



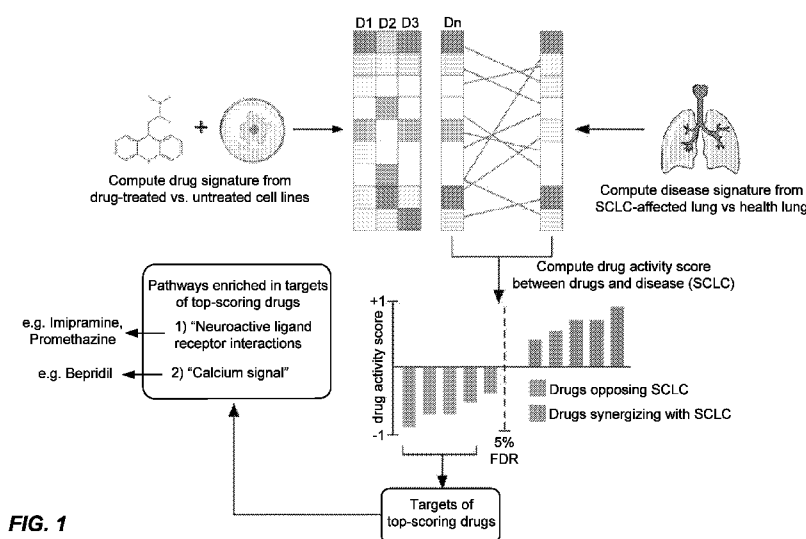
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(54) **Title:** METHODS FOR THE TREATMENT OF CANCER

(57) **Abstract:** Described herein are methods for the treatment of cancers, particularly neuroendocrine tumors. Also described herein are methods for selecting drug candidates for the treatment of cancers such as neuroendocrine tumors.

METHODS FOR THE TREATMENT OF CANCER

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/674,810, filed July 23, 2012, which application is fully incorporated by reference in its entirety.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant # T15 LM007033 awarded by the N.I.H. National Library of Medicine, and under grant # 5R01CA138256-04, awarded by the National Cancer Institute. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Cancer is a significant burden on human health, accounting for an estimated 13% of all deaths each year. A wide variety of tumors and cancers affecting cells and tissues of the endocrine and nervous system, classified as neuroendocrine tumors, can arise in different parts of the body, such as, for example, the brain, pancreas, and lung. Lung cancer is the number one cause of cancer deaths in the world, with more than 1.3 million deaths annually. More patients die from lung cancer every year in the United States than from colon, breast, bladder, and pancreas cancers combined. Lung cancer is divided into two major histopathological groups: non-small cell lung cancer (NSCLC, ~80-85% of cases) and small cell lung cancer (SCLC, ~15-20% of cases) (Heist and Engelman, 2012; van Meerbeeck et al., 2011). SCLC is a very deadly subtype of lung cancer characterized by the rapid expansion and metastasis of small cells with neuroendocrine features. Patients who receive no treatment only survive 2-4 months after the initial diagnosis. Patients with limited-stage disease at the time of diagnosis have a 5-year survival of less than 15%; overall, because most patients are diagnosed with advanced disease, the 5-year survival is only 5-10%. In addition to late detection, a major factor for the high mortality of SCLC patients is the paucity of effective therapies. SCLC patients are typically treated with 4-6 rounds of etoposide and a platinum-based agent, to which there is a 30-40% complete response rate; radiation therapy is often combined with chemotherapy, often as a preventive measure to slow the expansion of brain metastases.

[0004] However, these patients almost invariably relapse with disease that is resistant to their primary therapy (and other chemotherapeutic agents), and rapidly succumb to their

malignancy due to this chemoresistance. There is no approved targeted therapy for SCLC despite numerous attempts and clinical trials (Gustafsson et al., 2008; Neal et al., 2011; van Meerbeeck et al., 2011). Thus, a key issue in this field of research is to identify novel therapeutic strategies to block the growth of SCLC tumors. A better understanding of the biology of SCLC is needed to achieve this goal.

[0005] SCLC is even more challenging than other tumor types to study in patients because it is often detected late and because SCLC patients rarely undergo surgery (Lad et al., 1994); thus, primary human material is scarce, with the exception of a few established cell lines (Little et al., 1983) and rare primary cell lines (Daniel et al., 2009). To circumvent this problem, a mouse model was developed by deleting the *Rb* and *p53* tumor suppressor genes in the lung epithelium of adult conditional mutant mice (Meuwissen et al., 2003). This model is based on the observation that tumor cells in more than 90% of human SCLCs are mutant for both the *p53* and *RB1* tumor suppressor genes (Harbour et al., 1988; Wistuba et al., 2001). This pre-clinical mouse model and a similar mouse model in which tumors develop more rapidly (Schaffer et al., 2010) have been instrumental in identifying the cell of origin for SCLC (Park et al., 2011a; Sutherland et al., 2011), mechanisms of metastatic progression (Calbo et al., 2011), biomarkers for the disease (Taguchi et al., 2011), and Hedgehog pathway inhibitors as possible novel therapeutics in SCLC patients (Park et al., 2011b). However, there is still a lack of effective treatment options for SCLC. Furthermore, the identification of therapeutic approaches for the treatment of cancer is an arduous, costly, and often inefficient process. Even after a lead candidate has been identified, the compound must be tested for safety, dosage, and toxicity to be cleared for administration into human subjects. These tests can require several long years and potentially millions of dollars in cost.

SUMMARY OF THE INVENTION

[0006] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a histamine receptor antagonist.

[0007] In some embodiments, the histamine receptor antagonist is a histamine H1 receptor antagonist.

[0008] In some embodiments, the histamine H1 receptor antagonist is promethazine or azelastine.

[0009] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a monoamine oxidase inhibitor.

[0010] In some embodiments, the monoamine oxidase inhibitor is an irreversible monoamine oxidase inhibitor.

[0011] In some embodiments, the irreversible monoamine oxidase inhibitor is tranylcypromine.

[0012] In some embodiments, the monoamine oxidase inhibitor is an MAO-B inhibitor.

[0013] In some embodiments, the MAO-B inhibitor is pargyline.

[0014] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a serotonin receptor antagonist.

[0015] In some embodiments, the serotonin receptor antagonist is a serotonin receptor 2-type antagonist.

[0016] In some embodiments, the serotonin receptor 2-type antagonist is a serotonin receptor 2A antagonist.

[0017] In some embodiments, the serotonin receptor 2A antagonist is ritanserin.

[0018] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of an acetylcholine receptor antagonist.

[0019] In some embodiments, the acetylcholine receptor antagonist is a muscarinic acetylcholine receptor antagonist.

[0020] In some embodiments, the muscarinic acetylcholine receptor antagonist is an M3 acetylcholine receptor antagonist.

[0021] In some embodiments, the M3 acetylcholine receptor antagonist is 4-DAMP.

[0022] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of an adrenergic receptor antagonist.

- [0023] In some embodiments, the adrenergic receptor antagonist is an alpha-adrenergic receptor antagonist.
- [0024] In some embodiments, the alpha-adrenergic receptor antagonist is an alpha-1 adrenergic receptor antagonist.
- [0025] In some embodiments, the alpha-1 adrenergic receptor antagonist is doxazosin mesylate.
- [0026] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a monoamine reuptake inhibitor.
- [0027] In some embodiments, the monoamine reuptake inhibitor inhibits serotonin reuptake.
- [0028] In some embodiments, the monoamine reuptake inhibitor inhibits norepinephrine uptake.
- [0029] In some embodiments, the monoamine reuptake inhibitor inhibits both serotonin and norepinephrine uptake.
- [0030] In some embodiments, the monoamine reuptake inhibitor inhibits dopamine reuptake.
- [0031] In some embodiments, the monoamine reuptake inhibitor is a tricyclic antidepressant.
- [0032] In some embodiments, the tricyclic antidepressant is selected from the group consisting of amitriptyline, amitriptylin oxide, clomipramine, demexiptiline, desipramine, dibenzepin, dimetacrine, dosulepin/dothiepin, doxepin, imipramine, imipramin oxide, melitracen, metapramine, nitroxazepine, nortriptyline, pipofezine, propizepine, protriptyline, quinupramine, amineptine, opipramol, tianeptine, and trimipramine.
- [0033] In some embodiments, the tricyclic antidepressant is amitriptyline, desipramine, or imipramine.
- [0034] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a calcium channel blocker.
- [0035] In some embodiments, the calcium channel blocker inhibits voltage-gated and receptor-operated calcium channels.
- [0036] In some embodiments, the calcium channel blocker is bepridil.

[0037] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a biologically active agent that exhibits one or more biological activities selected from the group consisting of histamine receptor antagonism, monoamine oxidase inhibition, serotonin receptor antagonism, acetylcholine receptor antagonism, adrenergic receptor antagonism, monoamine reuptake inhibition, and calcium channel blockade.

[0038] In some embodiments, the biologically active agent exhibits two or more of said biological activities.

[0039] In some embodiments, the biologically active agent exhibits three or more of said biological activities.

[0040] In some embodiments, the biologically active agent exhibits four or more of said biological activities.

[0041] In some embodiments, the biologically active agent exhibits five or more of said biological activities.

[0042] In some embodiments, the biologically active agent exhibits six or more of said biological activities.

[0043] In some embodiments, the biologically active agent exhibits each of said biological activities.

[0044] In some aspects, the invention provides a method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a biologically active agent selected from Table S1

[0045] In some embodiments, the cancer or neoplasm is a neuroendocrine cancer or tumor.

[0046] In some embodiments, the neuroendocrine cancer or tumor is selected from the group consisting of pheochromocytoma, Merkel cell cancer, neuroendocrine carcinoma, endocrine tumors, carcinoid tumors, thymoma, thyroid cancer, pancreatic neuroendocrine tumor, and small cell lung carcinoma.

[0047] In some embodiments, the neuroendocrine cancer or tumor is small cell lung carcinoma.

[0048] In some embodiments, the pharmaceutical composition is not coadministered with another composition.

[0049] The method of any of the preceding claims wherein said cancer or tumor is resistant to chemotherapy.

[0050] In some aspects, the invention provides a method of developing a biologically active agent that reduces neuroendocrine tumors, comprising: computing a disease signature, comprising comparing a first gene expression profile from a neuroendocrine tumor tissue to a second gene expression profile from an equivalent non-tumor tissue, thereby computing said disease signature; computing a drug signature from a candidate agent, comprising (i) treating a first population of neuroendocrine tumor cells with said candidate agent, (ii) treating a second population of neuroendocrine tumor cells with an equivalent amount of control vehicle, (iii) generating a third and fourth gene expression profile from said first and second populations of neuroendocrine tumor cells, and (iv) comparing said third and fourth gene expression profiles, thereby computing said drug signature; computing a drug activity score between said candidate agent and disease, comprising comparing said drug signature to said disease signature; and selecting said candidate for development as a biologically active agent for the treatment of neuroendocrine tumors based on said drug activity score.

[0051] In some aspects, the invention provides a method of developing a biologically active agent for the treatment of neuroendocrine tumors, comprising: selecting a candidate agent from a library of agents, wherein said candidate agent exhibits one or more biological activities selected from the group consisting histamine receptor antagonism, monoamine oxidase inhibition, serotonin receptor antagonism, acetylcholine receptor antagonism, adrenergic receptor antagonism, monoamine reuptake inhibition, and calcium channel blockade; contacting said candidate agent with one or more neuroendocrine tumor cells; determining the viability of said one or more neuroendocrine tumor cells; and selecting said candidate for development as a biologically active agent for the treatment of neuroendocrine tumors if said candidate reduces the viability of said one or more neuroendocrine tumor cells.

[0052] In some embodiments, the candidate agent exhibits two or more of said biological activities.

[0053] In some embodiments, the candidate agent exhibits three or more of said biological activities.

[0054] In some embodiments, the candidate agent exhibits four or more of said biological activities.

[0055] In some embodiments, the candidate agent exhibits five or more of said biological activities.

[0056] In some embodiments, the candidate agent exhibits six or more of said biological activities.

[0057] In some embodiments, the candidate agent exhibits each of said biological activities.

[0058] In some aspects, the invention provides a method of treating a neuroendocrine cancer or tumor selected from the group consisting of pheochromocytoma, Merkel cell cancer, neuroendocrine carcinoma, endocrine tumors, carcinoid tumors, thymoma, thyroid cancer, pancreatic neuroendocrine tumor, and small cell lung carcinoma in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a tricyclic antidepressant selected from the group consisting of amitriptyline, amitriptylinoxide, clomipramine, demexiptiline, desipramine, dibenzepin, dimetacrine, dosulepin/dothiepin, doxepin, imipramine, imipraminoxide, melitracen, metapramine, nitroxazepine, Nortriptyline, pipofezine, propizepine, protriptyline, quinupramine, amineptine, opipramol, tianeptine, and trimipramine.

[0059] In some aspects, the invention provides a method of treating a cancer or tumor in a subject, comprising administering to said subject at least two biologically active agents, wherein at least one of said two biologically active agents exhibits one or more biological activities selected from the group consisting of histamine receptor antagonism, monoamine oxidase inhibition, serotonin receptor antagonism, acetylcholine receptor antagonism, adrenergic receptor antagonism, monoamine reuptake inhibition, and calcium channel blockade.

[0060] In some embodiments, at least two of said biologically active agents one or more biological activities exhibit selected from the group consisting of histamine receptor antagonism, monoamine oxidase inhibition, serotonin receptor antagonism, acetylcholine receptor antagonism, adrenergic receptor antagonism, monoamine reuptake inhibition, and calcium channel blockade.

[0061] In some embodiments, the at least two biologically active agents comprise a histamine receptor antagonist.

[0062] In some embodiments, the histamine receptor antagonist is azelastine or promethazine.

[0063] In some embodiments, the at least two biologically active agents comprise at least two histamine receptor antagonists.

[0064] In some embodiments, the at least two histamine receptor antagonists are azelastine and promethazine.

- [0065] In some embodiments, the at least two biologically active agents comprise a histamine receptor ligand and a tricyclic antidepressant.
- [0066] The method of any of the preceding claims, wherein the histamine receptor ligand is 2-(2-Pyridil)-ethylamine (PEA).
- [0067] The method of any of the preceding claims, wherein the tricyclic antidepressant is imipramine.
- [0068] The method of any of the preceding claims, wherein the histamine receptor ligand is a histamine receptor antagonist.
- [0069] In some embodiments, the at least two biologically active agents comprise a histamine receptor antagonist and a tricyclic antidepressant.
- [0070] In some embodiments, the at least two biologically active agents comprise azelastine and imipramine.
- [0071] In some embodiments, the at least two biologically active agents comprise an acetylcholine receptor antagonist and a tricyclic antidepressant.
- [0072] In some embodiments, the at least two biologically active agents comprise an acetylcholine receptor antagonist and imipramine.
- [0073] In some embodiments, the acetylcholine receptor antagonist is a muscarinic receptor antagonist.
- [0074] In some embodiments, the muscarinic receptor antagonist is an M3 receptor antagonist.
- [0075] In some embodiments, the M3 receptor antagonist is 4-DAMP.
- [0076] In some embodiments, the at least two biologically active agents comprise an alpha-adrenergic receptor antagonist and a tricyclic antidepressant.
- [0077] In some embodiments, the at least two biologically active agents comprise an alpha-adrenergic receptor antagonist and imipramine.
- [0078] In some embodiments, the alpha-adrenergic receptor antagonist is doxazosin mesylate.
- [0079] In some embodiments, the at least two biologically active agents comprise a serotonin (5-HT) receptor antagonist and a tricyclic antidepressant.
- [0080] In some embodiments, the at least two biologically active agents comprise a serotonin (5-HT) receptor antagonist and imipramine.
- [0081] In some embodiments, the 5-HT receptor antagonist is a 5-HT₂ receptor antagonist.
- [0082] In some embodiments, the 5-HT₂ receptor antagonist is ritanserin.

[0083] In some embodiments, the at least two biologically active agents comprise a neurotransmitter and imipramine.

[0084] In some embodiments, the neurotransmitter is serotonin.

[0085] In some embodiments, the neurotransmitter is acetylcholine.

[0086] In some embodiments, the cancer or tumor is selected from the group consisting of adrenal cortical cancer, anal cancer, aplastic anemia, bile duct cancer, bladder cancer, bone cancer, central nervous system tumors such as, e.g., glioblastoma, astrocytoma, neuroblastoma, and oligodendroglioma, breast cancer, Castleman Disease, cervical cancer, Non-Hodgkin's lymphoma, colorectal cancer, endometrial cancer, esophageal cancer, Ewing's tumors, eye cancer, gallbladder cancer, carcinoids, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin's disease, Kaposi's sarcoma, kidney cancer, laryngeal cancer, hypopharyngeal cancer, neuroendocrine tumors, acute lymphocytic leukemia, acute myeloid leukemia, children's leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, liver cancer, lung cancer, lung carcinoid tumors, male breast cancer, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal cancer, nasopharyngeal cancer, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, rhabdomyosarcoma, salivary gland cancer, sarcoma (adult soft tissue cancer), melanoma skin cancer, nonmelanoma skin cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Waldenstrom's macroglobulinemia, pheochromocytoma, Merkel cell cancer, endocrine tumors, thymoma, pancreatic cancer, pancreatic neuroendocrine tumor, non-small cell lung carcinoma, and small cell lung carcinoma.

[0087] In some embodiments, the cancer or tumor is a neuroendocrine cancer or tumor.

[0088] In some embodiments, the neuroendocrine cancer or tumor is selected from the group consisting of pheochromocytoma, Merkel cell cancer, neuroendocrine carcinoma, endocrine tumors, carcinoid tumors, thymoma, thyroid cancer, pancreatic neuroendocrine tumor, and small cell lung carcinoma.

[0089] In some embodiments, the neuroendocrine cancer or tumor is small cell lung carcinoma.

[0090] In some embodiments, the method comprises administering about 0.001- 100 mg/kg/day of said biologically active agent to said subject.

[0091] In some embodiments, the method comprises administering about 0.001- 1 mg/kg/day of said biologically active agent to said subject.

[0092] In some embodiments, the method comprises administering about 0.1- 10 mg/kg/day of said biologically active agent to said subject.

[0093] In some embodiments, the method comprises administering about 5-25 mg/kg/day of said biologically active agent to said subject.

[0094] In some embodiments, the method comprises administering about 10- 50 mg/kg/day of said biologically active agent to said subject.

[0095] In some embodiments, the method comprises administering about 40-100 mg/kg/day of said biologically active agent to said subject.

[0096] In some aspects, the invention provides a kit, comprising at least one biologically active agent of any of the preceding claims and instructions for use in the treatment of a cancer or tumor in a subject of any of the preceding claims.

INCORPORATION BY REFERENCE

[0097] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0098] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0099] **FIG. 1** depicts a flowchart of an exemplary drug candidate selection method for the treatment of small cell lung cancer (SCLC).

[00100] **FIG. 2** depicts representative viability assays of various lung cancer cell lines treated with clomipramine (A), imipramine (B), promethazine (C), tranylcypromine (D), repaglinide (E), bepridil (F), and pargyline (G).

[00101] **FIG. 3** depicts an exemplary strategy used for the treatment of mice with subcutaneous SCLC tumors (A), fold change in tumor volume of mice implanted with SCLC cell lines Kp1 and Kp3 (B), fold change in tumor volume of mice implanted with the human

SCLC cell line H187 (C), and representative images of SCLC xenografts 14 days after treatment with saline, imipramine, and promethazine (D).

[00102] FIG. 4A depicts a strategy used for the treatment of Rb/p53/p130 mutant mice (TKO mice) developing endogenous SCLC tumors (A), representative photographs of sections from mutant mice (B), tumor area/lung area in mice treated with saline, imipramine, and promethazine (C), and % distribution of tumor size (D).

[00103] FIG. 4B depicts an experimental strategy used for the treatment of Rb/p53/p130;Rosa26^{lox-Stop-lox-Luciferase} mice developing endogenous SCLC tumors and treated with Saline and Cisplatin weekly to generate chemonaïve and chemoresistant tumors, followed in some cases by implantation of the mouse SCLC tumor cells into NSG mice (A), fold change in tumor volume measured by luciferase activity in Saline- and Cisplatin-treated mice (B), fold change in tumor volume measured in the implanted NSG mice treated with imipramine (C), and representative images of the cisplatin- and/or saline-treated SCLC allografts collected 17 days after daily treatment.

[00104] FIG. 4C depicts relative increase in cell death of mouse and human SCLC cells treated with Imipramine and Promethazine (A), representative immunoblots of cleaved caspase 3 in mouse and human SCLC cell lines (B), representative immunostaining of cleaved caspase 3 in tumor sections from TKO mice with quantitation (C), representative immunostaining of phospho-Histone 3 in tumor sections from TKO mice with quantitation (D), effects of combined treatment of imipramine and a pan-caspase inhibitor on viability of mouse (E) and human (F) SCLC cell lines, representative immunoblots following treatment with imipramine (G), and effects of imipramine and a JNK inhibitor on viability of mouse SCLC cells (H).

[00105] FIG. 4D depicts effects of the combined treatment of Imipramine (50μM) and the necrosis inhibitor Necrostatin-1 (abbreviated as Necros) on the survival of mSCLC cells (Kp1) after 24 hours of treatment, as measured by the MTT viability assay.

[00106] FIG. 5A depicts heat maps showing normalized RNA expression levels for the Histamine 1 Receptor (H1R), the Muscarinic acetylcholine receptor isoform 3 (CHRM3), the Alpha1A and Alpha1B Adrenergic Receptors (ADRA1a and ADRA1b), and the Serotonin Receptor 2A (HTR2a) in human and mouse SCLC tumors from four independent studies (A), and heat maps showing normalized RNA expression levels of H1R, CHRM3, ADRA1a and ADRA1b, and HTR2a in 35 human primary Merkel Cell Carcinoma tumors, 42 Midgut carcinoid 36 tumors, 76 Pheochromocytoma tumors, and 88 Neuroblastoma tumors.

[00107] **FIG. 5B** depicts results of MTT viability assays of cells treated with the H1R antagonist Azelastine with or without promethazine (A), the CHRM3 antagonist 4-DAMP with or without imipramine (B), the ADRA1 antagonist Doxazosin Mesylate with or without imipramine (C), and the HTR2 antagonist Ritanserin with or without imipramine (D), MTT viability assay for mSCLC (Kp1) and hSCLC (H187) cells following 24hr of treatment with 50 μ M Forskolin (FSK), 100 μ M IBMX, or both drugs combined (E), effects of the combined treatment of 50 μ M Imipramine and 50 μ M FSK alone, 100 μ M IBMX alone, or FSK and IBMX together, as measured by the MTT viability assay (G).

[00108] **FIG. 5C** depicts results of MTT viability assays of cells treated with the H1 Histamine receptor ligand 2-(2-Pyridil)-ethylamine (PEA) with or without imipramine (A), acetylcholine with or without imipramine (B), and serotonin with or without imipramine (C).

[00109] **FIG. 6A** depicts representative phase contrast images of human Pancreatic Adenocarcinoma (PDAC), mouse Pancreatic Neuroendocrine tumors (PNET), human Neuroblastoma (NB), human Merkel Cell Carcinoma (MCC), and human carcinoid cells (CAR) cultured in low serum and treated with vehicle control, 50 μ M Imipramine, and 30 μ M Promethazine for 48 hours (A), and (C-D) MTT viability assays of PDAC, PNETs, NB, MCC, and CAR cells treated with increasing doses of Imipramine (C) and Promethazine (D).

[00110] **FIG. 6B** depicts representative viability assays of various neuroendocrine tumor cell lines after treatment with various concentrations of imipramine.

[00111] **FIG. 7** depicts (A) MTT viability assays of representative mouse (Kp1 in black, 2% serum) and human (H82 in grey, 0.5% serum) SCLC cell lines following treatment with increasing doses of Imipramine, Promethazine, and Bepridil. (B) Representative phase contrast images of NSCLC cells (A549), human SCLC cells (H82), and mouse SCLC cells (Kp1) cultured in 2% serum and treated with vehicle control, 50 μ M Imipramine, 30 μ M Promethazine, and 10 μ M Bepridil for 48 hours. (C) MTT survival assays of SCLC cells cultured in 2% serum (n=3 independent experiments) for 48 hours with Imipramine, Promethazine, and Bepridil.

[00112] **FIG. 8A** depicts representative H&E staining (A), pH3 immunostaining (B), and CC3 immunostaining (C) of tumor sections from NSG mice implanted subcutaneously with mSCLC cells and treated daily with Saline, Imipramine (25mg/kg), and Promethazine (25mg/kg) for 14 consecutive days. "N" depicts necrotic areas. (D) Representative phase contrast images of mSCLC (Kp1) cells treated with vehicle control (water) and 50 μ M Imipramine for the different times indicated.

[00113] **FIG. 8B** depicts representative FACS histograms and quantitation of fluorescence levels of the calcium indicator Fluo-3AM from mSCLC cells treated with imipramine and promethazine (A-C).

[00114] **FIG. 9** depicts heat maps showing normalized RNA expression levels for Histidine Decarboxylase (HDC) required for Histamine biosynthesis, Tryptophan Hydroxylase (TPH1) and DOPA Decarboxylase (DDC) for Serotonin biosynthesis, Phenylethanolamine N-methyltransferase (PNMT) and Dopamine B-hydroxylase (DBH) for Epinephrine biosynthesis, and Choline Acetyltransferase (HAT) for Acetylcholine biosynthesis in human and mouse SCLC tumors from four independent studies.

[00115] **FIG. 10** depicts (A) Representative phase contrast images of mouse SCLC cells (Kp3) and human SCLC cells (H187) cultured in 2% serum and treated with vehicle control, 50 μ M Desipramine, and 50 μ M Amitriptyline for 48 hours. (B) MTT viability assay of mouse and human SCLC cell lines following treatment with increasing doses of Desipramine and Amitriptyline at 2% serum and for 48 hours (n=3 independent experiments). ** P <0.01 and *** P <0.001. (C) Left: Representative phase contrast images of carcinoid cells (H727) cultured in 2% serum and treated with vehicle control, 50 μ M Imipramine, and 30 μ M Promethazine for 48 hours. Right: MTT viability assay of H727 carcinoid cells following treatment with 50 μ M Imipramine and 30 μ M Promethazine at 2% serum for 48 hours (n=3 independent experiments).

[00116] **FIG. 11** depicts representative viability assays of mouse and human SCLC cell lines treated with various concentrations of imipramine, desipramine, and amitriptyline.

[00117] **FIG. 12** depicts representative photographs of tumor sections taken from saline or desipramine treated TKO mice.

[00118] **FIG. 13** depicts expression levels of G-protein coupled receptors (GPCRs) and their ligands in SCLC cells.

[00119] **FIG. 14** depicts MTT viability assays of cells cultured at 2% serum (n $\geq$ 3 independent experiments) and treated with increasing doses of Epinephrine (Epi) in the absence or presence of 50 μ M Imipramine (Imip) or 30 μ M Promethazine (Prom).

DETAILED DESCRIPTION OF THE INVENTION

[00120] **Definitions**

[00121] As used in the specification and claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise.

[00122] The term “effective amount” or “therapeutically effective amount” refers to that amount of a biologically active agent described herein that is sufficient to effect the intended application including but not limited to disease treatment, as defined below. The therapeutically effective amount may vary depending upon the intended application (in vitro or in vivo), or the subject and disease condition being treated, e.g., the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will induce a particular response in target cells, e.g. reduction of platelet adhesion and/or cell migration. The specific dose will vary depending on the particular biologically active agents chosen, the dosing regimen to be followed, whether it is administered in combination with other biologically active agents, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried.

[00123] As used herein, “treatment” or “treating,” or “palliating” or “ameliorating” is used interchangeably. These terms refer to an approach for obtaining beneficial or desired results including but not limited to a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the subject, notwithstanding that the subject may still be afflicted with the underlying disorder. For prophylactic benefit, the compositions may be administered to a subject at risk of developing a particular disease, or to a subject reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made.

[00124] A “therapeutic effect,” as that term is used herein, encompasses a therapeutic benefit and/or a prophylactic benefit as described above. A prophylactic effect includes delaying or eliminating the appearance of a disease or condition, delaying or eliminating the onset of symptoms of a disease or condition, slowing, halting, or reversing the progression of a disease or condition, or any combination thereof.

[00125] The term “co-administration,” “administered in combination with,” and their grammatical equivalents, as used herein, encompass administration of two or more agents to an animal so that both agents and/or their metabolites are present in the subject at the same time. Co-administration includes simultaneous administration in separate compositions, administration at different times in separate compositions, or administration in a composition in which both agents are present.

[00126] The term “pharmaceutically acceptable salt” refers to salts derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like, when the molecule contains an acidic functionality; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate (methane sulfonate), ethane sulfonate, acetate, maleate, oxalate, phosphate, and the like. In a compound with more than one basic moiety, more than one of the basic moieties may be converted to the salt form, including but not limited to a bis- or tris-salt. Alternatively, a compound having more than one basic moiety may form a salt at only one of the basic moieties.

[00127] The terms “antagonist” and “inhibitor” are used interchangeably, and they refer to a compound having the ability to inhibit a biological function of a target protein, whether by inhibiting the activity or expression of the target protein. Accordingly, the terms “antagonist” and “inhibitors” are defined in the context of the biological role of the target protein. While preferred antagonists herein specifically interact with (e.g. bind to) the target, compounds that inhibit a biological activity of the target protein by interacting with other members of the signal transduction pathway of which the target protein is a member are also specifically included within this definition. A preferred biological activity inhibited by an antagonist is associated with the development, growth, or spread of a tumor.

[00128] The term “agonist” as used herein refers to a compound having the ability to initiate or enhance a biological function of a target protein, whether by inhibiting the activity or expression of the target protein. Accordingly, the term “agonist” is defined in the context of the biological role of the target polypeptide. While preferred agonists herein specifically interact with (e.g. bind to) the target, compounds that initiate or enhance a biological activity of the target polypeptide by interacting with other members of the signal transduction pathway of which the target polypeptide is a member are also specifically included within this definition.

[00129] As used herein, “agent” or “biologically active agent” refers to a biological, pharmaceutical, or chemical compound or other moiety. A biologically active agent can be, e.g., a compound. Non-limiting examples include a simple or complex organic or inorganic molecule, a peptide, a protein, an oligonucleotide, an antibody, an antibody derivative, antibody fragment, a vitamin derivative, a carbohydrate, a toxin, or a chemotherapeutic compound. Various compounds can be synthesized, for example, small molecules and oligomers (e.g., oligopeptides and oligonucleotides), and synthetic organic compounds based

on various core structures. In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like.

[00130] An “anti-cancer agent”, “anti-tumor agent” or “chemotherapeutic agent” refers to any agent useful in the treatment of a neoplastic condition. One class of anti-cancer agents comprises chemotherapeutic agents. “Chemotherapy” means the administration of one or more chemotherapeutic drugs and/or other agents to a cancer patient by various methods, including intravenous, oral, intramuscular, intraperitoneal, intravesical, subcutaneous, transdermal, buccal, or inhalation or in the form of a suppository.

[00131] The term “cell proliferation” refers to a phenomenon by which the cell number has changed as a result of division. This term also encompasses cell growth by which the cell morphology has changed (e.g., increased in size) consistent with a proliferative signal.

[00132] The term “selective inhibition” or “selectively inhibit” refers to a biologically active agent refers to the agent's ability to preferentially reduce the target signaling activity as compared to off-target signaling activity, via direct or indirect interaction with the target.

[00133] An “anti-cancer agent”, “anti-tumor agent” or “chemotherapeutic agent” refers to any agent useful in the treatment of a neoplastic condition. One class of anti-cancer agents comprises chemotherapeutic agents. “Chemotherapy” means the administration of one or more chemotherapeutic drugs and/or other agents to a cancer patient by various methods, including intravenous, oral, intramuscular, intraperitoneal, intravesical, subcutaneous, transdermal, buccal, or inhalation or in the form of a suppository.

[00134] “Prodrug” is meant to indicate a compound that may be converted under physiological conditions or by solvolysis to a biologically active compound described herein. Thus, the term “prodrug” refers to a precursor of a biologically active compound that is pharmaceutically acceptable. A prodrug may be inactive when administered to a subject, but is converted in vivo to an active compound, for example, by hydrolysis. The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in a mammalian organism (see, e.g., Bundgard, H., *Design of Prodrugs* (1985), pp. 7-9, 21-24 (Elsevier, Amsterdam). A discussion of prodrugs is provided in Higuchi, T., et al., “Pro-drugs as Novel Delivery Systems,” A.C.S. Symposium Series, Vol. 14, and in *Bioreversible Carriers in Drug Design*, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated in full by reference herein. The term “prodrug” is also meant to include any covalently bonded carriers, which release the active compound in vivo when such prodrug is administered to a mammalian subject. Prodrugs of an active compound, as described herein, may be prepared by modifying functional groups

present in the active compound in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent active compound. Prodrugs include compounds wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the active compound is administered to a mammalian subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of a hydroxy functional group, or acetamide, formamide and benzamide derivatives of an amine functional group in the active compound and the like.

