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(54) Title: METHODS OF TREATING CANCER USING LSD1 INHIBITORS AND/OR TGF-BETA INHIBITORS IN COMBINATION WITH IMMUNOTHERAPY

(57) Abstract: Provided herein are methods of treating cancer using LSD1 inhibitors and/or TGF β inhibitors in combination with immunotherapy.

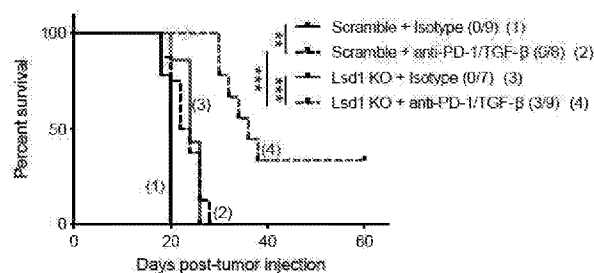


FIG. 21C

METHODS OF TREATING CANCER USING LSD1 INHIBITORS AND/OR TGF-beta INHIBITORS IN COMBINATION WITH IMMUNOTHERAPY

CLAIM OF PRIORITY

5 This application claims the benefit of U.S. Provisional Application No. 62/789,129, filed on January 7, 2019. The entire contents of the foregoing are incorporated herein by reference.

TECHNICAL FIELD

The present invention relates to the treatment of cancer.

10 BACKGROUND

Chromatin modifications play a broad and general role in regulating gene expression, and when they go awry, can lead to diseases. Consistent with this notion, recent cancer genome sequencing efforts have identified mutations in chromatin regulators, and in the case of hematopoietic cancers, chromatin regulators are one of
15 the most frequently mutated groups of genes.

SUMMARY

Without wishing to be bound by theory, the present results provide evidence that the histone H3K4 demethylase, lysine-specific demethylase 1A (LSD1, also known as KDM1A) plays a critical role in suppressing endogenous double stranded
20 RNA (dsRNA) levels and interferon responses in tumor cells, by regulating transcription of endogenous retroviral elements (ERVs) and dsRNA turnover mediated by the RNA-inducing silencing complex (RISC). dsRNA stress can lead to increased T cell infiltration and an enhanced anti-tumor T cell immunity to transplanted tumors cells lacking LSD1, as these tumors showed significant growth disadvantage only in
25 the immunocompetent mice. Furthermore, depletion of LSD1 rendered programmed cell death 1 (PD-1) blockade-refractory B16 tumors significantly responsive to anti-PD-1 therapy. Collectively, LSD1 was identified as a critical regulator of anti-tumor immunity, thereby suggesting that manipulating LSD1 can lead to a significant relief of tumor burden *in vivo*, especially in combination with anti-PD-1 therapy. These
30 findings have important implications for harnessing chromatin and epigenetic

regulators for onco-immunotherapy. In some embodiments, the immunotherapy is an antibody therapy (e.g., a monoclonal antibody, a conjugated antibody). This disclosure is based, *inter alia*, on the surprise results of administering a lysine-specific demethylase 1A (LSD1) inhibitor and/or a transforming growth factor beta (TGF β) inhibitor to a patient in need thereof. In some embodiments, a lysine-specific demethylase 1A (LSD1) inhibitor and/or a transforming growth factor beta (TGF β) inhibitor is administered with one or more of a programmed-cell death 1 (PD-1) inhibitor, a programmed-cell death ligand 1 (PD-L1) inhibitor, an immunotherapy, or combinations thereof. In some embodiments, disclosed herein is a method of inducing or increasing an immunological response to a cancer or tumor, the method comprising: administering to a patient in need of treatment a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor and/or a transforming growth factor beta (TGF β) inhibitor, and at least one of a programmed-cell death 1 (PD-1) inhibitor, a programmed-cell death ligand 1 (PD-L1) inhibitor, or an immunotherapy, to thereby induce or increase the immunological response to the cancer or tumor in the patient. In some embodiments, disclosed herein is a method of reducing or preventing recurrence of a cancer or tumor, the method comprising: administering to a patient in need of treatment a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor and/or a transforming growth factor beta (TGF β) inhibitor, and at least one of a programmed-cell death 1 (PD-1) inhibitor, a programmed-cell death ligand 1 (PD-L1) inhibitor, or an immunotherapy, to thereby reduce or prevent recurrence of the cancer or tumor in the patient.

In some embodiments, provided herein are methods of treating cancer in a patient that include: administering to a patient in need of cancer treatment therapeutically effective amounts of a lysine-specific demethylase 1A (LSD1) inhibitor, and/or a transforming growth factor beta (TGF β) inhibitor and at least one of a programmed-cell death 1 (PD-1) inhibitor and a programmed-cell death ligand 1 (PD-L1) inhibitor, to thereby treat cancer in the patient.

Also provided herein are methods of treating cancer in a patient that include: administering to a patient in need of cancer treatment therapeutically effective amounts of a lysine-specific demethylase 1A (LSD1) inhibitor, a transforming growth factor beta (TGF β) inhibitor and at least one immunotherapy, to thereby treat cancer in the patient.

In some embodiments of any of the methods described herein, the method further includes identifying the patient as having cancer prior to administering.

In some embodiments, the method includes administering a LSD1 inhibitor and a PD-1 inhibitor.

In some embodiments, the method includes administering a LSD1 inhibitor, a PD-1 inhibitor, and a PD-L1 inhibitor.

In some embodiments, the method includes administering a LSD1 inhibitor and a PD-L1 inhibitor.

- 5 In some embodiments, disclosed herein is a composition comprising a LSD1 inhibitor and/or a TGF- β inhibitor. In some embodiments, the composition is administered to a patient in need thereof. In some embodiments, the composition further comprises a PD-1 inhibitor and/or a PD-L1 inhibitor.

In some embodiments, the at least one immunotherapy is selected from the group consisting of: an antibody, an adoptive cellular therapy, an antibody-drug conjugate, a toxin, a cytokine therapy, a cancer vaccine, a checkpoint inhibitor. In some embodiments, the method includes the checkpoint inhibitor is a CTLA-4 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a PD-L2 inhibitor, an OX40 (TNFRSF4) inhibitor, a TIM3 (T Cell Immunoglobulin Mucin 3) inhibitor, or a LAG3 (Lymphocyte Activating 3) inhibitor. In some embodiments, the PD-1 inhibitors blocks the interaction of PD-1 with its ligands (e.g., PD-L1 or PD-L1).

In some embodiments of any of the methods described herein, the LSD1 inhibitor is selected from the group consisting of: a small molecule, an antibody, and an inhibitory nucleic acid. In some embodiments wherein the LSD1 inhibitor is an inhibitory nucleic acid, the inhibitory nucleic acid is a small interfering RNA or a short hairpin RNA. In some embodiments wherein the inhibitory nucleic acid is a short hairpin RNA, the short hairpin RNA includes SEQ ID NO: 2.

In some embodiments of any of the methods described herein, the LSD1 inhibitor is a small molecule selected from the group consisting of: tranylcypromine, RN 1 dihydrochloride, GSK-LSD1, GSK2879552, ORY1001, GSK690, namoline, Cpd 2d, S2101, OG-L002, SP2509, CBB2007 and IMG-7289.

In some embodiments of any of the methods described herein, the PD-1 inhibitor is selected from the group consisting of: a small molecule, an antibody, and an inhibitory nucleic acid.

In some embodiments wherein the PD-1 inhibitor is an inhibitory nucleic acid, the inhibitory nucleic acid is a small interfering RNA or a short hairpin RNA. In some embodiments wherein the inhibitory nucleic acid is a short hairpin RNA, the short hairpin RNA includes e.g., SEQ ID NO: 4.

5 In some embodiments wherein the PD-1 inhibitor is an antibody, the antibody is nivolumab or pembrolizumab.

In some embodiments of any of the methods described herein, the PD-L1 inhibitor is selected from the group consisting of: a small molecule, an antibody, and an inhibitory nucleic acid.

10 In some embodiments wherein the PD-L1 inhibitor is an inhibitory nucleic acid, the inhibitory nucleic acid is a small interfering RNA or a short hairpin RNA. In some embodiments wherein the inhibitory nucleic acid is a short hairpin RNA, the short hairpin RNA includes e.g., SEQ ID NO: 6.

In some embodiments of any of the methods described herein, the PD-L1 inhibitor is an antibody selected from the group consisting of: durvalumab, atezolizumab and avelumab.

In some embodiments of any of the methods described herein, the TGF β inhibitor is selected from the group consisting of: a small molecule, an antibody, an inhibitory nucleic acid, and a vaccine.

20 In some embodiments, the TGF β inhibitor is a small molecule (e.g., galunisertib).

In some embodiments, the TGF β inhibitor is a vaccine (e.g., gemogenovatucl-T).

25 In some embodiments wherein the TGF β inhibitor is an inhibitory nucleic acid, the inhibitory nucleic acid is a small interfering RNA, a short hairpin RNA or an antisense oligonucleotide.

In some embodiments wherein the TGF β inhibitor is an inhibitory nucleic acid, the inhibitory nucleic acid is a short hairpin RNA. In some embodiments, the inhibitory nucleic acid is a siRNA. In some embodiments, the siRNA comprises SEQ ID NO: 159.

30 In some embodiments wherein the TGF β inhibitor is an inhibitory nucleic acid, the inhibitory nucleic acid is an antisense oligonucleotide (e.g., belagenpumatucel-L, trabedersen)

In some embodiments of any of the methods described herein, the TGF β inhibitor is anti-TGF β antibody (e.g., fresolimumab).

In some embodiments of any of the methods described herein, the cancer is a primary tumor.

5 In some embodiments of any of the methods described herein, the cancer is a metastatic tumor.

In some embodiments of any of the methods described herein, the cancer is selected from the group consisting of: melanoma, acute myeloid leukemia (AML), squamous cell carcinoma, renal cell carcinoma, non-small cell lung cancer (NSCLC),
10 small cell lung cancer (SCLC), gastric cancer, bladder cancer, kidney cancer, head and neck cancer, Ewing sarcoma, Hodgkin's lymphoma, Merkel cell carcinoma, breast cancer and prostate cancer.

In some embodiments of any of the methods described herein, the cancer is a non-T-cell-infiltrating cancer.

15 In some embodiments of any of the methods described herein, the cancer is a PD-1 and/or PD-L1 refractory cancer.

In some embodiments of any of the methods described herein, the cancer is a PD-1 and/or PD-L1 resistant cancer.

20 In some embodiments of any of the methods described herein, the patient has previously received cancer treatment.

In some embodiments of any of the methods described herein, administering occurs at least once a week.

In some embodiments of any of the methods described herein, administering is via intravenous, subcutaneous, intraperitoneal, rectal, and/or oral administration.

25 In some embodiments of any of the methods described herein, the LSD1 inhibitor and the at least one PD-1 inhibitor or PD-L1 inhibitor are administered simultaneously to the patient.

In some embodiments of any of the methods described herein, the LSD1 inhibitor is administered to the patient prior to administration of the PD-1 inhibitor or
30 PD-L1 inhibitor.

In some embodiments of any of the methods described herein, the administration of the LSD1 inhibitor is stopped before the administration of the PD-1 inhibitor or the PD-L1 inhibitor.

In some embodiments of any of the methods described herein, the LSD1 inhibitor and the TGF β inhibitor are administered simultaneously to the patient.

In some embodiments of any of the methods described herein, the LSD1 inhibitor is administered to the patient prior to the administration of the TGF β inhibitor.

In some embodiments of any of the methods described herein, the administration of the LSD1 inhibitor is stopped before the administration of the TGF β inhibitor.

In some embodiments of any of the methods described herein, the TGF β inhibitor is administered to the patient prior to the administration of the LSD1 inhibitor or the at least one PD-1 inhibitor or PD-L1 inhibitor.

In some embodiments of any of the methods described herein, the LSD1 inhibitor, the TGF β inhibitor and the at least one PD-1 inhibitor or PD-L1 inhibitor are administered simultaneously to the patient.

In some embodiments of any of the methods described herein, the method further includes administering a chemotherapeutic agent.

In some embodiments of any of the methods described herein, treating includes reducing the volume of primary tumor in the patient.

In some embodiments of any of the methods described herein, treating includes delaying cancer progression in the patient.

In some embodiments of any of the methods described herein, treating includes modifying the tumor microenvironment of a cancer in the patient.

In some embodiments of any of the methods described herein, treating includes sensitizing a cancer to a checkpoint inhibitor therapy.

In some embodiments of any of the methods described herein, treating includes decreasing the risk of developing at least one metastatic tumor in the patient.

In some embodiments of any of the methods described herein, treating includes decreasing the rate of and/or delaying tumor growth at a metastatic site.

In some embodiments of any of the methods described herein, treating includes decreasing tumor cell migration.

In some embodiments of any of the methods described herein, treating includes decreasing tumor cell invasion.

In some embodiments of any of the methods described herein, treating includes decreasing the rate of tumor growth in the patient.

In some embodiments of any of the methods described herein, treating includes eliciting tumor-intrinsic double-stranded RNA stress in a cancer cell in the patient.

The present specification also provides compositions that are useful in the methods described herein, e.g., combined compositions that include a lysine-specific demethylase 1A (LSD1) inhibitor, a transforming growth factor beta (TGF β) inhibitor and at least one immunotherapy, e.g., at least one programmed-cell death 1 (PD-1) inhibitor and/or at least one programmed-cell death ligand 1 (PD-L1) inhibitor.

Also provided herein are methods of treating cancer in a patient that include: administering to a patient in need of cancer treatment therapeutically effective amounts of a lysine-specific demethylase 1A (LSD1) inhibitor, a transforming growth factor beta (TGF β) inhibitor and at least one immunotherapy, to thereby treat cancer in the patient.

In one aspect, the disclosure relates to a method of inducing or increasing an immunological response to a cancer or tumor, the method comprising: administering to a patient in need thereof a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor, and a transforming growth factor beta (TGF β) inhibitor, to thereby induce or increase the immunological response to the cancer or tumor in the patient.

In another aspect, the disclosure also relates to a method of reducing the likelihood of recurrence of a cancer or tumor in a patient, the method comprising: treating cancer in the patient by surgery, chemotherapy, or radiation therapy; and administering to the patient in need thereof a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor, and a transforming growth factor beta (TGF β) inhibitor, to thereby reduce or prevent recurrence of the cancer or tumor in the patient.

