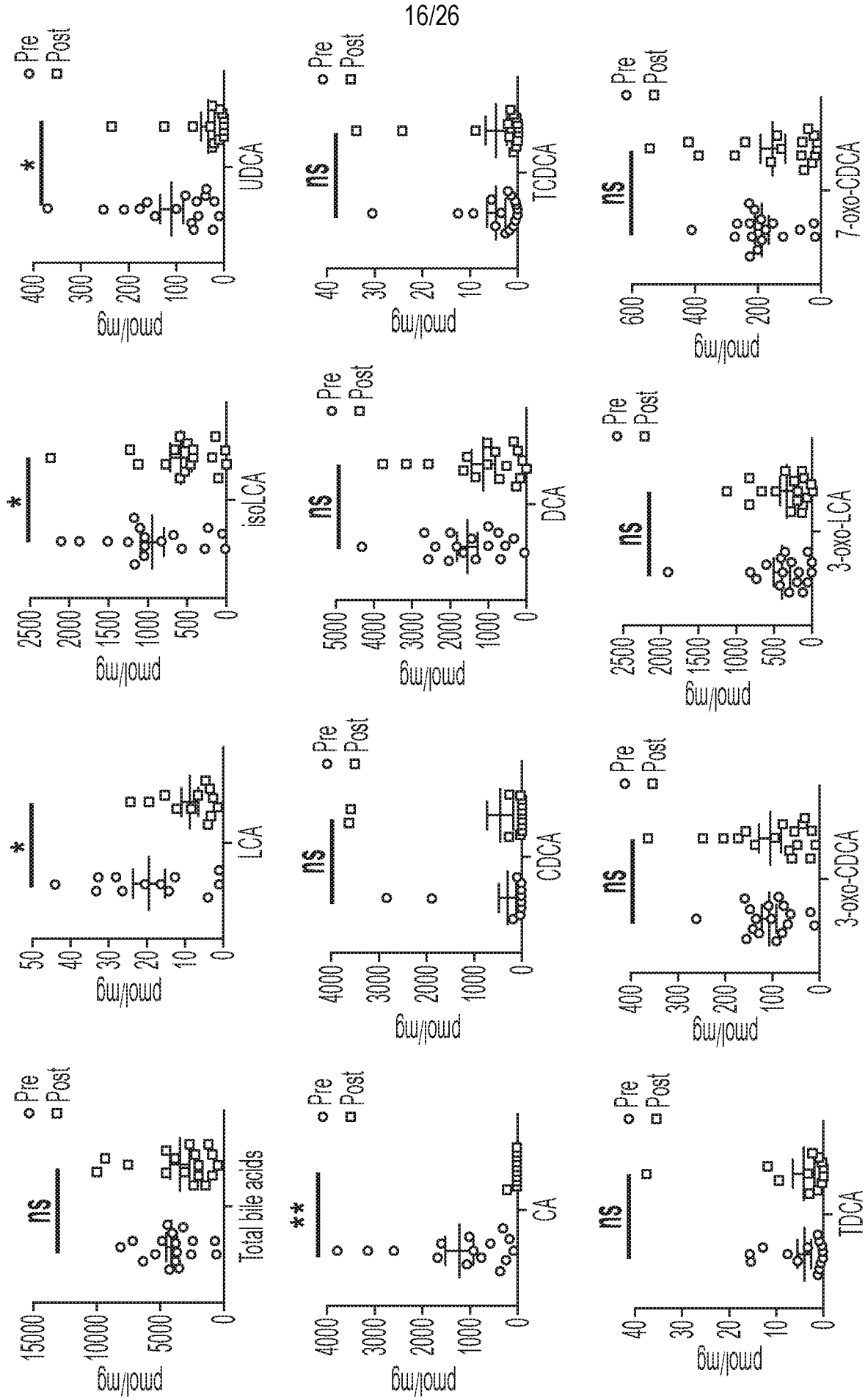


FIG. 13

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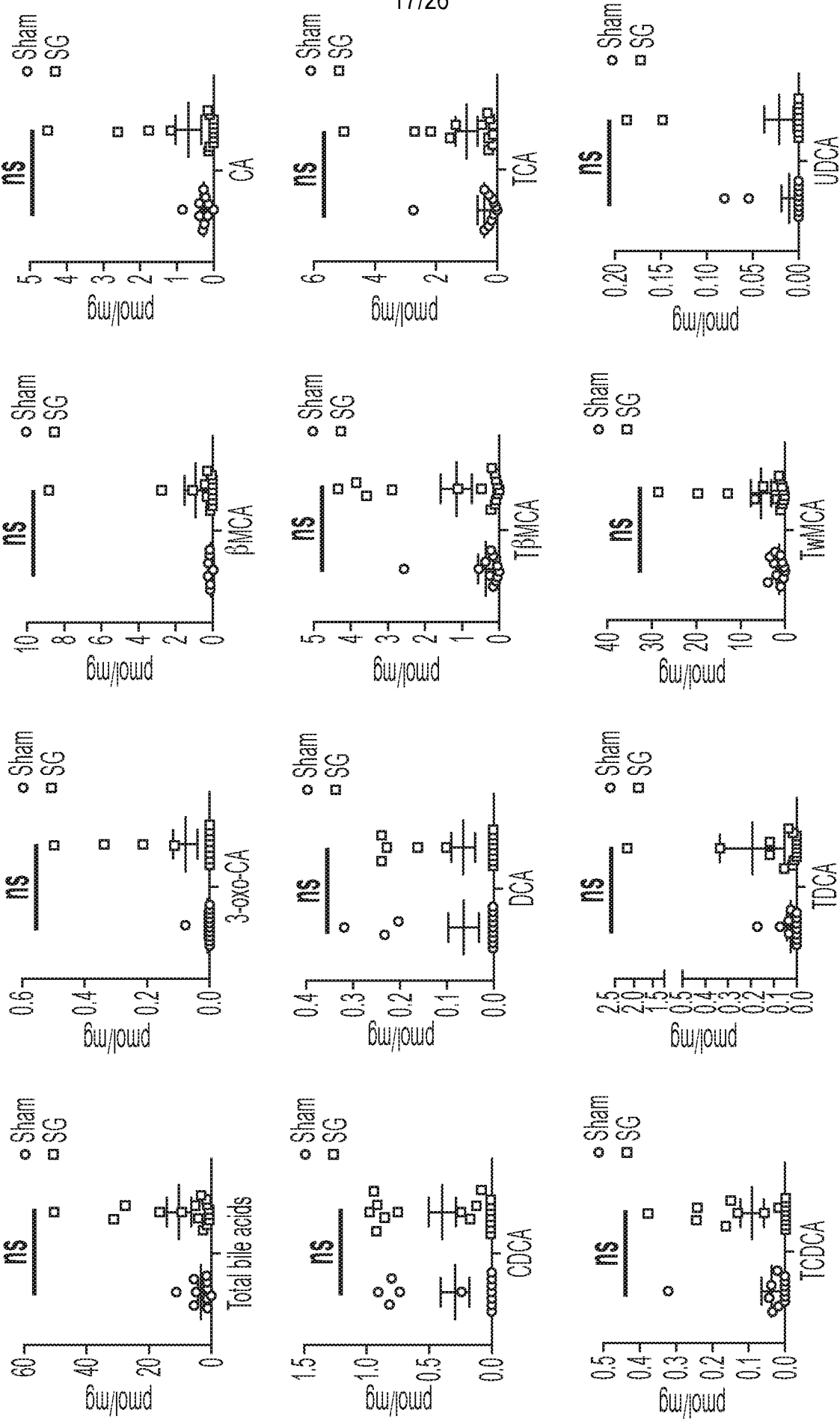


FIG. 14

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