[00135] The term “in vivo” refers to an event that takes place in a subject's body.

[00136] The term “in vitro” refers to an event that takes places outside of a subject's body. For example, an in vitro assay encompasses any assay run outside of a subject assay. In vitro assays encompass cell-based assays in which cells alive or dead are employed. In vitro assays also encompass a cell-free assay in which no intact cells are employed.

[00137] Described herein are methods for the treatment of cancer or neoplasm. A neoplasm can be a tumor or a cancer. The methods described herein can be applied to the treatment of several cancer or neoplasm types, such as, for example, adrenal cortical cancer, anal cancer, aplastic anemia, bile duct cancer, bladder cancer, bone cancer, central nervous system tumors such as, e.g., glioblastoma, astrocytoma, neuroblastoma, and oligodendroglioma, breast cancer, Castleman Disease, cervical cancer, Non-Hodgkin's lymphoma, colorectal cancer, endometrial cancer, esophageal cancer, Ewing's tumors, eye cancer, gallbladder cancer, carcinoids, gastrointestianl carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin's disease, Kaposi'sarcoma, kidney cancer, laryngeal cancer, hypopharyngeal cancer, neuroendocrine tumors, acute lymphocytic leukemia, acute myeloid leukemia, children's leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, liver cancer, lung cancer, lung carcinoid tumors, male breast cancer, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal cancer, nasopharyngeal cancer, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, rhabdomyosarcoma, salivary gland cancer, sarcoma (adult soft tissue cancer), melanoma skin cancer, nonmelanoma skin cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sacrcoma, vaginal cancer, vulvar cancer, and Waldenstrom's macroglobulinemia, pheochromocytoma, Merkel cell cancer, endocrine tumors, thymoma, pancreatic cancer, pancreatic neuroendocrine tumor, non-small cell lung carcinoma, and small cell lung carcinoma, among others.

[00138] In some embodiments, the method comprises administering a pharmaceutical composition to a subject. The subject can be an animal. The subject can be a mammal, e.g., a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee or baboon. In some cases, the subject is a human. The human can be diagnosed with or suspected of having a disease. The disease can be a cancer. Exemplary cancers are described herein.

[00139] Biologically active agents

[00140] The pharmaceutical composition can comprise a biologically active agent. The biologically active agent can be present in a therapeutically effective amount.

[00141] The biologically active agent can comprise histamine receptor antagonist activity. The agent can, for example, block or inhibit H1 receptor activity. The agent can be an H1 receptor inverse agonist, e.g., can bind to an H1 receptor and induce a pharmacological response that is opposite to a response induced by an agonist of the receptor. Exemplary H1 receptor antagonists and/or inverse agonists include, e.g., Acrivastine, Azelastine, Bilastine, Brompheniramine, Buclizine, Bromodiphenhydramine, Carbinoxamine, Cetirizine, Chlorpromazine, Cyclizine, Chlorpheniramine, Chlorodiphenhydramine, Clemastine, Cyproheptadine, Desloratadine, Dexbrompheniramine, Dexchlorpheniramine, Dimenhydrinate, Dimetindene, Diphenhydramine (Benadryl), Doxylamine, Ebastine, Embramine, Fexofenadine, Levocetirizine, Loratadine, Meclozine, Mirtazapine, Olopatadine, Orphenadrine, Phenindamine, Pheniramine, Phenyltoloxamine, Promethazine, Pyrilamine, Quetiapine, Rupatadine, Tripelennamine, and Triprolidine.

[00142] The agent can, for example, block or inhibit H2 histamine receptor activity. Exemplary agents that can block or inhibit H2 receptor activity include, but are not limited to: Cimetidine, Famotidine, Lafutidine, Nizatidine, Ranitidine, and Roxatidine.

[00143] The agent can block or inhibit H3 and/or H4 histamine receptor activity. Exemplary agents that can block or inhibit H2 receptor activity include, but are not limited to: A-349,821, ABT-239, Ciproxifan, Clobenpropit, Conessine, Thioperamide, JNJ 7777120, VUF-600.

[00144] A-349,821 can refer to (4'-[3-(2R,5R-dimethylpyrrolidin-1-yl)propoxy]biphenyl-4-yl)morpholin-4-ylmethanone.

[00145] ABT-239 can refer to 4-(2-{2-[(2R)-2-Methylpyrrolidin-1-yl]ethyl}-benzofuran-5-yl)benzonitrile.

- [00146] JNJ-7777120 can refer to 5-chloro-2-[(4-methylpiperazin-1-yl)carbonyl]-1H-indole.
- [00147] VUF-6002 can refer to 5-Chloro-2-[(4-methylpiperazin-1-yl)carbonyl]-1H-benzimidazole.
- [00148] Promethazine can refer to N,N-dimethyl-1-phenothiazin-10-ylpropan-2-amine.
- [00149] Azelastine can refer to: 4-[(4-chlorophenyl)methyl]-2-(1-methylazepan-4-yl)-1,2-dihydrophthalazin-1-one.
- [00150] The biologically active agent can be a monoamine oxidase inhibitor. Monoamine oxidase inhibitors generally refer to biologically active agents that inhibit the activity of the monoamine oxidase enzyme family. Exemplary monoamine oxidase enzymes include, but are not limited to Monoamine Oxidase A (MAO-A) and Monoamine Oxidase B (MAO-B). Exemplary MAO-A and/or MAO-B inhibitors include, e.g., Harmala alkaloids, Resveratrol, Curcumin, Rhodiola rosea, Ruta graveolens, Ginkgo biloba, Anthocyanins, Proanthocyanidin, Epicatechin, Emodin, Gentiana lutea, Hydrazines such as, e.g., Benmoxin (Nerusil, Neuralex), Hydralazine (Apresoline), Iproclozide (Sursum), Iproniazid (Marsilid, Iprozid, Ipronid, Rivivol, Propilniazida) Isocarboxazid (Marplan), Isoniazid (Laniazid, Nydrazid), Mebanazine (Actomol), Nialamide (Niamid), Octamoxin (Ximaol, Nimaol), Phenelzine (Nardil, Nardelzine), Pheniprazine (Catron), Phenoxypropazine (Drazine), Pivalylbenzhydrazine (Tersavid), Procarbazine (Matulane, Natulan, Indicarb), Safrazine (Safra), Non-hydrazine MAO inhibitors such as, e.g., Caroxazone (Surodil, Timostenil), Echinopsidine (Adepren), Furazolidone (Furoxone, Dependal-M), Linezolid (Zyvox, Zyvoxam, Zyvoxid), Tranylcypromine (Parnate, Jatrosom), and antibiotics such as, e.g., linezolid.
- [00151] Exemplary selective MAO-A inhibitors include, e.g., Brofaromine (Consonar), Metralindole (Inkazan), Minaprine (Cantor), Moclobemide (Aurorix, Manerix), Pirlindole (Pirazidol), Toloxatone (Humoryl), Amiflamine, Bazinaprine, Befloxatone, Cimoxatone, Clorgyline, Esuprone, Eprobemide (Befol), Methylene Blue, Pirlindole, Sercloramine, Tetrindole, Thesputaint, and CX157 (Tyrina). Exemplary selective MAO-B inhibitors include, e.g., Lazabemide (Pakio, Tempium), Pargyline (Eutonyl), Rasagiline (Azilect), Selegiline (Deprenyl, Eldepryl, Emsam), D-Deprenyl, Ladostigil, Milacemide, and Mofegiline.
- [00152] MAO inhibitors can also include various tryptamine and/or phenethylamine/amphetamine derivatives such as, e.g., α ET, α MT, amphetamine (itself), methamphetamine, MDMA, 4-MTA, PMA, 2C-T-7, and 2C-T-21.

[00153] The biologically active agent can be a serotonin receptor antagonist. The serotonin receptor antagonist can comprise serotonin receptor-2 type antagonist activity. Exemplary compounds with serotonin receptor-2 type antagonist activity include but are not limited to methysergide, aripiprazole, OSU-6162, metergoline, ketanserin, ritanserin, nefazodone, clozapine, olanzapine, quetiapine, risperidone and asenapine, MDL-100,907, cyproheptadine, pizotifen, 2-alkyl-4-aryl-tetrahydro-pyrimido-azepines, 9-Aminomethyl-9,10-dihydroanthracene, Hydroxyzine (Atarax), 5-MeO-NBpBrT, AC-90179, Nelotanserin, Eplivanserin (Sanofi Aventis), Pimavanserin (ACP-103), and Volinanserin.

[00154] The biologically active agent can be an acetylcholine receptor antagonist. The acetylchoine receptor antagonist can be a muscarinic acetylcholine receptor antagonist or anti-muscarinic agent. The muscarinic acetylcholine receptor antagonist can be an M3 receptor antagonist. Exemplary anti-muscarinic agents include, e.g., Atropine, Ipratropium, Scopolamine, Tiotropium, Mecamylamine, Hexamethonium, Trimethaphan, Bupropion, Dextromethorphan, and Diphenhydramine. Exemplary M3 muscarinic receptor antagonists include, but are not limited to atropine, Hyoscyamine, 4-DAMP (1,1-Dimethyl-4-diphenylacetoxypiperidinium iodide, CAS# 1952-15-4), DAU-5884 (8-Methyl-8-azabicyclo-3-endo[1.2.3]oct-3-yl-1,4-dihydro-2-oxo-3(2H)-quinazolinecarboxylic acid ester, CAS# 131780-47-7), dicycloverine, J-104,129 ((aR)-a-Cyclopentyl-a-hydroxy-N-[1-(4-methyl-3-pentenyl)-4-piperidinyl]benzeneacetamide, CAS# 244277-89-2), HL-031,120 ((3R,2'R)-enantiomer of EA-3167), tolterodine, oxybutynin, ipratropium, darifenacin, tiotropium, and Zamifenacin ((3R)-1-[2-(1,3-Benzodioxol-5-yl)ethyl]-3-(diphenylmethoxy)piperidine, CAS# 127308-98-9).

[00155] The biologically active agent can be an adrenergic receptor antagonist. The adrenergic receptor antagonist can be an alpha-adrenergic receptor antagonist. Exemplary alpha-adrenergic receptor antagonists include, e.g., Phenoxybenzamine, Phentolamine, Tolazoline, Trazodone, Typical and atypical antipsychotics, Alfuzosin, Prazosin, Doxazosin, Tamsulosin, Terazosin, Silodosin, Atipamezole, Idazoxan, Yohimbine, carvedilol and labetalol. Exemplary typical antipsychotics include, e.g., Chlorpromazine (Largactil, Thorazine), Chlorprothixene (Truxal), Thioridazine (Mellaril), Mesoridazine, Levomepromazine, Loxapine (Loxapac, Loxitane), Molindone (Moban), Perphenazine (Trilafon), Thiothixene (Navane), Trifluoperazine (Stelazine), Haloperidol (Haldol, Serenace), Fluphenazine (Prolixin), Droperidol, Zuclopenthixol (Clopixol), Flupentixol (Depixol), and Prochlorperazine.

[00156] Exemplary atypical antipsychotics include, e.g., Amisulpride, Aripiprazole, Asenapine, Blonanserin, Caripramine, Clocapramine, Clozapine, Iloperidone, Lurasidone, Mosapramine, Olanzapine, Paliperidone, Perospirone, Quetiapine, Remoxipride, Risperidone, Sertindole, Sulpiride, Ziprasidone, Zotepine, Bitopertin, Brexpiprazole, Cariprazine, Pimavanserin, Vabicaserin, or Zicronapine.

[00157] The biologically active agent can be a monoamine reuptake inhibitor. The monoamine reuptake inhibitor can inhibit the serotonin transporter, the norepinephrine transporter, the dopamine transporter, or any combination thereof.

[00158] The serotonin transporter inhibitor (e.g., serotonin reuptake inhibitor) can be, by way of example only, Alaproclate, Citalopram, Dapoxetine, Escitalopram, Femoxetine, Fluoxetine, Fluvoxamine, Ifooxetine, Indalpine, Omiloxetine, Panuramine, Paroxetine, Pirandamine, RTI-353, Sertraline, Zimelidine, Desmethylecitalopram, Desmethylesertraline, Didesmethylecitalopram, Seproxetine, Cianopramine, Litoxetine, Lubazodone, SB-649,915, Trazodone, Vilazodone, Dextromethorphan, Dimenhydrinate, Diphenhydramine, Mepyramine/Pyrimidine, Methadone, Propoxyphene, Mesembrine, or Roxindole. The dopamine transporter inhibitor (e.g., dopamine reuptake inhibitor) can be, by way of example only, Amineptine, Altropane, Amfonelic acid, BTCP, DBL-583, Difluoropine, GBR-12935, GYKI-52895, Iometopane, RTI-229, Vanoxerine, Medifoxamine, Chaenomeles speciosa, Hyperforin, Adhyperforin, Bupropion, Pramipexole, or Cabergoline. The norepinephrine transporter inhibitor, e.g., norepinephrine reuptake inhibitor, can be, by way of example only, Amedalin, Atomoxetine, CP-39,332, Daledalin, Edivoxetine, Esreboxetine, Lortalamine, Mazindol, Nisoxetine, Reboxetine, Talopram, Talsupram, Tandamine, Viloxazine, Maprotiline, Bupropion, Ciclazindol, Manifaxine, Radafaxine, Tapentadol, Teniloxazine, Ethanol, or Ginkgo biloba.

[00159] The biologically active agent can be a tricyclic antidepressant. The tricyclic antidepressant can be, e.g., amitriptyline, amitriptylinoxide, clomipramine, demexiptiline, desipramine, dibenzepin, dimetacrine, dosulepin/dothiepin, doxepin, imipramine, imipraminioxid, melitracen, metapramine, nitroxazepine, Nortriptyline, pipofezine, propizepine, protriptyline, quinupramine, amineptine, opipramol, tianeptine, or trimipramine.

[00160] The biologically active agent can be a calcium channel blocker. The biologically active agent can, for example, inhibit voltage-gated and/or receptor-operated calcium channels. Exemplary voltage gated calcium channel blockers include, e.g., Amlodipine, Aranidipine, Azelnidipine, Barnidipine, Benidipine, Cilnidipine, Clevidipine, Isradipine, Efonidipine, Felodipine, Lacidipine, Lercanidipine, Manidipine, Nicardipine,

Nifedipine, Nilvadipine, Nimodipine, Nisoldipine, Nitrendipine, Pranidipine, Verapamil, Gallopamil, Fendiline, Diltiazem, mibefradil, bepridil, fluspirilene, fendiline, or Ziconotide. Exemplary receptor-operated calcium channels include, e.g., AP5, AP7, CPPene, Selfotel, Amantadine, AZD6765, Dextrallorphan, Dextromethorphan, Dextrophan, Diphenidine, Dizocilpine, Ethanol, Eticyclidine, Gacyclidine, Ibogaine, Magnesium, Memantine, Methoxetamine, Nitrous oxide, Phencyclidine, Rolicyclidine, Tenocyclidine, Methoxydine, Tiletamine, Xenon, Neramexane, Eliprodil, Etoxadrol, Dexoxadrol, WMS-2539, NEFA, Remacemide, Delucemine, 8A-PDHQ, Aptiganel, HU-211, HU-210, Remacemide, Rhynchophylline, Ketamine, Ryanodine, Dantrolene, Ruthenium red, procaine, or tetracaine.

[00161] In some embodiments, the method comprises a combination therapy, e.g., administration of two or more biologically active agents. In some cases, sub-therapeutic amounts of one or both agents can be used. In other cases, therapeutically effective amounts of one or both agents can be used. In addition, the agents described herein may be administered either simultaneously or sequentially. If administered sequentially, an attending physician can decide on the appropriate sequence of administering the two or more agents.

[00162] The two or more agents can include any of the agents described herein. The two or more agents can include one agent as described herein and an additional anti-cancer agent. Non-limiting examples of additional anti-cancer agents include kinase inhibitors that can be combined with the subject agent include rapamycin, TOR-Kinase inhibitors, PI-103, BEZ235, Akt i, IC87114, and PIK-90, chemotherapeutic agents, cytotoxic agents, and non-peptide small molecules such as Gleevec (Imatinib Mesylate), Velcade (bortezomib), Casodex (bicalutamide), Iressa (gefitinib), and Adriamycin as well as a host of chemotherapeutic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine,

doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK.R®; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Rhone-Poulenc Rorer, Antony, France); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included as suitable chemotherapeutic cell conditioners are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; camptothecin-11 (CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO).

[00163] Exemplary pharmaceutical compositions

[00164] The pharmaceutical composition can be formulated for oral administration. For example, the pharmaceutical composition can be formulated as a solid dosage form. The solid dosage form can be, by way of example only, a tablet, capsule, caplet, pill, or thin film.

In some embodiments, the pharmaceutical composition may be a liquid pharmaceutical composition suitable for oral consumption. Pharmaceutical compositions of the invention suitable for oral administration can be presented as discrete dosage forms, such as capsules, cachets, or tablets, or liquids or aerosol sprays each containing a predetermined amount of an active ingredient as a powder or in granules, a solution, or a suspension in an aqueous or non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. Such dosage forms can be prepared by any of the methods of pharmacy, but all methods include the step of bringing the active ingredient into association with the carrier, which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation. For example, a tablet can be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets can be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as powder or granules, optionally mixed with an excipient such as, but not limited to, a binder, a lubricant, an inert diluent, and/or a surface active or dispersing agent. Molded tablets can be made by molding in a suitable machine a mixture of the powdered biologically active agent moistened with an inert liquid diluent.

[00165] This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising an active ingredient, since water can facilitate the degradation of some biologically active agents. For example, water may be added (e.g., 5%) in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms of the invention which contain lactose can be made anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected. An anhydrous pharmaceutical composition may be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions may be packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastic or the like, unit dose containers, blister packs, and strip packs.

[00166] An active ingredient can be combined in an intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques.

The carrier can take a wide variety of forms depending on the form of preparation desired for administration. In preparing the compositions for an oral dosage form, any of the usual pharmaceutical media can be employed as carriers, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (such as suspensions, solutions, and elixirs) or aerosols; or carriers such as starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents can be used in the case of oral solid preparations, in some embodiments without employing the use of lactose. For example, suitable carriers include powders, capsules, and tablets, with the solid oral preparations. If desired, tablets can be coated by standard aqueous or nonaqueous techniques.

[00167] Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, microcrystalline cellulose, and mixtures thereof.

[00168] Examples of suitable fillers for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof.

[00169] Disintegrants may be used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Too much of a disintegrant may produce tablets which may disintegrate in the bottle. Too little may be insufficient for disintegration to occur and may thus alter the rate and extent of release of the active ingredient(s) from the dosage form. Thus, a sufficient amount of disintegrant that is neither too little nor too much to detrimentally alter the release of the active ingredient(s) may be used to form the dosage forms of the biologically active agents disclosed herein. The amount of disintegrant used may vary based upon the type of formulation and mode of administration, and may be readily discernible to those of ordinary skill in the art. About 0.5 to about 15 weight percent of disintegrant, or about 1 to about 5 weight percent of disintegrant, may be used in the pharmaceutical composition. Disintegrants that can be used to form pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose,

croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other algin, other celluloses, gums or mixtures thereof.

[00170] Lubricants which can be used to form pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, or mixtures thereof. Additional lubricants include, for example, a syloid silica gel, a coagulated aerosol of synthetic silica, or mixtures thereof. A lubricant can optionally be added, in an amount of less than about 1 weight percent of the pharmaceutical composition.

[00171] When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient therein may be combined with various sweetening or flavoring agents, coloring matter or dyes and, if so desired, emulsifying and/or suspending agents, together with such diluents as water, ethanol, propylene glycol, glycerin and various combinations thereof.

[00172] The tablets can be uncoated or coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed. Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or olive oil.

[00173] Surfactant which can be used to form pharmaceutical compositions and dosage forms of the invention include, but are not limited to, hydrophilic surfactants, lipophilic surfactants, and mixtures thereof. That is, a mixture of hydrophilic surfactants may be employed, a mixture of lipophilic surfactants may be employed, or a mixture of at least one hydrophilic surfactant and at least one lipophilic surfactant may be employed.

[00174] A suitable hydrophilic surfactant may generally have an HLB value of at least 10, while suitable lipophilic surfactants may generally have an HLB value of or less than about 10. An empirical parameter used to characterize the relative hydrophilicity and hydrophobicity of non-ionic amphiphilic compounds is the hydrophilic-lipophilic balance

("HLB" value). Surfactants with lower HLB values are more lipophilic or hydrophobic, and have greater solubility in oils, while surfactants with higher HLB values are more hydrophilic, and have greater solubility in aqueous solutions. Hydrophilic surfactants are generally considered to be those compounds having an HLB value greater than about 10, as well as anionic, cationic, or zwitterionic compounds for which the HLB scale is not generally applicable. Similarly, lipophilic (i.e., hydrophobic) surfactants are compounds having an HLB value equal to or less than about 10. However, HLB value of a surfactant is merely a rough guide generally used to enable formulation of industrial, pharmaceutical and cosmetic emulsions.

[00175] Hydrophilic surfactants may be either ionic or non-ionic. Suitable ionic surfactants include, but are not limited to, alkylammonium salts; fusidic acid salts; fatty acid derivatives of amino acids, oligopeptides, and polypeptides; glyceride derivatives of amino acids, oligopeptides, and polypeptides; lecithins and hydrogenated lecithins; lysolecithins and hydrogenated lysolecithins; phospholipids and derivatives thereof; lysophospholipids and derivatives thereof; carnitine fatty acid ester salts; salts of alkylsulfates; fatty acid salts; sodium docusate; acyl lactylates; mono- and di-acetylated tartaric acid esters of mono- and di-glycerides; succinylated mono- and di-glycerides; citric acid esters of mono- and di-glycerides; and mixtures thereof.

[00176] Within the aforementioned group, ionic surfactants include, by way of example: lecithins, lysolecithin, phospholipids, lysophospholipids and derivatives thereof; carnitine fatty acid ester salts; salts of alkylsulfates; fatty acid salts; sodium docusate; acylactylates; mono- and di-acetylated tartaric acid esters of mono- and di-glycerides; succinylated mono- and di-glycerides; citric acid esters of mono- and di-glycerides; and mixtures thereof.

[00177] Ionic surfactants may be the ionized forms of lecithin, lysolecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidic acid, phosphatidylserine, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidic acid, lysophosphatidylserine, PEG-phosphatidylethanolamine, PVP-phosphatidylethanolamine, lactic esters of fatty acids, stearyl-2-lactylate, stearyl lactylate, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono/diglycerides, citric acid esters of mono/diglycerides, choly sarcosine, caproate, caprylate, caprate, laurate, myristate, palmitate, oleate, ricinoleate, linoleate, linolenate, stearate, lauryl sulfate, teracecyl sulfate, docusate, lauroyl carnitines, palmitoyl carnitines, myristoyl carnitines, and salts and mixtures thereof.

[00178] Hydrophilic non-ionic surfactants may include, but are not limited to, alkylglucosides; alkylmaltosides; alkylthioglucosides; lauryl macrogolglycerides; polyoxyalkylene alkyl ethers such as polyethylene glycol alkyl ethers; polyoxyalkylene alkylphenols such as polyethylene glycol alkyl phenols; polyoxyalkylene alkyl phenol fatty acid esters such as polyethylene glycol fatty acids monoesters and polyethylene glycol fatty acids diesters; polyethylene glycol glycerol fatty acid esters; polyglycerol fatty acid esters; polyoxyalkylene sorbitan fatty acid esters such as polyethylene glycol sorbitan fatty acid esters; hydrophilic transesterification products of a polyol with at least one member of the group consisting of glycerides, vegetable oils, hydrogenated vegetable oils, fatty acids, and sterols; polyoxyethylene sterols, derivatives, and analogues thereof; polyoxyethylated vitamins and derivatives thereof; polyoxyethylene-polyoxypropylene block copolymers; and mixtures thereof; polyethylene glycol sorbitan fatty acid esters and hydrophilic transesterification products of a polyol with at least one member of the group consisting of triglycerides, vegetable oils, and hydrogenated vegetable oils. The polyol may be glycerol, ethylene glycol, polyethylene glycol, sorbitol, propylene glycol, pentaerythritol, or a saccharide.

[00179] Other hydrophilic-non-ionic surfactants include, without limitation, PEG-10 laurate, PEG-12 laurate, PEG-20 laurate, PEG-32 laurate, PEG-32 dilaurate, PEG-12 oleate, PEG-15 oleate, PEG-20 oleate, PEG-20 dioleate, PEG-32 oleate, PEG-200 oleate, PEG-400 oleate, PEG-15 stearate, PEG-32 distearate, PEG-40 stearate, PEG-100 stearate, PEG-20 dilaurate, PEG-25 glyceryl trioleate, PEG-32 dioleate, PEG-20 glyceryl laurate, PEG-30 glyceryl laurate, PEG-20 glyceryl stearate, PEG-20 glyceryl oleate, PEG-30 glyceryl oleate, PEG-30 glyceryl laurate, PEG-40 glyceryl laurate, PEG-40 palm kernel oil, PEG-50 hydrogenated castor oil, PEG-40 castor oil, PEG-35 castor oil, PEG-60 castor oil, PEG-40 hydrogenated castor oil, PEG-60 hydrogenated castor oil, PEG-60 corn oil, PEG-6 caprate/caprylate glycerides, PEG-8 caprate/caprylate glycerides, polyglyceryl-10 laurate, PEG-30 cholesterol, PEG-25 phyto sterol, PEG-30 soya sterol, PEG-20 trioleate, PEG-40 sorbitan oleate, PEG-80 sorbitan laurate, polysorbate 20, polysorbate 80, POE-9 lauryl ether, POE-23 lauryl ether, POE-10 oleyl ether, POE-20 oleyl ether, POE-20 stearyl ether, tocopheryl PEG-100 succinate, PEG-24 cholesterol, polyglyceryl-10oleate, Tween 40, Tween 60, sucrose monostearate, sucrose monolaurate, sucrose monopalmitate, PEG 10-100 nonyl phenol series, PEG 15-100 octyl phenol series, and poloxamers.

[00180] Suitable lipophilic surfactants include, by way of example only: fatty alcohols; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lower alcohol fatty acids

esters; propylene glycol fatty acid esters; sorbitan fatty acid esters; polyethylene glycol sorbitan fatty acid esters; sterols and sterol derivatives; polyoxyethylated sterols and sterol derivatives; polyethylene glycol alkyl ethers; sugar esters; sugar ethers; lactic acid derivatives of mono- and di-glycerides; hydrophobic transesterification products of a polyol with at least one member of the group consisting of glycerides, vegetable oils, hydrogenated vegetable oils, fatty acids and sterols; oil-soluble vitamins/vitamin derivatives; and mixtures thereof. Within this group, preferred lipophilic surfactants include glycerol fatty acid esters, propylene glycol fatty acid esters, and mixtures thereof, or are hydrophobic transesterification products of a polyol with at least one member of the group consisting of vegetable oils, hydrogenated vegetable oils, and triglycerides.

[00181] In one embodiment, the composition may include a solubilizer to ensure good solubilization and/or dissolution of the biologically active agent of the present invention and to minimize precipitation of the biologically active agent of the present invention. This can be especially important for compositions for non-oral use, e.g., compositions for injection. A solubilizer may also be added to increase the solubility of the hydrophilic drug and/or other components, such as surfactants, or to maintain the composition as a stable or homogeneous solution or dispersion.

[00182] Examples of suitable solubilizers include, but are not limited to, the following: alcohols and polyols, such as ethanol, isopropanol, butanol, benzyl alcohol, ethylene glycol, propylene glycol, butanediols and isomers thereof, glycerol, pentaerythritol, sorbitol, mannitol, transcitol, dimethyl isosorbide, polyethylene glycol, polypropylene glycol, polyvinylalcohol, hydroxypropyl methylcellulose and other cellulose derivatives, cyclodextrins and cyclodextrin derivatives; ethers of polyethylene glycols having an average molecular weight of about 200 to about 6000, such as tetrahydrofurfuryl alcohol PEG ether (glycofuro) or methoxy PEG; amides and other nitrogen-containing compounds such as 2-pyrrolidone, 2-piperidone, 8-caprolactam, N-alkylpyrrolidone, N-hydroxyalkylpyrrolidone, N-alkylpiperidone, N-alkylcaprolactam, dimethylacetamide and polyvinylpyrrolidone; esters such as ethyl propionate, tributylcitrate, acetyl triethylcitrate, acetyl tributyl citrate, triethylcitrate, ethyl oleate, ethyl caprylate, ethyl butyrate, triacetin, propylene glycol monoacetate, propylene glycol diacetate, ϵ -caprolactone and isomers thereof, δ -valerolactone and isomers thereof, β -butyrolactone and isomers thereof; and other solubilizers known in the art, such as dimethyl acetamide, dimethyl isosorbide, N-methyl pyrrolidones, mono-octanoin, diethylene glycol monoethyl ether, and water.

[00183] Mixtures of solubilizers may also be used. Examples include, but not limited to, triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropyl methylcellulose, hydroxypropyl cyclodextrins, ethanol, polyethylene glycol 200-100, glycofurol, transcutool, propylene glycol, and dimethyl isosorbide. Particularly preferred solubilizers include sorbitol, glycerol, triacetin, ethyl alcohol, PEG-400, glycofurol and propylene glycol.

[00184] The amount of solubilizer that can be included is not particularly limited. The amount of a given solubilizer may be limited to a bioacceptable amount, which may be readily determined by one of skill in the art. In some circumstances, it may be advantageous to include amounts of solubilizers far in excess of bioacceptable amounts, for example to maximize the concentration of the drug, with excess solubilizer removed prior to providing the composition to a subject using conventional techniques, such as distillation or evaporation. Thus, if present, the solubilizer can be in a weight ratio of 10%, 25%, 50%, 100%, or up to about 200% by weight, based on the combined weight of the drug, and other excipients. If desired, very small amounts of solubilizer may also be used, such as 5%, 2%, 1% or even less. Typically, the solubilizer may be present in an amount of about 1% to about 100%, more typically about 5% to about 25% by weight.

[00185] The composition can further include one or more pharmaceutically acceptable additives and excipients. Such additives and excipients include, without limitation, detackifiers, anti-foaming agents, buffering agents, polymers, antioxidants, preservatives, chelating agents, viscomodulators, tonicifiers, flavorants, colorants, odorants, opacifiers, suspending agents, binders, fillers, plasticizers, lubricants, and mixtures thereof.

[00186] In addition, an acid or a base may be incorporated into the composition to facilitate processing, to enhance stability, or for other reasons. Examples of pharmaceutically acceptable bases include amino acids, amino acid esters, ammonium hydroxide, potassium hydroxide, sodium hydroxide, sodium hydrogen carbonate, aluminum hydroxide, calcium carbonate, magnesium hydroxide, magnesium aluminum silicate, synthetic aluminum silicate, synthetic hydrocalcite, magnesium aluminum hydroxide, diisopropylethylamine, ethanolamine, ethylenediamine, triethanolamine, triethylamine, triisopropanolamine, trimethylamine, tris(hydroxymethyl)aminomethane (TRIS) and the like. Also suitable are bases that are salts of a pharmaceutically acceptable acid, such as acetic acid, acrylic acid, adipic acid, alginic acid, alkanesulfonic acid, amino acids, ascorbic acid, benzoic acid, boric acid, butyric acid, carbonic acid, citric acid, fatty acids, formic acid, fumaric acid, gluconic

acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid, salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid, toluenesulfonic acid, uric acid, and the like. Salts of polyprotic acids, such as sodium phosphate, disodium hydrogen phosphate, and sodium dihydrogen phosphate can also be used. When the base is a salt, the cation can be any convenient and pharmaceutically acceptable cation, such as ammonium, alkali metals, alkaline earth metals, and the like. Example may include, but not limited to, sodium, potassium, lithium, magnesium, calcium and ammonium.

[00187] Suitable acids are pharmaceutically acceptable organic or inorganic acids. Examples of suitable inorganic acids include hydrochloric acid, hydrobromic acid, hydriodic acid, sulfuric acid, nitric acid, boric acid, phosphoric acid, and the like. Examples of suitable organic acids include acetic acid, acrylic acid, adipic acid, alginic acid, alkanesulfonic acids, amino acids, ascorbic acid, benzoic acid, boric acid, butyric acid, carbonic acid, citric acid, fatty acids, formic acid, fumaric acid, gluconic acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid, salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid, toluenesulfonic acid, uric acid and the like.

[00188] Pharmaceutical Compositions For Injection.

[00189] In some embodiments, the invention provides a pharmaceutical composition for injection containing a biologically active agent of the present invention and a pharmaceutical excipient suitable for injection. Components and amounts of agents in the compositions are as described herein.

[00190] The forms in which the novel compositions of the present invention may be incorporated for administration by injection include aqueous or oil suspensions, or emulsions, with sesame oil, corn oil, cottonseed oil, or peanut oil, as well as elixirs, mannitol, dextrose, or a sterile aqueous solution, and similar pharmaceutical vehicles.