In one aspect, the disclosure also provides a method of treating a patient at risk for developing cancer, the method comprising: administering to the patient in need of cancer treatment a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor, and a transforming growth factor beta (TGF β) inhibitor, to thereby treat cancer in the patient.

In some embodiments, the method further comprises administering an immunotherapy to the patient.

The term “treat” or “treatment” is used herein to denote delaying the onset of, inhibiting, alleviating the effects of, or prolonging the life of a patient suffering from, a condition, e.g., cancer. The terms “effective amount” and “amount effective to treat,” as used herein, refer to an amount or concentration of a composition or treatment described herein, e.g., an LSD1 inhibitor, a transforming growth factor beta (TGF β) inhibitor utilized for a period of time (including acute or chronic administration and periodic or continuous administration) that is effective within the context of its administration for causing an intended effect or physiological outcome. For example, effective amounts of a LSD1 inhibitor, a transforming growth factor beta (TGF β) inhibitor and an immunotherapy (e.g., any immunotherapy described herein) for use in the present disclosure include, for example, amounts that inhibit the growth of cancer, e.g., tumors and/or tumor cells, improve delay tumor growth, improve survival for a patient suffering from or at risk for cancer, and improve the outcome of other cancer treatments. As another example, effective amounts of a LSD1 inhibitor, a transforming growth factor beta (TGF β) inhibitor and an immunotherapy (e.g., any immunotherapy described herein) can include amounts that advantageously affect a tumor microenvironment.

The term “patient” or “subject” is used throughout the specification to describe an animal, human or non-human, to whom treatment according to the methods of the present disclosure is provided. Veterinary applications are clearly anticipated by the present disclosure. The term includes but is not limited to birds, reptiles, amphibians, and mammals, e.g., humans, other primates, pigs, rodents such as mice and rats, rabbits, guinea pigs, hamsters, cows, horses, cats, dogs, sheep and goats. Preferred subjects are humans, farm animals, and domestic pets such as cats and dogs.

Compositions and treatments described herein can be used to treat cellular proliferative and/or differentiation disorders. Examples of cellular proliferative and/or differentiation disorders include cancer, e.g., carcinoma, sarcoma, metastatic disorders and hematopoietic neoplastic disorders, e.g., leukemias.

The term “cancer” refers to cells having the capacity for autonomous growth. Examples of such cells include cells having an abnormal state or condition

characterized by rapidly proliferating cell growth. The term is meant to include cancerous growths, e.g., tumors; oncogenic processes, metastatic tissues, and malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Also included are malignancies of the various organ systems, such as respiratory, cardiovascular, renal, reproductive, hematological, neurological, hepatic, gastrointestinal, and endocrine systems; as well as adenocarcinomas, which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine, and cancer of the esophagus. Cancer that is “naturally arising” includes any cancer that is not experimentally induced by implantation of cancer cells into a subject, and includes, for example, spontaneously arising cancer, cancer caused by exposure of a patient to a carcinogen(s), cancer resulting from insertion of a transgenic oncogene or knockout of a tumor suppressor gene, and cancer caused by infections, e.g., viral infections. The term “carcinoma” is art recognized and refers to malignancies of epithelial or endocrine tissues. The term also includes carcinosarcomas, which include malignant tumors composed of carcinomatous and sarcomatous tissues. An “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

The term “sarcoma” is art recognized and refers to malignant tumors of mesenchymal derivation. The term “hematopoietic neoplastic disorders” includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin. A hematopoietic neoplastic disorder can arise from myeloid, lymphoid or erythroid lineages, or precursor cells thereof.

A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast, bone, and liver origin. Metastases develop, e.g., when tumor cells shed from a primary tumor adhere to vascular endothelium, penetrate into surrounding tissues, and grow to form independent tumors at sites separate from a primary tumor.

The term “PD-1 or PD-L1 refractory cancer” refers to a cancer characterized by resistance to PD-1 inhibitor or PD-L1 inhibitor treatment. In some embodiments, the cancer is characterized by a population of cells (e.g., cancer cells or immune cells such as T cells) that have a reduced level of PD-1 or PD-L1 on the surface, or a

reduced expression of PD-1 or PD-L1 (e.g., as compared to non-cancer cells, as compared to cells obtained from subjects without PD-1 or PD-L1 refractory cancer, or as compared to a reference level or value), and/or a genetic lesion in a PD-1 or PD-L1 gene. The terms “a reduced level” or “a decreased level” is a reduction or decrease
5 of PD-1 or PD-L1 of at least a 1% (e.g., at least 2%, at least 4%, at least 6%, at least 8%, at least 10%, at least 12%, at least 14%, at least 16%, at least 18%, at least 20%, at least 22%, at least 24%, at least 26%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%) reduction
10 as compared to a reference level or value.

The term “non-T-cell-infiltrating tumor” means a tumor that lacks T cells within its tumor microenvironment. In some embodiments, a non-T-cell-infiltrating tumor is characterized by a population of cancer cells that have down-regulated genes associated with T cell recognition, a reduced expression of polypeptides associated
15 with T cell recognition on its cell surface (e.g., a T-cell receptor), and/or T cell dysfunction.

The term “population” when used before a noun means two or more of the specific noun. For example, the phrase “a population of cancer cells” means “two or more cancer cells.” Non-limiting examples of cancer cells are described herein.

20 A “chemotherapeutic agent” refers to a chemical compound useful in the treatment of a cancer. Chemotherapeutic agents include, e.g., “anti-hormonal agents” or “endocrine therapeutics” which act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer. Additional classes, subclasses, and examples of chemotherapeutic agents are known in the art.

25 Individuals considered at risk for developing cancer may benefit from the present disclosure, e.g., because prophylactic treatment can begin before there is any evidence and/or diagnosis of the disorder. Individuals “at risk” include, e.g., individuals exposed to carcinogens, e.g., by consumption (e.g., by inhalation and/or ingestion), at levels that have been shown statistically to promote cancer in
30 susceptible individuals. Also included are individuals at risk due to exposure to ultraviolet radiation, or their environment, occupation, and/or heredity, as well as those who show signs of a precancerous condition such as polyps. Similarly, individuals in very early stages of cancer or development of metastases (i.e., only one

or a few aberrant cells are present in the individual's body or at a particular site in an individual's tissue) may benefit from such prophylactic treatment.

5 Skilled practitioners will appreciate that a patient can be diagnosed, e.g., by a medical professional, e.g., a physician or nurse (or veterinarian, as appropriate for the patient being diagnosed), as suffering from or at risk for a condition described herein, e.g., cancer, using any method known in the art, e.g., by assessing a patient's medical history, performing diagnostic tests, and/or by employing imaging techniques.

10 Skilled practitioners will also appreciate that treatment need not be administered to a patient by the same individual who diagnosed the patient (or the same individual who prescribed the treatment for the patient). Treatment can be administered (and/or administration can be supervised), e.g., by the diagnosing and/or prescribing individual, and/or any other individual, including the patient her/himself (e.g., where the patient is capable of self-administration).

15 Also contemplated by the present disclosure is administration of a LSD1 inhibitor, a transforming growth factor beta (TGF β) inhibitor and an immunotherapy (e.g., any immunotherapy described herein) to a patient in conjunction with at least one other treatment, e.g., chemotherapy, radiation therapy, gene therapy, and/or surgery, to treat conditions and disorders described herein (e.g., cancer).

20 Alternatively or in addition, treatments described herein can be administered in combination with chemotherapy. Chemotherapy can involve administration of any of the following classes of compounds: alkylating agents, antimetabolites, e.g., folate antagonists, purine antagonists and/or pyrimidine antagonists; spindle poisons, e.g., vincas (e.g., paclitaxel) and podophillotoxins; antibiotics, e.g., doxorubicin, bleomycin and/or mitomycin; nitrosoureas; inorganic ions, e.g., cisplatin; biologic response modifiers, e.g., tumor necrosis factor – α (TNF- α) and interferon; enzymes, e.g., asparaginase; protein toxins conjugated to targeting moieties; antisense molecules; and hormones, e.g., toremifene, leuprolide, flutamide, and megestrol.

25 Alternatively or in addition, treatments described herein can be administered in combination with radiation therapy, e.g., using γ -radiation, neutron beams, electron beams, and/or radioactive isotopes. Alternatively or in addition, treatments described herein can be administered to patients in combination with immunotherapies other than administering a PD-1 inhibitor, a PD-L1 inhibitor or a CTLA-4 inhibitor, e.g., administering specific effector cells, tumor antigens, and/or antitumor antibodies.

Alternatively or in addition, treatments described herein can be administered to patients in combination with gene therapy, e.g., the administration of DNA encoding tumor antigens and/or cytokines. Methods for treating cancer, e.g., surgery, chemotherapy, immunotherapy, and radiotherapy, are more fully described in *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 11, Chapters 143 and 144, the contents of which are expressly incorporated herein by reference in their entirety.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the disclosure will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1A is a bar graph showing quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis of selected endogenous retroviruses (ERVs) (HERV-E, HERV-F, HERV-K, HML-2, and ERVL), IFNs (IFN- α , IFN- β and IL-28) and ISGs (ISG15 and OASL) in human MCF-7 breast cancer cells treated with or without GSK-LSD1 for 6 days. The RT-qPCR data were normalized to GAPDH and then relative to DMSO. RT-qPCR was performed in duplicates and repeated two to three times. Error bars represent the standard error of mean (SEM). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 1B is a picture of immunoblots showing shRNA-mediated knockdown of LSD1 (sh-LSD1) in MCF-7 cells. Actin was used as a control for protein level.

FIG. 1C is a bar graph showing RT-qPCR analysis of LSD1, selected endogenous retroviruses (ERVs) (HERV-E, HERV-F, HERV-K, HML-2, and ERVL), IFNs (IFN- α , IFN- β and IL-28) and ISGs (ISG15 and OASL) in human

MCF-7 breast cancer cells transduced with shRNA against LSD1 (sh-LSD1) and shRNA against scramble (sh-Ctrl). The RT-qPCR data were normalized to GAPDH and then relative to sh-Ctrl. Error bars represent SEM from three experiments.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

5 FIG. 1D is a bar graph showing IFN- β secretion (pg/mL) in LSD1 knockdown (KD) MCF-7 cells detected by ELISA (n=3). Error bars represent standard deviation (SD) between triplicates in one of two experiments. n.d., not detected.

10 FIG. 1E is a picture of immunoblots showing LSD1 KD MCF-7 cells that were transduced with either wild type (WT) LSD1 or catalytically inactive LSD1 that harbors a K661A mutation (LSD1-K661A). Actin was used as a control for protein level.

15 FIG. 1F is a bar graph showing RT-qPCR analysis of selected ERVs (HERV-E, HERV-K, HML-2, and ERVL) in MCF-7 cells that ectopically expressed FH-EGFP, FH-LSD1 (resistant to shRNA) or FH-LSD1-K661A (resistant to shRNA), and were then transduced with shRNA against scramble (sh-Ctrl) or LSD1 (sh-LSD1). RT-qPCR was performed in duplicates and repeated two to three times. Error bars represent SEM from two experiments. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

20 FIG. 1G is a bar graph showing RT-qPCR analysis of IFN- α and IFN- β in MCF-7 cells cells that ectopically expressed FH-EGFP, FH-LSD1 (resistant to shRNA) or FH-LSD1-K661A (resistant to shRNA), and were then transduced with shRNA against scramble (sh-Ctrl) or LSD1 (sh-LSD1). RT-qPCR was performed in duplicates and repeated two to three times. Error bars represent the SEM from three experiments. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

25 FIG. 1H is a picture of immunoblots showing the protein expression of DNMT proteins in MCF-7 cells transduced with control shRNA or shRNA against LSD1.

FIG. 1I is bar graph showing 5-methylcytosine content in genomic DNA of control and LSD1 KD MCF-7 cells as determined by HPLC-MS analysis.

30 FIG. 1J is a picture of immunoblots showing the protein expression of ISG15 in MCF-7 cells with control shRNA, LSD1 KD, or LSD1 KD rescued with LSD1. Actin was used as a control for protein level.

FIG. 1K is a bar graph showing RT-qPCR analysis of LSD1, selected ERVs (HERV-E, HERV-F, HERV-K, HML-2, and ERVL) and IFNs (IFN- β and IL-28) in human T47D breast cancer cells transduced with shRNA against scramble or LSD1. The RT-qPCR data were normalized to GAPDH and then relative to sh-Ctrl. Error bars represent the standard deviation between triplicates. **p<0.01, ***p<0.001, ns, not significant, as determined by unpaired t-test.

FIG. 1L is a bar graph showing RT-qPCR analysis of LSD1, selected ERVs (HERV-E, HERV-K, HML-2, and ERVL), IFNs (IFN- α , IFN- β and IL-28) and ISGs (OASL and ISG15) in human embryonic 293T kidney cells transduced with shRNA against scramble or LSD1. The RT-qPCR data were normalized to GAPDH and then relative to sh-Ctrl. Error bars represent the standard deviation between duplicates. *p<0.05, **p<0.01, ***p<0.001, ns, not significant, as determined by unpaired t-test.

FIG. 1M is volcano and M-A plots showing differentially expressed genes in LSD1 KD versus control MCF-7 cells as determined by RNA-seq. Dots in grey represent significantly increased or decreased genes (FDR<0.05).

FIG. 1N is a representative dotmap showing the top 10 terms of a gene ontology (GO) analysis of upregulated genes ($\log_2(\text{FC}) > 1$ and FDR < 0.05) in LSD1 KD versus control MCF-7 cells. Dot size represents odds ratio.

FIG. 1O is representative dotmap showing the top 10 terms of a gene ontology (GO) analysis of downregulated genes ($\log_2(\text{FC}) < -1$ and FDR < 0.05) in LSD1 KD versus control MCF-7 cells. Dot size represents odds ratio.

FIG. 2A is a Gene Set Enrichment Analysis (GSEA) analysis for response to type 1 interferon (IFN) and antiviral response pathway in LSD1 KD versus WT control MCF-7 cells.

FIG. 2B is a plot showing LSD1 and H3K4me2 ChIP-seq signals at promoter regions of 125 induced interferon/antiviral responsive genes (IFN-gene, $\log_2(\text{FC}) > 0$ and FDR < 0.05) or 537 selected genes with LSD1 peaks as positive control (Pos-gene) in control (sh-Ctrl) and LSD1 KD (sh-LSD1) cells.

FIG. 2C is an IGV image of *TLR3* loci showing LSD1 and H3K4me2 levels in LSD1 KD and control MCF-7 cells.