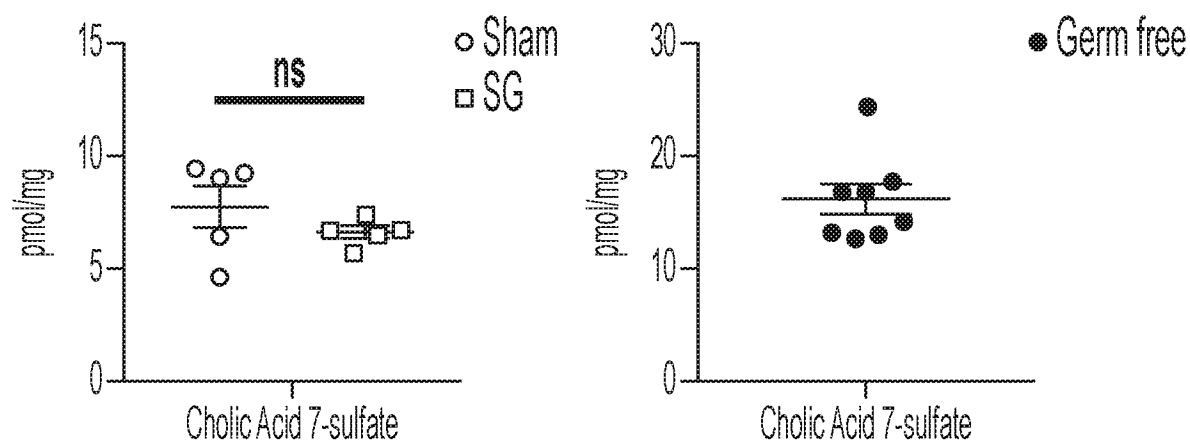


FIG. 15

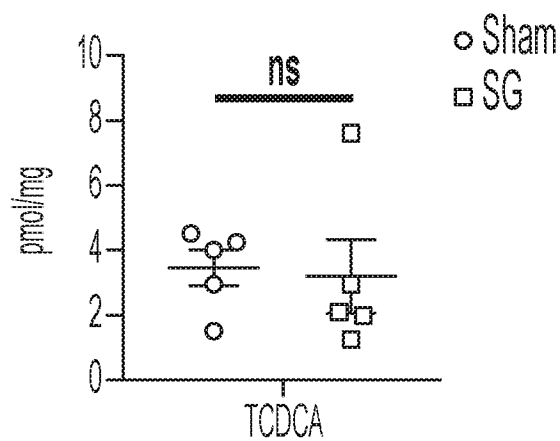


FIG. 16

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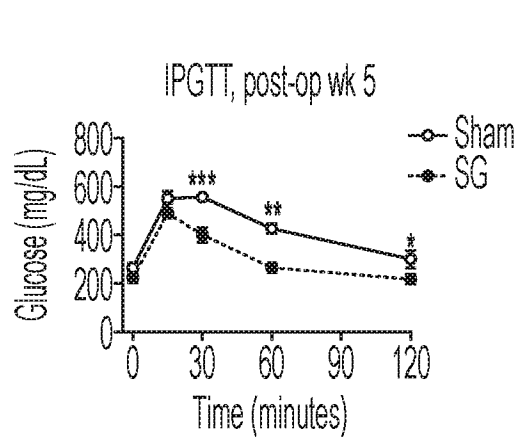