[00191] Aqueous solutions in saline are also conventionally used for injection. Ethanol, glycerol, propylene glycol, liquid polyethylene glycol, and the like (and suitable mixtures thereof), cyclodextrin derivatives, and vegetable oils may also be employed. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, for the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like.

[00192] Sterile injectable solutions are prepared by incorporating the biologically active agent of the present invention in the required amount in the appropriate solvent with various other ingredients as enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, certain desirable methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00193] Pharmaceutical Compositions for Topical (e.g., Transdermal) Delivery.

[00194] In some embodiments, the invention provides a pharmaceutical composition for transdermal delivery containing a biologically active agent of the present invention and a pharmaceutical excipient suitable for transdermal delivery.

[00195] Compositions of the present invention can be formulated into preparations in solid, semi-solid, or liquid forms suitable for local or topical administration, such as gels, water soluble jellies, creams, lotions, suspensions, foams, powders, slurries, ointments, solutions, oils, pastes, suppositories, sprays, emulsions, saline solutions, dimethylsulfoxide (DMSO)-based solutions. In general, carriers with higher densities are capable of providing an area with a prolonged exposure to the active ingredients. In contrast, a solution formulation may provide more immediate exposure of the active ingredient to the chosen area.

[00196] The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients, which are compounds that allow increased penetration of, or assist in the delivery of, therapeutic molecules across the stratum corneum permeability barrier of the skin. There are many of these penetration-enhancing molecules known to those trained in the art of topical formulation. Examples of such carriers and excipients include, but are not limited to, humectants (e.g., urea), glycols (e.g., propylene glycol), alcohols (e.g., ethanol), fatty acids (e.g., oleic acid), surfactants (e.g., isopropyl myristate and sodium lauryl sulfate), pyrrolidones, glycerol monolaurate, sulfoxides, terpenes (e.g., menthol), amines, amides, alkanes, alkanols, water, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[00197] Another exemplary formulation for use in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to

provide continuous or discontinuous infusion of a biologically active agent of the present invention in controlled amounts, either with or without another agent.

[00198] The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Pat. Nos. 5,023,252, 4,992,445 and 5,001,139. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

[00199] Pharmaceutical Compositions for Inhalation.

[00200] Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described supra. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices that deliver the formulation in an appropriate manner.

Other Pharmaceutical Compositions.

[00201] Pharmaceutical compositions may also be prepared from compositions described herein and one or more pharmaceutically acceptable excipients suitable for sublingual, buccal, rectal, intraosseous, intraocular, intranasal, epidural, or intraspinal administration. Preparations for such pharmaceutical compositions are well-known in the art. See, e.g., See, e.g., Anderson, Philip O.; Knoben, James E.; Troutman, William G, eds., Handbook of Clinical Drug Data, Tenth Edition, McGraw-Hill, 2002; Pratt and Taylor, eds., Principles of Drug Action, Third Edition, Churchill Livingstone, New York, 1990; Katzung, ed., Basic and Clinical Pharmacology, Ninth Edition, McGraw Hill, 20037ybg; Goodman and Gilman, eds., The Pharmacological Basis of Therapeutics, Tenth Edition, McGraw Hill, 2001; Remingtons Pharmaceutical Sciences, 20th Ed., Lippincott Williams & Wilkins., 2000; Martindale, The Extra Pharmacopoeia, Thirty-Second Edition (The Pharmaceutical Press, London, 1999); all of which are incorporated by reference herein in their entirety.

[00202] Administration of the biologically active agents or pharmaceutical composition of the present invention can be effected by any method that enables delivery of the biologically active agents to the site of action. These methods include oral routes, intraduodenal routes, parenteral injection (including intravenous, intraarterial, subcutaneous,

intramuscular, intravascular, intraperitoneal or infusion), topical (e.g. transdermal application), rectal administration, via local delivery by catheter or stent or through inhalation. Biologically active agents can also be administered intraadiposally or intrathecally.

[00203] The amount of the biologically active agent administered will be dependent on the subject being treated, the severity of the disorder or condition, the rate of administration, the disposition of the biologically active agent and the discretion of the prescribing physician. However, an effective dosage is in the range of about 0.001 to about 100 mg per kg body weight per day, preferably about 1 to about 35 mg/kg/day, in single or divided doses. For a 70 kg human, this would amount to about 0.05 to 7 g/day, preferably about 0.05 to about 2.5 g/day. In some instances, dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, e.g. by dividing such larger doses into several small doses for administration throughout the day.

[00204] In some embodiments, a biologically active agent of the invention is administered in a single dose. Typically, such administration will be by injection, e.g., intravenous injection, in order to introduce the agent quickly. However, other routes may be used as appropriate. A single dose of a biologically active agent of the invention may also be used for treatment of an acute condition.

[00205] In some embodiments, a biologically active agent of the invention is administered in multiple doses. Dosing may be about once, twice, three times, four times, five times, six times, or more than six times per day. Dosing may be about once a month, once every two weeks, once a week, or once every other day. In another embodiment a biologically active agent of the invention and another agent are administered together about once per day to about 6 times per day. In another embodiment the administration of a biologically active agent of the invention and an agent continues for less than about 7 days. In yet another embodiment the administration continues for more than about 6, 10, 14, 28 days, two months, six months, or one year. In some cases, continuous dosing is achieved and maintained as long as necessary.

[00206] Administration of the biologically active agents of the invention may continue as long as necessary. In some embodiments, a biologically active agent of the invention is administered for more than 1, 2, 3, 4, 5, 6, 7, 14, or 28 days. In some embodiments, a biologically active agent of the invention is administered for less than 28, 14, 7, 6, 5, 4, 3, 2,

or 1 day. In some embodiments, a biologically active agent of the invention is administered chronically on an ongoing basis, e.g., for the treatment of chronic effects.

[00207] An effective amount of a biologically active agent of the invention may be administered in either single or multiple doses by any of the accepted modes of administration of agents having similar utilities, including rectal, buccal, intranasal and transdermal routes, by intra-arterial injection, intravenously, intraperitoneally, parenterally, intramuscularly, subcutaneously, orally, topically, or as an inhalant.

[00208] The compositions of the invention may also be delivered via an impregnated or coated device such as a stent, for example, or an artery-inserted cylindrical polymer. Such a method of administration may, for example, aid in the prevention or amelioration of restenosis following procedures such as balloon angioplasty. Without being bound by theory, biologically active agents of the invention may slow or inhibit the migration and proliferation of smooth muscle cells in the arterial wall which contribute to restenosis. A biologically active agent of the invention may be administered, for example, by local delivery from the struts of a stent, from a stent graft, from grafts, or from the cover or sheath of a stent. In some embodiments, a biologically active agent of the invention is admixed with a matrix. Such a matrix may be a polymeric matrix, and may serve to bond the biologically active agent to the stent. Polymeric matrices suitable for such use, include, for example, lactone-based polyesters or copolyesters such as polylactide, polycaprolactoneglycolide, polyorthoesters, polyanhydrides, polyaminoacids, polysaccharides, polyphosphazenes, poly (ether-ester) copolymers (e.g. PEO-PLLA); polydimethylsiloxane, poly(ethylene-vinylacetate), acrylate-based polymers or copolymers (e.g. polyhydroxyethyl methacrylate, polyvinyl pyrrolidinone), fluorinated polymers such as polytetrafluoroethylene and cellulose esters. Suitable matrices may be nondegrading or may degrade with time, releasing the biologically active agent or biologically active agents. Biologically active agents of the invention may be applied to the surface of the stent by various methods such as dip/spin coating, spray coating, dip-coating, and/or brush-coating. The biologically active agents may be applied in a solvent and the solvent may be allowed to evaporate, thus forming a layer of biologically active agent onto the stent. Alternatively, the biologically active agent may be located in the body of the stent or graft, for example in microchannels or micropores. When implanted, the biologically active agent can diffuse out of the body of the stent to contact the arterial wall. Such stents may be prepared by dipping a stent manufactured to contain such micropores or microchannels into a solution of the biologically active agent of the invention in a suitable solvent, followed by evaporation of the solvent. Excess drug on the surface of the stent may

be removed via an additional brief solvent wash. In yet other embodiments, biologically active agents of the invention may be covalently linked to a stent or graft. A covalent linker may be used which degrades in vivo, leading to the release of the biologically active agent of the invention. Any bio-labile linkage may be used for such a purpose, such as ester, amide or anhydride linkages. Biologically active agents of the invention may additionally be administered intravascularly from a balloon used during angioplasty. Extravascular administration of the biologically active agents via the pericard or via advential application of formulations of the invention may also be performed to decrease restenosis.

[00209] A variety of stent devices which may be used as described are disclosed, for example, in the following references, all of which are hereby incorporated by reference: U.S. Pat. No. 5,451,233; U.S. Pat. No. 5,040,548; U.S. Pat. No. 5,061,273; U.S. Pat. No. 5,496,346; U.S. Pat. No. 5,292,331; U.S. Pat. No. 5,674,278; U.S. Pat. No. 3,657,744; U.S. Pat. No. 4,739,762; U.S. Pat. No. 5,195,984; U.S. Pat. No. 5,292,331; U.S. Pat. No. 5,674,278; U.S. Pat. No. 5,879,382; U.S. Pat. No. 6,344,053.

[00210] The biologically active agents of the invention may be administered in dosages. It is known in the art that due to intersubject variability agent pharmacokinetics, individualization of dosing regimen is necessary for optimal therapy. Dosing for a biologically active agent of the invention may be found by routine experimentation in light of the instant disclosure.

[00211] When a biologically active agent of the invention is administered in a composition that comprises one or more agents, and the agent has a shorter half-life than the agent of the invention unit dose forms of the agent and the biologically active agent of the invention may be adjusted accordingly.

[00212] The subject pharmaceutical composition may, for example, be in a form suitable for oral administration as a tablet, capsule, pill, powder, sustained release formulations, solution, suspension, for parenteral injection as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration as a suppository. The pharmaceutical composition may be in unit dosage forms suitable for single administration of precise dosages. The pharmaceutical composition will include a conventional pharmaceutical carrier or excipient and a biologically active agent according to the invention as an active ingredient. In addition, it may include other medicinal or pharmaceutical agents, carriers, adjuvants, etc.

[00213] Exemplary parenteral administration forms include solutions or suspensions of active agent in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired.

[00214] Also described herein are methods for developing a biologically active agent for the treatment of cancer. In particular embodiments, the methods are directed to developing a biologically active agent for the treatment of neuroendocrine tumors. Neuroendocrine tumors can generally be described as neoplasms arising from endocrine or nervous system cells. Non-limiting examples of neuroendocrine tumors include, e.g., pheochromocytoma, Merkel cell cancer, neuroendocrine carcinoma, endocrine tumors, carcinoid tumors, thymoma, thyroid cancer, pancreatic neuroendocrine tumor, small cell lung carcinoma.

[00215] Also provided herein are methods for developing a biologically active agent that reduces neuroendocrine tumors. The method can comprise selecting a candidate agent from a library of agents. In some embodiments, the library of agents can comprise a library of drugs approved by the United States Federal Food and Drug Administration (FDA). The drugs approved by the FDA can comprise drugs that are approved for treatment of a disease that is not a cancer. The drugs approved by the FDA can comprise drugs that are approved for treatment of a disease that is not a tumor. The drugs approved by the FDA can comprise drugs that are approved for treatment of a disease that is not a neuroendocrine tumor. In some cases, the drugs approved by the FDA can comprise drugs that are approved for the treatment of a cancer, a tumor, or a neuroendocrine tumor.

[00216] The library of agents can comprise agents that exhibit one or more biological properties. The one or more biological properties can comprise, e.g., antagonism of a receptor. The one or more biological properties can comprise antagonism of a G-protein coupled receptor (GPCR). The receptor can be, e.g., a histamine receptor, a serotonin receptor, an acetylcholine receptor, an adrenergic receptor. The one or more biological properties can include, e.g., monoamine oxidase inhibition, monoamine reuptake inhibition, and/or calcium channel blockade.

The method can comprise a systematic drug repositioning bioinformatics approach to identify novel FDA-approved candidate drugs to treat SCLC. Such a bioinformatics approach can enable systematic screening of compounds against an expression profile of human SCLC *in silico*.

EXAMPLES**[00217] Results**

[00218] *An integrative bioinformatics approach identifies candidate FDA-approved drugs for the treatment of small cell lung carcinoma.* To identify novel candidate drugs to treat SCLC, a bioinformatics approach that evaluates the therapeutic potential of a drug for a given disease by comparing gene expression profiles in response to FDA-approved drugs in multiple cell types across multiple diseases was used (FIG. 1-see Experimental Procedures). The list of identified candidate repositioned drugs with predicted efficacy against SCLC, along with annotations of their known targets, as well as the pathways enriched in the drug targets, are shown in Table 1 and Table S1.

[00219] Identified agents included drugs targeting molecules in the “Neuroactive ligand receptor interaction” and “Calcium Signaling” pathways. Exemplary identified agents include Imipramine, Clomipramine, Promethazine, Tranlycypromine, Pargyline, Bepridil. Finally, as a possible negative control, the drug Repaglinide was used.

[00220] *Treatment with the candidate drugs induces the death of SCLC cells in culture*

[00221] To evaluate the efficacy of the repositioned candidate drugs against SCLC in culture, cell viability assays after exposure to the drugs were performed. As a negative control, the non-small cell lung cancer (NSCLC) cell line A549 was used. Also tested were three established human SCLC lines (H82, H69, and H187) and three tumor cell lines from a mouse model of SCLC (Kp1, Kp2 and Kp3) (Park et al., 2011b). The doses and concentrations used were optimized for each drug and ranged from 100nM to 1μM for Repaglinide, 1-20μM for Bepridil and 10-100μM for Clomipramine, Promethazine, Imipramine, Tranlycypromine, and Pargyline; all these doses have been well documented in multiple cellular contexts. The IC₅₀ of these drugs in the human and mouse SCLC cells was confirmed (FIG. 7 and data not shown). The IC₈₀ of the selected drugs was used to determine the survival of each cell line compared to its vehicle-treated control. Treatment of SCLC cells with Imipramine, Clomipramine, Promethazine, and Bepridil, but not Tranlycypromine, Pargyline, or Repaglinide, significantly inhibited the growth of mouse and human SCLC but not NSCLC cells when cultured in 0.5-2% serum (FIG. 2, FIG. 7, and data not shown). Cells were also responsive to the drugs in higher serum conditions (5% and 10%, data not shown). Phase contrast images of control and treated SCLC cells suggested that Imipramine, Clomipramine, Promethazine, and Bepridil were inducing cell death rather than having cytostatic effects (FIG. 7, panel B).

[00222] *Imipramine and Promethazine inhibit the expansion of transplanted SCLC tumors*

[00223] The impact of the drugs (Imipramine, Promethazine, and Bepridil) on the growth of SCLC allografts and xenografts in immunocompromised mice was assessed. Once measurable tumors had formed 10-14 days following injection of Kp1 and Kp3 mouse SCLC cells, transplanted mice were treated for 2 weeks with Imipramine, Promethazine, and Bepridil (FIG 3, panel A). The drugs were administered daily by intraperitoneal (IP) injections at a concentration of 25mg/kg for Imipramine and Promethazine and 10mg/kg for Bepridil (e.g. (Breese and Traylor, 1971; Campos-Gonzalez and Kindy, 1992)). The three drugs markedly inhibited murine SCLC tumor growth as single agents throughout the course of the treatment (FIG 3, panel B). Imipramine and Promethazine treatment inhibited the growth of a human SCLC cell line in a similar xenograft assay to greater degrees than Bepridil (FIG 3C and 3D).

[00224] *Imipramine and Promethazine inhibit the expansion of endogenous mouse SCLC tumors*

[00225] The effects of two drug candidates, Imipramine and Promethazine, on endogenous SCLC tumors developing in the lung of *Rb/p53/p130* mutant mice was assessed. Five months after intra-tracheal instillation of Ad-Cre to initiate tumor development, at a time when mice have developed advanced lesions (Schaffer et al., 2010), daily intra-peritoneal (IP) injections of Imipramine and Promethazine were performed on groups of mutant mice; control mice were injected with saline. After 30 days of treatment, the lungs of all the mice were harvested and tumor development quantified (FIG. 4A, panel A). The analysis of whole lungs and hematoxylin and eosin-stained sections indicated that Imipramine- and Promethazine-treated mice had strikingly much fewer and smaller SCLC tumors than the control mice (FIG. 4A, panel B). Treatment with Imipramine and Promethazine significantly reduced tumor burden as measured by the total tumor area occupying the lungs and the size of the SCLC tumors (FIG. 4A, panels C-D). Histopathological analysis and staining with the neuroendocrine marker Synaptophysin confirmed that all tumors from the three cohorts displayed features of SCLC (data not shown), indicating that the treatment did not change tumor type. Together, these experiments identify the TCA Imipramine and the histamine H1 receptor antagonist Promethazine as potent inhibitors of the expansion of SCLC tumor cells in culture and *in vivo*.

[00226] Strong toxicity in both wild-type and tumor-bearing mice simultaneously treated with both Cisplatin and Etoposide was observed (data not shown). Consequently, to

determine the effects of the candidate drugs on chemoresistant tumors, Rb/p53/p130 mutant mice bearing SCLC tumors were treated with saline or Cisplatin only (FIG. 4B, panel A). Tumors that had survived chemotherapy and control chemonaïve tumors (FIG. 4B, panel B) were then grown in culture or transplanted into immunocompromised recipient mice (FIG. 4B, panel C). Chemoresistant mouse tumors were inhibited by Imipramine treatment similar to chemonaïve tumors both ex vivo (data not shown) and in primary allografts (FIG. 4B, panel D). Thus, tumor cells emerging from long-term treatment with a chemotherapeutic agent are still inhibited by this candidate drug.

[00227] Together, these experiments indicate that the expansion of SCLC cells is potentially inhibited by Imipramine and Promethazine and suggest that both chemoresistant tumors and disseminated tumors may respond to treatment in patients with advanced disease.

[00228] *Induction of apoptotic cell death by Imipramine and Promethazine in SCLC cells*

[00229] Surprisingly, treatment of SCLC cells with Imipramine and Promethazine led to apoptotic cell death, as assayed by Annexin V staining and the appearance of the cleaved form of Caspase 3 (CC3) in treated SCLC cells (FIG 4C, panels A and B). A similar induction of apoptotic cell death and a concomitant decrease in proliferation were observed in transplanted and endogenous tumors, as assayed by immunostaining for CC3 and the mitosis marker phospho-histone 3 (PH3) on tumor sections (FIG 8A, panels A-C and FIG 4C, panels C and D). To determine whether apoptosis was a major cause of death in SCLC cells treated with the two drugs, SCLC cells were pretreated for an hour with the pan-Caspase inhibitor zVAD-FMK at doses ranging from 30μM to 50μM, followed by treatment with Imipramine. As shown in FIG 4C, panels E and F, zVAD-FMK treatment rescued in a dose-dependent manner the cell death induced by Imipramine after 24 hours of exposure to the drug and zVAD-FMK. Washing out the drugs up to 6 hours after addition to the cells was enough to prevent the appearance of cell death and the decrease in viability observed 24 hours after treatment, while exposure of the cells to Imipramine for 8 hours or more was sufficient to induce an irreversible cell death in SCLC cells (FIG 8A, panel D). These observations led us to examine a number of signaling pathways in treated and control cells 1-12 hours after treatment (Figure 4C, panel G and data not shown). In particular, increased levels of phosphorylated JNK (c-Jun N-terminal kinase) and phosphorylated c-Jun in treated cells compared to control starting 1 hour after treatment (FIG. 4C, panel G) was found. These phosphorylation events can be associated with cellular stress and apoptosis. Combined treatment of SCLC cells with Imipramine and the selective JNK inhibitor SP600125 resulted

in a significant rescue of the apoptotic cell death induced by Imipramine, suggesting that activation of this kinase in response to the drug is at least partly responsible for the induction of cell death (FIG. 4C, panel H). These experiments indicate that TCAs induce cell death in SCLC cells by activating a Caspase 3- dependent apoptotic mechanism, preceded by the rapid activation of JNK and c-Jun. Changes in Calcium levels in response to Imipramine and Promethazine were assessed, and a rapid decrease in intracellular Calcium levels in SCLC cells after treatment was observed (FIG. 4C, panel I and FIG. 8B, panels A-B). Also observed were increased levels of reactive oxygen species (ROS) after drug treatment (FIG. 8B, panel C).

[00230] Induction of cell death by the candidate drugs is selective for neuroendocrine tumor cells

[00231] No induction of cell death was observed in human A549 and mouse LKR13 NSCLC cells in culture at the drug concentration used (FIG 7, panel C and data not shown) or in the lung epithelium of mice treated daily for one month with the candidate drugs, including lung neuroendocrine cells (FIG. 4D, panel A). Large areas of necrosis in treated tumors were apparent (FIG. 8A, panels A and C) and treatment of SCLC cells with an inhibitor of necrosis also partly rescued the cell death induced by the candidate drugs (FIG. 4D, panel B). Thus, the candidate drugs induce a rapid cell death specifically in neuroendocrine tumor cells.

[00232] *Potent induction of cell death in SCLC cells by concomittant inhibition of several GPCRs*

[00233] Analysis of microarray experiments with human (Bhattacharjee et al., 2001b) and mouse (Schaffer et al., 2010) primary tumors indicates that SCLC tumor cells always express several of these main targets of Imipramine and Promethazine (FIG 5A). The analysis of microarray experiments from multiple neuroendocrine human primary tumors indicates that Merkel cell carcinoma, midgut carcinoid tumors, pheochromocytoma, and neuroblastoma tumor cells nearly always express several of the five main GPCR targets of Imipramine and Promethazine, similar to that observed in SCLC human primary tumors (FIG. 5A). Our analysis of microarray experiments from human³⁴ and mouse²¹ SCLC, binding assays indicate that these G protein-coupled receptors (GPCRs) are expressed in SCLC cells (FIG. 5A, panel A and FIG. 13, panel A). To determine whether the effects of the candidate drugs may be due to blocking a specific GPCR or several/all of them at the same time, cells were treated with more selective antagonists of these GPCRs. Treatment of three mouse SCLC cell lines and three human SCLC cell lines with 10 μ M and 15 μ M of Azelastine, a selective H1R antagonist (Kempuraj et al., 2003), led to a significant reduction in cell survival (FIG. 5B, panel A).

These results suggested that the cell death induced by Promethazine and Imipramine is caused at least partly by antagonism of H1R. However, these data did not exclude the possibility that inhibition of CHRM3, HTR2, and ADRA1, or other receptors by the candidate drugs may also play a role in the inhibitory effects on SCLC. Results also indicate that 4-DAMP was efficient in significantly decreasing cell viability of most of the mouse and human SCLC cells tested (FIG. 5B, panel B). Similarly, specific inhibition of ADRA1 with Doxazosin Mesylate and of HTR2 with Ritanserin also significantly decreased cell viability of most mouse and human SCLC cells in a dose-dependent manner (FIG. 5B, panels C and D). While our bioinformatics analysis and its subsequent validation helped identify Imipramine and Promethazine as inhibitors of SCLC, the observation that canonical signaling pathways downstream from GPCRs are inhibited following treatment with these drugs suggested that other drugs not present in the databases initially queried but targeting the same GPCRs may have similar effects on SCLC cells. Indeed, treatment with Amitriptyline and Desipramine, two first-generation TCAs with high binding affinity to H1R, mAChR, HTR2, and ADRA1 caused a decrease in the survival of human and mouse SCLC cells, similar to that observed with Imipramine (FIG 10 A-B). These results (summarized in Table S2) indicate that the potent effects of TCAs in the induction of cell death in SCLC cell populations are mediated by the capacity of these drugs to antagonize several GPCRs simultaneously.

[00234] Addition of purified Epinephrine and of a selective agonist of the H1R to the culture medium was sufficient to increase survival and partially rescued the cell death phenotype induced by Promethazine and Imipramine (FIG. 14 and FIG. 5C). Acetylcholine and Serotonin were also able to partially rescue the cell death phenotype induced by Promethazine, which possesses fewer targets than Imipramine (FIG. 5C). Importantly, it was observed that SCLC cells express at high levels the enzymes required for the biosynthesis of the ligands that normally activate the main GPCRs inhibited by this drug (FIG. 9). A rapid production of Serotonin and Epinephrine in the supernatant of SCLC cells was also detected by mass spectrometry (FIG. 13 panels B and C). Competition by these ligands may explain the relatively high concentrations of drugs required to induce cell death in SCLC cells.

[00235] While the candidate drugs probably bind to multiple targets in SCLC cells, these experiments suggest that the potent effects of these drugs in the induction of cell death in SCLC cell populations are mediated at least in part by the capacity of these drugs to disrupt autocrine/paracrine survival loops between neurotransmitters and their receptors at the surface of SCLC cells.

[00236] Rare human neuroendocrine tumors such as Merkel cell carcinoma, midgut carcinoid tumors, pheochromocytoma, and neuroblastoma tumor cells also were found to express several of the main GPCR targets of Imipramine and Promethazine (FIG. 5A, panel B). Indeed, it was observed that both drugs are efficient in inducing rapid cell death in human cell lines from these cancer types as well as in mouse pancreatic neuroendocrine tumor cells, but not as strongly and efficiently as in non-neuroendocrine pancreatic adenocarcinoma cells (FIG. 6A, panels A and B).

[00237] It was found that SSRIs do not induce more cell death in SCLC cells versus NSCLC cells (data not shown). The monoamine oxidase inhibitors, Tranylcypromine and Pargyline, do not antagonize these GPCRs and were not efficient in inducing cell death in SCLC cells

[00238] *Inhibition of cAMP and PKA in SCLC cells following treatment with Imipramine and Promethazine*

[00239] Forskolin and the phosphodiesterase inhibitor IBMX were used to activate PKA. Upon addition of Forskolin and/or IBMX, alone or in combination, to vehicle (DMSO)- treated mouse and human SCLC cells, no significant increase in cell viability was observed after 24 hours (FIG. 5B, panel E). In contrast, addition of 50 μ M of Forskolin alone or 100 μ M of IBMX alone to Imipramine-treated SCLC cells, partially rescued the cell death phenotype; full rescue of viability was observed when Forskolin and IBMX were added together (FIG. 5B, panel F). Similarly, Promethazine-induced cell death was also completely rescued upon addition of both Forskolin and IBMX (data not shown). Phorbol 12-myristate 13-acetate (PMA) at doses ranging from 10nM to 1 μ M to was added to vehicle (DMSO)- and Imipramine-treated cells. While PMA did not increase the growth of the control cells significantly, it also did not rescue the cell death phenotype induced by Imipramine (data not shown). These results strongly suggest that Imipramine and Promethazine induce cell death of SCLC cells by targeting the Gs alpha subunit of the targeted GPCRs, thereby inhibiting the cAMP-dependent activation of PKA and inducing cell death *via* activation of the JNK/c-Jun module (Gerits et al., 2008).

[00240] *A wide range of neuroendocrine tumors are inhibited by Imipramine and Promethazine*

[00241] In addition, it was found that Imipramine and Promethazine are efficient in inducing rapid cell death in lung carcinoid cells, pancreas neuroendocrine tumor cells, neuroblastoma cells, and Merkel cell carcinoma cells. Similar to the absence of effects of TCAs on lung adenocarcinoma, these drugs had no apoptotic effects on pancreatic

adenocarcinoma cells at the same concentrations that induce cell death in neuroendocrine tumor cells (FIG 10, panel C and FIG. 6A, panels A-C and FIG. 6B). These observations suggest that a wide range of patients with neuroendocrine tumors may benefit from TCA treatment or from treatment with other inhibitors of the same GPCRs.

[00242] Experimental Procedures

[00243] *Drug repositioning and bioinformatics approach*

[00244] In brief, a SCLC disease signature was obtained using a method to identify human disease-associated experiments with normal controls in Gene Expression Omnibus (GEO) repository. This process identified experiments GSE11969 and GSE1037, which were analyzed using the RankProd method to identify a set of genes differentially expressed across the two experiments. All genes differentially expressed between *SCLC affected* and *control* classes having an estimated false discovery rate (FDR) $\leq 5\%$. The set of differentially expressed genes defined the SCLC signature, and therapeutic activity scores were estimated between the SCLC signatures and 1,300 drug signatures from the Connectivity Map. Top scoring drug hits were mapped to DrugBank records by a basic string-matching approach using the drug chemical name to identify drug targets associated with the compounds. Pathway enrichment analysis of targets was performed using the DAVID tool using the set of known drug targets in DrugBank as the background. P- values for the enrichment analysis were adjusted for multiple hypothesis-testing using the Benjamini-Hochberg (BH) method as implemented in DAVID.

[00245] *Mice, adenoviral infections, and subcutaneous xenografts*

[00246] The SCLC mouse model bearing deletions in *p53*, *Rb*, and *p130* was previously described (Schaffer et al., 2010). Ad-Cre (Baylor College of Medicine) infections were performed as previously described (Park et al., 2011c). Mice were maintained at the Stanford Research Animal Facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. NOD.SCID.Gamma mice were housed in the barrier facility at Stanford University. For subcutaneous injections, 0.5×10^6 mSCLC (Kp1 and Kp3) and 2×10^6 hSCLC (H187) cells were injected into the two flanks of each NSG mice with Matrigel (1:1) (BD Biosciences). Treatment with the drugs started once the SCLC tumors reached 100-150 mm³ (around 10-14 days after implantation). Imipramine (25mg/kg), Promethazine (25mg/kg), and Bepridil (10mg/kg) were administered intraperitoneally daily for 14 consecutive days. Tumor volume was measured every other day and calculated using the ellipsoid formula (length $\times$ width²).

[00247] *Drugs and inhibitors*

[00248] Imipramine, Promethazine, Clomipramine, Bepridil, and Azelastine were all purchased from

Sigma Aldrich, MO. Z-VAD-FMK, Ritanserin, 4-DAMP, and Doxazosin Mesylate were purchased from Tocris Bioscience, UK. The JNK inhibitor SP600125 was purchased from LC Laboratories, MA. All these powders were dissolved in the appropriate solvent according to the manufacturer's instructions. Forskolin and IBMX from Sigma Aldrich were a generous gift from the Rohatgi lab at Stanford University.

[00249] *Cell lines and tissue culture*

[00250] Mouse SCLC cells (Kp1, Kp2, and Kp3) were grown in RPMI 1640 media containing 10%

bovine growth serum (Schaffer et al., 2010). NCI-H82, NCI-H69, and NCI-H187 human SCLC cells (ATCC) and cultured in RPMI media containing 10% bovine growth serum. The NSCLC cell line A549 and lung carcinoid cells H727 were a generous gift from the Sweet-Cordero lab. Human pancreatic adenocarcinoma cell line (PANC1) and human Neuroblastoma cell line (HTB1) were obtained from ATCC and cultured in the same conditions as described above. The Merkel cell carcinoma cell line was a generous gift from Dr. Paul Nghiem. The neuroendocrine mouse pancreatic cancer cells (MIN-6 and β -TC) were a generous gift from Dr. Seung Kim and were cultured in DMEM containing high glucose (Thermo Scientific) and 15% serum.

[00251] *MTT assays and Annexin V staining*

[00252] For 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays (Roche), cells were seeded at 8×10^4 (2% serum) or 1×10^5 (0.5% serum) per well in 96-well plates at day 0 and drugs were added on day 1. MTT reagents 1 and 2 were added on day 2 or day 3 depending on the experiments. The percentage survival was determined as the ratio of treated cells versus vehicle control. Quantification of cell death was performed using AnnexinV-FITC and Propidium Iodide (PI) according to the manufacturer's instructions (BD Biosciences). FACS data were analyzed using FlowJo software and the percentage of cell death was determined as the sum of AnnexinV positive cells, PI positive cells, and double positive cells.

[00253] *Immunoblot analysis and immunostaining*

[00254] For immunoblotting, SCLC cells were homogenized using lysis buffer containing 1% NP40, 50mM HEPES-KOH pH 7.8, 150mM NaCl, 10mM EDTA and a cocktail of protease inhibitors (Jahchan et al., 2010). The antibodies used were JNK, p-JNK,

c-Jun, phospho-c-Jun (p-c-Jun), and cleaved Caspase 3 (all purchased from Cell signaling), Karyopherin β 1 (Santa Cruz), and α -Tubulin (Sigma). We used 5 μ m paraffin sections for H&E staining and immunostaining. Paraffin sections were de-waxed and rehydrated in the Trilogy reagent (Cell Marque). The primary antibodies used were phospho-histone 3 Ser10 (PH3; Millipore) and Cleaved Caspase 3 (CC3; Cell signaling). Alexa Fluor secondary antibodies (Invitrogen) were used for antibody detection. Fluorescent images were captured on the Leica fluorescent microscope. For quantification of the number of CC3 and PH3 positive cells, tumor of similar size and area ranging between 1000 to 30,000 pixel units were included. Very small and very large tumors of area measuring below or above this range were excluded.