FIG. 2D is an IGV image of *SED1A* loci showing LSD1 and H3K4me2 levels in LSD1 KD and control MCF-7 cells.

FIG. 2E is a heatmap for differential transcript expression of repetitive elements between LSD1 KD and WT control.

FIG. 2F is heatmaps showing differential expression of sense or antisense transcripts of ERVs between LSD1 KD and WT control.

5 FIG. 2G is plots showing LSD1 and H3K4me2 ChIP-seq signals at genomic loci of 8593 individual ERVs from 279 ERV subfamilies in control and LSD1 KD cells.

FIG. 2H is plots of LSD1 and H3K4me2 ChIP-seq signals at genomic loci of *HERV-E* subfamily in control and LSD1 KD cells.

10 FIG. 2I is a bar graph showing fold changes of reverse complementary sense-/antisense transcripts (overlapping) and extra sense or antisense transcripts (extra) of a number of retrotransposons between LSD1 KD and control cells determined by directional RNA-seq.

FIG. 2J is a picture of a PCR gel showing PCR amplification of selected ERVs using strand specific primers in MCF-7 cells with sh-Ctrl or sh-LSD1. An asterisk indicates non-specific bands.

FIG. 2K is a bar graph showing RT-qPCR analysis of EGFP, engineered HERV-(K+E) in MCF-7 cells transduced with pHAGE-EGFP or pHAGE-HERV-(K+E). The RT-qPCR data were normalized to GAPDH and then relative to untransduced cells. Error bars represent SEM from two experiments.

FIG. 2L is a bar graph showing RT-qPCR analysis of IFN- α , IFN- β , IL-28, ISG15 and OASL in MCF-7 cells transduced with pHAGE-EGFP or pHAGE-HERV-(K+E). The RT-qPCR data were normalized to GAPDH and then relative to untransduced cells. Error bars represent SEM from two experiments. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

FIG. 2M is a bar graph showing double-stranded RNA (dsRNA) enrichment of selected retrotransposons (HERV-E, HERV-F, HERV-K, ERVL, Syn-1, Line1 and AluYA5) in control (sh-Ctrl) and LSD1 KD (sh-LSD1) MCF-7 cells by RT-qPCR. Total RNA extract from control or LSD1 KD MCF-7 cells was digested with RNase A versus mock under high salt condition (350 mM NaCl), followed by a second round of RNA extraction with TRIzol. The ratios of (retrotransposon/GAPDH)_{RNase}/(retrotransposon/GAPDH)_{mock} were calculated as enrichment fold. GAPDH was used as an internal control. Error bars represent SEM

from three experiments. $p < 0.05$, $**p < 0.01$, ns, not significant, as determined by unpaired t-test.

FIG. 2N is a bar graph showing RT-qPCR analysis of selected retrotransposon transcripts (HERV-E, HERV-F, HERV-K, ERVL, Syn-1, Line1, AluYA5) and GAPDH captured by a dsRNA-specific antibody (J2) pulldown assay in MCF-7 cells with sh-Ctrl or sh-LSD1. Error bars represent SD between duplicates. $*p < 0.05$, $**p < 0.01$, ns, not significant, as determined by unpaired t-test. n.d., not detected.

FIG. 2O is a heatmap showing the expression nucleic acid receptors in control and LSD1 KD MCF-7 cells as determined by RNA-seq.

FIG. 2P is a representative immunoblot of TLR3, MDA5 and RIG-I in control and LSD1 KD cells. Actin was used as a control for protein level.

FIG. 3A is a picture of immunoblots showing TLR3, MDA5 and RIG-I expression in control (sh-Ctrl), LSD1 KD (sh-LSD1), LSD1/TLR3 DKO (sh-LSD1 + sh-TLR3), LSD1/MDA5 DKO (sh-LSD1 + sh-MDA5), or LSD1/RIG-I DKO (sh-LSD1 + sh-RIG-I) MCF-7 cells.

FIG. 3B is a bar graph showing RT-qPCR analysis of TLR3, selected ERVs (HERV-E, HERV-K and HML-2), IFNs (IFN- β and IL-28) and ISGs (ISG15 and OASL) in MCF-7 cells transduced with shRNA against scramble, LSD1 or LSD1 and TLR3. RT-qPCR was performed in duplicates and repeated two to three times. Error bars represent standard deviation (SD). $p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns, not significant as determined by unpaired t-test.

FIG. 3C is a bar graph showing RT-qPCR analysis of MDA5, selected ERVs (HERV-E, HERV-K and HML-2), IFNs (IFN- β and IL-28) and ISGs (ISG15 and OASL) in MCF-7 cells transduced with shRNA against scramble, LSD1 or LSD1 and MDA5. RT-qPCR was performed in duplicates and repeated two to three times. Error bars represent standard deviation (SD). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns, not significant as determined by unpaired t-test.

FIG. 3D is a bar graph showing RT-qPCR analysis of RIG-I, selected ERVs (HERV-E, HERV-K and HML-2), IFNs (IFN- β and IL-28) and ISGs (ISG15 and OASL) in MCF-7 cells transduced with shRNA against scramble, LSD1 or LSD1 and RIG-I. RT-qPCR was performed in duplicates and repeated two to three times. Error bars represent standard deviation (SD). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns, not significant as determined by unpaired t-test.

FIG. 3E is a bar graph showing RT-qPCR analysis of MAVS, selected ERVs (HERV-E, HERV-K and HML-2), IFNs (IFN- β and IL-28) and ISGs (ISG15 and OASL) in MCF-7 cells transduced with shRNA against scramble, LSD1 or LSD1 and MAVS. Error bars represent standard deviation between duplicates. * $p < 0.05$,
 5 ** $p < 0.01$, *** $p < 0.001$, ns, not significant as determined by unpaired t-test.

FIG. 3F is a picture of immunoblots showing cGAS and STING proteins in MCF-7 transduced with shRNA against scramble (sh-Ctrl), LSD1 (sh-LSD1), LSD1 and cGAS (sh-LSD1 + sh-cGAS) or LSD1 and STING (sh-LSD1 + sh-STING).

FIG. 3G is a bar graph showing RT-qPCR analysis of HERV-E, HERV-K,
 10 HML-2, IFN- β , IL-28, ISG15 and OASL in MCF-7 cells transduced with shRNA against scramble (sh-Ctrl), LSD1 (sh-LSD1), LSD1 and cGAS (sh-LSD1 + sh-cGAS) or LSD1 and STING (sh-LSD1 + sh-STING). The RT-qPCR data were normalized to GAPDH and then relative to sh-Ctrl. Error bars represent SEM from three experiments. ns, not significant, as determined by unpaired t-test.

FIG. 3H is a bar graph showing RT-qPCR analysis of IFN- β , IL-28, OASL and ISG15 in control, cGAS KD (sh-cGAS) and STING KD (sh-STING) MCF-7 transfected with fragmented genomic DNA from mammalian cells or mock transfection. The RT-qPCR data were normalized to GAPDH and then relative to sh-Ctrl + mock. Error bars represent SD between duplicates in one of two experiments.
 20 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 4A is a picture of immunoblots showing protein expression of core components (DICER, AGO2 and TRBP2) of the RISC complex in MCF-7 cells transduced with shRNA against scramble (sh-Ctrl), LSD1 (sh-LSD1), and rescued with WT LSD1 or catalytically inactive LSD1-K661A. Actin was used as a control
 25 for protein level.

FIG. 4B is a picture of immunoblots showing protein expression of LSD1 and Drosha in MCF-7 cells transduced with control shRNA (sh-Ctrl) or LSD1 shRNA (sh-LSD1). Actin was used as a control for protein level.

FIG. 4C is a picture of immunoblots showing GFP and GFPL protein
 30 expression in U2OS cells expressing dual reporters GFPL/GFP-*let-7* and transduced with shRNA against scramble, LSD1 or AGO2. Actin was used as a control for protein level.

FIG. 4D is a picture of immunoblots showing ISG15 protein expression in MCF-7 + sh-Ctrl cells, MCF-7 + sh-LSD1 cells, MCF-7 + sh-LSD1 + sh-TLR3 cells, MCF-7 + sh-LSD1 + sh-RIG-I cells, and MCF-7 + sh-LSD1 + sh-MDA5 cells. Actin was used as a control for protein level.

5 FIG. 4E is a picture of immunoblots showing MAVS protein expression in MCF-7 + sh-Ctrl cells, MCF-7 + sh-LSD1 cells, MCF-7 + sh-LSD1 + sh1-MAVS cells, and MCF-7 + sh-LSD1 + sh2-MAVS cells. Actin was used as a control for protein level.

10 FIG. 4F is a bar graph showing RT-qPCR analysis of GFP, GFPL, LSD1 and AGO2 in U2OS cells expressing dual reporters GFPL/GFP-let-7 and transduced with shRNA against scramble, LSD1 or AGO2. Error bars represent SD between duplicates.

15 FIG. 4G is a bar graph showing relative let-7 miRISC activity by quantifying GFP and GFPL protein signals in U2OS cells expressing dual reporters GFPL/GFP-let-7 and transduced with shRNA against scramble, LSD1 or AGO2. Ratios of GFPL over GFP protein in different samples from five repeats for sh-LSD1 and two repeats for sh-AGO2 were calculated. The ratio in control shRNA sample was considered as 100% miRISC activity. *** $p < 0.001$.

20 FIG. 4H is a bar graph showing double-stranded RNA (dsRNA) enrichment of selected retrotransposons (HERV-E, HERV-F, HERV-K, ERVL, Syn-1, Line1 and AluYA5) in control (sh-Ctrl) and AGO2 KD (sh-AGO2) MCF-7 cells by RT-qPCR. Total RNA extract from control or LSD1 KD MCF-7 cells was digested with RNase A versus mock under high salt condition (350 mM NaCl), followed by a second round of RNA extraction with TRIzol. The ratios of
25 (retrotransposon/GAPDH)RNase/(retrotransposon/GAPDH)mock were calculated as enrichment fold. GAPDH was used as an internal control. RT-qPCR was performed in duplicates and repeated two to three times. Error bars represent SD between duplicates. $p < 0.05$, ** $p < 0.01$, ns, not significant, as determined by unpaired t-test.

30 FIG. 4I is a bar graph showing RT-qPCR analysis of selected IFNs (IFN- β and IL-28) and ISGs (OASL, ISG15, TLR3, MDA5 and RIG-I) in MCF-7 cells transduced with shRNA against scramble or AGO2. RT-qPCR was performed in duplicates and repeated twice. Error bars represent standard deviation. ** $p < 0.01$, *** $p < 0.001$, as determined by unpaired t-test.

FIG. 4J is a picture of immunoblots showing protein expression of MDA5, RIG-I and ISG15 in the same cells used in FIG. 4I. Actin was used as a control for protein level.

FIG. 4K is bar graph showing RT-qPCR analysis of IFN- β , IL-28, ISG15, OASL, TLR3, MDA5 and RIG-I in MCF-7 cells transduced with shRNA against scramble (sh-Ctrl) or DICER (sh4-DICER). The RT-qPCR data were normalized to GAPDH and then relative to sh-Ctrl. Error bars represent SD between duplicates in one experiment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 4L is a picture of immunoblots showing protein expression of DICER in MCF-7 + sh-Ctrl, MCF-7 + sh1-DICER, MCF-7 + sh4-DICER cells. Actin was used as a control for protein level.

FIG. 4M is bar graph showing RT-qPCR analysis of IFN- β , IL-28, ISG15, OASL, TLR3, MDA5 and RIG-I in MCF-7 cells transduced with shRNA against scramble (sh-Ctrl) or TRBP2 (sh4-TRBP2). The RT-qPCR data were normalized to GAPDH and then relative to sh-Ctrl. Error bars represent SD between duplicates in one experiment. ** $p < 0.01$, *** $p < 0.001$, as determined by unpaired t-test.

FIG. 4N is a picture of immunoblots showing protein expression of TRBP2 in MCF-7 + sh-Ctrl, MCF-7 + sh1-TRBP2, MCF-7 + sh4-TRBP2 cells. Actin was used as a control for protein level.

FIG. 4O is a picture of immunoblots showing protein expression of AGO2 and LSD1 in MCF-7 cells, MCF-7 + sh-LSD1 cells, MCF-7 + FH-AGO2 cells and MCF-7 + FH-AGO2 + sh-LSD1 cells. Actin was used as a control for protein level.

FIG. 4P is a bar graph showing dsRNA enrichment of retrotransposons (HERV-E, HERV-F, HERV-K, ERVL, Line1 and AluYA5) in MCF-7 + sh-control cells, MCF-7 + sh-LSD1 cells, MCF-7 + FH-AGO2 + sh-control cells, and MCF-7 + FH-AGO2 + sh-LSD1 cells. Error bars represent standard error of the mean (SEM) from five experiments. * $p < 0.05$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 4Q is a bar graph showing RNA levels of HERV-E, HERV-K, HML-2, IFN β , IL-28, ISG15 and OASL in MCF-7 + sh-control cells, MCF-7 + sh-LSD1 cells, MCF-7 + FH-AGO2 + sh-control cells, and MCF-7 + FH-AGO2 + sh-LSD1 cells.

Error bars represent SD between triplicates. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

FIG. 5A is a picture of immunoblots showing the protein expression of core components of the RISC complex (DICER, AGO2 and TRBP2) and LSD1 in MCF-7 + sh-Ctrl cells, MCF-7 + sh-LSD1 cells, MCF-7 + sh-LSD1 + sh-TLR3 cells, MCF-7 + sh-LSD1 + sh-MDA5 cells, MCF-7 + sh-LSD1 + sh-RIG-I cells, and MCF-7 + sh-LSD1 + sh-MAVS cells. Actin was used as a control for protein level.

FIG. 5B is a bar graph showing RT-qPCR analysis of AGO1, AGO2, AGO3, AGO4, DICER and TRBP2 in control and LSD1 KD MCF-7 cells. Data was normalized to GAPDH and relative to sh-Ctrl. Error bars represent SEM from two experiments. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

FIG. 5C is a picture of immunoblots showing the protein expression of AGO2 in MCF-7 cells treated with 50 $\mu\text{g/ml}$ cycloheximide (CHX) in the presence of absence of 2 μM GSK-LSD1 at 0, 3, 6, 9, 12, hours. Actin was used as a control for protein level.