FIG. 17A

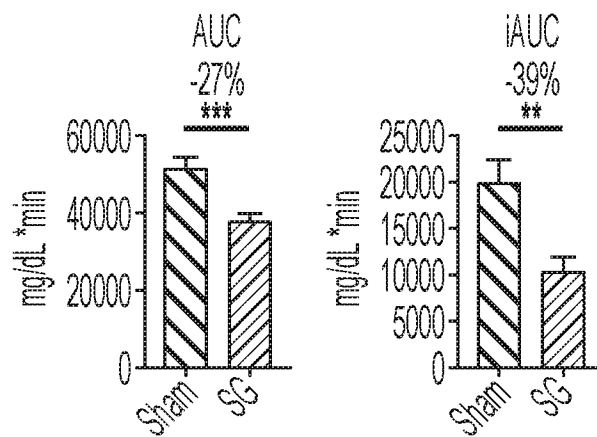


FIG. 17B

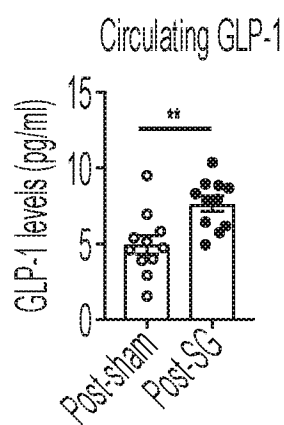
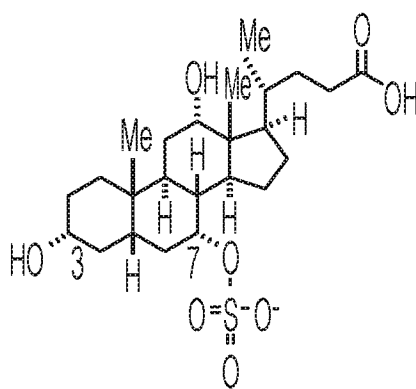


FIG. 17C



cholic acid-7-sulfate (CA7S)

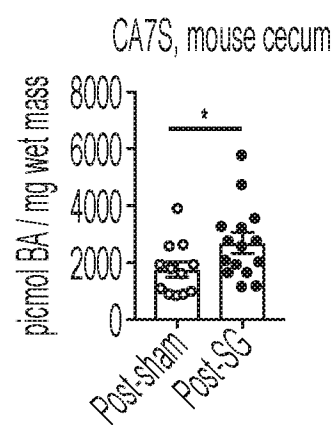


FIG. 17E

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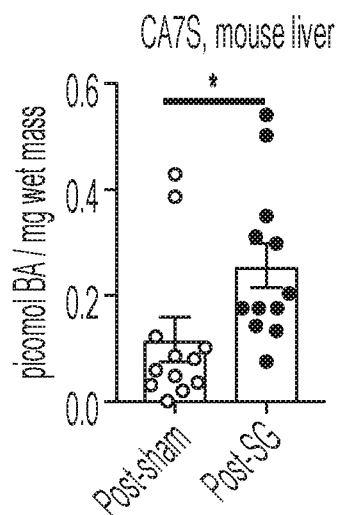