[00255] *Microarray analyses*

[00256] The normalized human SCLC datasets used in this study were Bhattacharjee (Bhattacharjee et al., 2001a) and GSE6044 (Rohrbeck et al., 2008) for primary tissue samples, and Wooster (Twentyman et al., 1992) for SCLC cell lines. Primary mouse SCLC microarrays were described elsewhere (Schaffer et al., 2010). The normalized neuroendocrine tumors datasets used were as follows: GSE22396 for Merkel Cell Carcinoma (Paulson et al., 2011), GSE16476 for neuroblastoma (Molenaar et al., 2012), GSE2841 for pheochromocytoma (Dahia et al., 2005), and GSE271162 for midgut carcinoid tumors (Edfeldt et al., 2011).

[00257] *Image analysis and statistics*

[00258] Analysis of the tumor area and fluorescent images was done using ImageJ software by measuring pixel units. Statistical significance was assayed by Student's t test with the Prism GraphPad software (two-tailed unpaired t-test). *: p-value<0.05; **: p-value<0.01; ***: p-value<0.001; ns: not significant. Data are represented as mean +/- standard error of the mean.

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[00336] **Figure Legends**

[00337] **FIG. 1: A bioinformatics-based drug repositioning approach identifies candidate drugs to inhibit SCLC.** (A) Schematic representation of the bioinformatics workflow for the repositioning approach used to identify potential candidate drugs for the treatment of SCLC.

[00338] **FIG. 2: (A-F) Representative MTT viability assays of cells cultured in 0.5% serum (n≥3 independent experiments).** A549 are NSCLC cells, H82, H69, and H187 are human SCLC cell lines, and Kp1, Kp2, and Kp3 are mouse SCLC cell lines. Cells were treated two days with 20μM Clomipramine (A), 50μM Imipramine (B), 30μM Promethazine (C), 100μM Tranylcypromine (D), 100μM Repaglinide (E), and 10μM Bepridil (F), and 100 μM Pargyline (G). **P*<0.05, ***P*<0.01, and ****P*<0.001.

[00339] **FIG. 3: Inhibitory effects of Imipramine, Promethazine, and Bepridil on SCLC allografts and xenografts.** (A) Strategy used for the treatment of mice growing SCLC tumors under their skin. NSG immunocompromised mice were subcutaneously implanted with 2 different mouse SCLC cell lines (Kp1 and Kp3) (B) and one human SCLC

cell line (H187) (C) and the fold change of the tumor volume was measured at the times indicated of daily IP injections with vehicle control (Saline and corn oil; n=10 in (B) and n=4 in (C)), Imipramine (25mg/kg; n=7 in (B) and n=4 in (C)), Promethazine (25mg/kg; n=8 in (B) and n=4 in (C)), and Bepridil (10mg/kg; n=7 in (B) and n=3 in (C)) (3 independent experiments in (B) and 1 experiment in (C)). Values are shown as mean $\pm$ s.e.m. The unpaired t-test was used to calculate the p-values of treated versus control tumors at different days of treatment. * P <0.05, ** P <0.01, and *** P <0.001. Values that are not significant are not indicated. (D) Representative images of SCLC xenografts (H187) collected 14 days after daily treatment with Saline, Imipramine, and Promethazine.

[00340] FIG. 4A: Imipramine and Promethazine inhibit the growth of SCLC tumors in a pre-clinical mouse model. (A) Strategy used for the treatment of *Rb/p53/p130* mutant mice developing endogenous SCLC tumors. (B) Representative photographs of the lungs from mutant 6 months after Ad-Cre infection, one month after the beginning of treatment with Saline, Imipramine (25mg/kg), or Promethazine (25mg/kg). Representative photographs of Hematoxylin and eosin (H&E) stained sections from mutant mice in (B). (C) Quantification of the tumor surface area (pixel area units quantified by ImageJ) of mutant mice treated with Saline (n=10), Imipramine (n=9), and Promethazine (n=6). (5 independent experiments). The unpaired t-test was used to calculate the p-values of Imipramine-treated (P = 0.0017) and Promethazine-treated (P =0.0008) mice compared to control TKO mice. (D) Bar graph showing the percentage size distribution of the tumors from mutant mice injected with Saline (n=10), Imipramine (n=9), and Promethazine (n=5). Values are shown as mean $\pm$ s.e.m. * P <0.05, ** P <0.01, *** P <0.001, ns, not significant.

FIG. 4B. (A), Strategy used for the treatment of *Rb/p53/p130;Rosa26lox-Stop-lox*-Luciferase mice developing endogenous SCLC tumors and treated with Saline and Cisplatin weekly to generate chemonaïve and chemoresistant tumors. Deletion of the lox-Stop-lox cassette by Cre allows expression of the reporter and measurement of tumor volume. (B), Fold change of the tumor volume measured by luciferase activity in Saline- and Cisplatin-treated mice. (C), NSG mice were subcutaneously implanted with the Saline-treated and Cisplatin-treated mouse SCLC cells and the fold change of the tumor volume was measured at the times indicated of daily IP injections with vehicle control (Saline n=4) and Imipramine (25mg/kg; n=4). Values are shown as mean $\pm$ s.e.m. The unpaired t-test was used to calculate the p-values of Imipramine-treated versus Saline-treated chemonaïve and chemoresistant tumors at different days of treatment. * P <0.05, ** P <0.01, and *** P <0.001. Values that are

not significant are not indicated. (D), Representative images of Cisplatin- and Saline-treated SCLC allografts collected 17 days after daily treatment.

[00341] FIG. 4C: Imipramine and Promethazine induce the apoptotic cell death of SCLC cells through activation of Caspase 3. (A) Relative increase in cell death based on quantification of Annexin V and PI staining by FACS analysis of mouse (mSCLC, Kp1) and human (hSCLC, H82) cells cultured in 2% serum and treated for 24 hours with 50 μ M Imipramine and 30 μ M Promethazine. Values from three independent experiments are shown as mean $\pm$ s.e.m. Values from three independent experiments are shown as mean $\pm$ s.e.m. * P <0.05 and *** P <0.001. (B) Representative immunoblotting of cleaved caspase 3 (CC3) in mSCLC (Kp1) and hSCLC (H82) cells treated with 50 μ M Imipramine for 12h. Karyopherin was used as a loading control. (C) Representative immunostaining of CC3 in tumor sections from Rb/p53/p130 mutant mice treated daily with Saline, Imipramine, and Promethazine for 30 consecutive days. Right: Quantification of the percentage of CC3 positive cells per tumor area of saline (n=142 tumors from 10 mice), Imipramine- (n=153 tumors from 9 mice; P <0.0001), and Promethazine- (n=103 from 6 mice; P <0.0001) treated tumors. (D) Representative Immunostaining of phospho-Histone 3 (PH3) in tumor sections from Rb/p53/p130 mutant mice treated daily with Saline, Imipramine, and Promethazine for 30 consecutive days. Right: Quantification of the percentage of PH3 positive cells per tumor area of saline (n=180 tumors from 10 mice), Imipramine- (n=160 from 9 mice; P =0.0006), and Promethazine-treated tumors (n=83 from 6 mice; P =0.0011). (E-F) Effects of the combined treatment of Imipramine (50 μ M) and the pan-caspase inhibitor Z-VAD-FMK on the survival of mSCLC (E) and hSCLC (F) after 24 hours of treatment, as measured by the MTT viability assay. Values from three independent experiments are shown as mean $\pm$ s.e.m. The paired t-test was used to calculate the p-values of Imipramine-treated cells versus control DMSO-treated cells and of Imipramine-treated cells versus Z-VAD-FMK- treated cells combined with Imipramine. * P <0.05, ** P <0.01, *** P <0.001, ns, not significant. (G) Representative immunoblotting of p-c-Jun, total c-Jun, p-JNK, and total JNK in mSCLC cells (Kp1) treated with 50 μ M Imipramine for the indicated times. Tubulin was used as a loading control. (H) Effects of the combined treatment of 50 μ M Imipramine and 500nM of the JNK inhibitor SP600125 on mSCLC cells after 24 hours of treatment, as measured by the MTT viability assay. Values from three independent experiments are shown as mean $\pm$ s.e.m. The unpaired t-test was used to calculate the p-values of Imipramine-treated cells versus control DMSO-treated cells and of Imipramine-treated cells versus SP600125-treated cells combine with Imipramine. * P <0.05 and *** P <0.001. (I) Quantification of the percentage of mSCLC

cells (Kp1 and Kp3) with low Ca²⁺ levels by FACS analysis of Control untreated cells (Ctrl) and Imipramine-treated cells at the times indicated. Values from three independent experiments for each cell line are shown as mean $\pm$ s.e.m. The unpaired t-test was used to calculate the p-values of Imipraminetreated cells versus the untreated control cells at the times indicated. *P<0.05, **P<0.01, ***P<0.001, ns, not significant.

[00342] FIG. 4D. Induction of cell death by candidate agents is selective. (A) Representative immunostaining of Synaptophysin and CC3 in lung sections from Rb/p53/p130 mutant mice treated daily with Imipramine for 30 days. Normal lung neuroendocrine cells are indicated with white arrows. Note the absence of signal for CC3 in the lungs. The pale green staining is the autofluorescence background of the paraffin-embedded tissue. (B) Effects of the combined treatment of Imipramine (50 μ M) and the necrosis inhibitor Necrostatin-1 (abbreviated as Necros) on the survival of mSCLC cells (Kp1) after 24 hours of treatment, as measured by the MTT viability assay. Values from three independent experiments are shown as mean $\pm$ s.e.m. The unpaired t-test was used to calculate the p-values of Imipramine-treated cells versus control DMSO-treated cells and of Imipramine-treated cells versus Necrostatin- treated cells combined with Imipramine. *P<0.05, **P<0.01, ***P<0.001, ns, not significant.

[00343] FIG. 5A: Heat Maps of RNA expression. (A) Heat maps showing normalized RNA expression levels for the Histamine 1 Receptor (H1R), the Muscarinic acetylcholine receptor isoform 3 (CHRM3), the Alpha1a and Alpha1b Adrenergic Receptors (ADRA1a and ADRA1b), and the Serotonin Receptor 2A (HTR2) in human primary tumors and in mouse primary tumors. (B) Heat maps showing the normalized RNA expression levels of the Histamine 1 Receptor (H1R), the Muscarinic acetylcholine receptor isoform 3 (CHRM3), the Alpha1a and Alpha1b Adrenergic Receptors (ADRA1a and ADRA1b), and the Serotonin Receptor 2A (HTR2) in 35 human primary Merkel Cell Carcinoma tumors, 42 Midgut carcinoid tumors, 76 Pheochromocytoma tumors, and 88 Neuroblastoma tumors.

[00344] FIG. 5B depicts results of MTT viability assays of cells treated with various agents. (A-D) MTT viability assays of cells cultured at 2% serum ($n \geq 3$ independent experiments) and treated with the H1R antagonist Azelastine (A), the CHRM3 antagonist 4-DAMP (B), the ADRA1 antagonist Doxazosin Mesylate (C), and the HTR2 antagonist Ritanserin (D), in comparison to treatment with Promethazine (Prom - in A) or Imipramine (Imip - in B, C, and D). *P<0.05, **P<0.01, and ***P<0.001. (E) MTT viability assay for mSCLC (Kp1) and hSCLC (H187) cells following 24hr of treatment with 50 μ M Forskolin (FSK), 100 μ M IBMX, or both drugs combined. ns, not significant. (F) Effects of the

combined treatment of 50 μ M Imipramine and 50 μ M FSK alone, 100 μ M IBMX alone, or FSK and IBMX together, as measured by the MTT viability assay. Values from at least three independent experiments are shown as mean $\pm$ s.e.m. The unpaired t-test was used to calculate the p-values of Imipramine-treated cells versus control DMSO-treated cells and of Imipramine- treated cells versus FSK-, IBMX-, and FSK+IBMX-treated cells combined with Imipramine. * P <0.05, ** P <0.01, *** P <0.001, ns, not significant.

[00345] **FIG. 5C** depicts results of MTT viability assays of cells treated with increasing doses of the specific H1R ligand 2-(2-Pyridil)-ethylamine (PEA) (A), Acetylcholine (Ace) (B), and Serotonin (Ser) (C), in the absence or presence of 50 μ M Imipramine (Imip) and 30 μ M Promethazine (Prom) for 24 hours. The paired t-test was used to calculate the p-values of each ligand, Imipramine- and Promethazine- treated cells versus control cells and of Imipramine- and Promethazine- treated cells versus ligand- treated cells combined with Imipramine or Promethazine.

[00346] **FIG. 6A: Tricyclic antidepressants inhibit the growth of several other types of neuroendocrine tumors.** (A) Representative phase contrast images of Pancreatic Adenocarcinoma (PDAC), Pancreatic Neuroendocrine tumors (PNET), Neuroblastoma (NB), and Merkel Cell Carcinoma (MCC) cells cultured in low serum and treated with vehicle control, 50 μ M Imipramine, and 30 μ M Promethazine for 48 hours. (B-D) MTT viability assays of PDAC, PNETs, NB, and MCC cells treated with increasing doses of Imipramine (B Promethazine (C) and Imipramine (**FIG. 6B**). Values from three independent experiments are shown as mean $\pm$ s.e.m. * P <0.05, ** P <0.01, *** P <0.001, ns, not significant.

[00347] **FIG. 7** (A) MTT viability assays of representative mouse (Kp1 in black, 2% serum) and human (H82 in grey, 0.5% serum) SCLC cell lines following treatment with increasing doses of Imipramine, Promethazine, and Bepridil. (B) Representative phase contrast images of NSCLC cells (A549), human SCLC cells (H82), and mouse SCLC cells (Kp1) cultured in 2% serum and treated with vehicle control, 50 μ M Imipramine, 30 μ M Promethazine, and 10 μ M Bepridil for 48 hours. (C) MTT survival assays of NSCLC (A549 and LKR13) and SCLC cells (H82, H69, H187, Kp1, Kp2, and Kp3) cultured in 2% serum (n>3 independent experiments) for 48 hours with 50 μ M Imipramine, 30 μ M Promethazine, and 10 μ M Bepridil. Similar results were obtained in cells growing in dialyzed serum (data not shown). The black bars represent the vehicle-treated cells normalized to 100%. * P <0.05, ** P <0.01, and *** P <0.001.

[00348] FIG. 8A. Representative H&E staining (A), pH3 immunostaining (B), and CC3 immunostaining (C) of tumor sections from NSG mice implanted subcutaneously with mSCLC cells and treated daily with Saline, Imipramine (25mg/kg), and Promethazine (25mg/kg) for 14 consecutive days. “N” depicts necrotic areas. (D) Representative phase contrast images of mSCLC (Kp1) cells treated with vehicle control (water) and 50μM Imipramine for the different times indicated.

[00349] FIG. 8B. (A) Representative FACS histograms showing the fluorescence levels of the Ca²⁺ indicator Fluo-3AM from mSCLC cells (Kp3) treated with 50μM Imipramine and 30μM Promethazine for the indicated times. The left peak represents low Ca²⁺ levels. (B), Quantification of b for Promethazine (C), Representative FACS histograms showing the fluorescence levels of the ROS indicator DCF from hSCLC cells (H82) after Imipramine treatment at the times indicated. Similar results were obtained in mouse SCLC cells and in response to Bepridil (not shown).

[00350] FIG. 9 depicts heat maps showing normalized RNA expression levels for Histidine Decarboxylase (HDC) required for Histamine biosynthesis, Tryptophan Hydroxylase (TPH1) and DOPA Decarboxylase (DDC) for Serotonin biosynthesis, Phenylethanolamine Nmethyltransferase (PNMT) and Dopamine B-hydroxylase (DBH) for Epinephrine biosynthesis, and CholineAcetyltransferase (HAT) for Acetylcholine biosynthesis in human and mouse SCLC tumors from four independent studies.

[00351] FIG. 10 (A) Representative phase contrast images of mouse SCLC cells (Kp3) and human SCLC cells (H187) cultured in 2% serum and treated with vehicle control, 50μM Desipramine, and 50μM Amitriptyline for 48 hours. (B) MTT viability assay of mouse and human SCLC cell lines following treatment with increasing doses of Desipramine and Amitriptyline at 2% serum and for 48 hours (n=3 independent experiments). **P<0.01 an***P<0.001. (C) Left: Representative phase contrast images of carcinoid cells (H727) cultured in 2% serum and treated with vehicle control, 50μM Imipramine, and 30μM Promethazine for 48 hours. Right: MTT viability assay of H727 carcinoid cells following treatment with 50μM Imipramine and 30μM Promethazine at 2% serum for 48 hours (n=3 independent experiments). ***P<0.001.

[00352] FIG. 11 depicts representative viability assays of mouse and human SCLC cell lines treated with various concentrations of imipramine, desipramine, and amitriptyline.

[00353] FIG. 12 depicts representative photographs of tumor sections taken from saline or despiramine treated TKO mice.

[00354] FIG. 13 Expression of GPCRs and their ligands in SCLC cells. (A), Quantification of the number of muscarinic receptors (mAChR) per mSCLC (Kp3) and hSCLC (H69) cell using an in vitro binding assay. (B), Examples of the LC-MS/MS analysis for KP3 cells. 20 fmol standards are shown and the peaks for epinephrine and serotonin are marked. (C), Summary of the results of two mass spectrometry independent experiments, indicating whether the ligands of interest were detected or not in the supernatant of SCLC cells after 4 hours of incubation in PBS. Note that the absence of detection does not indicate that the ligands are not produced by the tumor cells but rather that the method used may not have been sensitive enough.

[00355] FIG. 14 depicts MTT viability assays of cells cultured at 2% serum ($n \geq 3$ independent experiments) and treated with increasing doses of Epinephrine (Epi) in the absence or presence of 50 μ M Imipramine (Imip) or 30 μ M Promethazine (Prom). The paired t-test was used to calculate the p-values of Epinephrine-, Imipramine- and Promethazine-treated cells versus control cells and of Imipramine- and Promethazine- treated cells versus Epinephrine- treated cells combined with Imipramine or Promethazine. * $P < 0.05$, ** $P < 0.01$, ns, not significant.

[00356] Table 1: Pathways significantly enriched among top-scoring SCLC repositioning hits.

Pathway name (KEGG ID)	Fold-enrichment	P-value	Gene targets
Neuroactive ligand-receptor interaction (hsa04080)	6.75	1.66×10^{-8}	<i>GABRA1, THRA, THRB, DRD2, GRIN3A, ADORA1, H1R, CHRM5, CHRM4, HTR1A, CHRM3, CHRM2, CHRM1, F2, ADRA1B, ADRA2A, ADRA1A, HTR2A</i>
Calcium signaling pathway (hsa04020)	5.99	3.13×10^{-4}	<i>CHRM5, HRH1, CHRM3, TNNC1, CHRM2, CHRM1, ADRA1B, ADRA1A, CACNA1A, CALM1, HTR2A</i>
Complement and coagulation cascades (hsa04610)	8.3	1.52×10^{-2}	<i>F10, F2, F9, F7, PROS1, PROC</i>

[00357] The unique set of canonical targets associated with the top-scoring SCLC repositioning hits was evaluated for biological enrichment in KEGG pathways using DAVID. The enrichment statistic p-values were adjusted for multiple testing using the Benjamini-Hochberg method, and pathways with

and adjusted p-value < 0.05 are reported as being enriched for targets of top-scoring SCLC repositioning hits.

[00358] Table S1: Top 100 FDA-approved drugs identified by a bioinformatics drug-repositioning approach.

name	average_score	average (p_value)	average (q_valu
dexverapamil	-0.58685731	<1.00E-6	<1.00E-6
5109870	-0.563708646	<1.00E-6	<1.00E-6
MS-275	-0.562830202	<1.00E-6	<1.00E-6
fisetin	-0.55714395	<1.00E-6	<1.00E-6
celastrol	-0.550486901	<1.00E-6	<1.00E-6
DL-thiorphan	-0.548556783	<1.00E-6	<1.00E-6
GW-8510	-0.548163237	<1.00E-6	<1.00E-6
menadione	-0.544402549	<1.00E-6	<1.00E-6
alsterpaulone	-0.537165639	<1.00E-6	<1.00E-6
camptothecin	-0.532230869	<1.00E-6	<1.00E-6
doxorubicin	-0.525977537	<1.00E-6	<1.00E-6
trifluridine	-0.512970553	<1.00E-6	<1.00E-6
tyrphostin AG-825	-0.507905359	<1.00E-6	<1.00E-6
phenoxybenzamine	-0.505701052	<1.00E-6	<1.00E-6
0175029-0000	-0.501597715	<1.00E-6	<1.00E-6
thioguanosine	-0.499539557	<1.00E-6	<1.00E-6
0297417-0002B	-0.496859399	<1.00E-6	<1.00E-6
spiperone	-0.49504838	<1.00E-6	<1.00E-6
apigenin	-0.488593524	<1.00E-6	<1.00E-6
H-7	-0.483719412	<1.00E-6	<1.00E-6
methacholine chloride	-0.480147679	<1.00E-6	<1.00E-6
azacitidine	-0.480091515	<1.00E-6	<1.00E-6
milrinone	-0.47848454	<1.00E-6	<1.00E-6
irinotecan	-0.477529925	<1.00E-6	<1.00E-6
trazodone	-0.473433798	<1.00E-6	<1.00E-6
N-acetyl-L-leucine	-0.468534396	<1.00E-6	<1.00E-6
MG-262	-0.467145553	<1.00E-6	<1.00E-6
trioxysalen	-0.465587897	<1.00E-6	<1.00E-6
rimexolone	-0.464860278	<1.00E-6	<1.00E-6
monobenzene	-0.463978919	<1.00E-6	<1.00E-6
quinostatin	-0.460897534	<1.00E-6	<1.00E-6
tranylcypromine	-0.459539839	<1.00E-6	<1.00E-6
daunorubicin	-0.455659657	<1.00E-6	<1.00E-6
5286656	-0.450460489	<1.00E-6	<1.00E-6
imipramine	-0.449778317	<1.00E-6	<1.00E-6
3-aminobenzamide	-0.443897068	<1.00E-6	<1.00E-6
clomipramine	-0.441855744	<1.00E-6	<1.00E-6
1,4-chrysenequinone	-0.440809626	<1.00E-6	<1.00E-6
sanguinarine	-0.417481903	<1.00E-6	<1.00E-6
exemestane	-0.417132074	<1.00E-6	<1.00E-6
repaglinide	-0.412826605	<1.00E-6	<1.00E-6
imipenem	-0.404825841	<1.00E-6	<1.00E-6
cantharidin	-0.399721302	<1.00E-6	<1.00E-6
triflusal	-0.399406963	<1.00E-6	<1.00E-6
pararosaniline	-0.390603335	<1.00E-6	<1.00E-6
5152487	-0.390492402	<1.00E-6	<1.00E-6
MG-132	-0.389287627	<1.00E-6	<1.00E-6
phthalylsulfathiazole	-0.442927127	2.00E-06	4.79E-06
meticrane	-0.436113424	2.00E-06	4.79E-06
estriol	-0.442368329	2.50E-06	5.99E-06

gliclazide	-0.436767189	2.50E-06	5.99E-06
promethazine	-0.436372959	2.50E-06	5.99E-06
sulconazole	-0.431600399	2.50E-06	5.99E-06
semustine	-0.402933065	2.50E-06	5.99E-06
mitoxantrone	-0.483429633	3.33E-06	7.98E-06
Prestwick-559	-0.440904438	3.33E-06	7.98E-06
antimycin A	-0.47260708	4.00E-06	9.01E-06
eucalropine	-0.418305974	5.00E-06	1.20E-05
pargyline	-0.405574181	5.00E-06	1.13E-05
etamsylate	-0.40478686	5.00E-06	1.13E-05
talampicillin	-0.451058186	7.50E-06	1.72E-05
medrysone	-0.482309651	8.33E-06	1.77E-05
luteolin	-0.435341141	1.00E-05	2.17E-05
Prestwick-1084	-0.42987771	1.00E-05	2.25E-05
thiostrepton	-0.434935936	1.25E-05	2.65E-05
procaine	-0.41577906	1.60E-05	3.42E-05
bepidil	-0.419246851	1.75E-05	3.75E-05
4,5-dianilinophthalimide	-0.445977737	2.00E-05	4.35E-05
tobramycin	-0.397019119	2.00E-05	4.38E-05
ronidazole	-0.391770951	2.33E-05	5.04E-05
ipratropium bromide	-0.456688744	2.67E-05	5.29E-05
5248896	-0.408340346	4.00E-05	7.93E-05
skimmianine	-0.404504975	4.00E-05	7.93E-05
anabasine	-0.401405389	4.67E-05	8.99E-05
imidurea	-0.40171475	7.33E-05	1.41E-04
etomidate	-0.407257433	9.00E-05	1.67E-04
roxarsone	-0.407162439	1.38E-04	2.46E-04
methazolamide	-0.414310806	1.78E-04	3.02E-04
PHA-00816795	-0.40622862	1.85E-04	3.29E-04
omeprazole	-0.410536548	2.25E-04	3.80E-04
lithyronine	-0.392278481	2.45E-04	4.17E-04
ifenprodil	-0.416664891	3.33E-04	5.66E-04
parthenolide	-0.414300742	3.33E-04	5.43E-04
pyrazinamide	-0.405285328	3.48E-04	5.76E-04
depropine	-0.407780219	3.78E-04	6.12E-04
clemizole	-0.411678816	3.86E-04	6.19E-04
cortisone	-0.395238885	4.13E-04	6.81E-04
riboflavin	-0.44034217	5.20E-04	8.22E-04
piperidolate	-0.390810385	5.57E-04	9.37E-04
alfaxalone	-0.411471354	1.46E-03	2.22E-03
norcyclobenzaprine	-0.409828282	1.64E-03	2.43E-03
halcinonide	-0.38979023	1.65E-03	2.47E-03
chrysin	-0.435010559	1.92E-03	2.87E-03
propafenone	-0.390753021	2.00E-03	2.94E-03
puromycin	-0.390343991	2.27E-03	3.42E-03
sulfafurazole	-0.396092549	9.32E-03	1.31E-02
resveratrol	-0.45232584	1.11E-01	1.11E-01
pyrvinium	-0.392675747	1.67E-01	1.67E-01
8-azaguanine	-0.407540366	2.50E-01	2.50E-01
ellipticine	-0.393933909	2.50E-01	2.50E-01

Table S2: Summary of the effects of specific GPCR inhibitors on different cell lines. (+++) and (–) indicate highest efficacy and no effect of the drug, respectively.

Cell line	Imipramine	Promethazine	Azelastine	4-DAMP	Doxazosin	Ritanserin
Receptor(s) targeted	H1R, HTR1, CHRM3, ADRA1	H1R, HTR1, CHRM3, ADRA1	H1R	CHRM3	ADRA1	HTR1
B82	+	+	+	–	–	+++
B69	++	++	+	+++	++	–
B187	++	++	++	+++	+++	+++
KP1	+++	+++	++	++	+++	++
KP2	+++	+++	++	–	++	–
KP3	+++	+++	+++	+++	+++	++
A549	–	–	–	–	–	–

[00359] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a histamine receptor antagonist.
2. The method of claim 1, wherein said histamine receptor antagonist is a histamine H1 receptor antagonist.
3. The method of any of the preceding claims, wherein said histamine H1 receptor antagonist is promethazine or azelastine.
4. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a monoamine oxidase inhibitor.
5. The method of any of the preceding claims, wherein said monoamine oxidase inhibitor is an irreversible monoamine oxidase inhibitor.
6. The method of any of the preceding claims, wherein said irreversible monoamine oxidase inhibitor is tranylcypromine.
7. The method of any of the preceding claims, wherein said monoamine oxidase inhibitor is an MAO-B inhibitor.
8. The method of any of the preceding claims, wherein said MAO-B inhibitor is pargyline.
9. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a serotonin receptor antagonist.
10. The method of any of the preceding claims, wherein said serotonin receptor antagonist is a serotonin receptor 2-type antagonist.
11. The method of any of the preceding claims, wherein said serotonin receptor 2-type antagonist is a serotonin receptor 2A antagonist.
12. The method of any of the preceding claims, wherein said serotonin receptor 2A antagonist is ritanserin.
13. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of an acetylcholine receptor antagonist.
14. The method of any of the preceding claims, wherein said acetylcholine receptor antagonist is a muscarinic acetylcholine receptor antagonist.

15. The method of any of the preceding claims, wherein said muscarinic acetylcholine receptor antagonist is an M3 acetylcholine receptor antagonist.
16. The method of any of the preceding claims, wherein said M3 acetylcholine receptor antagonist is 4-DAMP.
17. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of an adrenergic receptor antagonist.
18. The method of any of the preceding claims, wherein said adrenergic receptor antagonist is an alpha-adrenergic receptor antagonist.
19. The method of any of the preceding claims, wherein said alpha-adrenergic receptor antagonist is an alpha-1 adrenergic receptor antagonist.
20. The method of any of the preceding claims, wherein said alpha-1 adrenergic receptor antagonist is doxazosin mesylate.
21. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a monoamine reuptake inhibitor.
22. The method of any of the preceding claims, wherein said monoamine reuptake inhibitor inhibits serotonin reuptake.
23. The method of any of the preceding claims, wherein said monoamine reuptake inhibitor inhibits norepinephrine uptake.
24. The method of any of the preceding claims, wherein said monoamine reuptake inhibitor inhibits both serotonin and norepinephrine uptake.
25. The method of any of the preceding claims, wherein said monoamine reuptake inhibitor inhibits dopamine reuptake.
26. The method of any of the preceding claims, wherein said monoamine reuptake inhibitor is a tricyclic antidepressant.
27. The method of any of the preceding claims, wherein said tricyclic antidepressant is selected from the group consisting of amitriptyline, amitriptylinoxide, clomipramine, demexiptiline, desipramine, dibenzepin, dimetacrine, dosulepin/dothiepin, doxepin, imipramine, imipraminoxide, melitracen, metapramine, nitroxazepine, nortriptyline, pipofezine, propizepine, protriptyline, quinupramine, amineptine, opipramol, tianeptine, and trimipramine.
28. The method of any of the preceding claims, wherein said tricyclic antidepressant is amitriptyline, desipramine, or imipramine.

29. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a calcium channel blocker.
30. The method of any of the preceding claims, wherein said calcium channel blocker inhibits voltage-gated and receptor-operated calcium channels.
31. The method of any of the preceding claims, wherein said calcium channel blocker is bepridil.
32. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a biologically active agent that exhibits one or more biological activities selected from the group consisting of histamine receptor antagonism, monoamine oxidase inhibition, serotonin receptor antagonism, acetylcholine receptor antagonism, adrenergic receptor antagonism, monoamine reuptake inhibition, and calcium channel blockade.
33. The method of any of the preceding claims, wherein said biologically active agent exhibits two or more of said biological activities.
34. The method of any of the preceding claims, wherein said biologically active agent exhibits three or more of said biological activities.
35. The method of any of the preceding claims, wherein said biologically active agent exhibits four or more of said biological activities.
36. The method of any of the preceding claims, wherein said biologically active agent exhibits five or more of said biological activities.
37. The method of any of the preceding claims, wherein said biologically active agent exhibits six or more of said biological activities.
38. The method of any of the preceding claims, wherein said biologically active agent exhibits each of said biological activities.
39. A method of treating cancer or neoplasm in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a biologically active agent selected from Table S1
40. The method of any of the preceding claims, wherein said cancer or neoplasm is a neuroendocrine cancer or tumor.
41. The method of any of the preceding claims, wherein said neuroendocrine cancer or tumor is selected from the group consisting of pheochromocytoma, Merkel cell cancer,

neuroendocrine carcinoma, endocrine tumors, carcinoid tumors, thymoma, thyroid cancer, pancreatic neuroendocrine tumor, and small cell lung carcinoma.