FIG. 5D is a graph showing the quantification of AGO2 signal from five experiments of MCF-7 cells treated with 50 $\mu\text{g/ml}$ cycloheximide (CHX) in the presence of absence of 2 μM GSK-LSD1 at 0, 3, 6, 9, 12, hours. Error bars represent SEM from five experiments. ** $p < 0.01$, as determined by unpaired t-test.

FIG. 5E is a picture of immunoblots showing the physical interaction between LSD1 and AGO2 by co-immunoprecipitation assay using whole cell lysate (WCL) of MCF-7 cells stably expressing FH-AGO2.

FIG. 5F is a picture of immunoblots showing the physical interaction between LSD1 and TRBP2 by co-immunoprecipitation assay using whole cell lysate (WCL) of MCF-7 cells stably expressing FH-TRBP2.

FIG. 5G is a picture of immunoblots showing protein expression of DICER, AGO2, TRBP2, LSD1 and Tubulin in whole cell lysate (WCL), cytoplasm (CytoE) and nuclear lysate (NE).

FIG. 5H is a picture of immunoblots showing the physical interaction between LSD1 and AGO2 by co-immunoprecipitation assay using whole cell lysate (WCL) or nuclear lysate (NE) of MCF-7 cells stably expressing FH-LSD1.

FIG. 5I is a picture of immunoblots showing purified FH-AGO2 from MCF-7 cells treated by LSD1 KD or GSK-LSD1 as determined by mass spectrometry for the

identification of lysine methylation. DMSO, sh-Ctrl (VGKSGNIPAGTTVDTK; SEQ ID NO: 156) and sh-LSD1, GSK-LSD1 (VGK(me)SGNIPAGTTVDTK; SEQ ID NO: 157).

FIG. 5J is a dot plot detecting the reactivity of K726me1-specific antibody
5 against un-, mono- or di-methylated AGO2 peptides.

FIG. 5K is a picture of immunoblots showing ectopically expressed wild type FH-AGO2, FH-AGO2-K726R and FH-AGO2-K726A in MCF-7 cells treated with LSD1 KD co-immunoprecipitated by α -Flag and immunoblotted with mono-methyl AGO2 specific antibody.

FIG. 5L is a picture of immunoblots showing ectopically expressed wild type FH-AGO2, FH-AGO2-K726R and FH-AGO2-K726A in MCF-7 cells treated with GSK-LSD1 co-immunoprecipitated by α -HA and immunoblotted with mono-methyl AGO2 specific antibody.

FIG. 5M is a picture of immunoblots showing K726me1 on endogenous
15 AGO2 in control or LSD1 KD MCF-7 cells.

FIG. 5N is a picture of immunoblots showing AGO2 mono-methylation status at K726 in *in vitro* demethylation assay with immunoprecipitated proteins from MCF-7 cells..

FIG. 5O is a bar graph showing signal intensities of AGO2 mono-methylation
20 status at K726 in *in vitro* demethylation assay with immunoprecipitated proteins from MCF-7 cells. Error bars represent SEM from three experiments. * $p < 0.05$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 5P is a graph showing protein stability of transiently expressed wild type FH-AGO2 and FH-AGO2-K726R in 293T cells measured using CHX chase assay in
25 the presence or absence of 2 μ M GSK-LSD1. The averaged AGO2 quantification from two experiments was shown.

FIG. 6A is bar graph showing RT-qPCR analysis of selected retrotransposons (MuSD, MuERV-L, Line1 and IAP), IFNs (IFN- α , IFN- β and IL-28) and ISG15, OASL, TLR3, MDA5 and RIG-I in murine B16 melanoma cells transfected with
30 gRNA against scramble (scramble) or LSD1 (LSD1 KO, clone g5-4). Data was normalized to GAPDH and then relative to scramble. Error bars represent SEM from three experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns, not significant, as determined by unpaired t-test.

FIG. 6B is bar graph showing RT-qPCR analysis of MuERV-L, Line 1, IFN- β and ISG15 in murine Lewis lung carcinoma (LLC) cells transduced with gRNA against scramble (scramble) or LSD1 (LSD1 KO1 and LSD1 KO2). The RT-qPCR data were normalized to GAPDH and then relative to scramble. Error bars represent SEM from three experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, as determined by unpaired t-test.

FIG. 6C is bar graph showing RT-qPCR analysis of MuERV-L, Line 1, IFN- α , IFN- β , IL-28, ISG15 and OASL in D4m.3A cells transduced with gRNA against scramble (scramble) or LSD1 (LSD1 KO1 and LSD1 KO2). The RT-qPCR data were normalized to GAPDH and then relative to scramble. Error bars represent SEM from three experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 6D is bar graph showing RT-qPCR analysis of MuSD, MuERV-L, Line 1, IAP, IFN- α , IFN- β and IL-28 in B16 cells transfected with gRNA against scramble (scramble) or LSD1 (LSD1 KO, clone g4-7). The RT-qPCR data were normalized to GAPDH and then relative to scramble. Error bars represent SEM from three experiments.

FIG. 6E is a bar graph showing double-stranded RNA (dsRNA) enrichment of selected retrotransposons (MuSD, MuERV-L, Line1 and IAP) in control or LSD1 KO B16 cells. Total RNA extract from control or LSD1 KO B16 cells was digested with RNase A versus mock under high salt condition (350 mM NaCl), followed by a second round of RNA extraction with TRIzol. The ratios of (retrotransposon/Actin)RNase/(retrotransposon/Actin)mock were calculated as enrichment fold. Error bars represent SEM from three experiments. * $p < 0.05$, as determined by unpaired t-test.

FIG. 6F is a picture of Hybond N+ membranes immunoblotted with a dsRNA-specific antibody (J2) using total RNA extracted from scramble or LSD1 KO B16 cells and treated with mock, RNase T1, RNase III or RNase A (350 mM NaCl), followed by a second round of RNA extraction with TRIzol.

FIG. 6G is a bar graph showing double-stranded RNA (dsRNA) enrichment of MuERV-L, MuSD, IAP and Line1 in control and LSD1 KO D4m cells by RT-qPCR. Total RNA extract from control or LSD1 KO D4m cells was digested with RNase A versus mock under high salt condition (350 mM NaCl), followed by a second round

of RNA extraction with TRIzol. The ratios of (retrotransposon/GAPDH)/RNase/(retrotransposon/GAPDH)mock were calculated as enrichment fold. GAPDH was used as an internal control. Error bars represent SEM from three experiments. $p < 0.05$, ns, not significant, as determined by unpaired t-test.

5 FIG. 6H is a picture of a crystal violet cell proliferation assay of B16 + sh-Ctrl cells, B16 + sh1-LSD1 cells and B16 + sh3-LSD1 cells, after 6 days of growth before crystal violet staining.

FIG. 6I is a bar graph showing the relative colony area of the proliferation assay of FIG. 6H relative to B16 + sh-Ctrl. Error bars represent SD between
10 triplicates in one of two experiments. $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$, as determined by unpaired t-test.

FIG. 6J is a picture of a crystal violet cell proliferation assay of scramble and LSD1 KO B16 cells (clone g4-7 and clone g5-4), after 6 days of growth before crystal violet staining.

15 FIG. 6K is a bar graph showing the relative colony area of the proliferation assay of FIG. 6J relative to scramble. Error bars represent SD between triplicates in one of two experiments. $**p < 0.01$, as determined by unpaired t-test.

FIG. 6L is a representative image of sequencing results of genomic *Lsd1*, *Mda5*, *Ifnar1*, *Ifnb* and *Tlr3* exons targeted by gRNAs in corresponding B16 clones and in alignment with reference sequences. From top to bottom: B16 CRISPR-LSD1, clone gRNA4-A7 (SEQ ID NO: 122), reference (SEQ ID NO: 123); B16 CRISPR-LSD1, clone gRNA5-4 (SEQ ID NO: 124), reference (SEQ ID NO: 125); B16 CRISPR-MDA5, clone gRNA4-16 (SEQ ID NO: 126), reference (SEQ ID NO: 127); B16 CRISPR-MDA5, clone gRNA4-16 (SEQ ID NO: 128), reference (SEQ ID NO: 129); B16 CRISPR-LSD1/MDA5, clone gRNA4-19 (SEQ ID NO: 130), reference (SEQ ID NO: 131); B16 CRISPR-LSD1/MDA5, clone gRNA4-19 (SEQ ID NO: 132), reference (SEQ ID NO: 133); B16 CRISPR-IFNAR1, clone gRNA1-10 (SEQ ID NO: 134), reference (SEQ ID NO: 135); B16 CRISPR-IFNAR1, clone gRNA1-10 (SEQ ID NO: 136), reference (SEQ ID NO: 137); B16 CRISPR-LSD1/IFNAR1, clone gRNA1-16 (SEQ ID NO: 138), reference (SEQ ID NO: 139); B16 CRISPR-IFN β , clone gRNA3-14 (SEQ ID NO: 140), reference (SEQ ID NO: 141); B16 CRISPR-LSD1/IFN β , clone gRNA3-16 (SEQ ID NO: 142), reference (SEQ ID NO: 25
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143); B16 CRISPR-LSD1/TLR3, clone gRNA6-7 (SEQ ID NO: 144), reference (SEQ ID NO: 145).

FIG. 6M is a picture of immunoblots showing LSD1 and MDA5 expression in CRISPR/Cas9-modified B16 cells (scramble, LSD1 KO, and LSD1/MDA5 DKO).

5 FIG. 6N is a picture of a crystal violet cell proliferation assay of B16 scramble cells, B16 LSD1 KO cells and B16 LSD1/MDA5 KO cells, after 6 days of growth before crystal violet staining.

FIG. 6O is a bar graph showing the relative colony area of the proliferation assay of FIG. 6N relative to B16 scramble. Error bars represent SD between quadruplicates in one of two experiments. *** $p < 0.001$, **** $p < 0.0001$, as determined by unpaired t-test.

FIG. 6P is a bar graph showing RT-qPCR analysis of selected retrotransposons (MuERV-L and Line1) and IFNs (IFN- α , IFN- β and IL-28), OASL, ISG15, TLR3 and RIG-I in B16 scramble cells, B16 LSD1 KO cells and B16 LSD1/MDA5 KO cells. Error bars represent SEM between duplicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 6Q is a picture of Hybond N+ membranes immunoblotted with a dsRNA-specific antibody (J2) using total RNA extracted from scramble, LSD1 KO or LSD1/MDA5 DKO B16 cells.

20 FIG. 7A is a representative image of sequencing results of genomic *Lsd1* exon targeted by gRNA5 in corresponding LLC clones and in alignment with reference sequences. From top to bottom: LLC CRISPR-LSD1, clone gRNA5-A29 (SEQ ID NO: 146), reference (SEQ ID NO: 147); LLC CRISPR-LSD1, clone gRNA5-B30 (SEQ ID NO: 148), reference (SEQ ID NO: 149).

25 FIG. 7B is a picture of immunoblots of LSD1 in CRISPR/Cas9-modified LLC clones.

FIG. 7C is a representative image of sequencing results of genomic *Lsd1* exons targeted by two gRNAs in corresponding D4m clones and in alignment with reference sequences. From top to bottom: D4m CRISPR-LSD1, clone gRNA5-B37 (SEQ ID NO: 150), reference (SEQ ID NO: 151); D4m CRISPR-LSD1, clone gRNA3-8 (SEQ ID NO: 152), reference (SEQ ID NO: 153) and D4m CRISPR-LSD1, clone gRNA3-8 (SEQ ID NO: 154), reference (SEQ ID NO: 155).

FIG. 7D is a picture of immunoblots of LSD1 in CRISPR/Cas9-modified D4m clones.

FIG. 7E is a picture of immunoblots of LSD1 in CRISPR/Cas9-modified B16 clones transfected with different gRNAs targeting *Lsd1*.

5 FIG. 7F is a picture of immunoblots of LSD1 in CRISPR/Cas9-modified B16 clones transfected with different gRNAs targeting *Lsd1*.

FIG. 8A is a bar graph showing RT-qPCR analysis of MuERV-L, Line1, IFN- α , IFN- β , IL-28, OASL and ISG15 in B16 scramble cells and MDA5 KO B16 cells. The RT-qPCR data were normalized to GAPDH and then relative to scramble. Error bars represent SD between duplicates. ns, not significant, as determined by unpaired t-test.

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FIG. 8B is a picture of a crystal violet cell proliferation assay of B16 scramble cells, B16 LSD1 KO cells and B16 MDA5 KO cells, after 6 days of growth before crystal violet staining.

15 FIG. 8C is a bar graph showing the relative colony area of the proliferation assay of FIG. 8B relative to B16 scramble. Error bars represent SD between quadruplicates in one of two experiments. **** $p < 0.0001$, ns, not significant, as determined by unpaired t-test.

FIG. 8D is a bar graph showing RT-qPCR analysis of IFN- α , IFN- β , IL-28, OASL and ISG15 in B16 scramble cells, LSD1 KO B16 cells and LSD1/TLR3 DKO B16 cells. The RT-qPCR data were normalized to GAPDH and then relative to scramble. Error bars represent SD between duplicates. ns, not significant, as determined by unpaired t-test.

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FIG. 8E is a bar graph showing RT-qPCR analysis of IFN- α , IFN- β , IL-28 OASL and ISG15 in B16 scramble cells, B16 LSD1 KO cells and B16 LSD1/IFNAR1 DKO cells. Error bars represent SD between duplicates. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

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FIG. 8F is a picture of a crystal violet cell proliferation assay of B16 scramble cells, B16 LSD1 KO cells and B16 LSD1/IFNAR1 DKO cells, after 6 days of growth before crystal violet staining.

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FIG. 8G is a bar graph showing the relative colony area of the proliferation assay of FIG. 8F relative to B16 scramble. Error bars represent SD between

triplicates in one of two experiments. * $p < 0.05$, ** $p < 0.01$, ns, not significant, as determined by unpaired t-test.

FIG. 8H is a picture of immunoblots showing IFNAR1 expression in CRISRP/Cas9-modified B16 cells as indicated.

5 FIG. 8I is a picture of a crystal violet cell proliferation assay of B16 scramble cells, B16 LSD1 KO cells, B16 IFN- β KO cells and B16 LSD1/IFN- β DKO cells, after 6 days of growth before crystal violet staining.