FIG. 17F

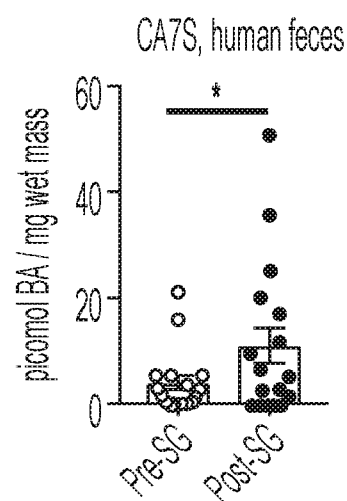


FIG. 17G

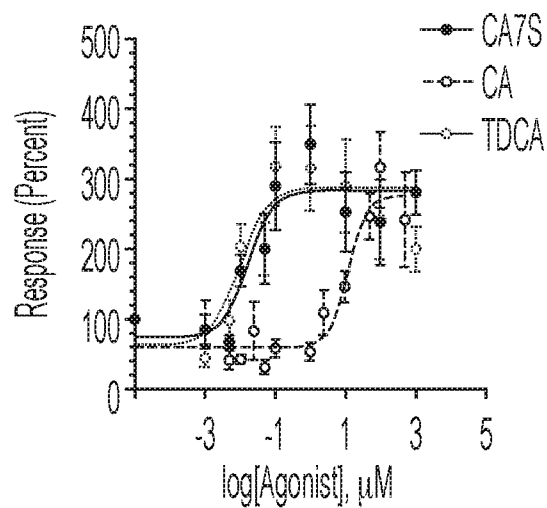


FIG. 17H

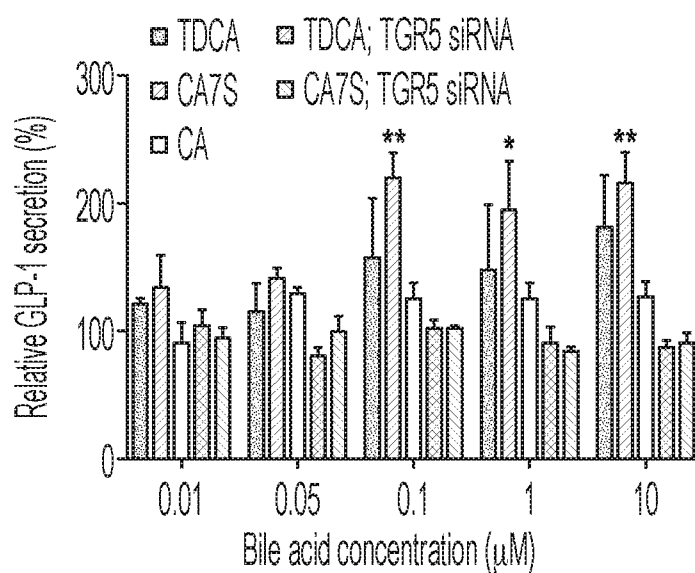


FIG. 17I

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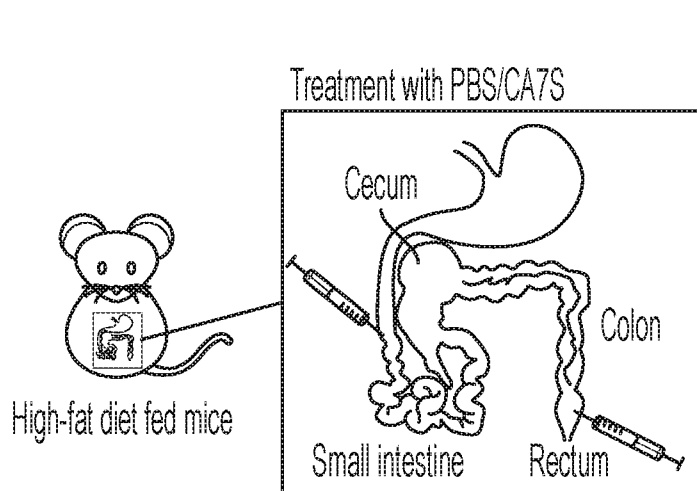


FIG. 18A

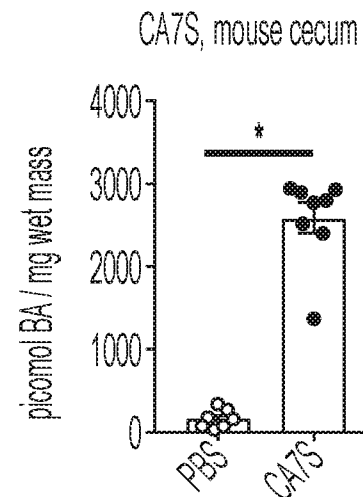


FIG. 18B

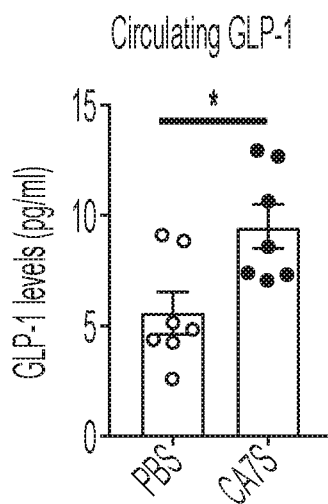


FIG. 18C

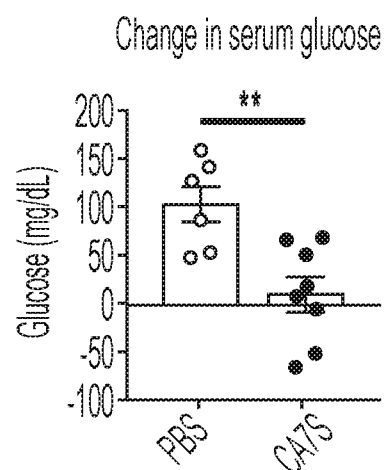


FIG. 18D

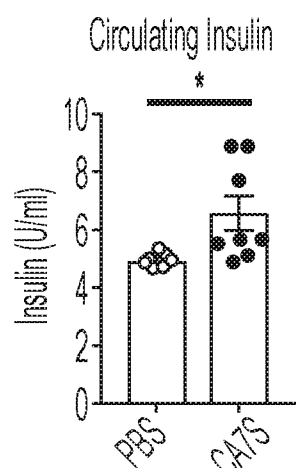
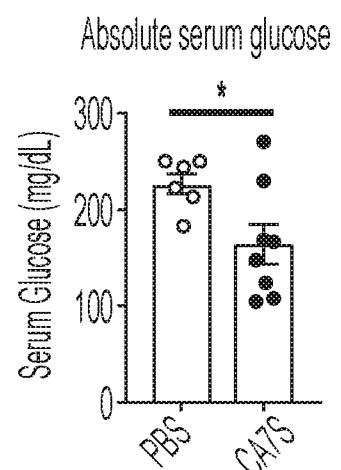