42. The method of any of the preceding claims, wherein said neuroendocrine cancer or tumor is small cell lung carcinoma.
43. The method of any of the preceding claims, wherein said pharmaceutical composition is not coadministered with another composition.
44. The method of any of the preceding claims wherein said cancer or tumor is resistant to chemotherapy.
45. A method of developing a biologically active agent that reduces neuroendocrine tumors, comprising:
 - a) computing a disease signature, comprising comparing a first gene expression profile from a neuroendocrine tumor tissue to a second gene expression profile from an equivalent non-tumor tissue, thereby computing said disease signature;
 - b) computing a drug signature from a candidate agent, comprising (i) treating a first population of neuroendocrine tumor cells with said candidate agent, (ii) treating a second population of neuroendocrine tumor cells with an equivalent amount of control vehicle, (iii) generating a third and fourth gene expression profile from said first and second populations of neuroendocrine tumor cells, and (iv) comparing said third and fourth gene expression profiles, thereby computing said drug signature;
 - c) computing a drug activity score between said candidate agent and disease, comprising comparing said drug signature to said disease signature; and
 - d) selecting said candidate for development as a biologically active agent for the treatment of neuroendocrine tumors e based on said drug activity score.
46. A method of developing a biologically active agent for the treatment of neuroendocrine tumors, comprising
 - a) selecting a candidate agent from a library of agents, wherein said candidate agent exhibits one or more biological activities selected from the group consisting histamine receptor antagonism, monoamine oxidase inhibition, serotonin receptor antagonism, acetylcholine receptor antagonism, adrenergic receptor antagonism, monoamine reuptake inhibition, and calcium channel blockade;
 - b) contacting said candidate agent with one or more neuroendocrine tumor cells;
 - c) determining the viability of said one or more neuroendocrine tumor cells; and

- d) selecting said candidate for development as a biologically active agent for the treatment of neuroendocrine tumors if said candidate reduces the viability of said one or more neuroendocrine tumor cells.
47. The method of any of the preceding claims, wherein said candidate agent exhibits two or more of said biological activities.
48. The method of any of the preceding claims, wherein said candidate agent exhibits three or more of said biological activities.
49. The method of any of the preceding claims, wherein said candidate agent exhibits four or more of said biological activities.
50. The method of any of the preceding claims, wherein said candidate agent exhibits five or more of said biological activities.
51. The method of any of the preceding claims, wherein said candidate agent exhibits six or more of said biological activities.
52. The method of any of the preceding claims, wherein said candidate agent exhibits each of said biological activities.
53. A method of treating a neuroendocrine cancer or tumor selected from the group consisting of pheochromocytoma, Merkel cell cancer, neuroendocrine carcinoma, endocrine tumors, carcinoid tumors, thymoma, thyroid cancer, pancreatic neuroendocrine tumor, and small cell lung carcinoma in a subject in need thereof, comprising administering to said subject a pharmaceutical composition comprising a therapeutically effective amount of a tricyclic antidepressant selected from the group consisting of amitriptyline, amitriptylinoxide, clomipramine, demexiptiline, desipramine, dibenzepin, dimetacrine, dosulepin/dothiepin, doxepin, imipramine, imipraminoxide, melitracen, metapramine, nitroxazepine, Nortriptyline, pipofezine, propizepine, protriptyline, quinupramine, amineptine, opipramol, tianeptine, and trimipramine.
54. A method of treating a cancer or tumor in a subject, comprising administering to said subject at least two biologically active agents, wherein at least one of said two biologically active agents exhibits one or more biological activities selected from the group consisting of histamine receptor antagonism, monoamine oxidase inhibition, serotonin receptor antagonism, acetylcholine receptor antagonism, adrenergic receptor antagonism, monoamine reuptake inhibition, and calcium channel blockade.
55. The method of any of the preceding claims, wherein at least two of said biologically active agents one or more biological activities exhibit selected from the group consisting

of histamine receptor antagonism, monoamine oxidase inhibition, serotonin receptor antagonism, acetylcholine receptor antagonism, adrenergic receptor antagonism, monoamine reuptake inhibition, and calcium channel blockade.

56. The method of any of the preceding claims, wherein said at least two biologically active agents comprise a histamine receptor antagonist.
57. The method of any of the preceding claims, wherein said histamine receptor antagonist is azelastine or promethazine.
58. The method of any of the preceding claims, wherein said at least two biologically active agents comprise at least two histamine receptor antagonists.
59. The method of any of the preceding claims, wherein said at least two histamine receptor antagonists are azelastine and promethazine.
60. The method of any of the preceding claims, wherein said at least two biologically active agents comprise a histamine receptor ligand and a tricyclic antidepressant.
61. The method of any of the preceding claims, wherein the histamine receptor ligand is 2-(2-Pyridil)-ethylamine (PEA).
62. The method of any of the preceding claims, wherein the tricyclic antidepressant is imipramine.
63. The method of any of the preceding claims, wherein the histamine receptor ligand is a histamine receptor antagonist.
64. The method of any of the preceding claims, wherein said at least two biologically active agents comprise a histamine receptor antagonist and a tricyclic antidepressant.
65. The method of any of the preceding claims, wherein said at least two biologically active agents comprise azelastine and imipramine.
66. The method of any of the preceding claims, wherein said at least two biologically active agents comprise an acetylcholine receptor antagonist and a tricyclic antidepressant.
67. The method of any of the preceding claims, wherein said at least two biologically active agents comprise an acetylcholine receptor antagonist and imipramine.
68. The method of any of the preceding claims, wherein said acetylcholine receptor antagonist is a muscarinic receptor antagonist.
69. The method of any of the preceding claims, wherein said muscarinic receptor antagonist is an M3 receptor antagonist.
70. The method of any of the preceding claims, wherein said M3 receptor antagonist is 4-DAMP.

71. The method of any of the preceding claims, wherein said at least two biologically active agents comprise an alpha-adrenergic receptor antagonist and a tricyclic antidepressant.
72. The method of any of the preceding claims, wherein said at least two biologically active agents comprise an alpha-adrenergic receptor antagonist and imipramine.
73. The method of any of the preceding claims, wherein said alpha-adrenergic receptor antagonist is doxazosin mesylate.
74. The method of any of the preceding claims, wherein said at least two biologically active agents comprise a serotonin (5-HT) receptor antagonist and a tricyclic antidepressant.
75. The method of any of the preceding claims, wherein said at least two biologically active agents comprise a serotonin (5-HT) receptor antagonist and imipramine.
76. The method of any of the preceding claims, wherein said 5-HT receptor antagonist is a 5-HT₂ receptor antagonist.
77. The method of any of the preceding claims, wherein said 5-HT₂ receptor antagonist is ritanserin.
78. The method of any of the preceding claims, wherein said at least two biologically active agents comprise a neurotransmitter and imipramine.
79. The method of any of the preceding claims, wherein said neurotransmitter is serotonin.
80. The method of any of the preceding claims, wherein said neurotransmitter is acetylcholine.
81. The method of any of the preceding claims, wherein said cancer or tumor is selected from the group consisting of adrenal cortical cancer, anal cancer, aplastic anemia, bile duct cancer, bladder cancer, bone cancer, central nervous system tumors such as, e.g., glioblastoma, astrocytoma, neuroblastoma, and oligodendroglioma, breast cancer, Castleman Disease, cervical cancer, Non-Hodgkin's lymphoma, colorectal cancer, endometrial cancer, esophageal cancer, Ewing's tumors, eye cancer, gallbladder cancer, carcinoids, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, gestational trophoblastic disease, Hodgkin's disease, Kaposi's sarcoma, kidney cancer, laryngeal cancer, hypopharyngeal cancer, neuroendocrine tumors, acute lymphocytic leukemia, acute myeloid leukemia, children's leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, liver cancer, lung cancer, lung carcinoid tumors, male breast cancer, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal cancer, nasopharyngeal cancer, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, rhabdomyosarcoma, salivary gland cancer, sarcoma (adult soft tissue cancer),

melanoma skin cancer, nonmelanoma skin cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Waldenstrom's macroglobulinemia, pheochromocytoma, Merkel cell cancer, endocrine tumors, thymoma, pancreatic cancer, pancreatic neuroendocrine tumor, non-small cell lung carcinoma, and small cell lung carcinoma.

82. The method of any of the preceding claims, wherein said cancer or tumor is a neuroendocrine cancer or tumor.
83. The method of any of the preceding claims, wherein said neuroendocrine cancer or tumor is selected from the group consisting of pheochromocytoma, Merkel cell cancer, neuroendocrine carcinoma, endocrine tumors, carcinoid tumors, thymoma, thyroid cancer, pancreatic neuroendocrine tumor, and small cell lung carcinoma.
84. The method of any of the preceding claims, wherein said neuroendocrine cancer or tumor is small cell lung carcinoma.
85. The method of any of the preceding claims, comprising administering about 0.001- 100 mg/kg/day of said biologically active agent to said subject.
86. The method of any of the preceding claims, comprising administering about 0.001- 1 mg/kg/day of said biologically active agent to said subject.
87. The method of any of the preceding claims, comprising administering about 0.1- 10 mg/kg/day of said biologically active agent to said subject.
88. The method of any of the preceding claims, comprising administering about 5-25 mg/kg/day of said biologically active agent to said subject.
89. The method of any of the preceding claims, comprising administering about 10- 50 mg/kg/day of said biologically active agent to said subject.
90. The method of any of the preceding claims, comprising administering about 40-100 mg/kg/day of said biologically active agent to said subject.
91. A kit, comprising at least one biologically active agent of any of the preceding claims and instructions for use in the treatment of a cancer or tumor in a subject of any of the preceding claims.

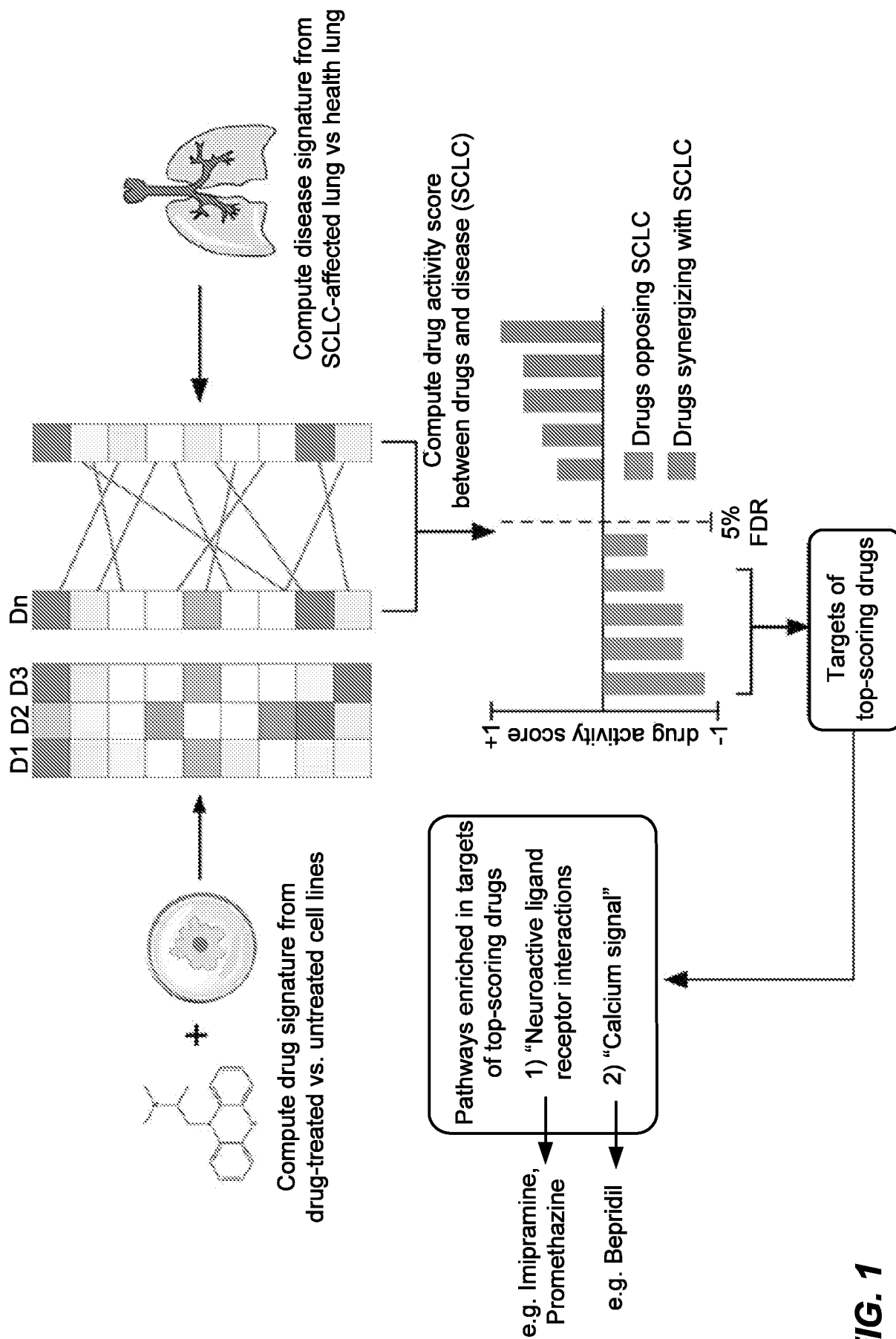
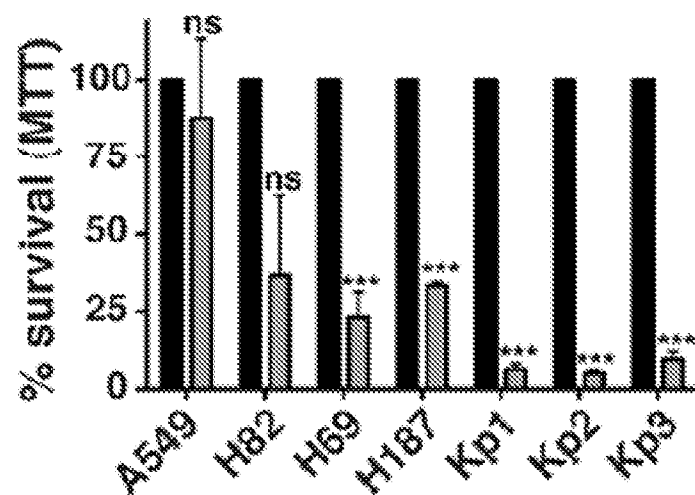
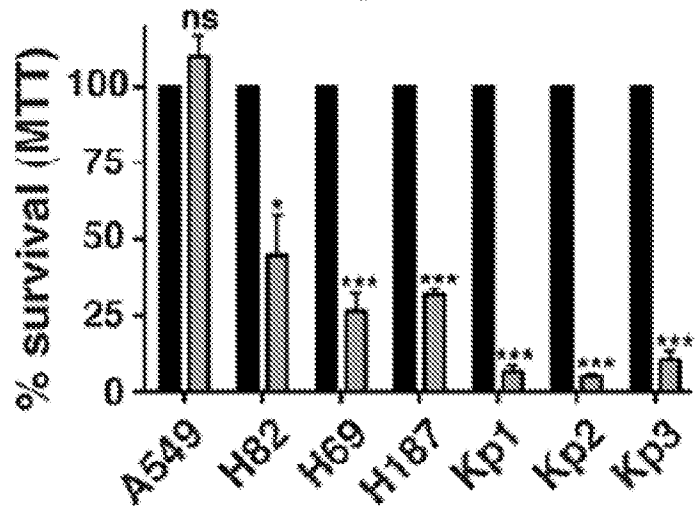
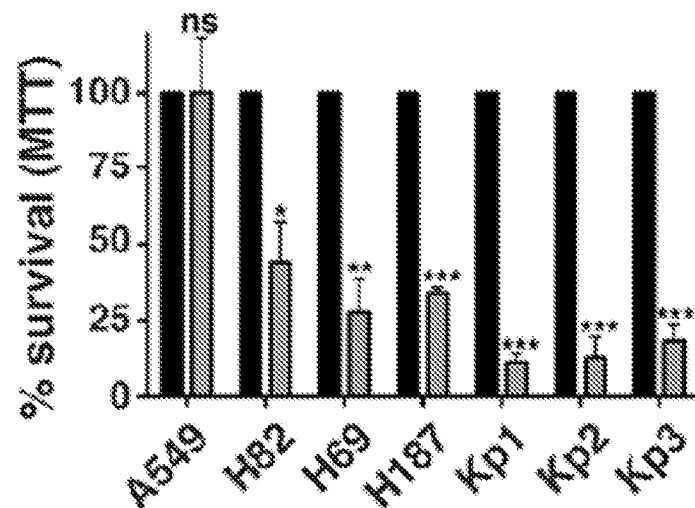


FIG. 1

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A**Clomipramine****B****Imipramine****C****Promethazine****FIG. 2**

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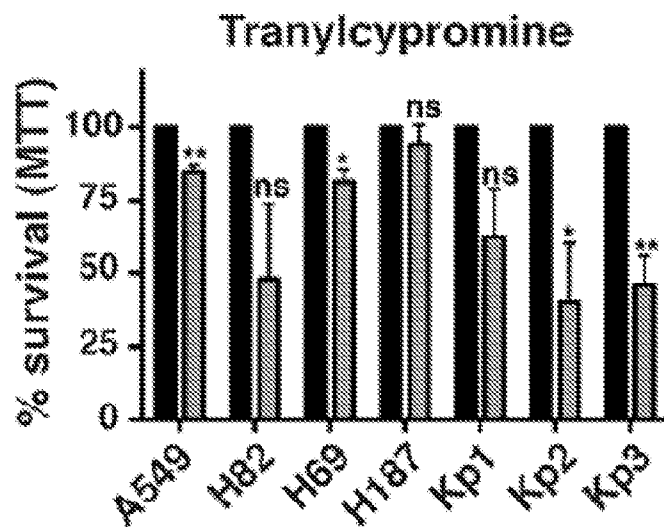
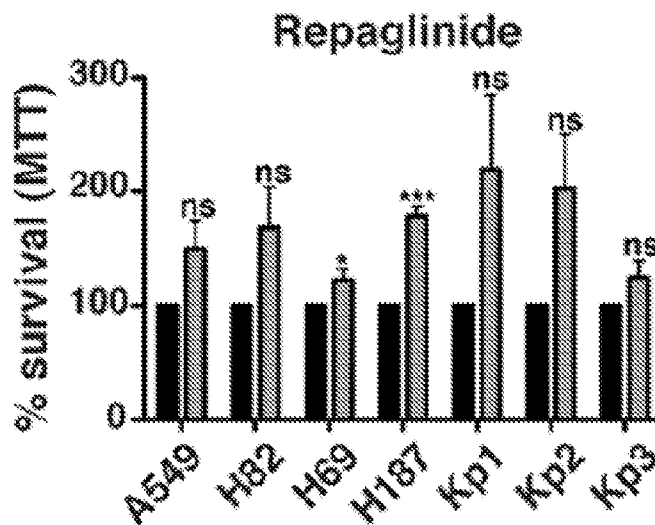
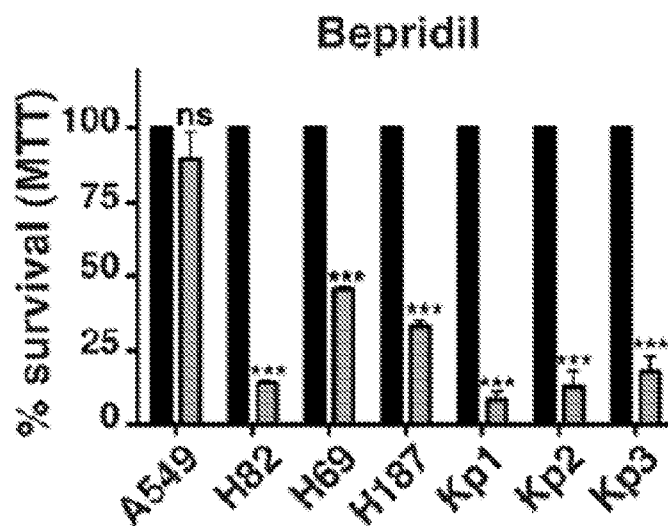
D**E****F**

FIG. 2
(Continued)

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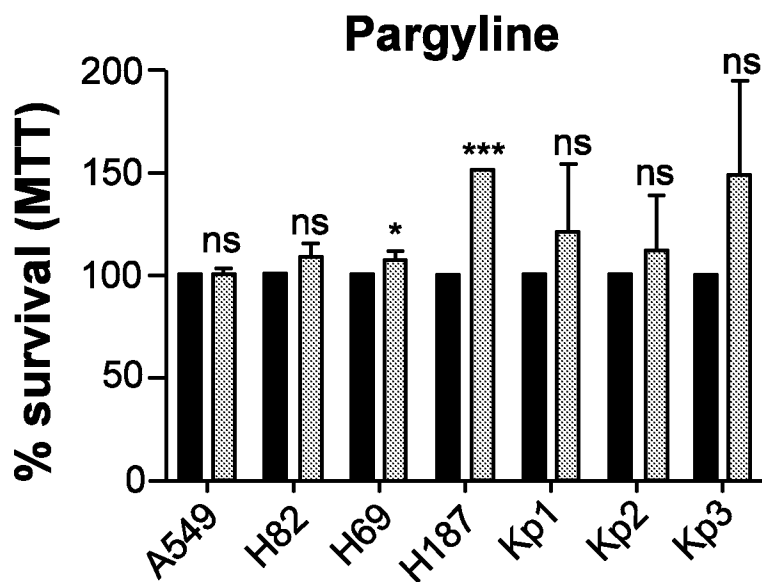
G

FIG. 2
(Continued)

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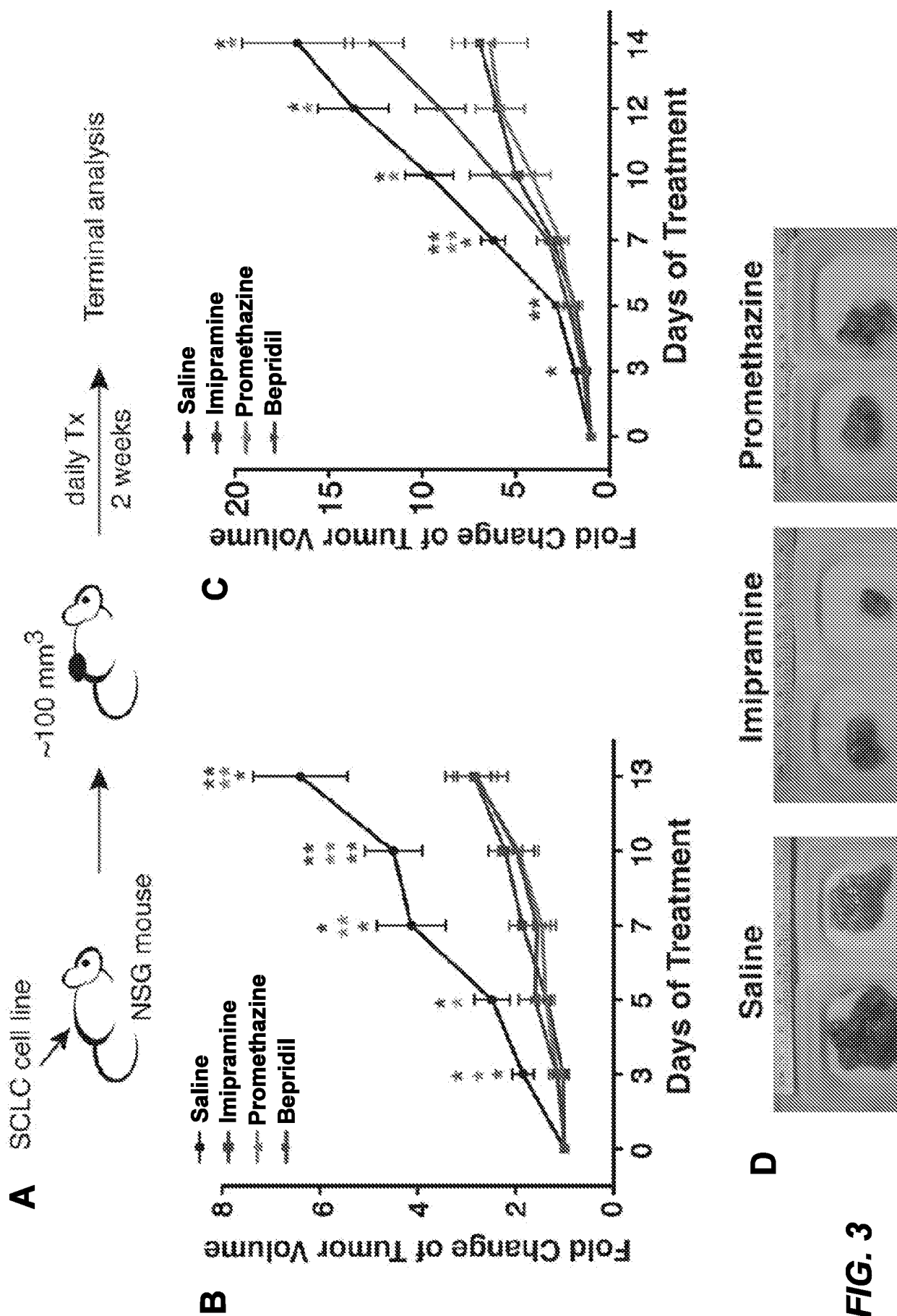


FIG. 3

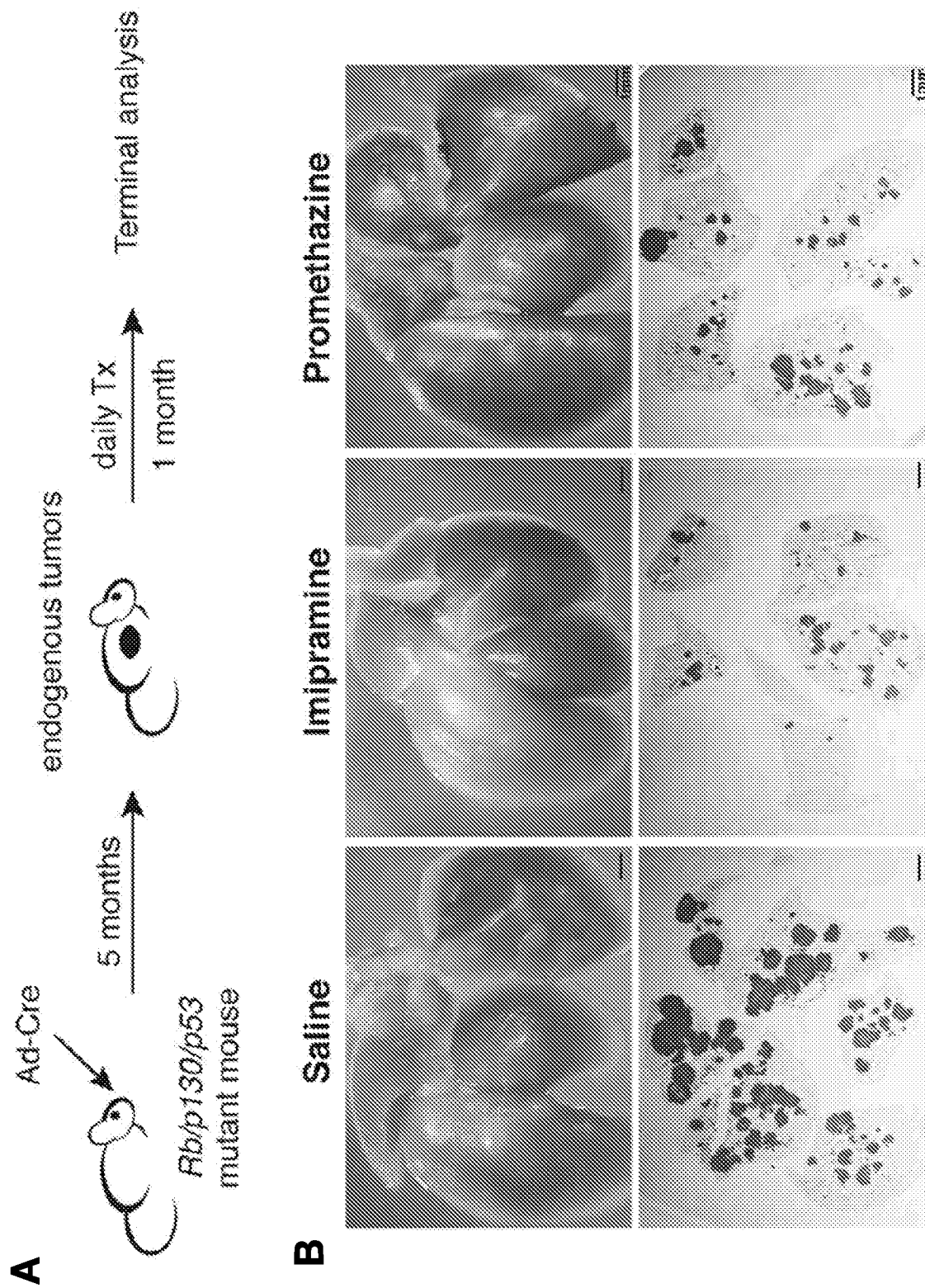


FIG. 4A

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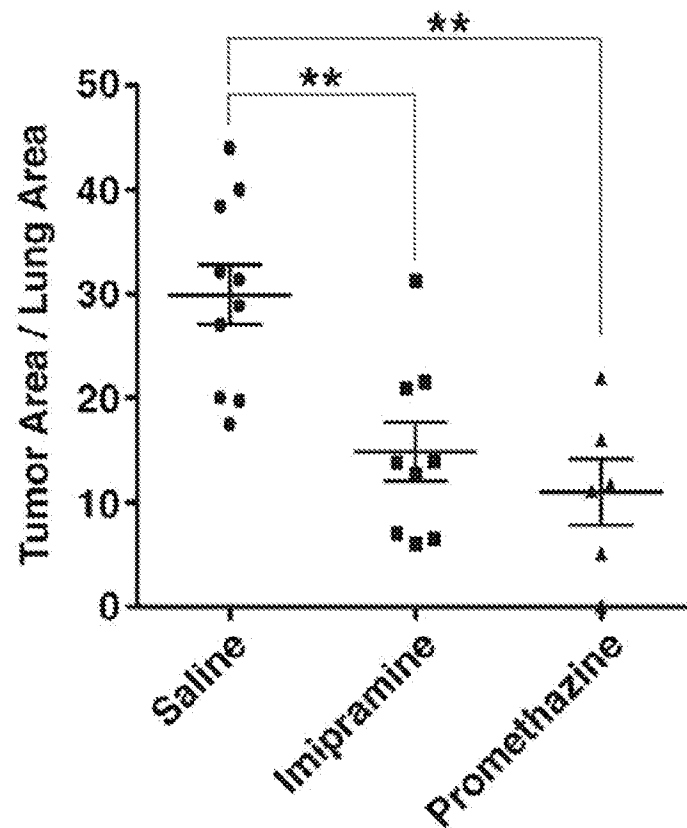
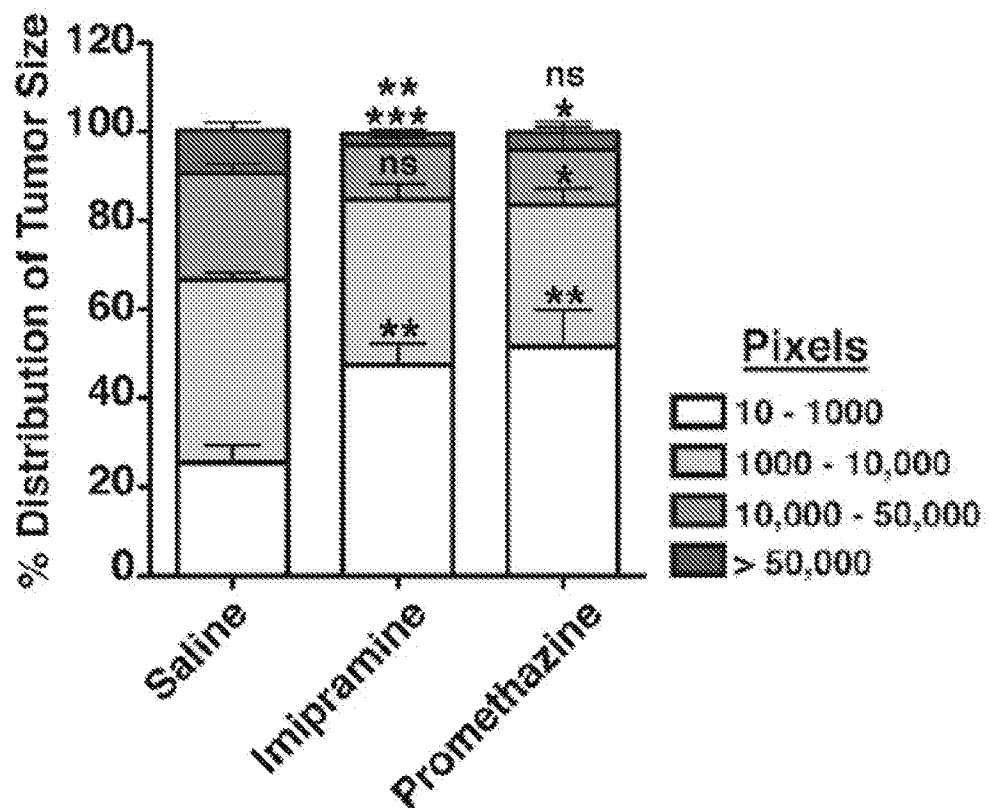
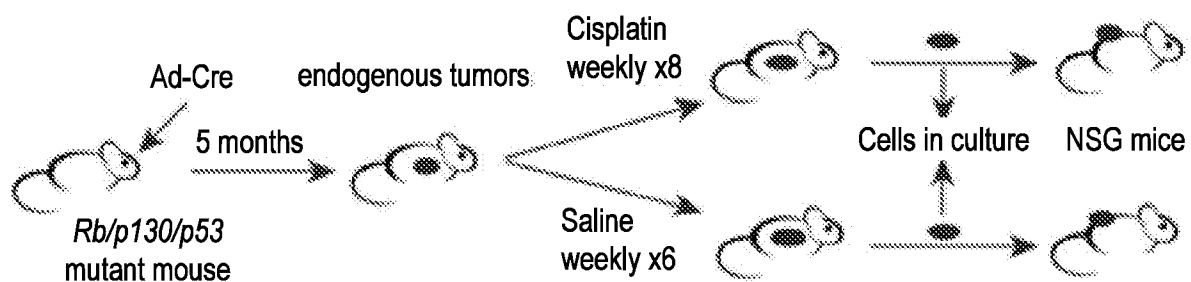
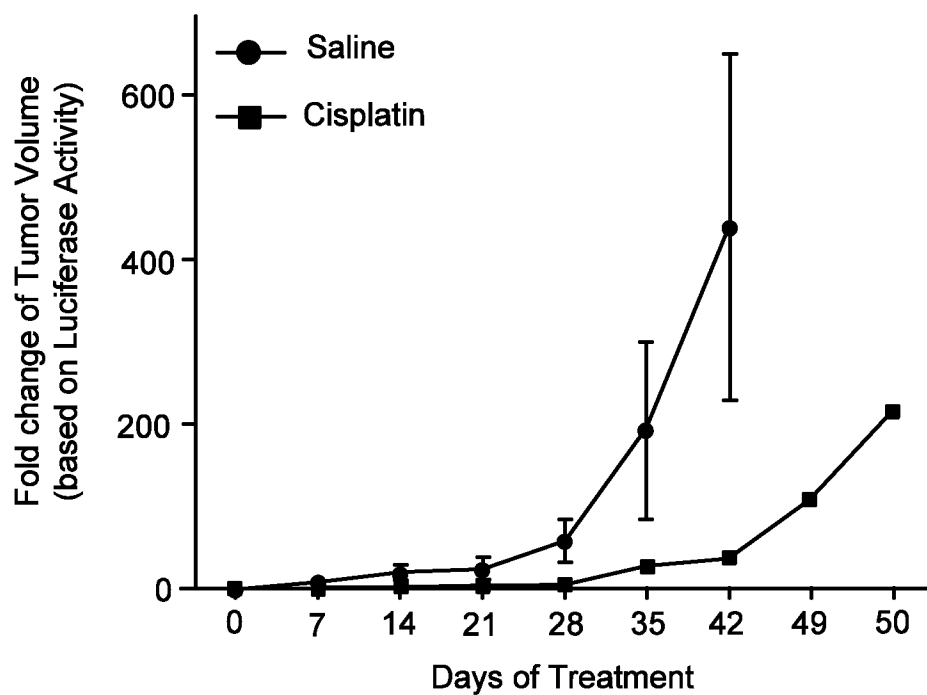
C**D**