FIG. 8J is a bar graph showing the relative colony area of the proliferation assay of FIG. 8J relative to B16 scramble. Error bars represent SD between triplicates
10 in one of two experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 8K is a bar graph showing RT-qPCR analysis of MuERV-L, Line1, IFN- α , IL-28, ISG15, OASL, TLR3, MDA5, RIG-I in B16 scramble cells, LSD1 KO B16 cells and LSD1/IFN- β DKO B16 cells. The RT-qPCR data were normalized to
15 GAPDH and then relative to scramble. Error bars represent SD between duplicates. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

FIG. 8L is bar graph showing mouse IFN- β levels in scramble, IFN- β KO, LSD1/IFN- β DKO B16 cells challenged by poly(I:C), as determined by enzyme-linked immunosorbent assay (ELISA). n.d., not detected.

20 FIG. 9A is a line graph showing tumor growth of immunocompetent mice inoculated with 500k scramble (n=14) or LSD1 KO B16 cells (n=11). Error bars represent SEM of individual mice in one experiment. Data represents two independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, as determined by unpaired t-test.

25 FIG. 9B is a line graph showing survival of immunocompetent mice inoculated with 500k scramble or LSD1 KO B16 cells. Data represents two independent experiments. Error bars represent SEM of individual mice in one experiment. *** $p < 0.001$, as determined by log-rank test.

FIG. 9C is a line graph showing tumor growth of immunodeficient mice (TCR α KO) or immunocompetent mice inoculated with 500k scramble or LSD1 KO
30 B16 cells. Error bars represent SEM of individual mice in one experiment. Data represents two independent experiments. ns, not significant, as determined by ANOVA.

FIG. 9D is a line graph showing survival of immunodeficient mice (TCR α KO) or immunocompetent mice inoculated with 500k scramble or LSD1 KO B16 cells. Data represents two independent experiments. Error bars represent SEM of individual mice in one experiment. * $p < 0.05$, ns, not significant, as determined by log-rank test.

FIG. 9E is a line graph showing tumor growth of immunocompetent mice inoculated with 500k scramble B16 cells, LSD1 KO B16 cells, MDA5 KO B16 cells, or LSD1/MDA5 DKO B16 cells. Error bars represent SEM of individual mice in one experiment. Data represents two independent experiments. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns, not significant, as determined by ANOVA.

FIG. 9F is a line graph showing survival of immunocompetent mice inoculated with 500k scramble B16 cells, LSD1 KO B16 cells, MDA5 KO B16 cells, or LSD1/MDA5 DKO B16 cells. Data represents two independent experiments. Error bars represent SEM of individual mice in one experiment. *** $p < 0.001$, **** $p < 0.0001$, ns, not significant, as determined by log-rank test.

FIG. 9G is a line graph showing tumor growth of immunocompetent mice inoculated with 500k scramble B16 cells, LSD1 KO B16 cells, IFN- β KO B16 cells, or LSD1/IFN- β DKO B16 cells. Error bars represent SEM of individual mice in one experiment. Data represents two independent experiments. **** $p < 0.0001$, ns, not significant, as determined by ANOVA.

FIG. 9H is representative images of lung metastasis in immunocompetent mice receiving 200k scramble or LSD1 KO B16 cells intravenously taken 14 days post-injection.

FIG. 9I is a dot plot showing the quantification of lung metastasis in immunocompetent mice receiving 200k scramble or LSD1 KO B16 cells intravenously.

FIG. 10A is bar graphs showing the number of tumor infiltrating lymphocytes (TILs) per gram of B16 tumor in immunocompetent mice ($n=5$ for scramble, $n=5$ for LSD1 KO and $n=6$ for LSD1/MDA5 DKO) as determined by flow cytometry at day 14 when tumor sizes were comparable among the tested groups. Data represents two independent experiments. Error bars represent SEM of individual mice in one experiment. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

FIG. 10B is bar graphs showing T cells in draining lymph nodes (dNLs) of B16 tumor-bearing immunocompetent mice (n=5 for scramble, n=5 for LSD1 KO and n=6 for LSD1/MDA5 DKO). *p<0.05, ns, not significant, as determined by unpaired t-test.

FIG. 10C is bar graphs showing the percentage of granzyme B positive (GzmB+) or Ki-67+ CD8+ TILs as in 10A. Error bars represent SEM of individual mice in one experiment. ns, not significant, as determined by unpaired t-test.

FIG. 10D is bar graphs showing the clonality and entropy of CD8+ TILs in transplanted B16 tumors (n=5 for scramble, n=3 for LSD1 KO) as determined by TCRseq. ns, not significant, as determined by unpaired t-test.

FIG. 10E is volcano and M-A plots showing differentially expressed genes in GFP-labeled B16 tumor cells (n=3 for scramble and LSD1 KO) isolated from tumor-bearing immunocompetent mice (referred to as *ex vivo* cells hereafter), as determined by RNA-seq. Dots in grey represent significantly increased or decreased genes (FDR<0.05) in LSD1 KO versus scramble cells.

FIG. 10F is volcano and M-A plots showing differentially expressed genes in GFP-labeled B16 tumor cells (n=3 for scramble and LSD1/MDA5 DKO) isolated from tumor-bearing immunocompetent mice (referred to as *ex vivo* cells hereafter), as determined by RNA-seq. Dots in grey represent significantly increased or decreased genes (FDR<0.05) in LSD1/MDA5 DKO versus scramble cells.

FIG. 10G is a heatmap showing differential expression (FDR<0.05) of ERVs between scramble and LSD1 KO cells (n=3).

FIG. 10H is plots showing LSD1 and H3K4me2 ChIP-seq signals at genomic loci of 74 ERV subfamilies in control and LSD1 KD cells in *ex vivo* scramble and LSD1 KO B16 cells.

FIG. 10I is plots showing LSD1 and H3K4me2 ChIP-seq signals at genomic loci of a representative ERVK10C subfamily in control and LSD1 KD cells in *ex vivo* scramble and LSD1 KO B16 cells.

FIG. 10J is a representative dotmap showing the top 10 terms of a GO analysis of upregulated genes ($\log_2(\text{FC}) > 1$ and $\text{FDR} < 0.05$) in LSD1 KO versus control scramble B16 cells. Dot size represents odds ratio.

FIG. 10K is GSEA analysis for inflammatory response and cytokine production in *ex vivo* LSD1 KO versus scramble B16 cells.

FIG. 10L is a representative box and whisker plot showing log₂(FC) of upregulated genes in the top 10 terms (170 in total) in LSD1 KO and LSD1/MDA5 DKO versus scramble B16 cells.

FIG. 10M is GSEA analysis for positive regulation of cell proliferation in *ex vivo* LSD1 KO versus scramble B16 cells.

FIG. 10N is a heatmap showing all genes categorized in GO term “MHC protein complex”.

FIG. 10O is a bar graph showing mean fluorescent intensity (MFI) of MHC-1⁺ B16 cells isolated from scramble, LSD1 KO and LSD1/MDA5 DKO B16 tumors from immunocompetent mice. Data represents two independent experiments. Error bars represent SEM of individual mice in one experiment. ***p<0.001, ****p<0.0001, ns, not significant, as determined by unpaired t-test.

FIG. 10P is a line graph showing tumor growth of immunocompetent mice and TCRα KO mice inoculated with 500k scramble D4m.3A cells or LSD1 KO D4m.3A cells. Error bars represent SEM of individual mice in one experiment. Data represents two independent experiments. **p<0.01, ****p<0.0001, as determined by ANOVA.

FIG. 10Q is a line graph showing survival of immunocompetent mice and TCRα KO mice inoculated with 500k scramble D4m.3A cells or LSD1 KO D4m cells. Error bars represent SEM of individual mice in one experiment. ***p<0.001, ****p<0.0001, ns, not significant, as determined by log-rank test.

FIG. 10R is bar graphs showing the number of CD4⁺ and CD8⁺ TILs per gram of D4m.3A tumor in immunocompetent mice (n=3 in each group) as determined by flow cytometry. Data represents two independent experiments. Error bars represent SEM of individual mice in one experiment. *p<0.05, as determined by unpaired t-test.

FIG. 10S is a bar graph showing mean fluorescent intensity (MFI) of MHC-1⁺ *ex vivo* D4m.3A cells (n=3). Data represents two independent experiments. Error bars represent SEM of individual mice in one experiment. *p<0.05, as determined by unpaired t-test.

FIG. 10T is a bar graph showing counts per million (CPM) of PD-L1 of tumor-extracted B16 cells (*ex vivo*; n=3) as determined by RNA-seq. Data represents

two independent experiments. Error bars represent SEM of individual mice in one experiment. *** $p < 0.001$, ns, not significant, as determined by unpaired t-test.

FIG. 10U is a bar graph showing MFI of PD-L1⁺ B16 cells isolated from scramble, LSD1 KO and LSD1/MDA5 DKO B16 tumors from immunocompetent mice. Data represents two independent experiments. Error bars represent SEM of individual mice in one experiment. * $p < 0.05$, ns, not significant, as determined by unpaired t-test.

FIG. 11A is a line graph showing tumor growth of immunocompetent mice inoculated with 250k scramble B16 cells or LSD1 KO B16 cells, and treated with PD-1 blocking antibody or isotype control based on a set time (day 14) for initial treatment. Arrow bars indicate time points of anti-PD-1 injection. Error bars represent SEM of individual mice in one experiment. **** $p < 0.0001$, ns, not significant, as determined by ANOVA.

FIG. 11B is a line graph showing survival of immunocompetent mice inoculated with 250k scramble B16 cells or LSD1 KO B16 cells, and treated with PD-1 blocking antibody or isotype control based on a set time (day 14) for initial treatment. Error bars represent SEM of individual mice in one experiment. **** $p < 0.001$, **** $p < 0.0001$, ns, not significant, as determined by log-rank test.

FIG. 11C is a line graph showing tumor growth of immunocompetent mice inoculated with 500k scramble B16 cells or LSD1 KO B16 cells, and treated with PD-1 blocking antibody or isotype control based on a set tumor size ($\sim 200 \text{ mm}^3$) for initial treatment. Arrow bars indicate time points of initial anti-PD-1 injection (black arrow (at day 14) - into scramble tumor-bearing mice; grey arrow (at day 18) - into LSD1 KO tumor-bearing mice), followed by continuous injection every other day until the end of experiment. Error bars represent SEM of individual mice in one experiment. **** $p < 0.0001$, ns, not significant, as determined by ANOVA.

FIG. 11D is a line graph showing survival of immunocompetent mice inoculated with 500k scramble B16 cells or LSD1 KO B16 cells, and treated with PD-1 blocking antibody or isotype control based on a set tumor size ($\sim 200 \text{ mm}^3$) for initial treatment. Error bars represent SEM of individual mice in one experiment. *** $p < 0.001$, ns, not significant, as determined by log-rank test.

FIG. 12A is bar graph showing the frequencies of mutation, amplification and deletion of LSD1 across a panel of cancer types were analyzed using cBioPortal by selecting all listed studies.

FIG. 12B is a representative plot showing analysis of LSD1 RNA expression in cancerous tissues versus normal tissues from various types of cancer patients in The Cancer Genome Atlas (TCGA) dataset.

FIG. 12C is survival curves of LSD1-low and LSD1-high patient groups dichotomously divided by LSD1 median in two cancer types using TCGA dataset.

FIG. 12D is a representative correlation analysis for LSD1 expression versus IFN/antiviral response in cancerous tissues from various types of cancer patients in The Cancer Genome Atlas (TCGA) dataset.

FIG. 12E is a representative correlation analysis for LSD1 expression versus CD8⁺ T cell infiltration in cancerous tissues from various types of cancer patients in The Cancer Genome Atlas (TCGA) dataset.

FIG. 12F is survival curves of LSD1-low (first tertile, n=113) and LSD1-int/high (second and third tertiles, n=210) SKCM patient groups divided based on LSD1 expression using TCGA dataset.

FIG. 12G is a plot of top 10 GO terms based on p value generated by DAVID functional annotation of differentially expressed genes (FC > 1.5 or FC < 0.67, and FDR < 0.05) in LSD1-low group versus LSD1-int/high group of SKCM (increased genes – black(top), decreased genes – grey(bottom)).

FIG. 12H is a plot comparing CD8a expression between LSD1-low group and LSD1-intermediate (int)/high group of SKCM.

FIG. 12I is a plot comparing GzmB expression between LSD1-low group and LSD1-intermediate (int)/high group of SKCM.

FIG. 13A is a bar graph showing the RNA expression of TGFB subfamily (TGFB1, TGFB2 and TGFB3) in *in vitro* cultured control (Ctrl) and LSD1 knockdown (KD) MCF-7 cells, as analyzed by RNA-seq. *p<0.05, **p<0.01, ns, not significant.

FIG. 13B is a bar graph showing RNA expression of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in scramble and Lsd1 knockout (KO) B16 cells isolated from tumor-bearing mice, as analyzed by RNA-seq. **p<0.01, ***p<0.001, ns, not significant.

FIG. 13C is a bar graph showing the RNA expression of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in in vitro cultured B16 cells of scramble, Lsd1 KO or Lsd1 KO rescued with ectopic LSD1 (Lsd1 KO + hLSD1), as measured by qPCR. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant.

5 FIG. 13D is a bar graph showing the RNA expression of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in in vitro cultured B16 cells of scramble, Lsd1 KO or Lsd1/Ifnb double KO (DKO), as measured by qPCR. * $p < 0.05$, ns, not significant.

FIG. 13E is a bar graph showing the RNA expression of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in in vitro cultured D4m cells of scramble or Lsd1 KO, as measured by qPCR. * $p < 0.05$, ** $p < 0.01$.

FIG 13F is a bar graph showing the RNA expression of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in in vitro cultured scramble LLC cells and two LLC lines deficient of Lsd1, as measured by qPCR. ** $p < 0.01$, *** $p < 0.001$, ns, not significant.

FIG. 14A is a bar graph showing ChIP-PCR analysis of LSD1 occupancy at the promoter regions of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in scramble and Lsd1 KO B16 cells. ** $p < 0.01$.

FIG. 14B is a bar graph showing ChIP-PCR analysis of H3K4me2 enrichment at the promoter regions of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in scramble and Lsd1 KO B16 cells. * $p < 0.05$, ns, not significant.

20 FIG. 14C is a bar graph showing ChIP-PCR analysis of H3K27ac enrichment at the promoter regions of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in scramble and Lsd1 KO B16 cells. * $p < 0.05$, ns, not significant.