FIG. 18E

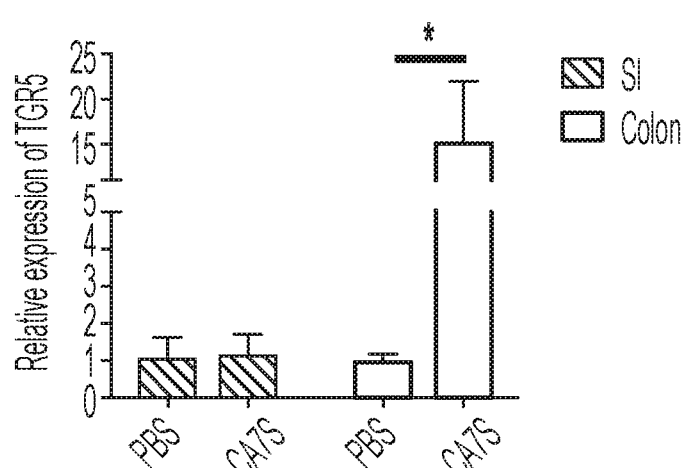
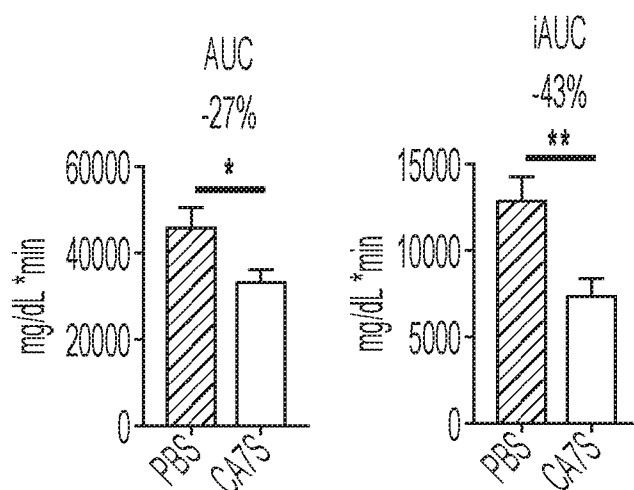
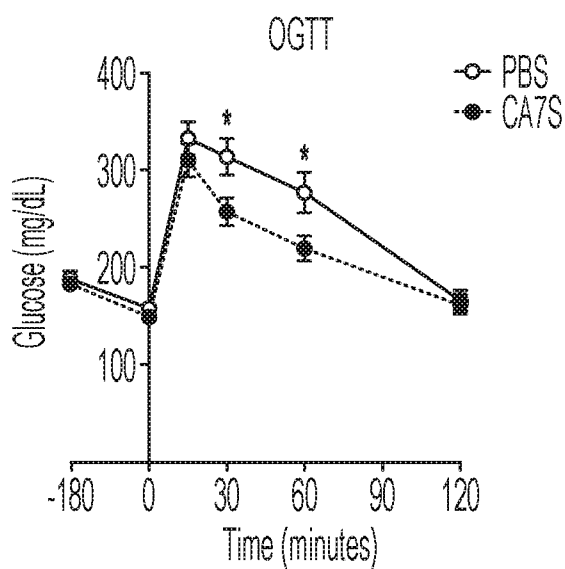
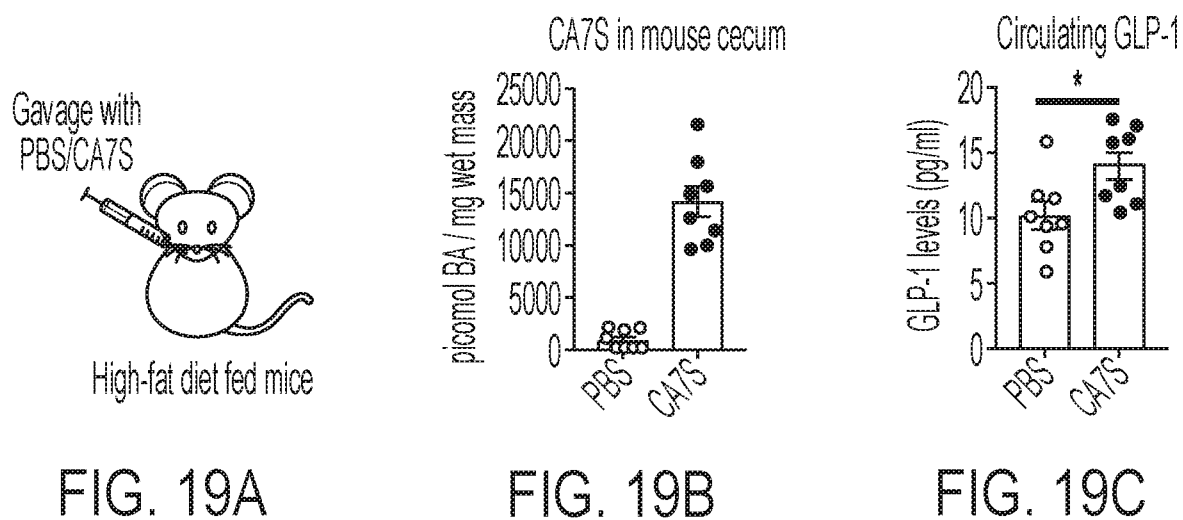