FIG. 4A
(Continued)

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A**B****FIG. 4B**

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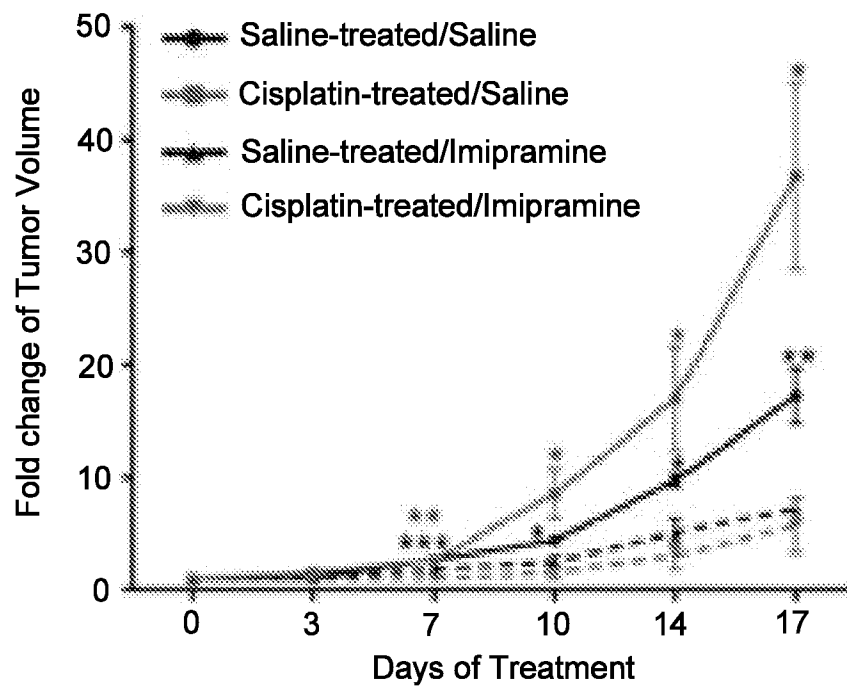
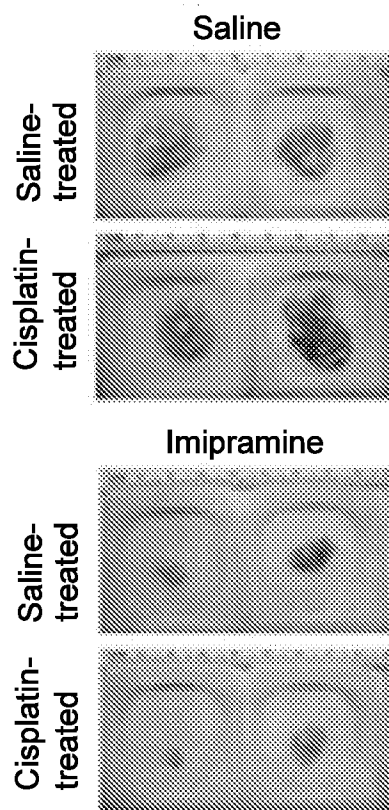
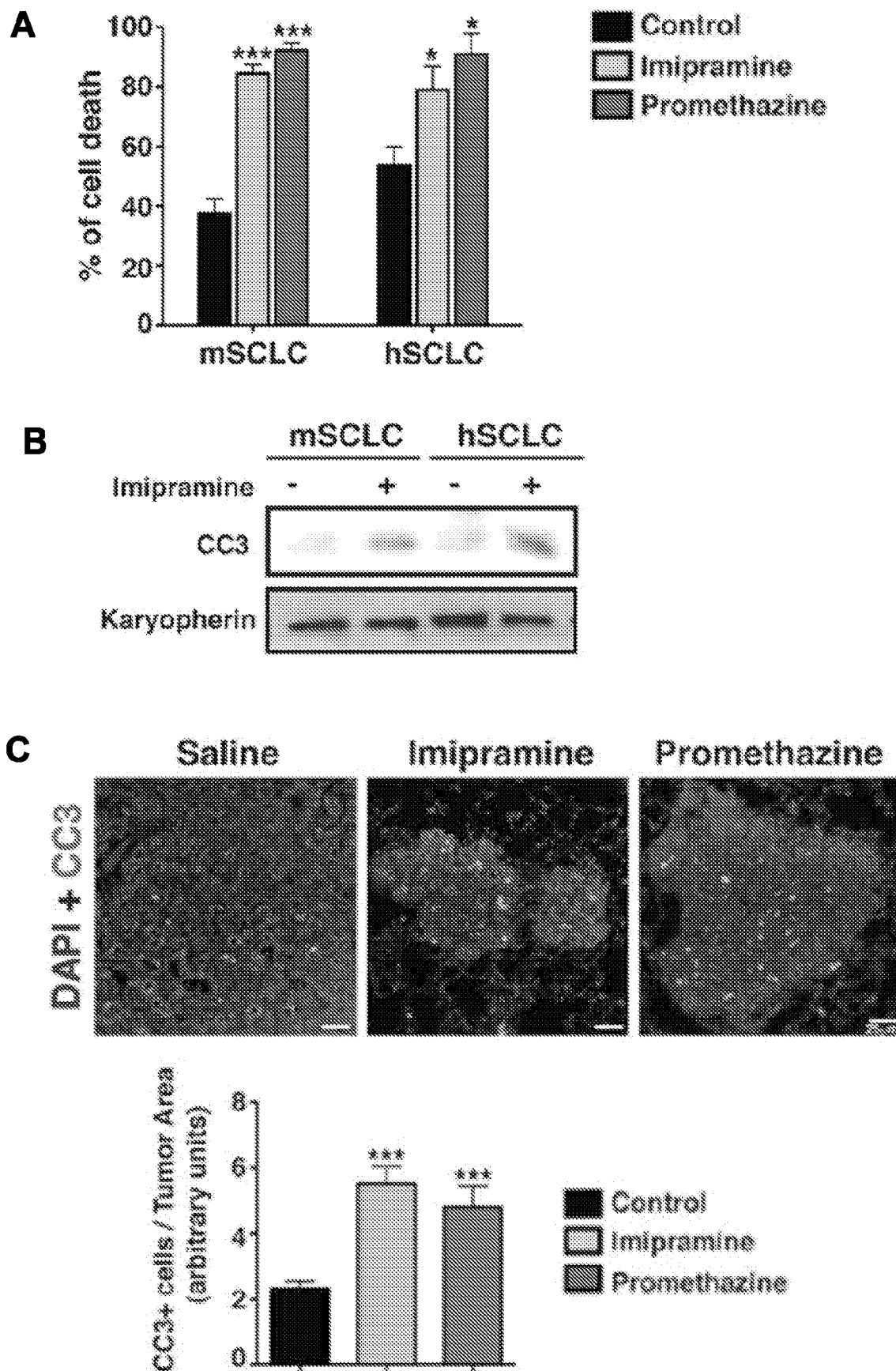
C**D**

FIG. 4B
(Continued)

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**FIG. 4C**

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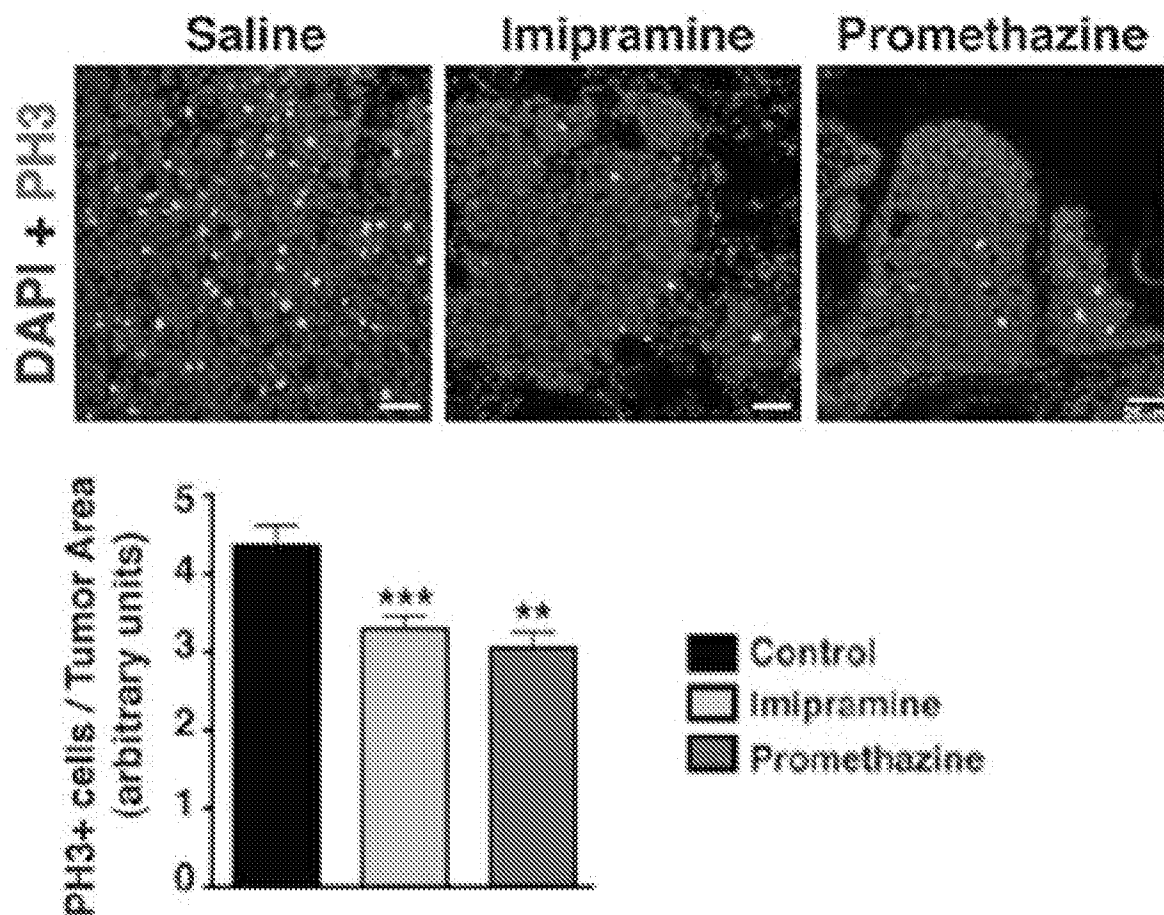
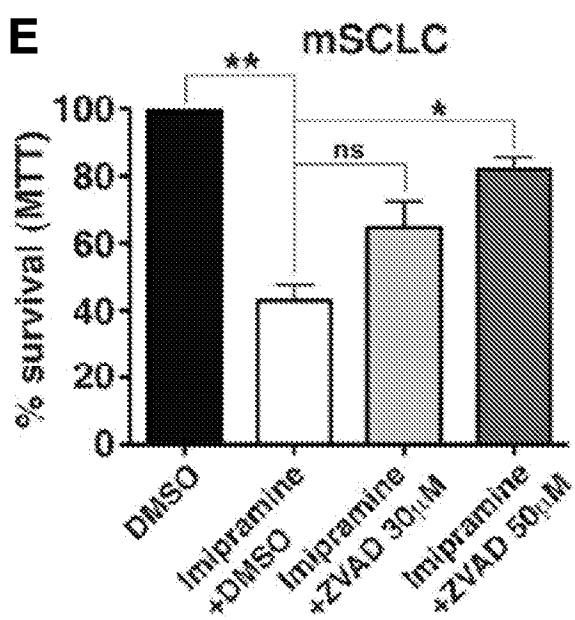
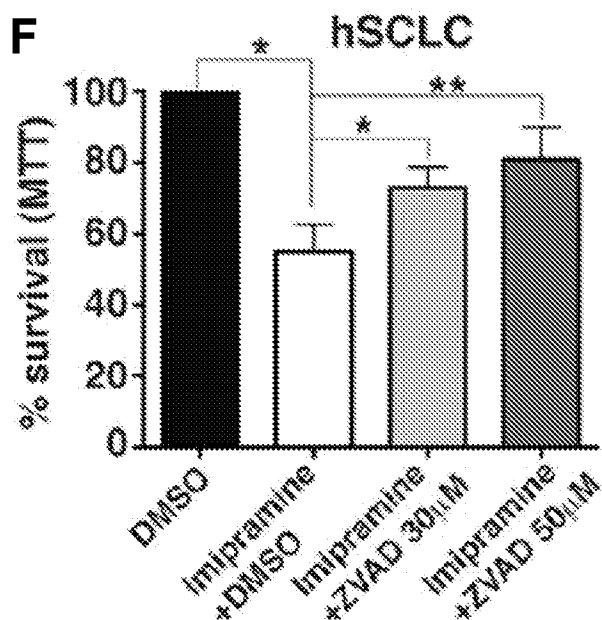
D**E****F**

FIG. 4C
(Continued)

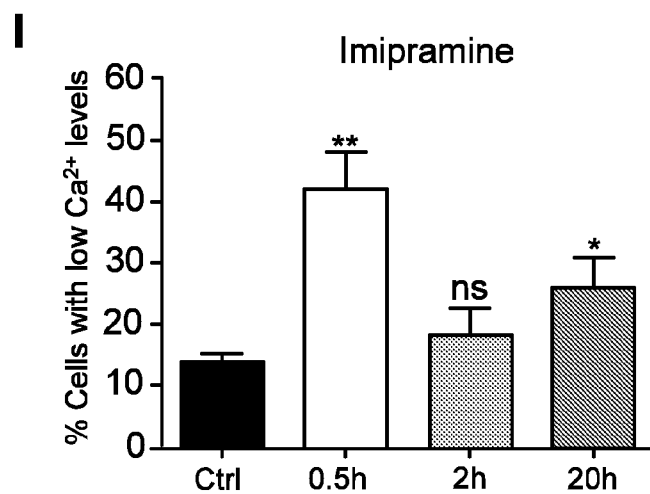
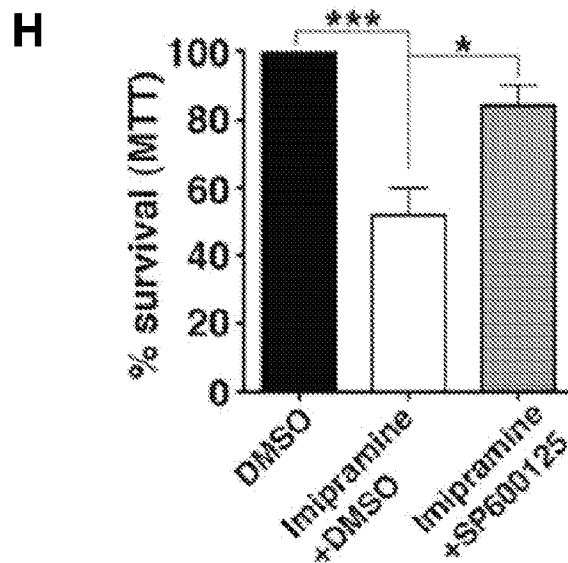
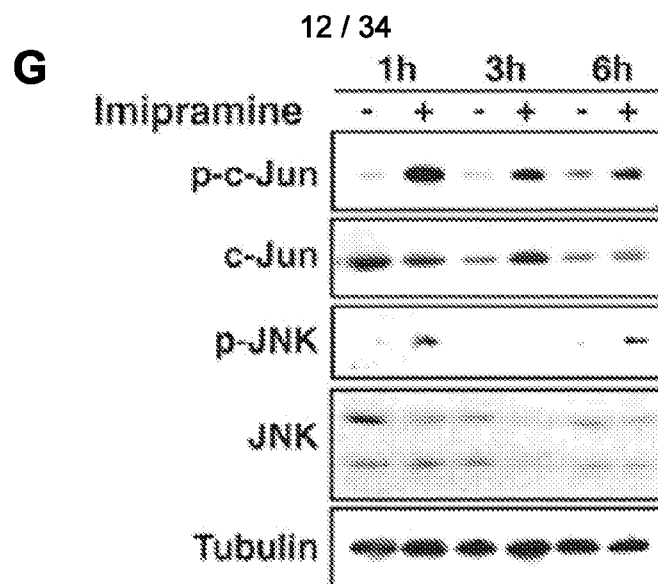
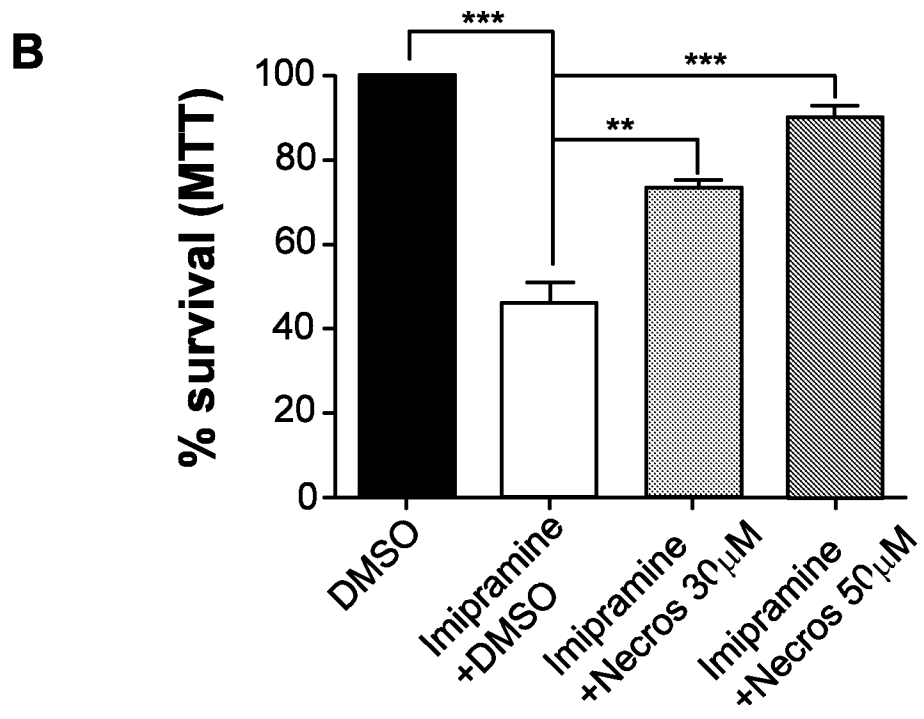
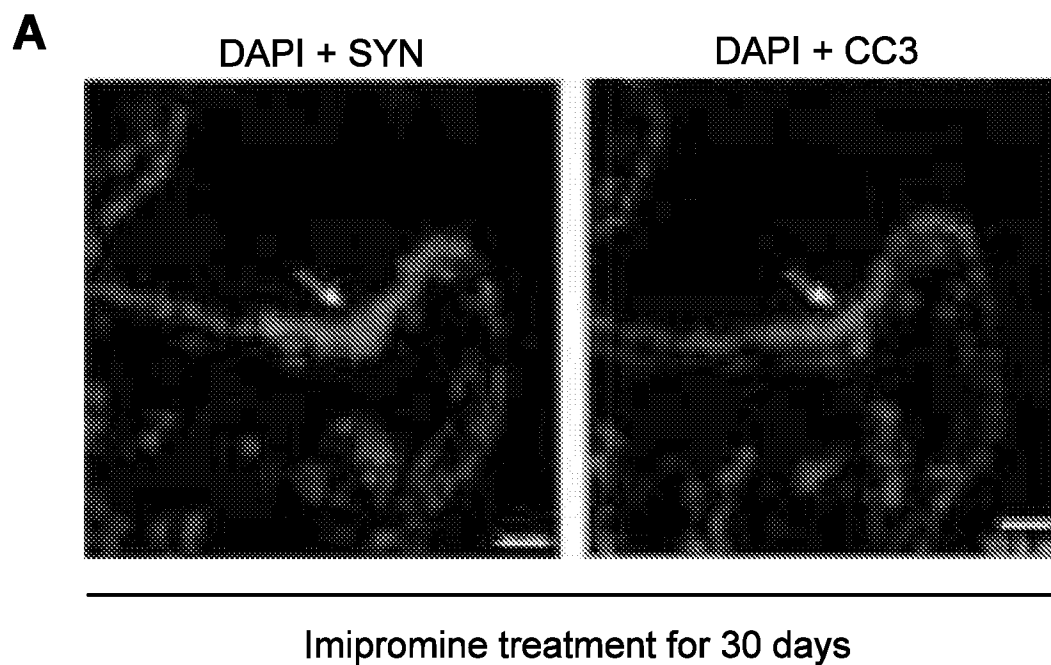


FIG. 4C
(Continued)

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**FIG. 4D**

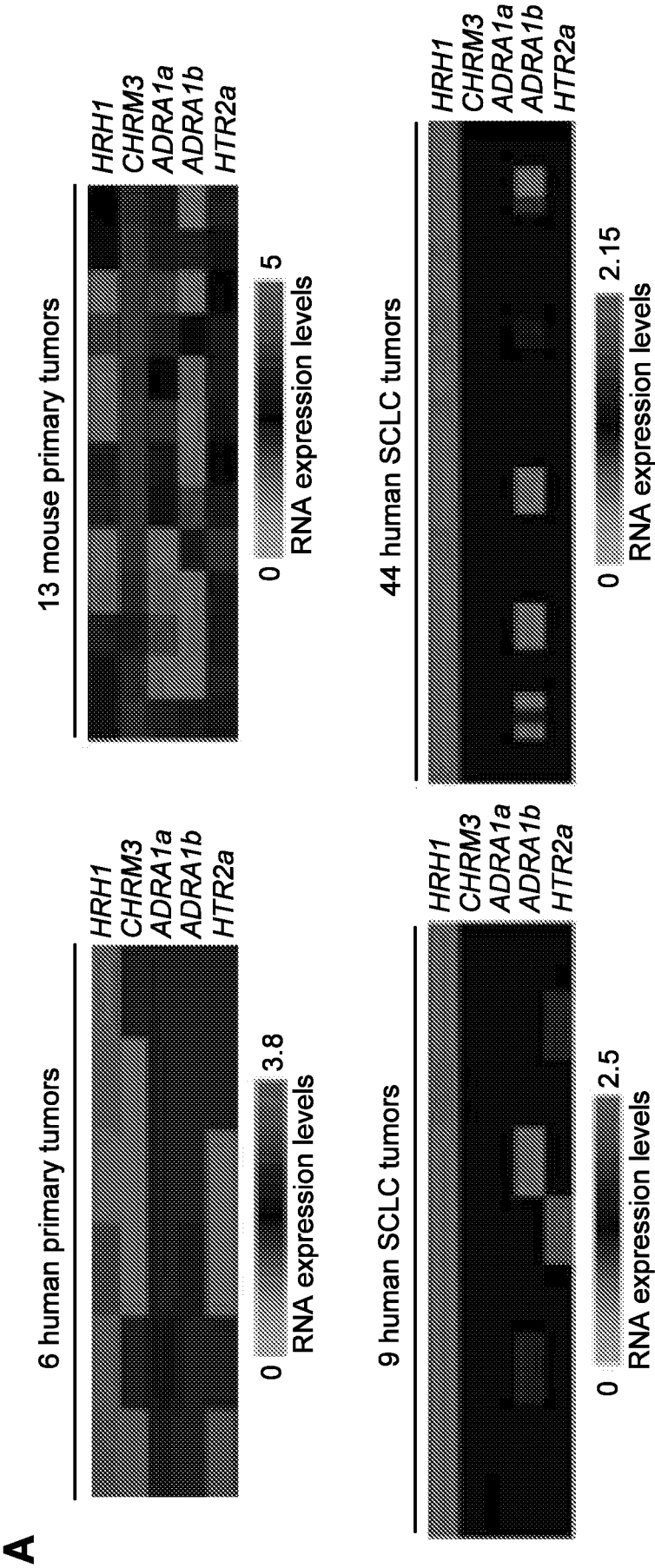


FIG. 5A

B

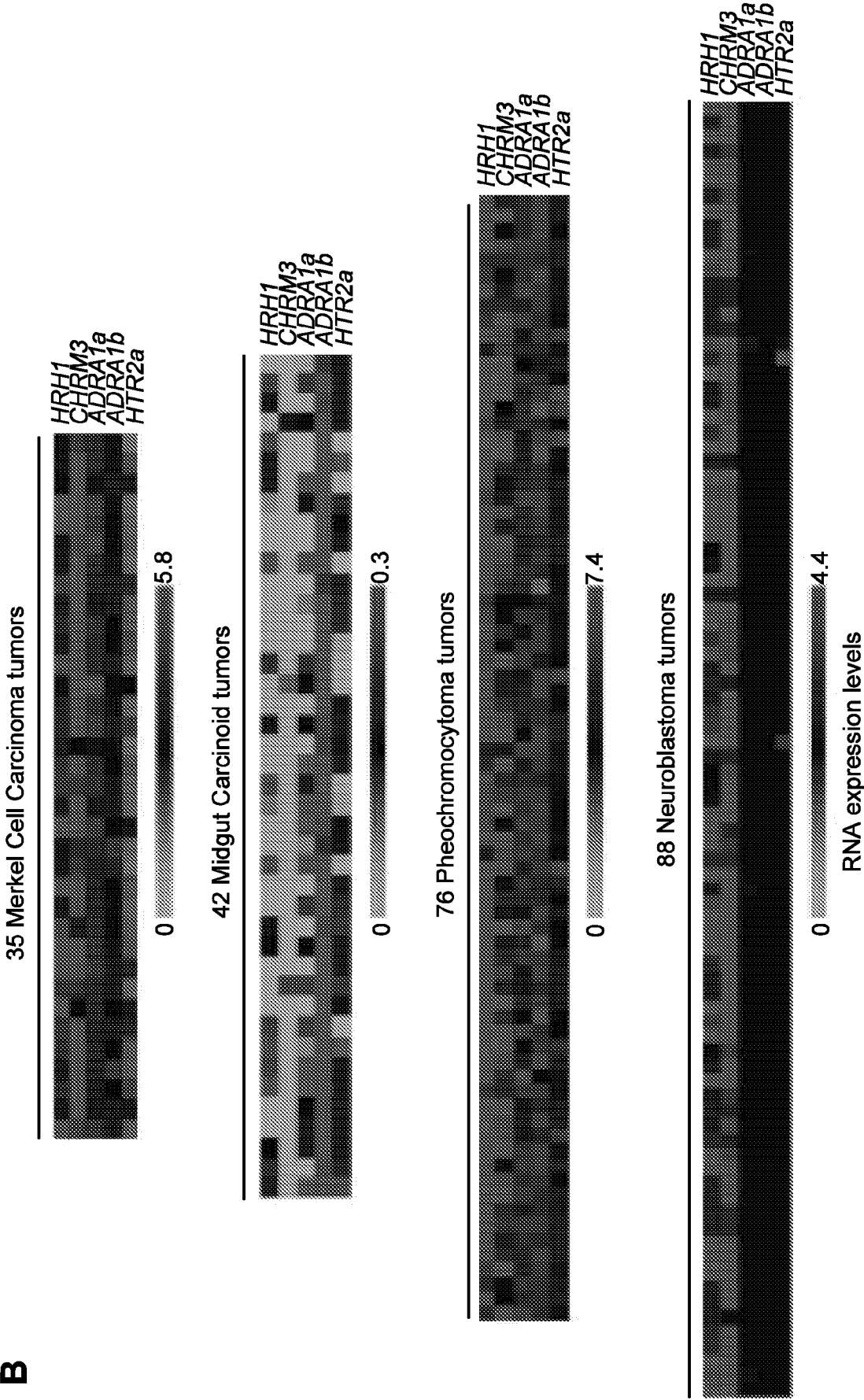
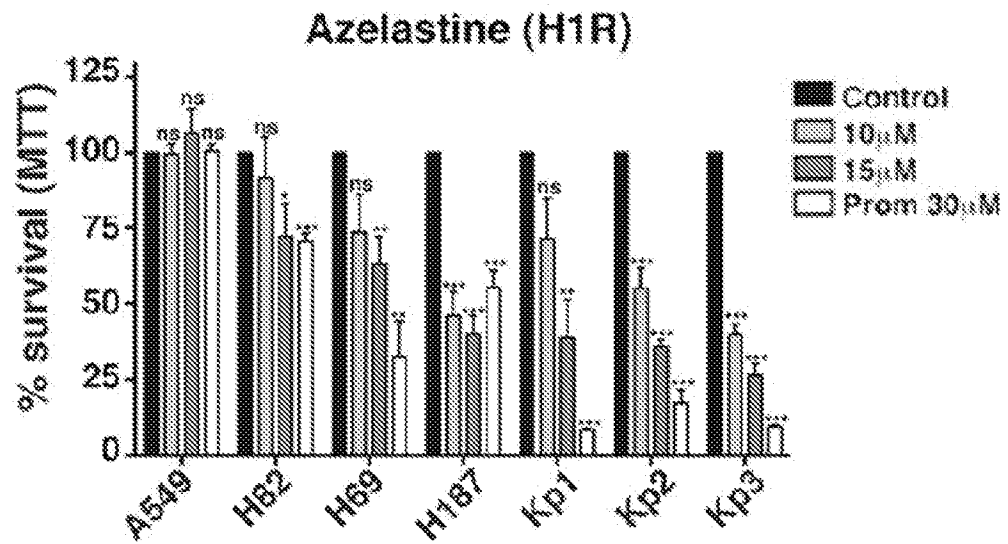
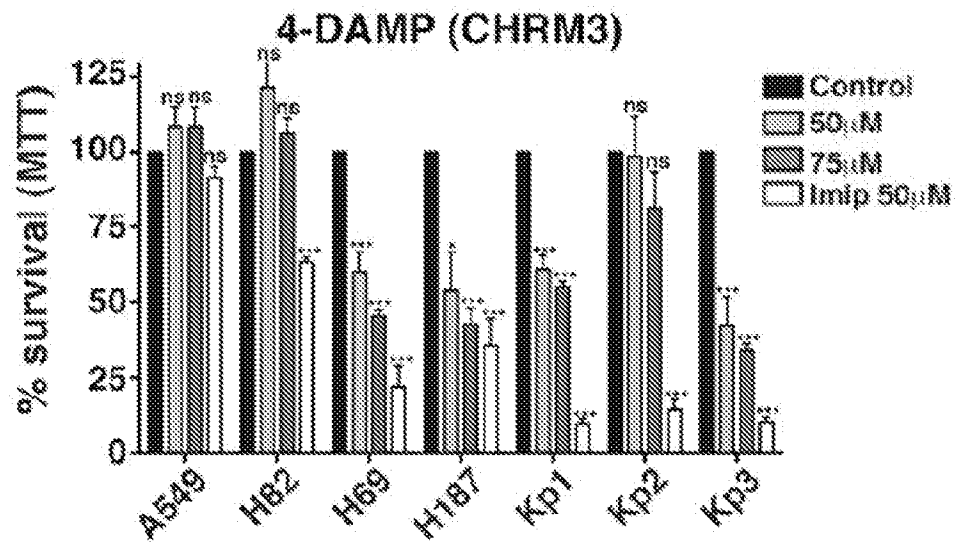
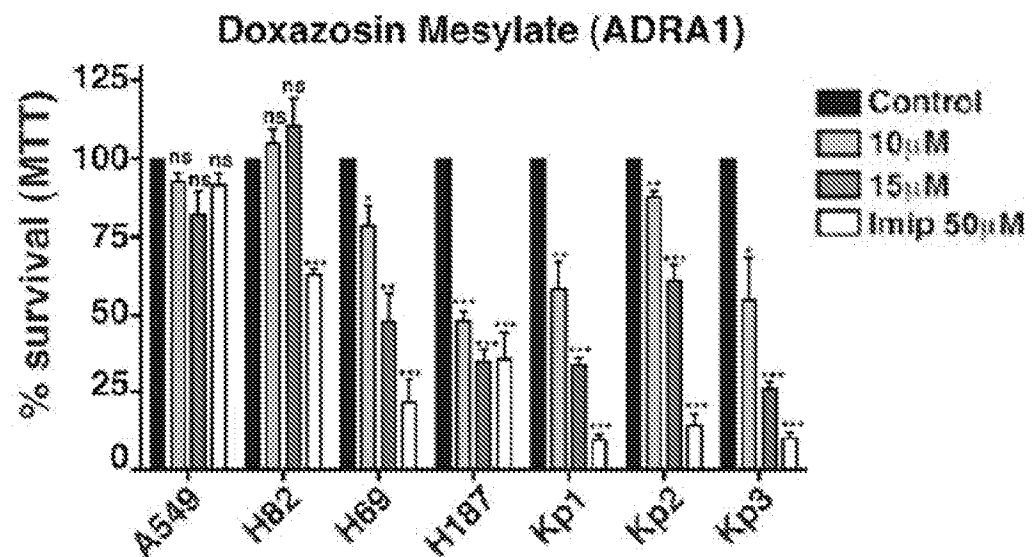