FIG. 15A is a bar graph showing the RNA level of Tgfb subfamily (Tgfb1, Tgfb2 and Tgfb3) in scramble, Lsd1 KO and Lsd1/Tgfb1/Tgfb2/Tgfb3 quadruple knockout (Lsd1/Tgfb QKO) B16 cells.

FIG. 15B is a bar graph showing the RNA expression of Serpine1 in scramble, Lsd1 KO, Lsd1 KO + hLSD1, and Lsd1/Tgfb QKO B16 cells. ** $p < 0.01$.

FIG. 15C is a bar graph showing the RNA expression of a panel of IFNs and ISGs in scramble, Lsd1 KO and Lsd1/Tgfb QKO B16 cells. * $p < 0.05$, ** $p < 0.01$, ns, not significant.

FIG. 15D is a picture of immunoblots showing the expression of RIG-I in scramble, Lsd1 KO, Lsd1 KO + hLSD1, and Lsd1/Tgfb QKO B16 cells.

FIG. 16A is a line graph showing tumor growth in immunocompetent mice inoculated with scramble, Lsd1 KO, Lsd1/Tgfb QKO or Tgfb1/Tgfb2/Tgfb3 triple knockout (Tgfb TKO) B16 cells over time (in days post-tumor injection). ** $p < 0.01$, ns, not significant.

5 FIG. 16B is a line graph showing survival curve of immunocompetent mice inoculated with scramble, Lsd1 KO, Lsd1/Tgfb QKO and Tgfb TKO B16 cells over time (in days post-tumor injection). * $p < 0.05$, ns, not significant.

FIG. 16C is a line graph showing tumor growth in immunocompetent versus immunodeficient TCR α KO mice inoculated with scramble or Lsd1/Tgfb QKO B16
10 cells over time (in days post-tumor injection). ** $p < 0.01$, ns, not significant.

FIG. 16D is a line graph showing survival curve of immunocompetent versus immunodeficient TCR α KO mice inoculated with scramble or Lsd1/Tgfb QKO B16 cells over time (in days post-tumor injection). ns, not significant.

FIG. 17A is a picture of mouse lungs colonized with scramble, Lsd1 KO,
15 Lsd1/Tgfb QKO and Tgfb TKO B16 tumors at day 14 after tail vein injection of 0.2 million cells.

FIG. 17B is a dot graph showing the number of tumor nodules in immunocompetent mice receiving scramble, Lsd1 KO, Lsd1/Tgfb QKO and Tgfb TKO B16 cells at day 14 after tail vein injection of 0.2 million cells. * $p < 0.05$,
20 ** $p < 0.01$.

FIG. 18A is a dot graph showing the number of infiltrated CD4 $^{+}$ T cells in scramble, Lsd1 KO and Lsd1/Tgfb QKO B16 tumors at day 14 after tumor implantation into immunocompetent mice. * $p < 0.05$, ns, not significant.

FIG. 18B is a dot graph showing the number of infiltrated CD8 $^{+}$ T cells in
25 scramble, Lsd1 KO and Lsd1/Tgfb QKO B16 tumors at day 14 after tumor implantation into immunocompetent mice. ** $p < 0.01$, ns, not significant.

FIG. 18C is a dot graph showing the percentage of Granzyme-B $^{+}$ (GzmB $^{+}$) cells among tumor-infiltrating CD3 $^{+}$ CD8 $^{+}$ T cells in scramble, Lsd1 KO and Lsd1/Tgfb QKO B16 tumors at day 14 after tumor implantation into
30 immunocompetent mice. * $p < 0.05$, ** $p < 0.01$, ns, not significant.

FIG. 18D is a dot graph showing the percentage of Ki-67 $^{+}$ cells among tumor-infiltrating CD3 $^{+}$ CD8 $^{+}$ T cells in scramble, Lsd1 KO and Lsd1/Tgfb QKO B16

tumors at day 14 after tumor implantation into immunocompetent mice. * $p < 0.05$, ** $p < 0.01$, ns, not significant.

FIG. 18E is a dot graph showing the percentage of PD-1+ cells among tumor-infiltrating CD3+CD8+ T cells in scramble, Lsd1 KO and Lsd1/Tgfb QKO B16 tumors at day 14 after tumor implantation into immunocompetent mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant.

FIG. 19A is a line graph showing tumor growth of immunocompetent mice inoculated with scramble B16 cells and treated with isotype control over time (in days post-tumor injection). Arrows indicate the injection of isotype control at a dose of 100 μ g per mouse per injection.

FIG. 19B is a line graph showing tumor growth of immunocompetent mice inoculated with scramble B16 cells and treated with anti-PD-1 over time (in days post-tumor injection). Arrows indicate the injection of anti-PD-1 at a dose of 100 μ g per mouse per injection.

FIG. 19C is a line graph showing tumor growth of immunocompetent mice inoculated with Lsd1/Tgfb QKO B16 cells and treated with isotype control over time (in days post-tumor injection). Arrows indicate the injection of isotype control at a dose of 100 μ g per mouse per injection.

FIG. 19D is a line graph showing tumor growth of immunocompetent mice inoculated with Lsd1/Tgfb QKO B16 cells and treated with anti-PD-1 over time (in days post-tumor injection). Arrows indicate the injection of anti-PD-1 at a dose of 100 μ g per mouse per injection.

FIG. 20A is a dot graph showing the protein level of TGF β 1 in implanted B16 tumors. ** $p < 0.01$.

FIG. 20B is a line graph showing tumor growth of wildtype (WT) and CD4-dnT β RII transgenic mice subcutaneously inoculated with Lsd1 KO or Lsd1/Tgfb QKO B16 cells. **** $p < 0.0001$, ns, not significant.

FIG. 20C is a dot graph showing percentages of GzmB+ cells among CD8+ TILs analyzed by intracellular staining and flow cytometry. * $p < 0.05$, ** $p < 0.01$, ns, not significant.

FIG. 20D is a bar graph showing the RNA expression of Tgfb1 and Serpine 1 in scramble, Lsd1 KO + empty vector (EV), Lsd1 KO + dominant negative TGFBR2 (DN), and Lsd1/Tgfb QKO B16 cells. *** $p < 0.001$, ns, not significant

FIG. 20E is a line graph showing tumor growth of immunocompetent mice subcutaneously inoculated with Lsd1 KO B16 cells stably expressing T β R2-DN (DN) or empty vector (EV). * $p < 0.05$.

FIG. 20F is a bar graph showing ovalbumin (Ova)-mediated relative killing of genetically modified B16 cells by OT-I cells. * $p < 0.05$, ns, not significant.

FIG. 21A is a line graph showing survival curves of immunocompetent mice implanted with 500k scramble or Lsd1/Tgfb QKO B16 tumor cells. The anti-PD-1 or isotype control treatment was initiated based on a set volume ($\sim 250 \text{ mm}^3$) and continued every other day for a total for four injections. **** $p < 0.0001$, ns, not significant.

FIG. 21B is a line graph showing survival curves of immunocompetent mice implanted with 250k scramble or Lsd1/Tgfb QKO B16 tumor cells. The anti-PD-1 or isotype control treatment was initiated based on a set time (day 10) and continued every other day for a total for four injections. **** $p < 0.0001$, ns, not significant.

FIG. 21C is a line graph showing survival curves of immunocompetent mice implanted with 500k scramble or Lsd1 KO B16 tumor cells. The anti-PD-1/anti-TGF- β or isotype control treatment was initiated based on a set time (day 13) and continued every other day for a total for three injections. ** $p < 0.01$, *** $p < 0.001$.

FIG. 22A is a line graph showing tumor growth curves of tumor-free mice from (FIG. 21B) or age-matched naïve mice re-challenged with 500k WT B16 tumor cells. ** $p < 0.01$.

FIG. 22B is a line graph showing survival curves of tumor-free mice from (FIG. 21B) or age-matched naïve mice re-challenged with 500k WT B16 tumor cells. *** $p < 0.001$.

FIG. 22C is a line graph showing tumor growth curves of tumor-free mice from (FIG. 22B) or age-matched naïve mice re-challenged with 500k WT MC38 tumor cells. ns, not significant.

FIG. 22D is a line graph showing survival curves of tumor-free mice from (FIG. 22B) or age-matched naïve mice re-challenged with 500k WT MC38 tumor cells. ns, not significant.

DETAILED DESCRIPTION

Chromatin regulators play a broad role in regulating gene expression. When gene regulation goes awry, this can lead to the development of cancer. Without

wishing to be bound by theory, the present disclosure demonstrated that ablation of the histone demethylase lysine-specific demethylase 1A (LSD1) in human and mouse cells leads to double-stranded RNA (dsRNA) stress, through elevating the transcript level of certain repetitive elements and impairing the small RNA machinery, i.e.,
5 RNA-induced silencing complex (RISC), which triggers type I interferon activation. Significantly, LSD1 deletion in mouse B16 melanoma cells leads to the activation of potent anti-tumor adaptive immunity, which restrained tumor growth *in vivo*. Importantly, LSD1 depletion also elicited dramatic responses of checkpoint blockade-refractory B16 tumors to anti-PD-1 therapy. The present disclosure describes the
10 potent impact of LSD1 on tumor responses to host immunity and immunotherapy and describes LSD1 inhibition combined with PD-1 and/ or PD-L1 (sometimes collectively referred to herein as “PD-(L)1”) blockade as a strategy for cancer treatment.

Cancer immunotherapy, including anti-PD(L)1 therapy, has achieved
15 successful clinical outcomes in controlling tumor progression (Sharma and Allison (2015) Cell 161(2): 205-214). Recent human clinical trial using PD-1 or PD-L1 directed immunotherapy have reported promising results, leading to FDA approval of PD-1 pathway inhibitors for multiple tumor types including melanoma, non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), renal cell
20 carcinoma (RCC), Hodgkin’s lymphoma, bladder cancer, Merkel cell carcinoma, and microinstability high (MSI^{hi}) or mismatch repair deficient adult and pediatric solid tumors (Pauken and Sharpe (2018) Nat Rev Immunol 18(3): 153-167). However, a majority of cancer patients do not respond to anti-PD-(L)-1 therapy, due to multiple mechanisms including dysfunctional T cells and lack of T cell infiltration or
25 recognition by T cells (Sharma et al. (2017) Cell 168: 707-723). The broad roles of chromatin regulators in controlling cancer and T cell functions raise the possibility of their involvement in regulating tumor responses or resistance to immunotherapy. Indeed, a recent study found that inhibition of DNA methylation leads to tumor interferon pathway activation, and increased responses to cancer immunotherapy
30 (Chiappinelli et al. (2015) Cell 162(5): 974-986). On the other hand, blocking de novo DNA methylation in T cells enhances anti-PD-L1-mediated T cells rejuvenation and tumor control (Ghoneim et al. (2017) Cell 170:142-157 e119). However, how the full spectrum of chromatin regulators regulates cancer cells and impacts their

responses to the immune system are still poorly understood. Moreover, the therapeutic potential of manipulating these factors to remodel the cancer chromatin landscape for onco-immunotherapy is under-explored.

Naturally occurring dsRNAs derived from a variety of sources including retrotransposons are processed into endo-siRNA by RISC (Watanabe et al. (2008) Nature 453(7194): 539-543). The epigenetic regulation of retrotransposon (such as ERVs) transcription in mammal germ cells and early embryonic development are well documented (Leung and Lorincz (2012) Trends Biochem Sci 37(4): 127-133; Song et al. (2012) Nat Rev Mol Cell Biol 13(5): 283-296), but much less is known in differentiated somatic cells. Without wishing to be bound by theory, the present disclosure shows that LSD1 represses the transcription of a subset of ERVs in human cancer cells, consistent with a previous report showing LSD1 is involved in regulating ERV expression in mESCs (Macfarlan et al. (2011) Genes Dev 25(6): 594-607). Although ERV transcript induction by LSD1 inhibition is not dramatic, their dsRNA forms are much more significantly elevated. Additionally, intracellular dsRNAs can be derived from different categories of transcripts, including ERVs, LINEs, SINEs and gene/pseudogene duplexes (Carthew and Sontheimer 2009 Cell 136: 642-655), many of which are also up-regulated in the LSD1 null tumor cells. Importantly, it is the dsRNA forms, rather than the overall transcripts, that are directly recognized by dsRNA sensors to induce IFN activation. LSD1 inhibition also compromises the expression of RISC proteins and subsequently RISC activity, thus blocking dsRNA from entering the RNA interference pathway. By coordinating these two processes, LSD1 inhibition reinforces dsRNA stress and subsequent cellular responses.

Cancer immunotherapy, particularly PD-1-directed or PD-L1-directed checkpoint blockade therapy, has revolutionized cancer treatment. PD-(L)1 inhibitors have been approved by FDA to treat a wide range of cancers, including melanoma, Hodgkin disease, non-small cell lung carcinoma, kidney cancer, head and neck squamous carcinoma, bladder cancer and microsatellite instability high or mismatch repair-deficient cancers (Sharpe (2018) Nat Rev Immunol 18(3): 153-167). However, a majority of cancer patients show only partial or no response, or temporary responses to PD-(L)1-targeted therapy. Thus, there is unmet clinical need to extend the benefits to non-responders and to achieve a long-lasting therapeutic effect. Tumors that show no responses to the initial treatment with PD-(L)1 inhibitors are generally thought to

be “cold” tumors, which could attribute to multiple mechanisms in either tumor cells or T cells including low antigenic mutations, defects in antigen processing and presentation due to genetic mutations or epigenetic alterations, lack of T cell infiltration or recognition, etc. (Sharma et al. (2017) Cell 168.4: 707-723). Tumors that initially show responses to PD-(L)1 blockade therapy for a period of time may acquire resistance in spite of continuous treatment, which may be caused by new genetic mutations or epigenetic alterations that disable T cell recognitions or functions. A better understanding on how tumor response to T cell immunity and PD-(L)1 blockade therapy is regulated at both genetic and epigenetic levels will reveal new therapeutic strategies for extending the benefits to non-responders and achieving long-lasting responses.