FIG. 18F

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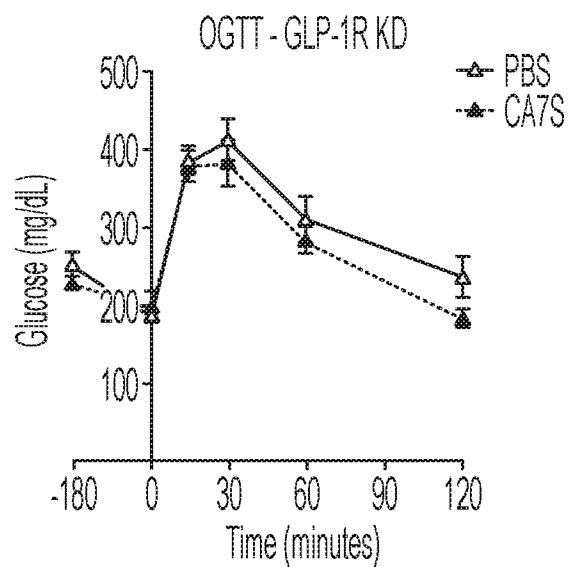


FIG. 19F

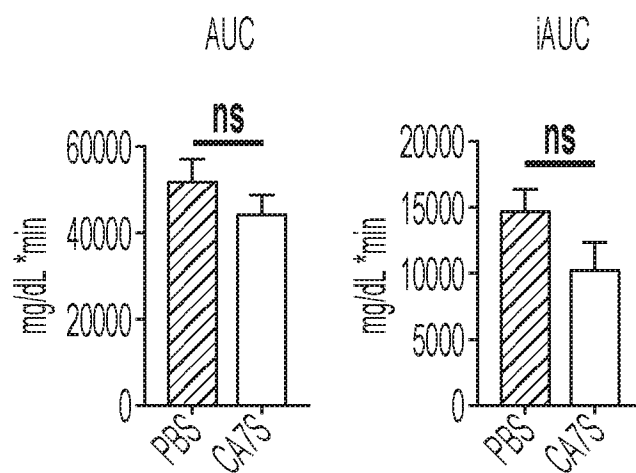


FIG. 19G

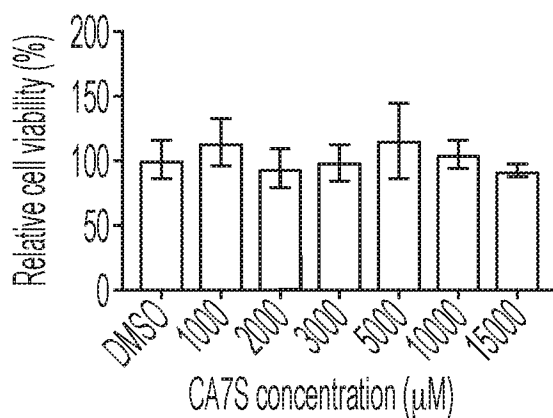


FIG. 19H

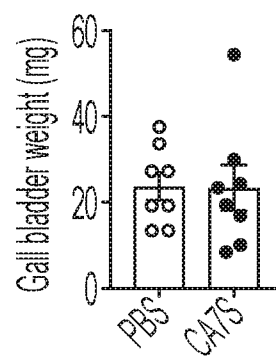


FIG. 19I