FIG. 5A
(Continued)

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A**B****C****FIG. 5B**

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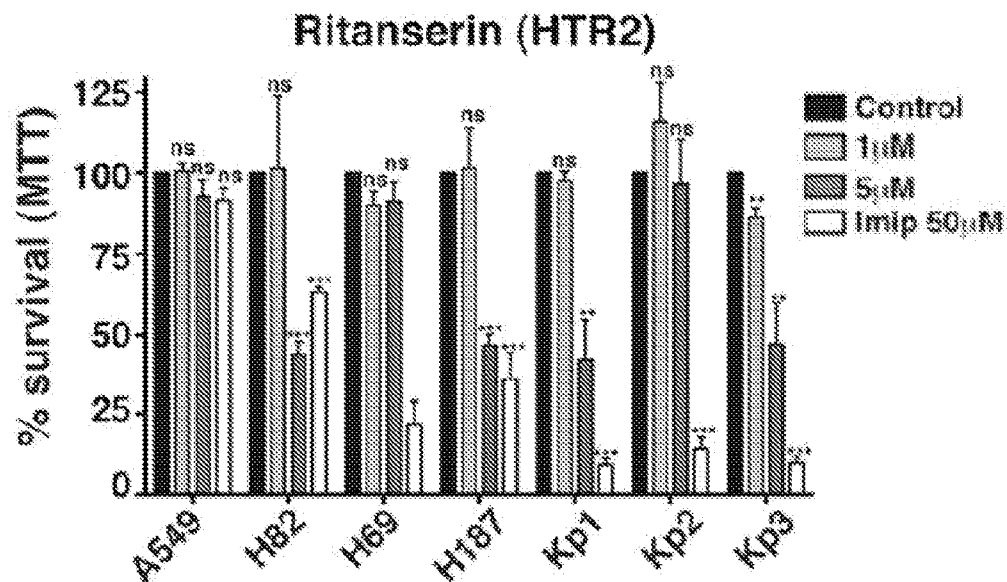
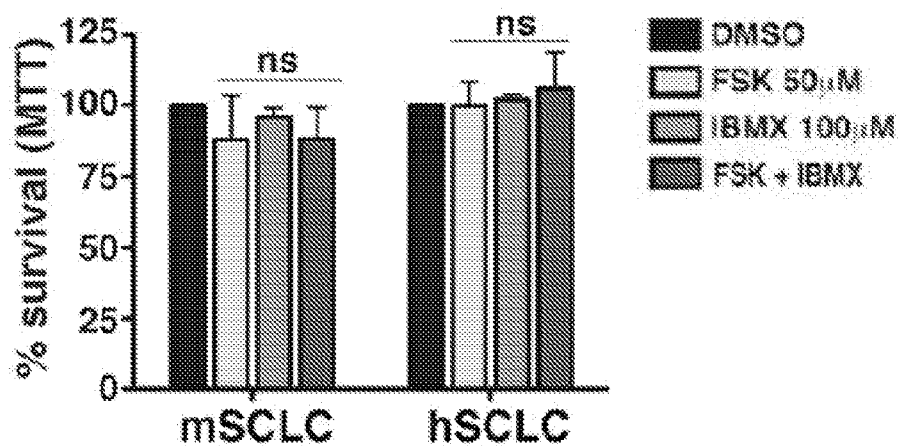
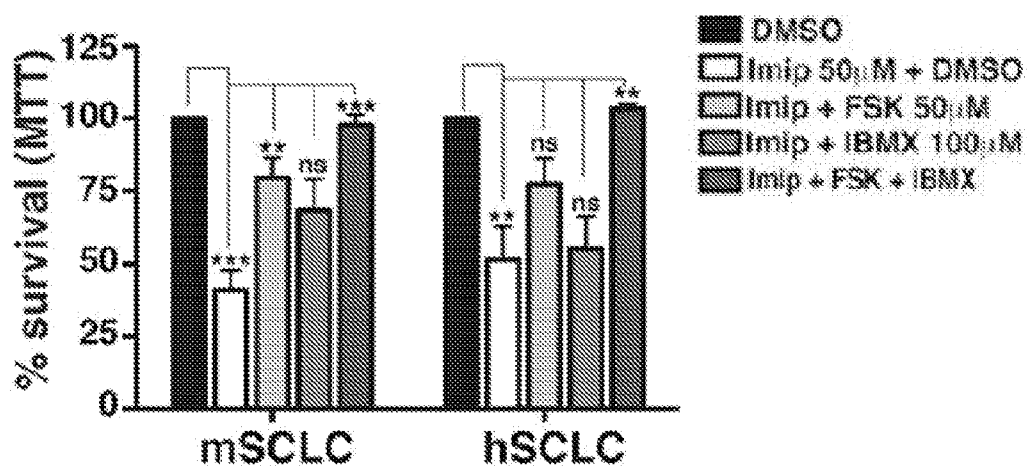
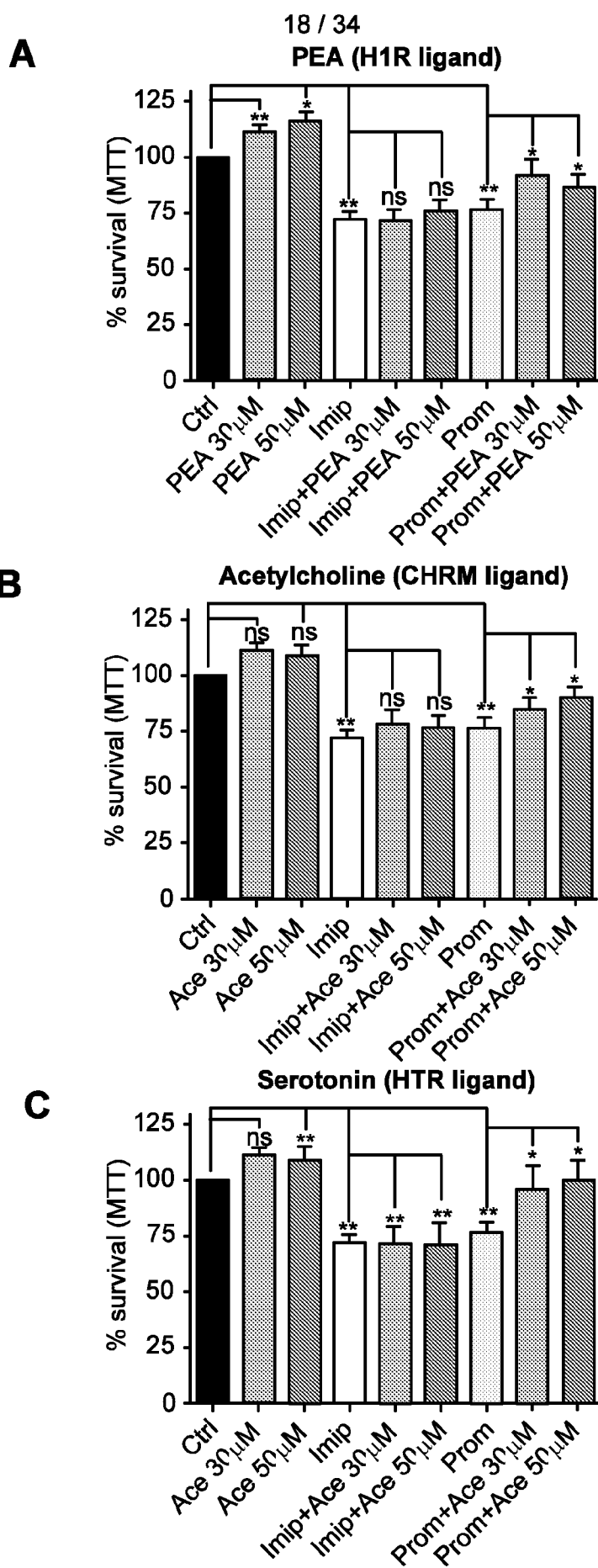
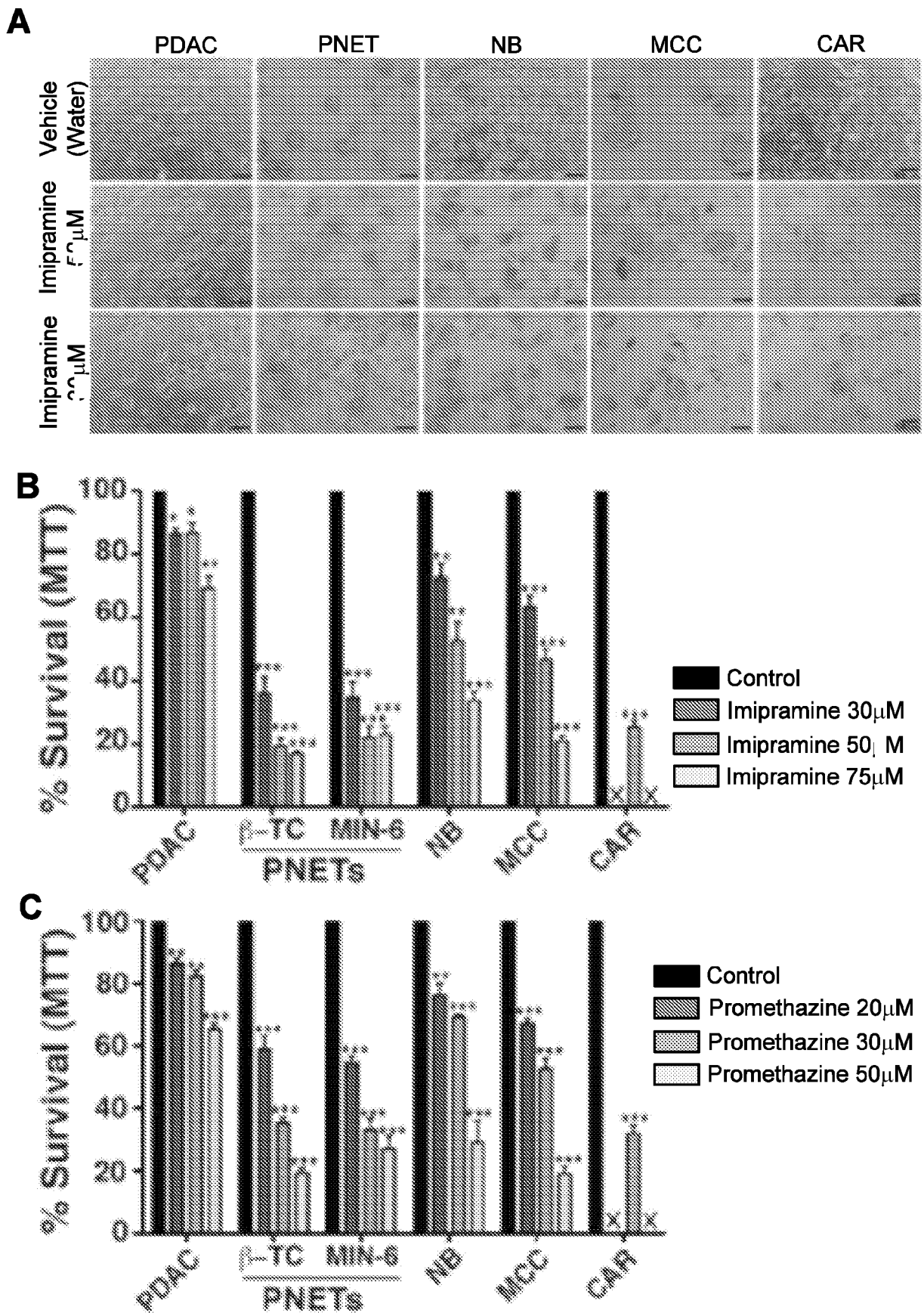
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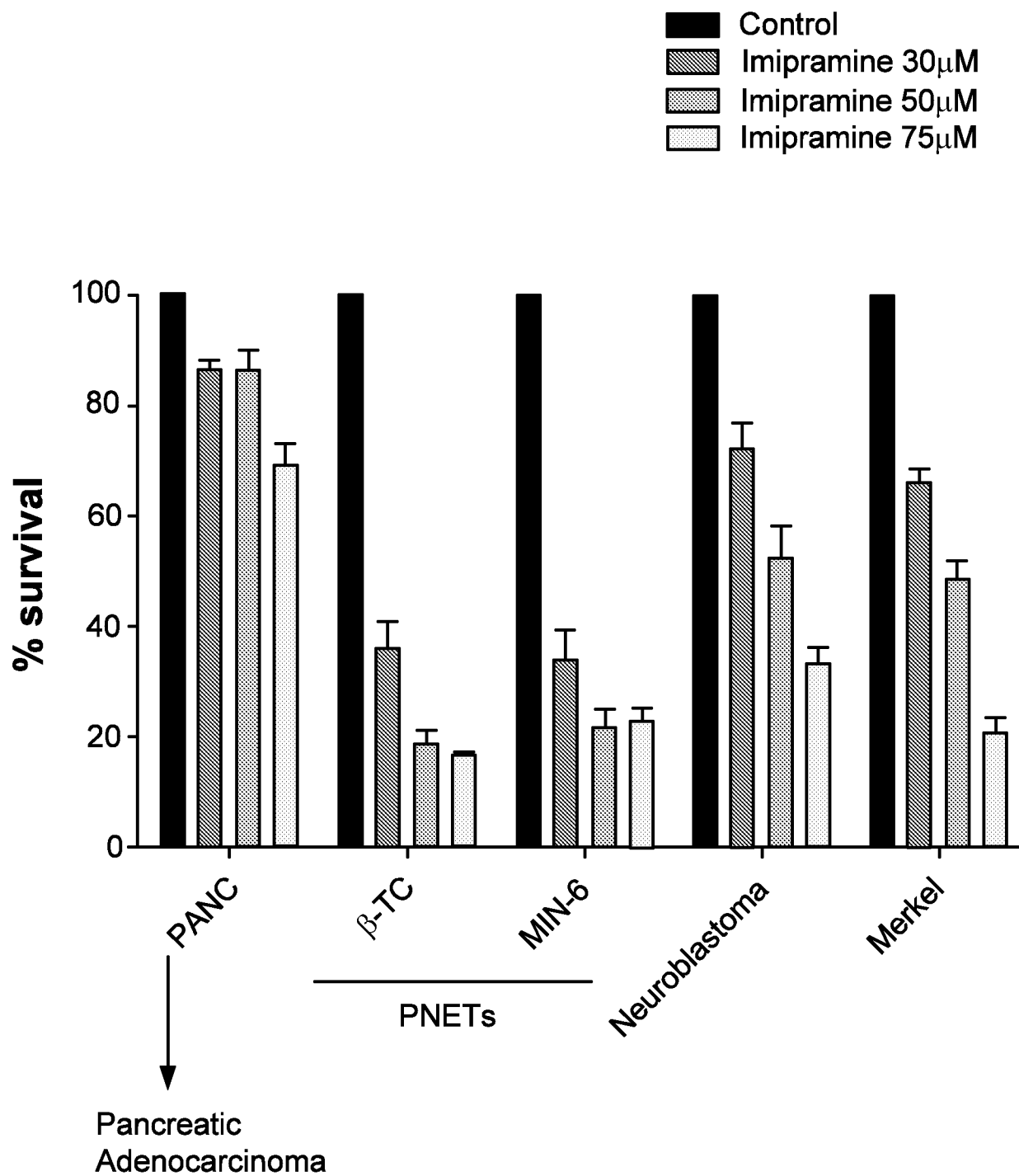
FIG. 5B
(Continued)

**FIG. 5C**

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**FIG. 6A**

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**FIG. 6B**

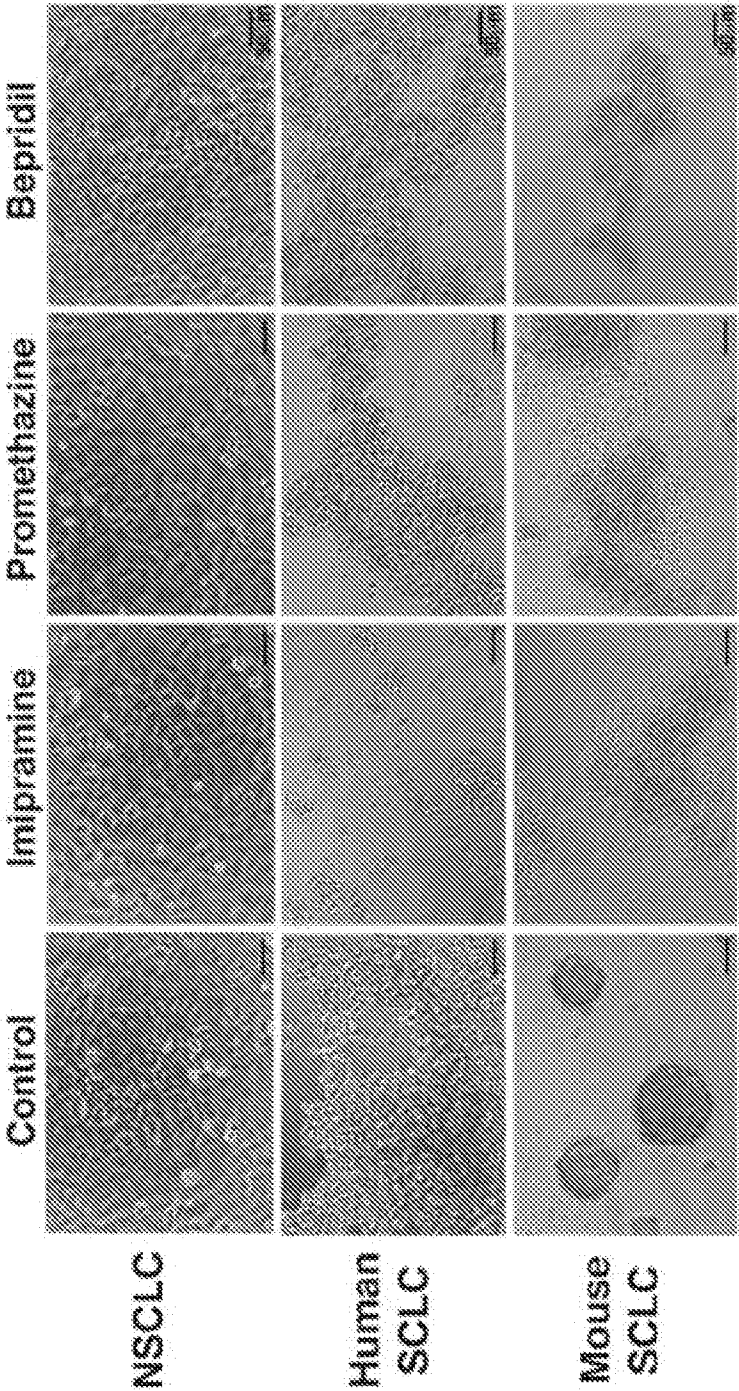
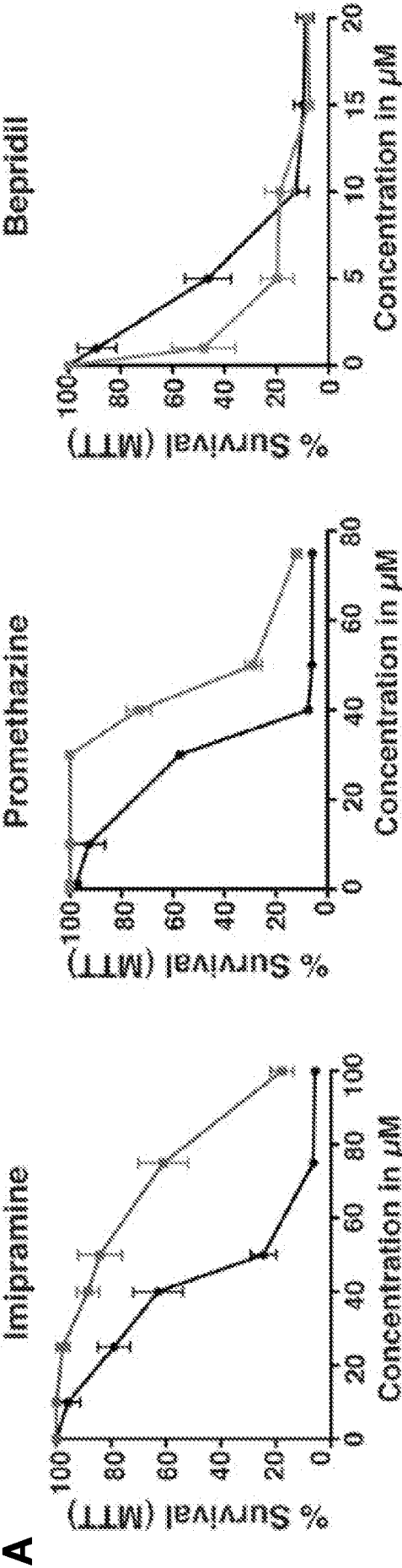


FIG. 7

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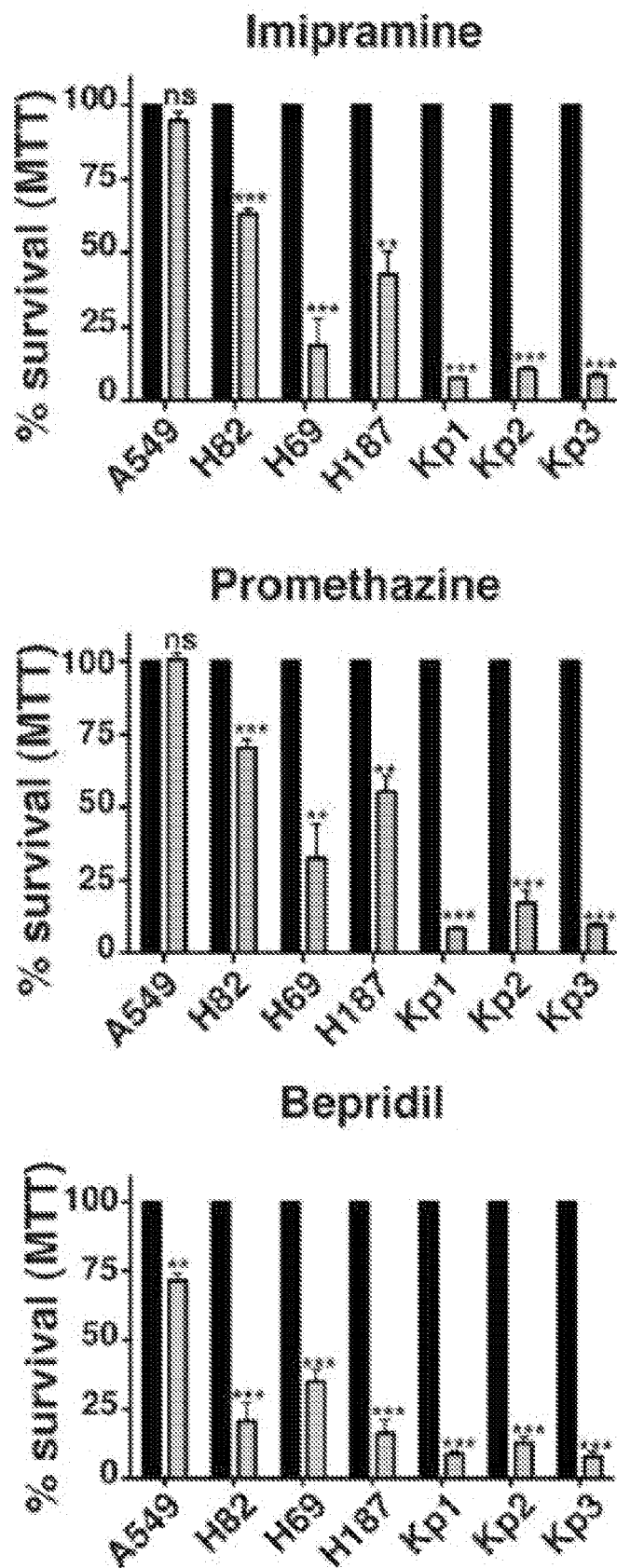
C

FIG. 7
(Continued)

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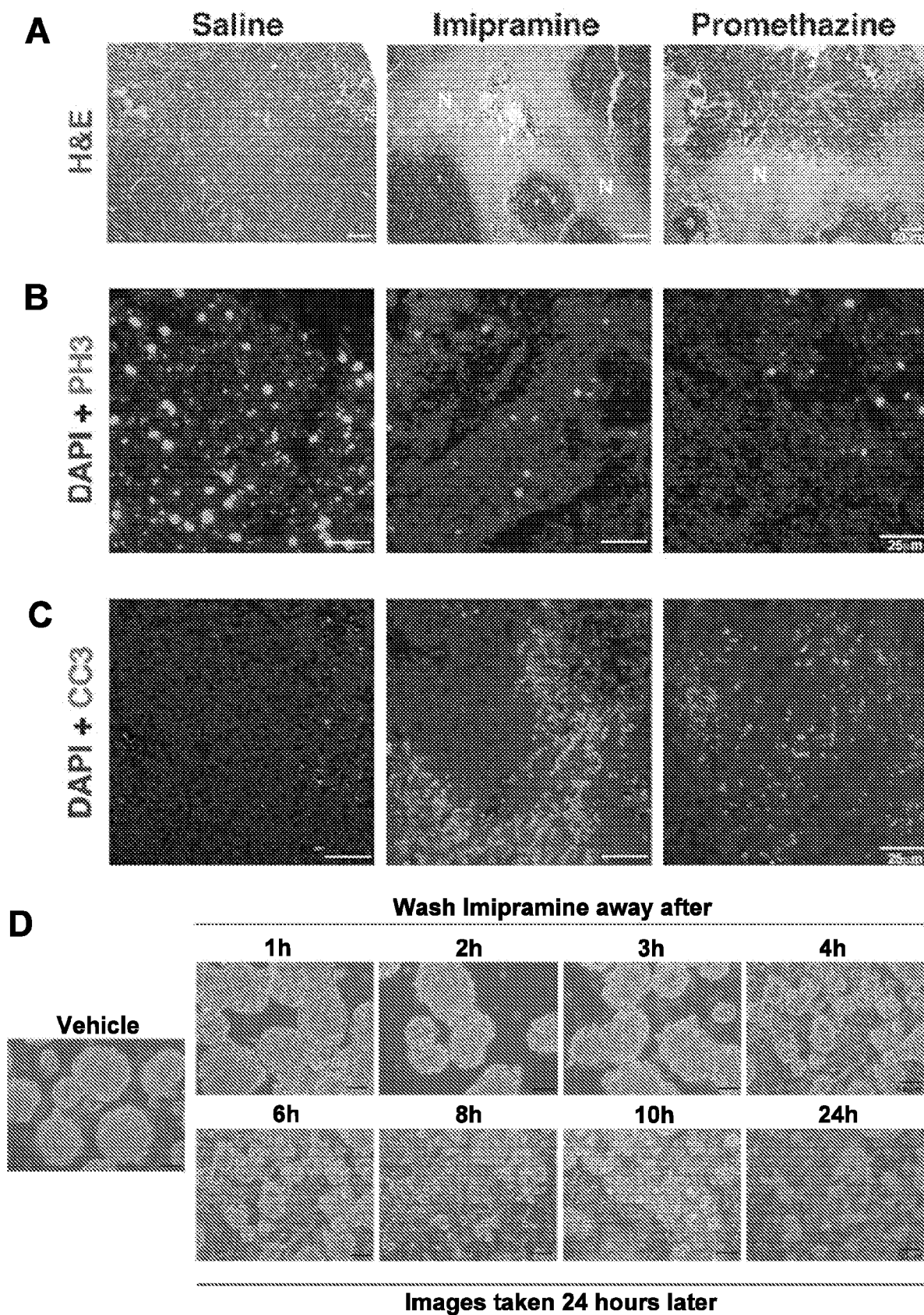
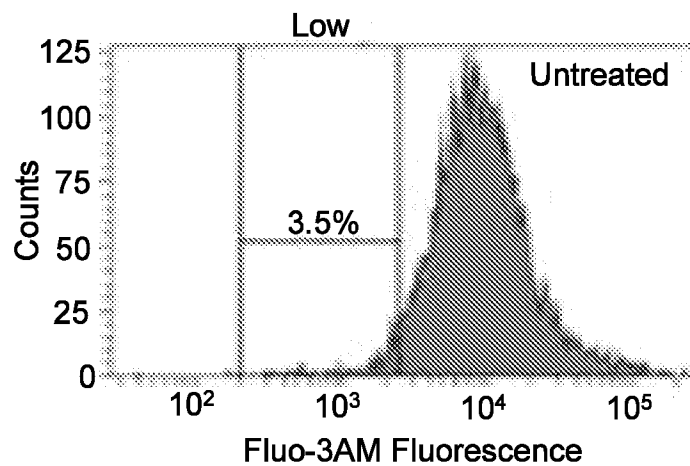
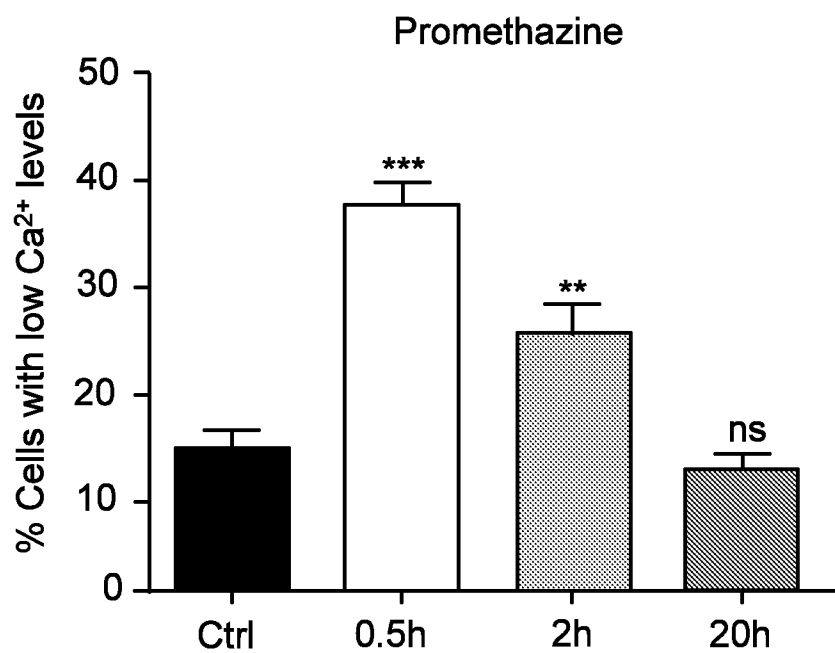
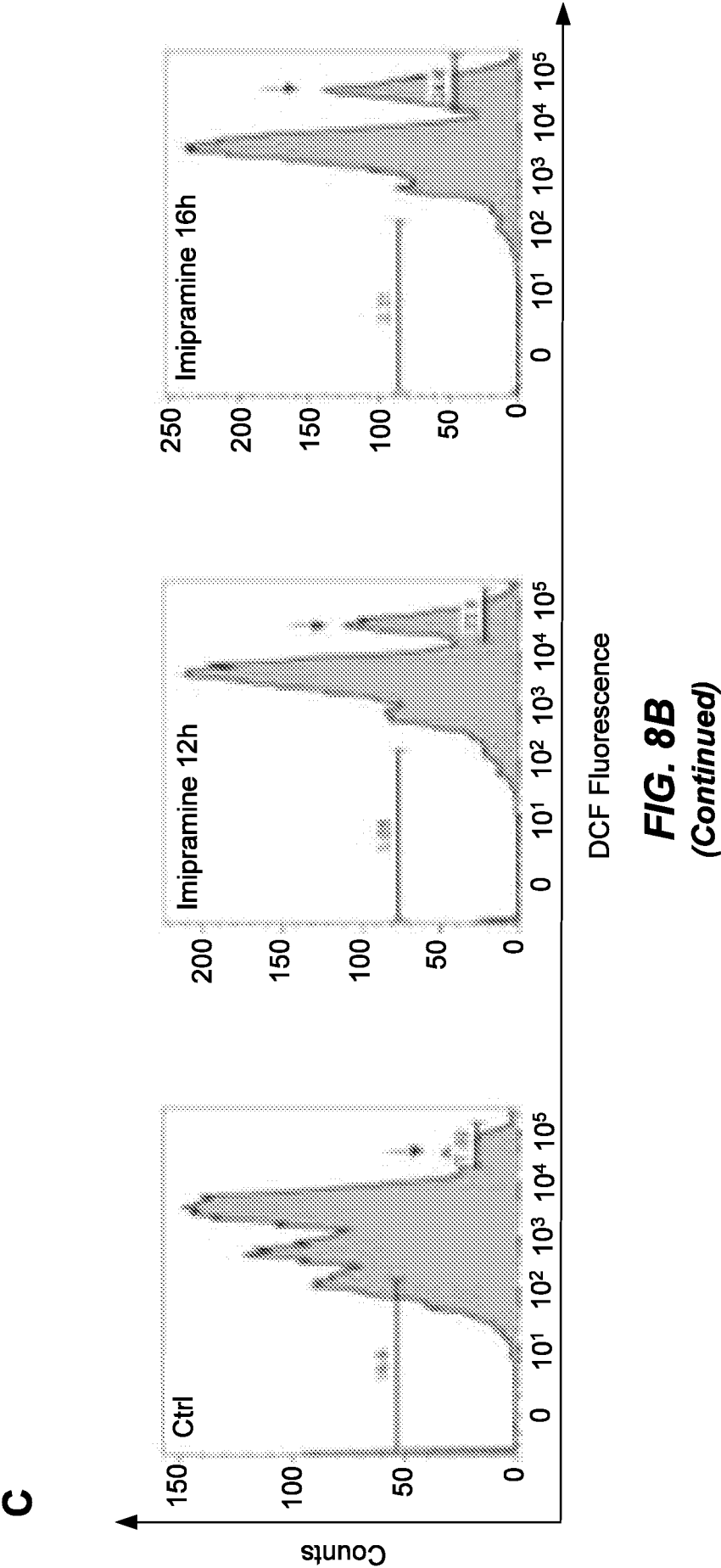