Recently, histone specific demethylase LSD1 was identified and characterized as a potent inhibitor of antitumor T cell immunity and tumor responsiveness to anti-PD-1 therapy (Sheng et al. (2018) Cell 174: 549-563). Briefly, the inhibition of LSD1, an enzyme that catalyzes the removal of methyl group from H3K4me2 or H3K4me1, leads to the accumulation of H3K4me2 at certain endogenous retrovirus (ERV) loci and their transcriptional activation in tumor cells. In addition, LSD1 inhibition decreases the expression of RNA-induced silencing complex (RISC). Both effects result in double-stranded RNA (dsRNA) stress and activation of type 1 interferon in tumor cells, which consequently stimulates antitumor T cell immunity and restrains tumor growth. Moreover, LSD1 ablation in “cold” tumors enhances tumor immunogenicity and T cell infiltration, and elicits significant responses of checkpoint blockade-refractory mouse melanoma to anti-PD-1 therapy. The analysis of TCGA data also consistently shows an inverse correlation between LSD1 expression and CD8+ T cell infiltration in various human tumors, suggesting LSD1 inhibition combined with PD-(L)1 blockade as a promising cancer treatment strategy. This study together with a few other reports on epigenetic regulators, including DNMTs (Chiappinelli (2015) Cell 162(5): 974-986; Roulois (2015) Cell 162(5): 961-973; Topper (2017) Cell 171(6): 1284-1300; Ghoneim (2017) Cell 170(1): 142-157), TET2 (Pan (2017) Immunity 47(2): 284-297; Fraietta (2018) Nature 558(7709): 307-312), EZH2 [Peng et al. (2015) Nature 527: 249-253; Canadas et al. (2018) Nat Med 24: 1143-1150], BRDs [DO et al. (2017) Cancer Discov 8: 852-867] SWI/SNF chromatin remodeler [Miao et al. (2018) Science 359: 801-806; Pan et al. (2018) Science 359:

770-775], strongly indicated that epigenetic/chromatin regulators play critical roles in modulating antitumor immunity and tumor responses to immunotherapy, and represent a class of genes (consisting of ~600 genes) under-explored in this specific context.

5 To further characterize LSD1 as a potential target for priming “cold” tumors and enhancing the efficacy of PD-(L)1 blockade therapy, multiple additional pathways were investigated to determine whether they were essentially regulated by LSD1 and could affect LSD1 ablation-stimulated antitumor immunity. While T cell infiltration was elevated by LSD1 ablation, the cytotoxicity and proliferation of CD8⁺ tumor-infiltrating lymphocytes (TILs) remained unaltered in LSD1 null tumors, demonstrated by the unaltered expression of Granzyme B (GzmB) and Ki-67, even though the “cold” tumors were inflamed. Therefore, certain immune suppressive molecules derived from tumor cells could also be induced by LSD1 ablation, which might compromise the cytotoxicity of CD8⁺ TILs. The breakdown of the detrimental side of LSD1 inhibition could potentiate its immune stimulatory effect in cancer immunotherapy.

Non-limiting aspects of these methods are described below, and can be used in any combination without limitation. Additional aspects of these methods are known in the art.

20 ***Methods of Treatment***

Provided herein are methods of treating cancer in a patient. Exemplary methods include administering to a patient in need of cancer treatment therapeutically effective amounts of a lysine-specific demethylase 1A (LSD1) inhibitor, a transforming growth factor beta (TGFβ) inhibitor and a programmed-cell death 1 (PD-1) inhibitor or a programmed-cell death ligand 1 (PD-L1) inhibitor, or both, to thereby treat cancer in the patient. In some embodiments, a LSD1 inhibitor and a TGFβ inhibitor are administered to the subject.

Also provided herein are methods of treating cancer in a patient that include, e.g. administering to a patient in need of cancer treatment therapeutically effective amounts of a lysine-specific demethylase 1A (LSD1) inhibitor, a transforming growth factor beta (TGFβ) inhibitor and/or at least one immunotherapy (e.g., a PD-1 or PD-L1 inhibitor), to thereby treat cancer in the patient. In some embodiments, an effective amount of LSD1 inhibitor and an effective amount of TGFβ inhibitor are

administered to the subject. In some embodiments, an effective amount of LSD1 inhibitor and an effective amount of immunotherapy are administered to the subject. The effective amount is an amount or dosage sufficient to effect beneficial or desired results including halting, slowing, retarding, or inhibiting progression of a disease, e.g., a cancer. An effective amount will vary depending upon, e.g., an age and a body weight of a subject to which the agent is to be administered, a severity of symptoms and a route of administration, and thus administration can be determined on an individual basis. An effective amount can be administered in one or more administrations. By way of example, an effective amount is an amount sufficient to ameliorate, stop, stabilize, reverse, inhibit, slow and/or delay progression of a cancer in a patient or is an amount sufficient to ameliorate, stop, stabilize, reverse, slow and/or delay proliferation of a cell (e.g., a biopsied cell, any of the cancer cells described herein, or cell line (e.g., a cancer cell line)) in vitro. As is understood in the art, an effective amount of a therapeutic agent may vary, depending on, inter alia, patient history as well as other factors such as the type (and/or dosage) of the agent used.

In one aspect, the disclosure also provides methods of reducing the rate of the increase of volume of a tumor in a subject over time, methods of reducing the risk of developing a metastasis, or methods of reducing the risk of developing an additional metastasis in a subject. In some embodiments, the treatment can halt, slow, retard, or inhibit progression of a cancer. In some embodiments, the treatment can result in the reduction of in the number, severity, and/or duration of one or more symptoms of the cancer in a subject.

In some embodiments, the compositions and methods disclosed herein can be used for treatment of patients at risk for a cancer. Patients with cancer can be identified with various methods known in the art.

In methods described herein, the cancer can be, e.g., a primary tumor, a metastatic tumor, or a non-T-cell-infiltrating tumor.

In any of the presently-described methods, the cancer can be, e.g., melanoma, acute myeloid leukemia (AML), squamous cell carcinoma, renal cell carcinoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), gastric cancer, bladder cancer, kidney cancer, head and neck cancer, Ewing sarcoma, Hodgkin's lymphoma, Merkel cell carcinoma, breast cancer or prostate cancer. Treatment of

multiple cancer types at the same time is contemplated by and within the present disclosure.

A cancer described herein can be, e.g., a PD-1 and/or PD-L1 refractory or resistant cancer. In some instances, the patient having the cancer may have
5 previously received cancer treatment (e.g., any of the cancer treatment described herein).

Administering may be performed, e.g., at least once (e.g., at least 2-times, at least 3-times, at least 4-times, at least 5-times, at least 6-times, at least 7-times, at least
10 8-times, at least 9-times, at least 10-times, at least 11-times, at least 12-times, at least 13-times, or at least 14-times) a week. Also contemplated are monthly treatments, e.g. administering at least once, twice, three times, or four times per month for at least 1 month (e.g., at least two, three, four, five, or six or more months, e.g., 12 or more months), and yearly treatments (e.g., administration once a year for one or more years). Administration can be via any art-known means, e.g., intravenous,
15 subcutaneous, intraperitoneal, oral, and/or rectal administration, or any combination of known administration methods.

Administration can include administering compositions in any useful format. For example, skilled practitioners will appreciate that a number of compositions are within the present invention. One useful composition may be a combination
20 composition comprising an LSD1 inhibitor, a transforming growth factor beta (TGF β) inhibitor and a PD-1 and/or PD-L1 inhibitor. Such a combined composition can be administered to the patient in any useful dosing regimen. When using separate compositions, e.g., a first composition comprising an LSD1 inhibitor, a second composition comprising a transforming growth factor beta (TGF β) inhibitor and a
25 third composition comprising a PD-1 and/or PD-L1 inhibitor, the compositions can be administered in any order. For example, the first composition can be administered followed by administration of the second composition, and the third composition, the first composition can be administered followed by the third composition and the second composition; the second composition can be administered before the first
30 composition and before the third composition, the third composition can be administered before the first and/or the second composition, or the first, the second and the third compositions can be administered essentially simultaneously.

In one aspect of any of the methods described herein, a first composition comprising an LSD1 inhibitor is administered prior to the administration of a second composition comprising a transforming growth factor beta (TGF β) inhibitor, a PD-1 and/or PD-L1 inhibitor. For example, a patient can receive at least one dose (e.g., at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven doses, or at least twelve doses) of first composition comprising an LSD1 inhibitor prior to the administration of a second composition comprising a transforming growth factor beta (TGF β) inhibitor, a PD-1 and/or PD-L1 inhibitor.

In some embodiments, the second composition (e.g., TGF β inhibitor and/or immunotherapy) can be administered to the subject prior to, during, or after administering the first composition (e.g., LSD1 inhibitor). In some embodiments, the one or more additional therapeutic agents and the LSD1 inhibitor are administered to the subject such that there is an overlap in the bioactive period of the one or more additional therapeutic agents and the LSD1 inhibitor in the subject. In some embodiments, a third composition (e.g., an immunotherapy) can be administered to the subject prior to, during, or after administering the first or the second composition.

In one aspect of any of the methods described herein, a first composition comprising a transforming growth factor beta (TGF β) inhibitor is administered prior to the administration of a second composition comprising an LSD1 inhibitor, a PD-1 and/or PD-L1 inhibitor. For example, a patient can receive at least one dose (e.g., at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven doses, or at least twelve doses) of first composition comprising a TGF β inhibitor prior to the administration of a second composition comprising an LSD1 inhibitor, a PD-1 and/or PD-L1 inhibitor.

In one aspect of any of the methods described herein, a first composition comprising a transforming growth factor beta (TGF β) inhibitor is administered prior to the administration of a second composition comprising an LSD1 inhibitor, and prior to the administration of a third composition comprising a PD-1 and/or PD-L1 inhibitor. For example, a patient can receive at least one dose (e.g., at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven

doses, or at least twelve doses) of first composition comprising a TGF β inhibitor prior to the administration of a second composition comprising an LSD1 inhibitor, and can received at least one dose (e.g., at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven doses, or at least twelve doses) of the second composition comprising an LSD1 inhibitor prior to the administration of a third composition comprising a PD-1 and/or PD-L1 inhibitor.

In one aspect of any of the methods described herein, a first composition comprising an LSD1 inhibitor is administered prior to the administration of a second composition comprising a transforming growth factor beta (TGF β) inhibitor, and prior to the administration of a third composition comprising a PD-1 and/or PD-L1 inhibitor. For example, a patient can receive at least one dose (e.g., at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven doses, or at least twelve doses) of first composition comprising an LSD1 inhibitor prior to the administration of a second composition comprising a transforming growth factor beta (TGF β) inhibitor, and can received at least one dose (e.g., at least two doses, at least three doses, at least four doses, at least five doses, at least six doses, at least seven doses, at least eight doses, at least nine doses, at least ten doses, at least eleven doses, or at least twelve doses) of the second composition comprising a TGF β inhibitor prior to the administration of a third composition comprising a PD-1 and/or PD-L1 inhibitor.

As used herein, treating includes “prophylactic treatment”, which means reducing the incidence of or preventing (or reducing the risk of) a sign or symptom of a cancer in a patient at risk of developing a cancer. The term “therapeutic treatment” refers to reducing signs or symptoms of a cancer, reducing cancer progression, reducing severity of a cancer, and/or re-occurrence in a cancer patient.

The methods described herein can in some instances include administering a composition, e.g., a sterile composition, comprising an inhibitory nucleic acid that is complementary to LSD1, TGF β or PD-1 as described herein. A composition may include a LSD1 inhibitory nucleic acid, a TGF β inhibitory nucleic acid, or a PD-1 inhibitory nucleic acid, or both. Inhibitory nucleic acids for use in practicing the methods described herein are described below.

Inhibitory nucleic acids have been employed as therapeutic moieties in the treatment of disease states in subjects, including humans. Inhibitory nucleic acids can be useful therapeutic modalities that can be configured to be useful in treatment regimens for the treatment of cells, tissues and animals, especially humans.

5 For therapeutics, a subject, e.g., a human, having cancer or suspected of having cancer, or at increased risk of developing a cancer (e.g., by virtue of family history, genetic testing, or presence of other identified risk factor), can be treated by administering an inhibitory nucleic acid in accordance with this disclosure. For example, in one non-limiting embodiment, the methods comprise the step of
10 administering to the subject in need of treatment a therapeutically effective amount of one or more of a LSD1 inhibitory nucleic acid (e.g., a LSD1 antisense molecule, a LSD1 small interfering RNA, a LSD1 small hairpin RNA), a TGF β inhibitory nucleic acid (e.g., a TGF β antisense molecule, a TGF β small interfering RNA, a TGF β small hairpin RNA), a PD-1 inhibitory nucleic acid (e.g., a PD-1 antisense molecule, a PD-1
15 small interfering RNA, a PD-1 small hairpin RNA) or a PD-L1 inhibitory nucleic acid (e.g., a PD-L1 antisense molecule, a PD-L1 small interfering RNA, a PD-L1 small hairpin RNA) as described herein.

In some embodiments, one or more additional therapeutic agents can be administered to the subject. The additional therapeutic agent can comprise one or
20 more inhibitors selected from the group consisting of an inhibitor of B-Raf, an EGFR inhibitor, an inhibitor of a MEK, an inhibitor of ERK, an inhibitor of K-Ras, an inhibitor of c-Met, an inhibitor of anaplastic lymphoma kinase (ALK), an inhibitor of a phosphatidylinositol 3-kinase (PI3K), an inhibitor of an Akt, an inhibitor of mTOR, a dual PI3K/mTOR inhibitor, an inhibitor of Bruton's tyrosine kinase (BTK), and an
25 inhibitor of Isocitrate dehydrogenase 1 (IDH1) and/or Isocitrate dehydrogenase 2 (IDH2). In some embodiments, the additional therapeutic agent is an inhibitor of indoleamine 2,3-dioxygenase-1 (IDO1) (e.g., epacadostat).

In some embodiments, the additional therapeutic agent can comprise one or more inhibitors selected from the group consisting of an inhibitor of HER3, an
30 inhibitor of MDM2, an inhibitor of BCL2, an inhibitor of CHK1, an inhibitor of activated hedgehog signaling pathway, and an agent that selectively degrades the estrogen receptor.

In some embodiments, the additional therapeutic agent can comprise one or more therapeutic agents selected from the group consisting of Trabectedin, nab-paclitaxel, Trebananib, Pazopanib, Cediranib, Palbociclib, everolimus, fluoropyrimidine, IFL, regorafenib, Reolysin, Alimta, Zykadia, Sutent, temsirolimus, axitinib, everolimus, sorafenib, Votrient, Pazopanib, IMA-901, AGS-003, cabozantinib, Vinflunine, an Hsp90 inhibitor, Ad-GM-CSF, Temazolomide, IL-2, IFN α , vinblastine, Thalomid, dacarbazine, cyclophosphamide, lenalidomide, azacytidine, lenalidomide, bortezomid, amrubicine, carfilzomib, pralatrexate, and enzastaurin.