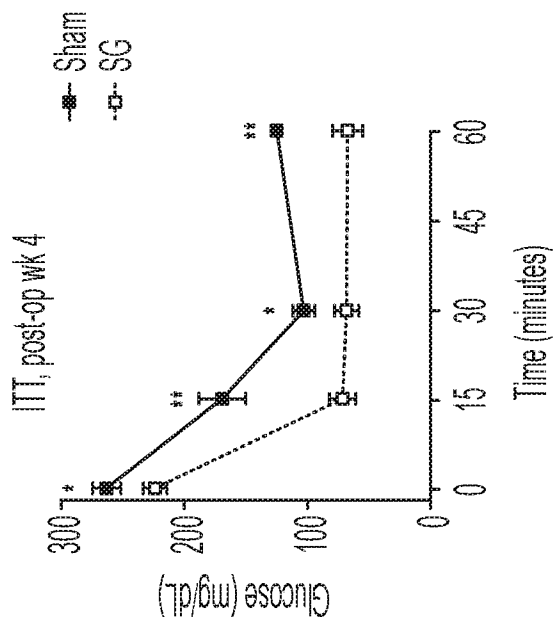


FIG. 20A

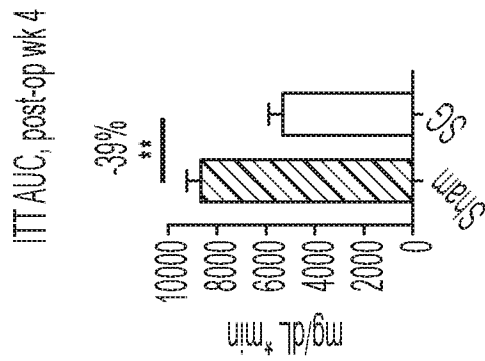


FIG. 20B

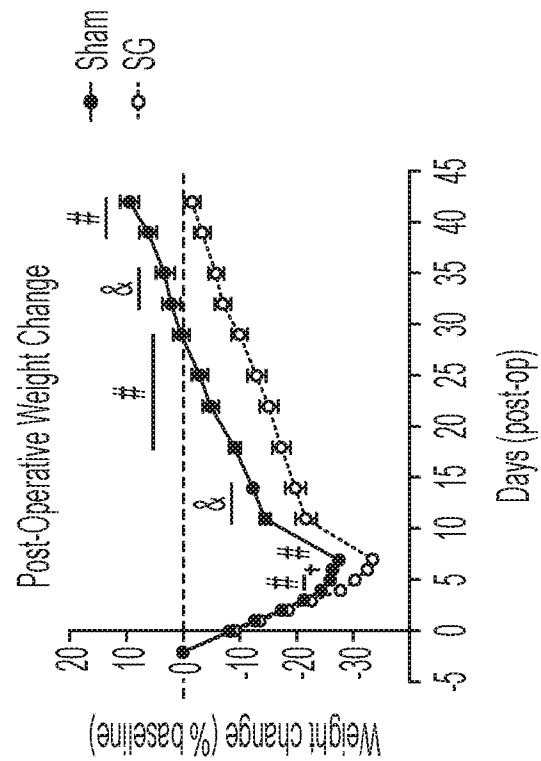


FIG. 20C

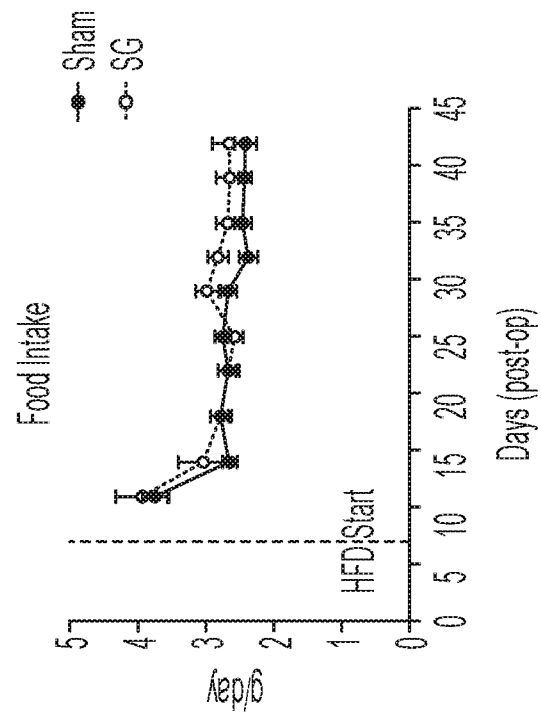


FIG. 20D

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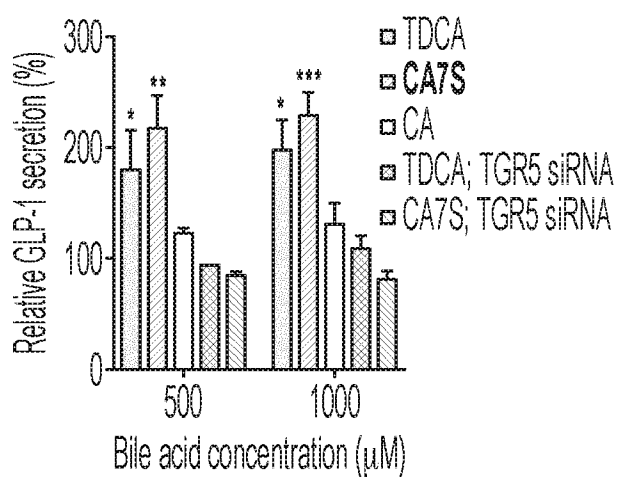