FIG. 8

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A**B****FIG. 8B**



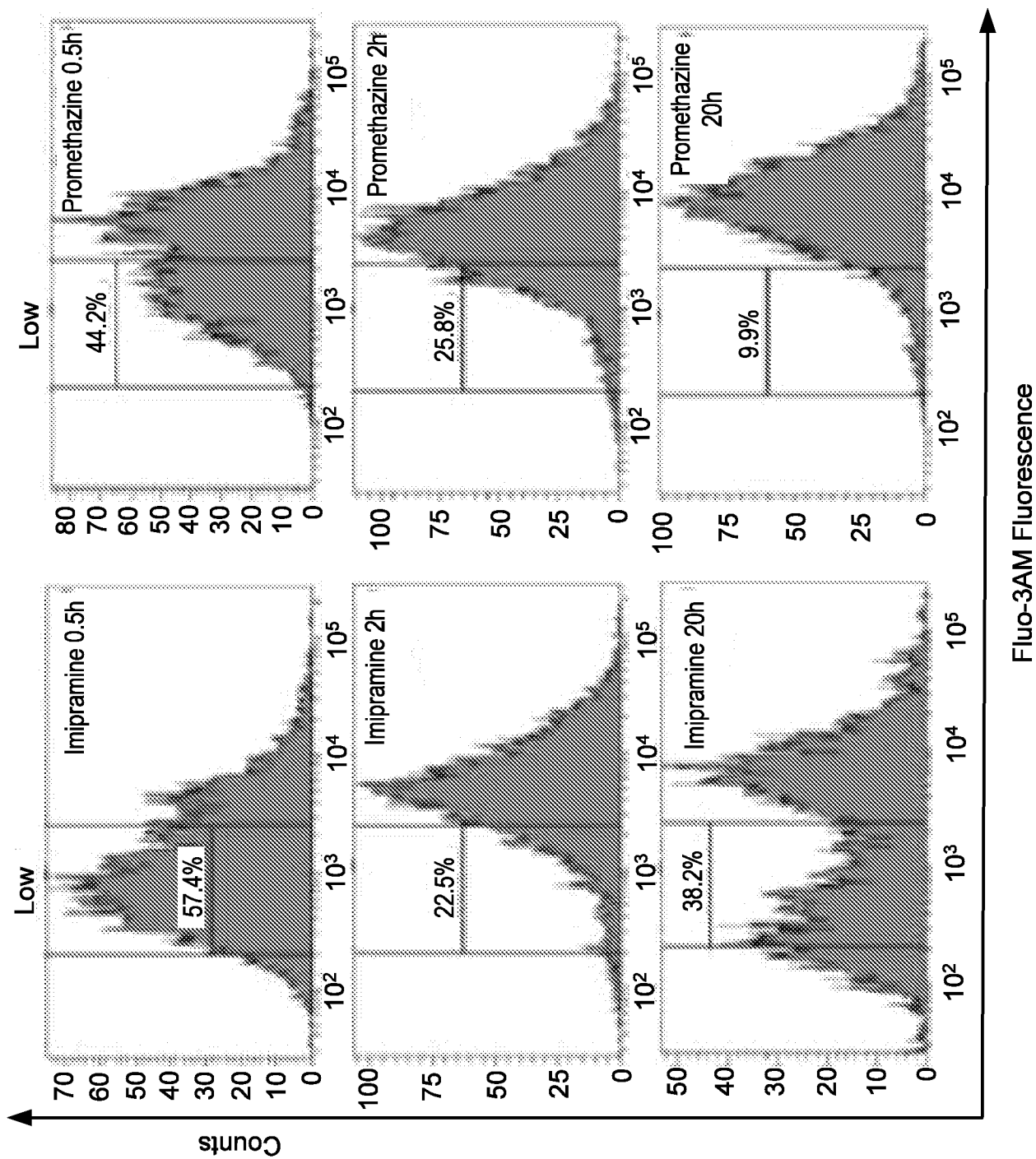


FIG. 8B
(Continued)

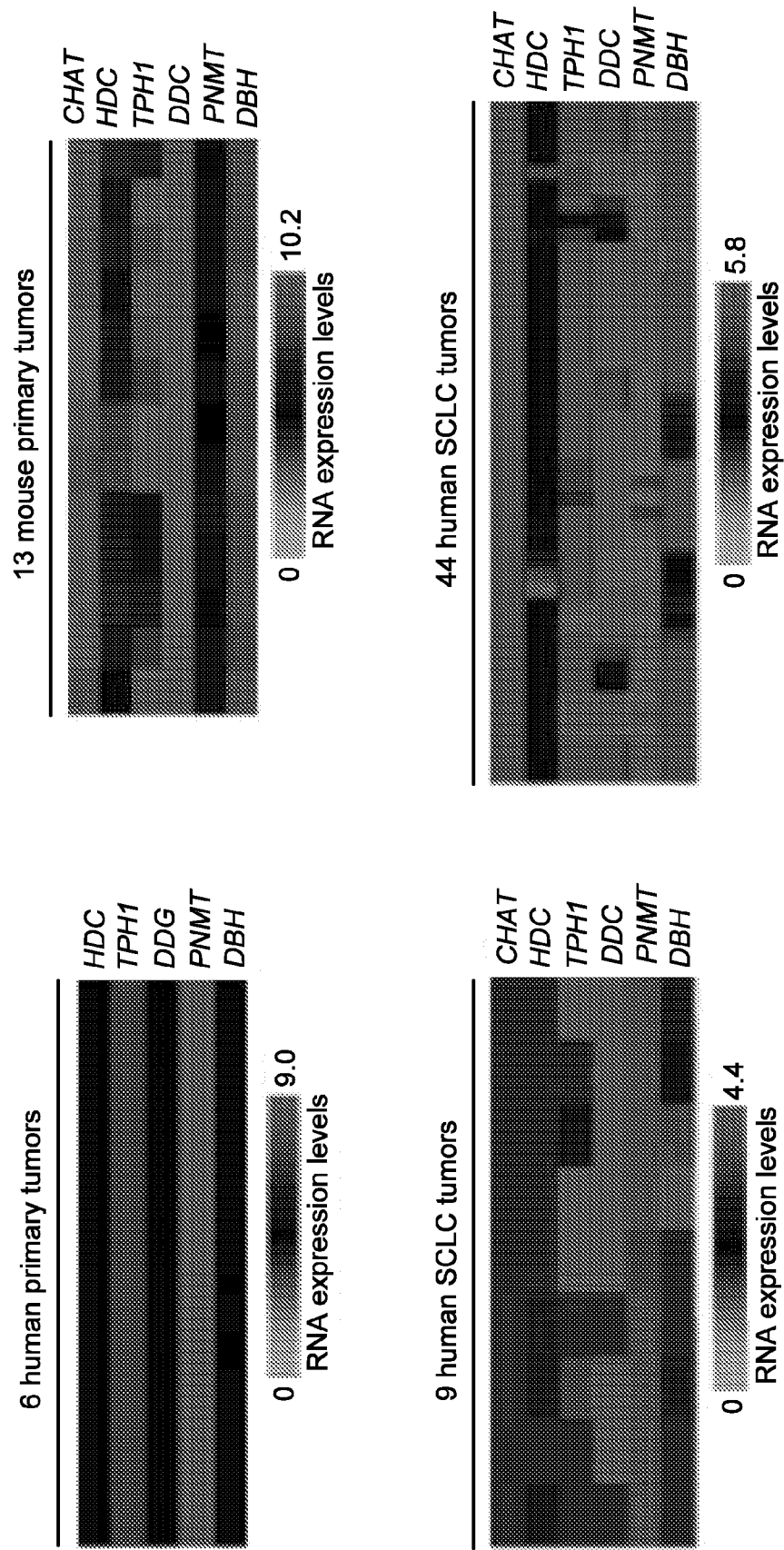
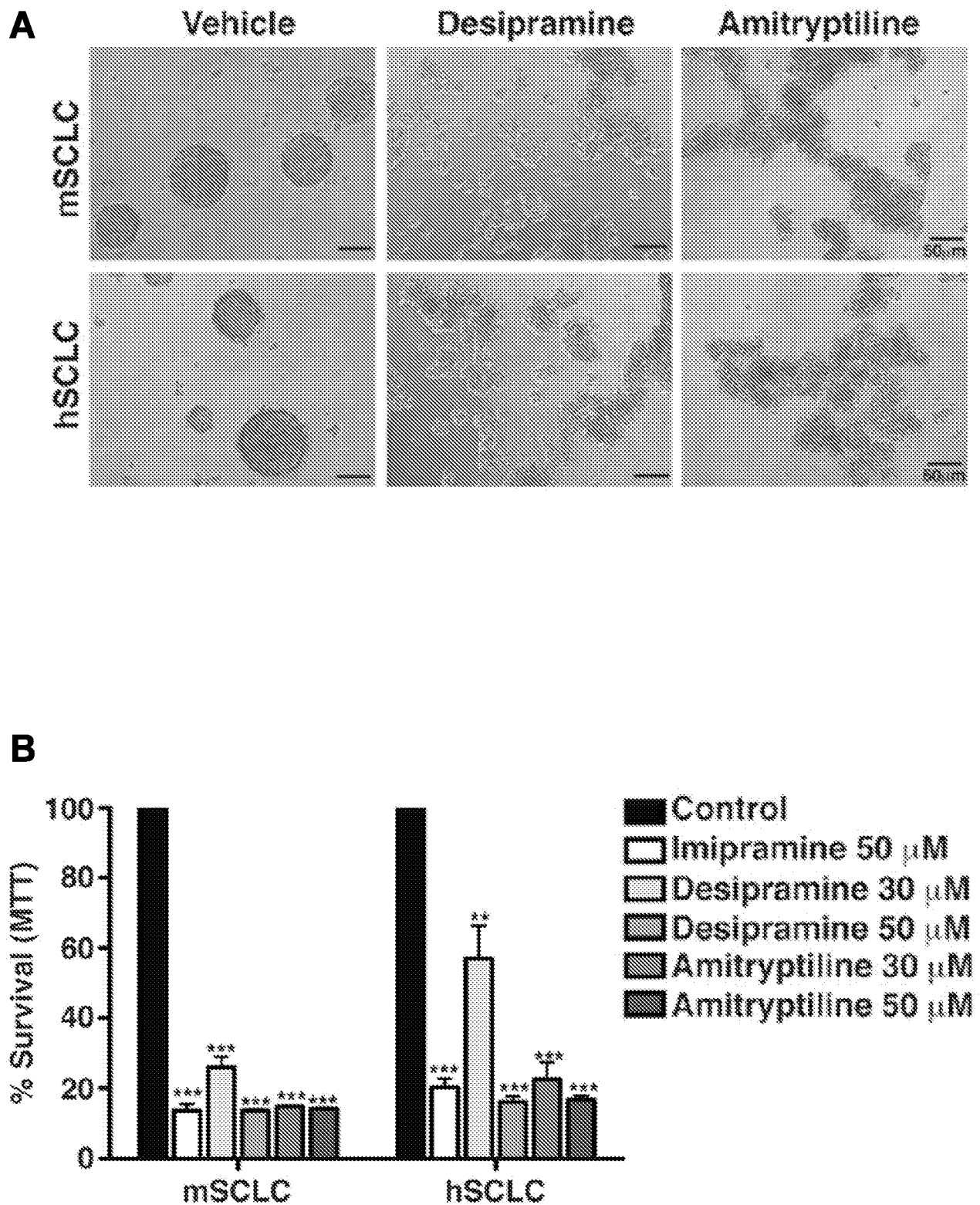


FIG. 9

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**FIG. 10**

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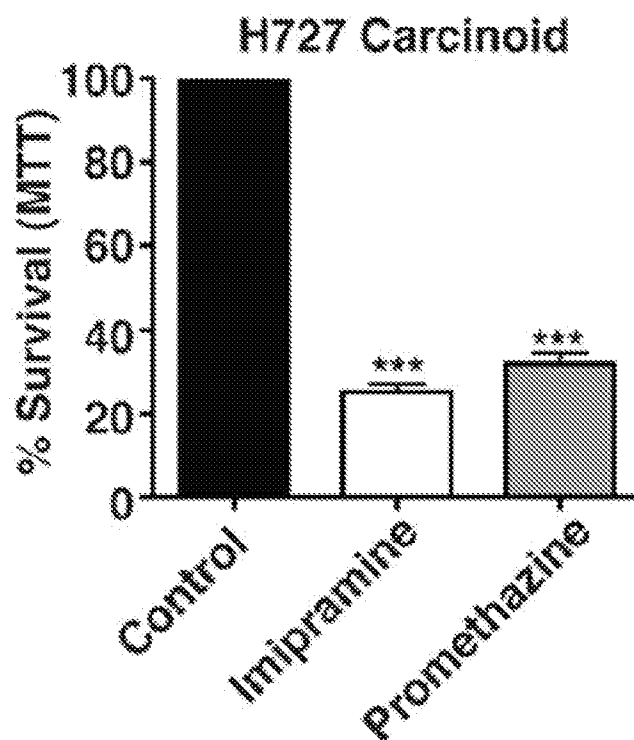
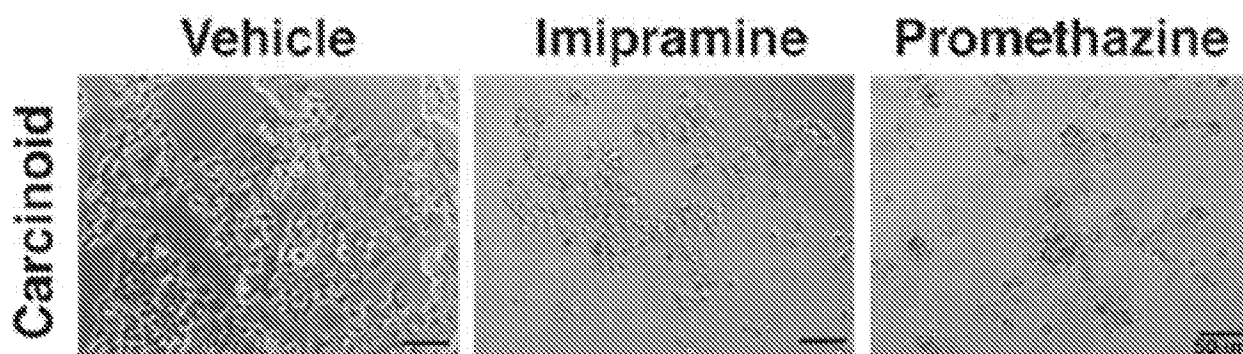
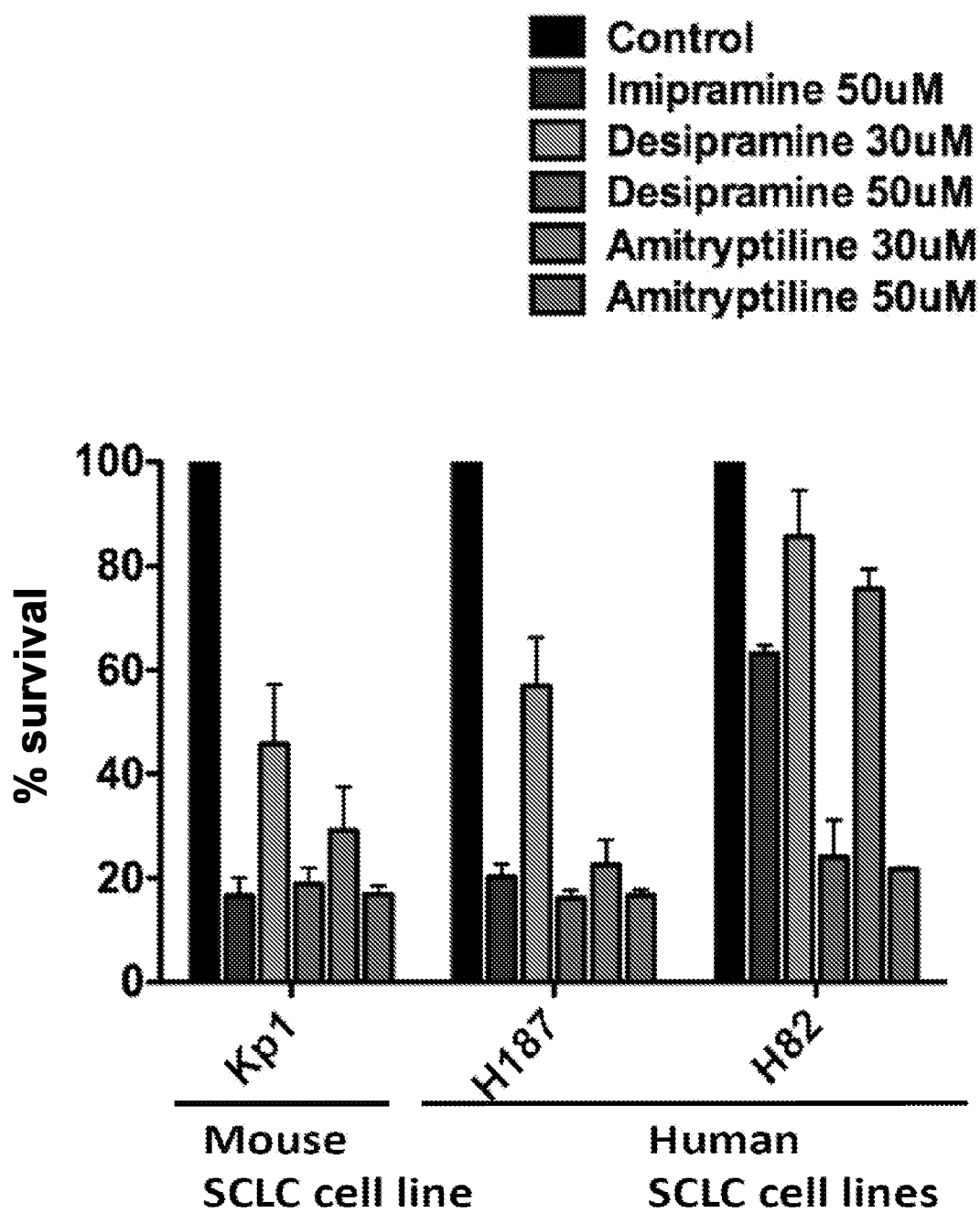
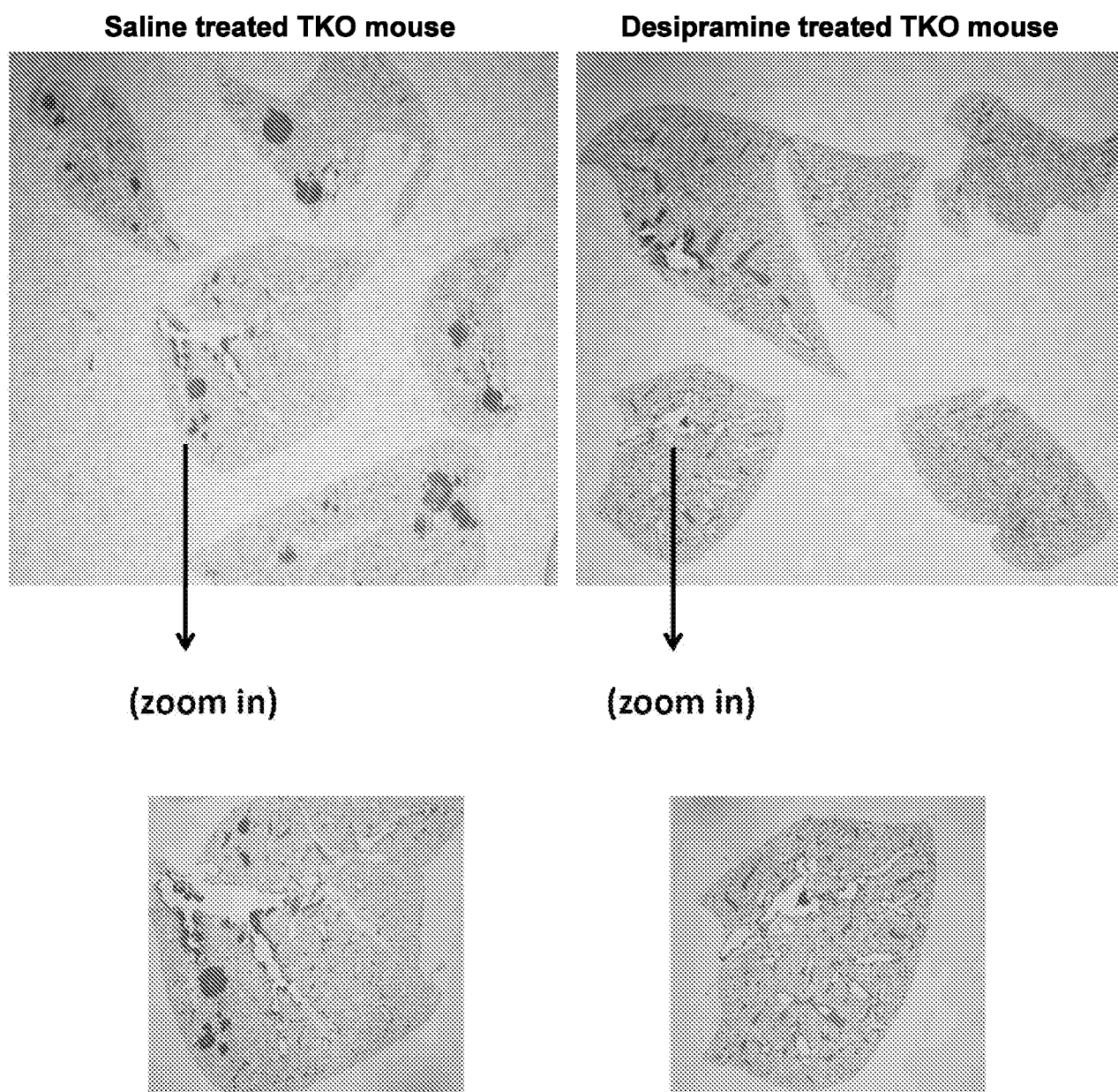
C

FIG. 10
(Continued)

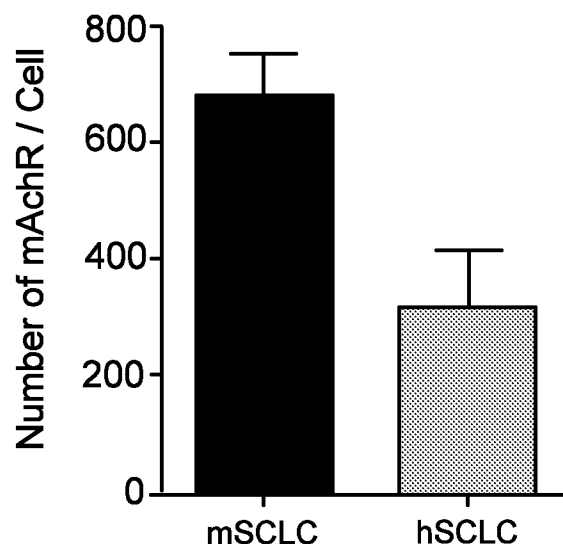
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**FIG. 11**

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**FIG. 12**

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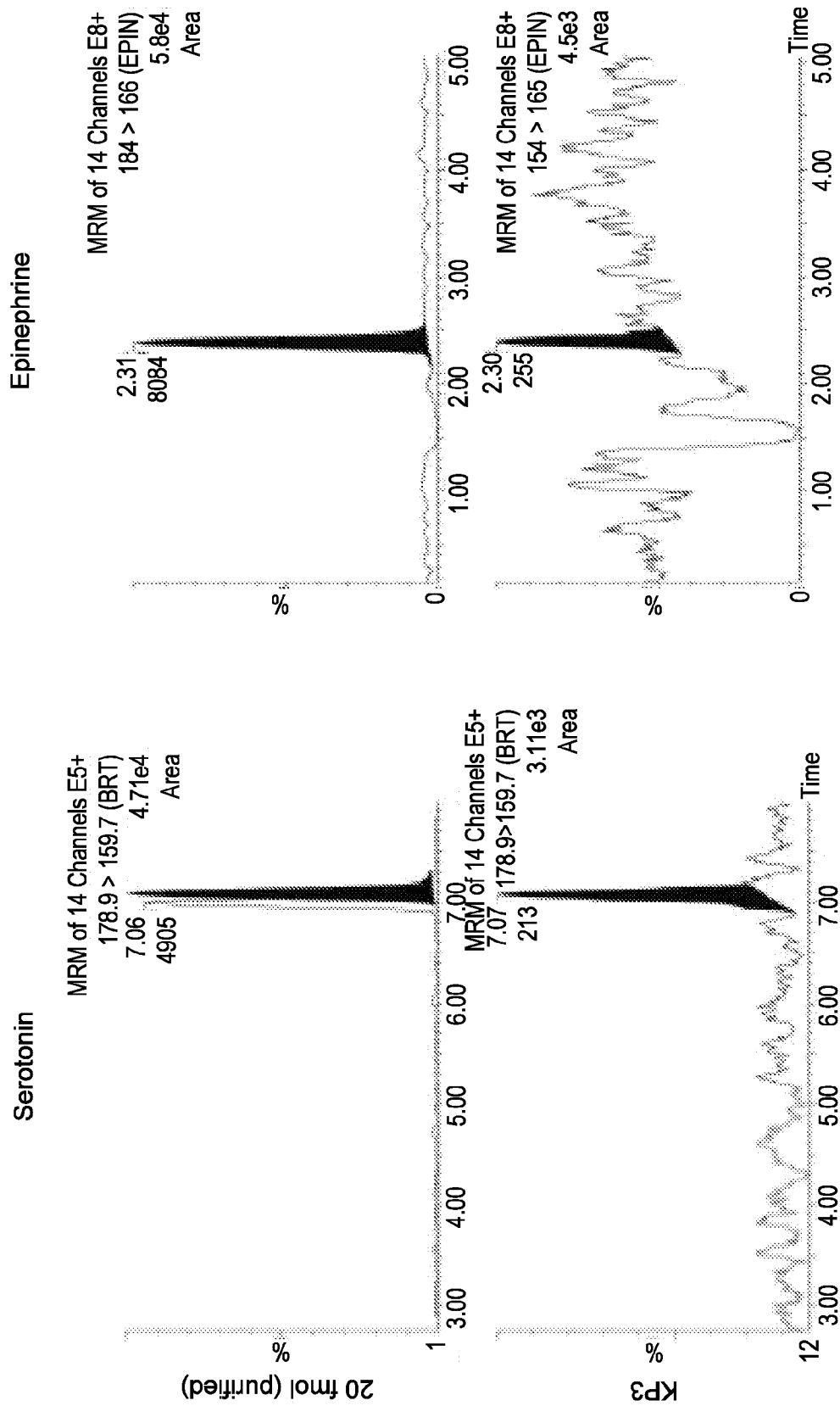
A**C**

	Norepinephrine	Epinephrine	Histamine	Serotonin
Experiment #1 H82	nd	+	nd	+
Experiment #1 KP1	nd	nd	nd	+
Experiment #1 KP3	nd	+	nd	+
Experiment #1 KP1	nd	+	nd	+
Experiment #1 KP3	nd	+	nd	+

Expression of GPCRs and their ligands in SCLC cells. A. Quantification of the number of muscarinic receptors (mAChR) per mSCLC (Kp3) and hSCLC (H69) cell using an in vitro binding assay. C. Summary of the results of two mass spectrometry independent experiments, indicating whether the ligands of interest were detected or not in the supernatant of SCLC cells after 4 hours of incubation in PBS. Note that the absence of detection does not indicate that the ligands are not produced by the tumor cells but rather that the method used may not have been sensitive enough.

FIG. 13

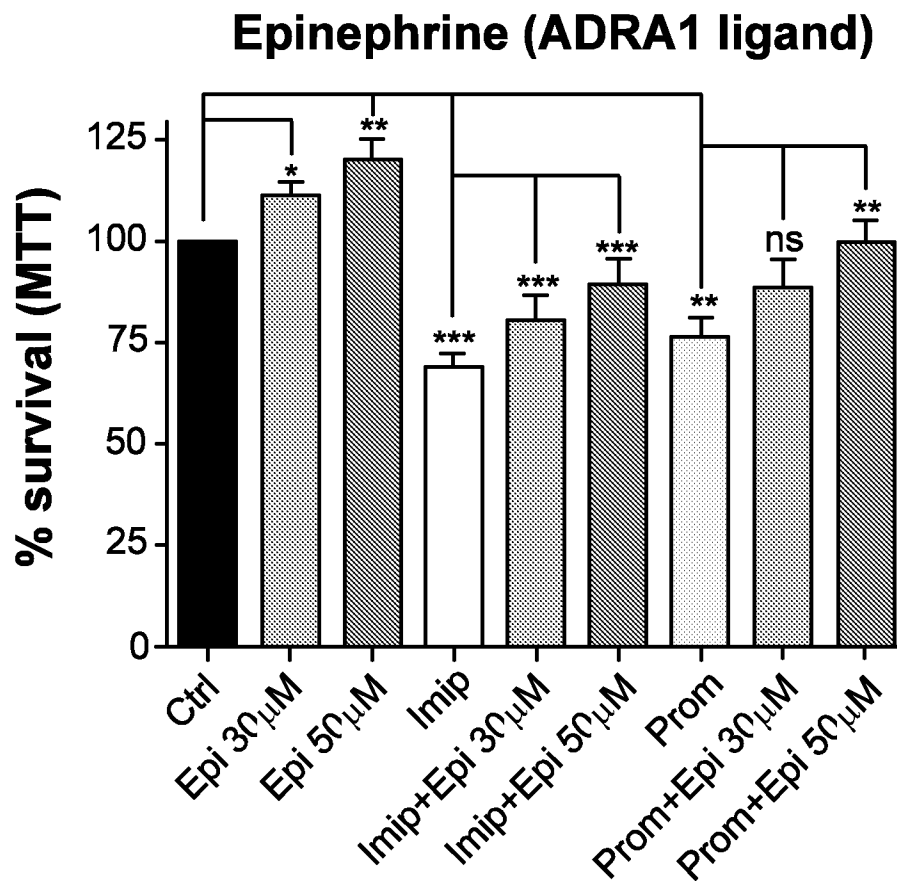
33 / 34



Expression of GPCRs and their ligands in SCLC cells. B. Examples of the LC-MS/MS analysis for KP3 cells.
20 fmol standards are shown and the peaks for epinephrine and serotonin are marked.

FIG. 13
(Continued)

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**FIG. 14**