FIG. 21A

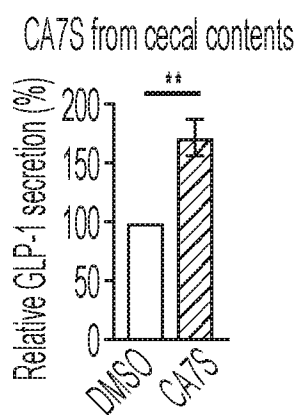


FIG. 21B

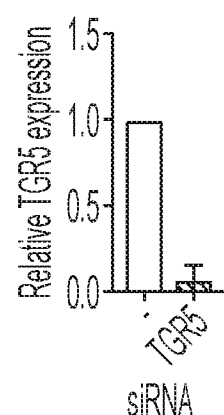


FIG. 21C

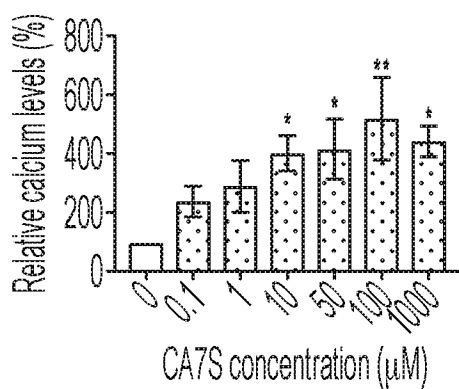


FIG. 21D

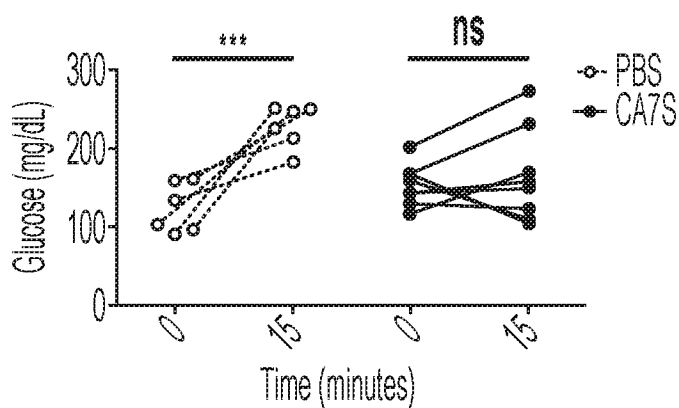


FIG. 21E

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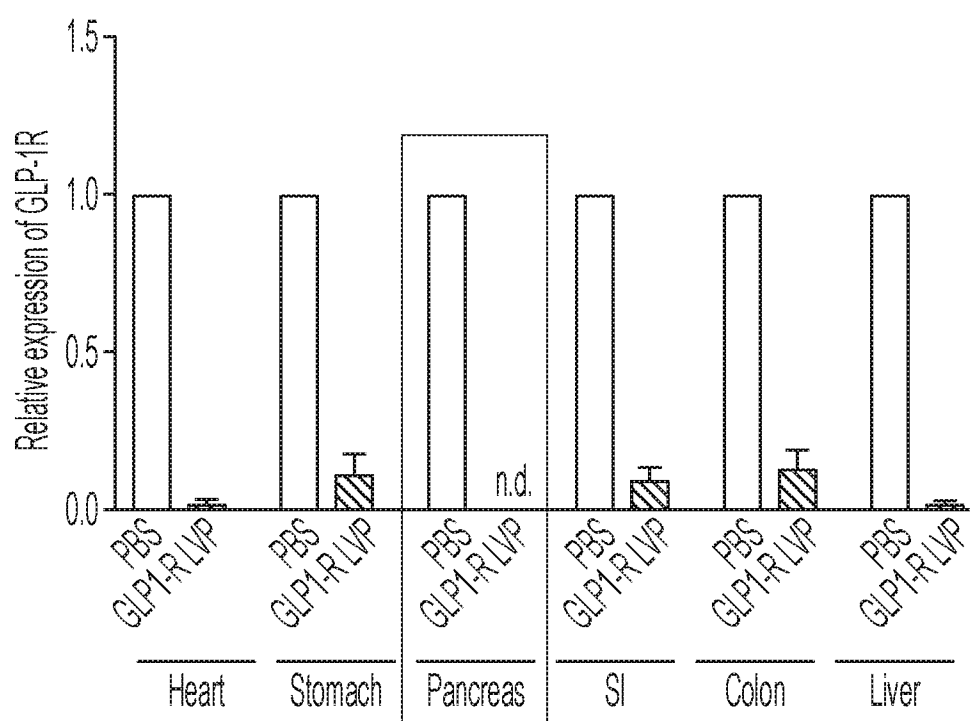


FIG. 22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/47856

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/22, 38/28; A61P 3/00, 5/48, 5/50 (2019.01)

CPC - CPC: A61K 9/02, 9/06, 9/08, 9/0031, 9/122, 9/284, 9/2054, 9/2866, 31/575, 31/4985, 35/413, 45/06; A61P 5/48, 5/50

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 document

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

See Search History document

Electronic data base consulted during the international search (name of data base 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 ----- Y ----- A	TW 201700447 A (Intercept Pharmaceuticals Inc et al.) 01 January 2017; page 2, paragraphs 2-3; page 7, paragraphs 14, 16; page 27, second paragraph; claim 25	1 --- 34 --- 2, 18, 20, 27-28, 40-49
X ----- A	ZHANG et al. 'Effects Of Feeding Bile Acids And A Bile Acid Sequestrant On Hepatic Bile Acid Composition In Mice' Journal of Lipid Research. Volume 51, 2010, pp. 3230-3242; abstract; figure 2; figure 2 legend. DOI 10.1194/jlr.M007641	13 --- 1, 2, 18, 20, 27-28, 40-49
A	US 2010/0130426 A1 (YOUNG et al.) 27 May 2010; abstract; paragraph [0110]; claims 13, 16	2, 18, 20, 27-28, 40-49
A	HUIJGHEBAERT et al. 'Specificity of Bile Salt Sulfatase Activity from Clostridium sp. Strain S1' Applied And Environmental Microbiology, Nov. 1982, Vol. 44, No. 5, p. 1030-1034; page 1031, column 2, sixth paragraph.	27-28, 40-49
A	US 2014/0234256 A1 (MARCH et al.) 21 August 2014; abstract; paragraphs [0032], [0046]	18, 20
Y	JACOBS et al. 'A Disease-Associated Microbial and Metabolomics State in Relatives of Pediatric Inflammatory Bowel Disease Patients' Cell Mol Gastroenterol Hepatol. 2016, Vol. 2; pages 750-766; page 758, second column, first paragraph; page 760, second column, second paragraph - page 761, first column. DOI: 10.1016/j.jcmgh.2016.06.004	34

☐ 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

"D" document cited by the applicant in the international application

"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

18 November 2019 (18.11.2019)

Date of mailing of the international search report

10 DEC 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/47856

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

An Invitation to Furnish Nucleotide and/or Amino Acid Sequence Listing and to Pay, Where Applicable, Late Furnishing Fee ("ISA/225") was mailed on 05 September 2019 (05.09.2019). The applicant did not, within the prescribed time limit, pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b). Accordingly, ISA/US cannot consider the sequence listing submitted on 04 October 2019 (04.10.2019). Therefore, the international search has been carried out only to the extent possible.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/47856

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.: 3-12, 14-17, 19, 21-26, 29-33, 35-39, 50
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.