In some embodiments, the additional therapeutic agent can comprise one or more therapeutic agents selected from the group consisting of an adjuvant, a TLR agonist, tumor necrosis factor (TNF) α , IL-1, HMGB1, an IL-10 antagonist, an IL-4 antagonist, an IL-13 antagonist, an IL-17 antagonist, an HVEM antagonist, an ICOS agonist, a treatment targeting CX3CL1, a treatment targeting CXCL9, a treatment targeting CXCL10, a treatment targeting CCL5, an LFA-1 agonist, an ICAM1 agonist, and a Selectin agonist.

In some embodiments, carboplatin, nab-paclitaxel, paclitaxel, cisplatin, pemetrexed, or gemcitabine is administered to the subject.

Immunotherapy

An immunotherapy can be administered to the patient in methods described herein. The term “immunotherapy” refers to a therapeutic treatment that involves administering to a patient an agent that modulates the immune system. For example, an immunotherapy can increase the expression and/or activity of a regulator of the immune system. In other instances, an immunotherapy can decrease the expression and/or activity of a regulator of the immune system. In some instances, an immunotherapy can recruit and/or enhance the activity of an immune cell. An example of an immunotherapy is a therapeutic treatment that involves administering at least one, e.g., two or more, immune checkpoint inhibitors. Exemplary immune checkpoint inhibitors useful in the presently-described methods are CTLA-4 inhibitors, PD-1 inhibitors, PD-L1 inhibitors, PD-L2 inhibitors, OX40 inhibitor, TIM3 inhibitors, or LAG3 inhibitors, or combinations thereof.

The immunotherapy can be a cellular immunotherapy (e.g., adoptive T-cell therapy, dendritic cell therapy, natural killer cell therapy). For example, the cellular

immunotherapy can be sipuleucel-T (APC8015; Provenge™; Plosker (2011) Drugs 71(1): 101-108). In some instances, the cellular immunotherapy includes cells that express a chimeric antigen receptor (CAR). In some instances, the cellular immunotherapy can be a CAR-T cell therapy, e.g., tisagenlecleucel (Kymriah™).

5 Immunotherapy can be, e.g., an antibody therapy (e.g., a monoclonal antibody, a conjugated antibody). In some embodiments, the antibody is an anti-CTLA-4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-OX40 antibody, an anti-TIM3 antibody, or an anti-LAG3 antibody. Exemplary antibody therapies are bevacizumab (Mvasti™, Avastin®), trastuzumab
 10 (Herceptin®), avelumab (Bavencio®), rituximab (MabThera™, Rituxan®), edrecolomab (Panorex), daratumumab (Darzalex®), olaratumab (Lartruvo™), ofatumumab (Arzerra®), alemtuzumab (Campath®), cetuximab (Erbix®), oregovomab, pembrolizumab (Keytruda®), dinutuximab (Unituxin®), obinutuzumab (Gazyva®), tremelimumab (CP-675,206), ramucirumab (Cyramza®), ublituximab
 15 (TG-1101), panitumumab (Vectibix®), elotuzumab (Empliciti™), avelumab (Bavencio®), necitumumab (Portrazza™), cirmtuzumab (UC-961), ibritumomab (Zevalin®), isatuximab (SAR650984), nimotuzumab, fresolimumab (GC1008), lirilumab (INN), mogamulizumab (Poteligeo®), ficlatuzumab (AV-299), denosumab (Xgeva®), ganitumab, urelumab, pidilizumab or amatuximab.

20 An immunotherapy described herein can involve administering an antibody-drug conjugate to a patient. The antibody-drug conjugate can be, e.g., gemtuzumab ozogamicin (Mylotarg™), inotuzumab ozogamicin (Besponsa®), brentuximab vedotin (Adcetris®), ado-trastuzumab emtansine (TDM-1; Kadcyla®), mirvetuximab soravtansine (IMGN853) or anetumab ravtansine.

25 In some instances, the immunotherapy includes blinatumomab (AMG103; Blincyto®) or midostaurin (Rydapt).

An immunotherapy can include administering to the patient a toxin. For example, the immunotherapy can including administering denileukin diftitox (Ontak®).

30 In some instances, the immunotherapy can be a cytokine therapy. The cytokine therapy can be, e.g., an interleukin 2 (IL-2) therapy, an interferon alpha (IFN-α) therapy, a granulocyte colony stimulating factor (G-CSF) therapy, an interleukin 12 (IL-12) therapy, an interleukin 15 (IL-15) therapy, an interleukin 7 (IL-

7) therapy or an erythropoietin-alpha (EPO) therapy. In some embodiments, the IL-2 therapy is aldesleukin (Proleukin®). In some embodiments, the IFN- α therapy is IntronA® (Roferon-A®). In some embodiments, the G-CSF therapy is filgrastim (Neupogen®).

5 In some instances, the immunotherapy is an immune checkpoint inhibitor. For example, the immunotherapy can include administering one or more immune checkpoint inhibitors. In some embodiments, the immune checkpoint inhibitor is a CTLA-4 inhibitor, a PD-1 inhibitor or a PD-L1 inhibitor. An exemplary CTLA-4 inhibitor would be, e.g., ipilimumab (Yervoy®) or tremelimumab (CP-675,206). In
10 some embodiments, the PD-1 inhibitor is pembrolizumab (Keytruda®) or nivolumab (Opdivo®). In some embodiments, the PD-L1 inhibitor is atezolizumab (Tecentriq®), avelumab (Bavencio®) or durvalumab (Imfinzi™).

In some instances, the immunotherapy is mRNA-based immunotherapy. For example, the mRNA-based immunotherapy can be CV9104 (see, e.g., Rausch et al.
15 (2014) Human Vaccin Immunother 10(11): 3146-52; and Kubler et al. (2015) J. Immunother Cancer 3:26).

In some instances, the immunotherapy can involve bacillus Calmette-Guerin (BCG) therapy.

In some instances, the immunotherapy can be an oncolytic virus therapy. For
20 example, the oncolytic virus therapy can involve administering talimogene alherparepvec (T-VEC; Imlygic®).

In some instances, the immunotherapy is a cancer vaccine, e.g., a human papillomavirus (HPV) vaccine. For example, an HPV vaccine can be Gardasil®, Gardasil9® or Cervarix®. In some instances, the cancer vaccine is a hepatitis B virus
25 (HBV) vaccine. In some embodiments, the HBV vaccine is Engerix-B®, Recombivax HB® or GI-13020 (Tarmogen®). In some embodiments, the cancer vaccine is Twinrix® or Pediarix®. In some embodiments, the cancer vaccine is BiovaxID®, Oncophage®, GVAX, ADXS11-001, ALVAC-CEA, PROSTVAC®, Rindopepimut®, CimaVax-EGF, lapuleucel-T (APC8024; Neuvenge™), GRNVAC1,
30 GRNVAC2, GRN-1201, hepcortespenlisimut-L (Hepko-V5), DCVAX®, SCIB1, BMT CTN 1401, PrCa VBIR, PANVAC, ProstAtak®, DPX-Survivac, or viagenpumatucl-L (HS-110).

The immunotherapy can involve, e.g., administering a peptide vaccine. For example, the peptide vaccine can be nelipepimut-S (E75) (NeuVax™), IMA901, or SurVaxM (SVN53-67). In some instances, the cancer vaccine is an immunogenic personal neoantigen vaccine (see, e.g., Ott et al. (2017) Nature 547: 217-221; Sahin et al. (2017) Nature 547: 222-226). In some embodiments, the cancer vaccine is RGS4K, or NEO-PV-01. In some embodiments, the cancer vaccine is a DNA-based vaccine. In some embodiments, the DNA-based vaccine is a mammaglobin-A DNA vaccine (see, e.g., Kim et al. (2016) OncoImmunology 5(2): e1069940).

10 *Cancer*

The methods described herein can be used in cancer treatments. Non-limiting examples of cancer that can be treated by the methods described herein include: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adrenocortical carcinoma, anal cancer, appendix cancer, astrocytoma, basal cell carcinoma, brain tumor, bile duct cancer, bladder cancer, bone cancer, breast cancer, bronchial tumor, Burkitt Lymphoma, carcinoma of unknown primary origin, cardiac tumor, cervical cancer, chordoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloproliferative neoplasm, colon cancer, colorectal cancer, craniopharyngioma, cutaneous T-cell lymphoma, ductal carcinoma, embryonal tumor, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, fibrous histiocytoma, Ewing sarcoma, eye cancer, germ cell tumor, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gestational trophoblastic disease, glioma, head and neck cancer, hairy cell leukemia, hepatocellular cancer, histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, islet cell tumor, Kaposi sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, leukemia, lip and oral cavity cancer, liver cancer, lobular carcinoma in situ, lung cancer, lymphoma, macroglobulinemia, malignant fibrous histiocytoma, melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, midline tract carcinoma involving NUT gene, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma, mycosis fungoides, myelodysplastic syndrome, myelodysplastic/myeloproliferative neoplasm, nasal cavity and para-nasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, non-

small cell lung cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytomas, pituitary tumor, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell cancer, renal pelvis and ureter cancer, retinoblastoma, rhabdoid tumor, salivary gland cancer, Sezary syndrome, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, spinal cord tumor, stomach cancer, T-cell lymphoma, teratoid tumor, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, and Wilms' tumor.

For example, any of the methods described herein can be used to treat a cancer selected from the group consisting of: melanoma, acute myeloid leukemia (AML), squamous cell carcinoma, renal cell carcinoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), gastric cancer, bladder cancer, kidney cancer, head and neck cancer, Ewing sarcoma, Hodgkin's lymphoma, Merkel cell carcinoma, breast cancer and prostate cancer.

In some embodiments, the cancer is a solid tumor (e.g., breast cancer or kidney cancer). In some embodiments, the cancer is melanoma.

In some embodiments, the cancer does not respond to or is resistant to an immunotherapy (e.g., anti-PD1 antibody) or a chemotherapy. Thus, in one aspect, the disclosure provides a method of treating cancer, involve determining that the subject is resistant to an immunotherapy or a chemotherapy.

LSD1 inhibitor

As used herein, the term "LSD1 inhibitor" refers to a therapeutic agent that reduces, decreases, blocks or inhibits the expression or activity of LSD1. For example, the LSD1 inhibitor can block or disrupt the catalytic active site of LSD1. The LSD1 inhibitor can be, e.g., a selective LSD1 inhibitor or a non-selective LSD1 inhibitor.

The LSD1 inhibitor can be a small molecule, an antibody, or an inhibitory nucleic acid. A non-exhaustive list of small molecule LSD1 inhibitors is provided in Table 1.

Table 1. Exemplary list of small molecule LSD1 inhibitors

Chemical name	Generic name or name as used in clinical trials	CAS#
trans-2-Phenylcyclopropylamine hemisulfate salt	Tranylcypromine	13492-01-8
1-(4-methylpiperazin-1-yl)-2-[[2-(4-phenylmethoxyphenyl)cyclopropyl]amino]ethanone	RN 1 dihydrochloride	1781835-13-9
rel- N-[(1R,2S)-2-Phenylcyclopropyl]-4-Piperidinamine hydrochloride (1:2)	GSK-LSD1	1431368-48-7
4-[[4-[[[(1R,2S)-2-phenylcyclopropyl]amino]methyl]-1-piperidinyl]methyl]-benzoic acid	GSK2879552	1401966-69-5
rel-N1-[(1R,2S)-2-phenylcyclopropyl]-1,4-cyclohexanediamine, dihydrochloride	ORY1001	1431326-61-2
(R)-4-[5-(Pyrrolidin-3-ylmethoxy)-2-p-tolylpyridin-3-yl]-benzonitrile Bis-TFA Salt	GSK690	2101305-84-2
3-Chloro-6-nitro-2-(trifluoromethyl)-4H-1-benzopyran-4-one	Namoline	342795-11-3
1,15-bis{N5-[3,3-(diphenyl)propyl]-N1-biguanido}-4,12-diazapentadecane	Cpd 2d	
(1R,2S)-rel-2-[3,5-Difluoro-2-(phenylmethoxy)phenyl]cyclopropanamine hydrochloride	S2101	1239262-36-2
4'-[(1R,2S)-2-aminocyclopropyl]-[1,1'-biphenyl]-3-ol	OG-L002	1357302-64-7
3-(4-morpholinylsulfonyl)-benzoic acid (2E)-2-[1-(5-chloro-2-hydroxyphenyl)ethylidene]hydrazide	SP2509	1423715-09-6
Methyl-3-(4-(4-carbamimidoylbenzoyl)piperazine-1-carbonyl)-5-((4-carbamimidoylpiperazin-1-yl)methyl)benzoate	CBB1007	1379573-92-8
N-[(2S)-5-[[[(1R, 2S)-2-(4-fluorophenyl)cyclopropyl]amino]-1-(4-methylpiperazin-1-yl)-1-oxopentan-2-yl]-4-(1H-1,2,3-triazol-1-yl)benzamide, bis-tosylate salt	IMG-7289	

In some instances, the LSD1 inhibitor is an inhibitory nucleic acid.

SEQ ID NO: 1 is an exemplary human sequence of LSD1:

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5      a  tgttatctgg gaagaaggcg gcagccgcgg
      cggcggcggc tgcagcggca gcaaccggga cggaggctgg ccctgggaca gcaggcggct

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ccgagaacgg gtctgaggtg gccgcgcagc ccgcgggcct gtcggggcca gccgaggtcg
 ggccggggggc ggtgggggag cgcacacccc gcaagaaaga gcctccgcgg gcctcgcccc
 ccggggggcct ggcggaaccg ccgggggtccg cagggcctca ggccggccct actgtcgtgc
 ctgggtctgc gacccccatg gaaactggaa tagcagagac tccggagggg cgtcggacca
 5 gccggcgcaa gcgggcgaag gtagagtaca gagagatgga tgaaagcttg gccaacctct
 cagaagatga gtattattca gaagaagaga gaaatgccaa agcagagaag gaaaagaagc
 tccccccacc accccctcaa gcccacctg aggaagaaaa tgaaagttag cctgaagaac
 catcggggca agcaggagga cttcaagacg acagttcttg agggtaggga gacggccaag
 catcaggtgt ggagggcgca gctttccaga gccgacttcc tcatgaccgg atgacttctc
 10 aagaagcagc ctgttttcca gatattatca gtggaccaca acagaccagc aagggtttttc
 ttttcttagg aaaccgcaca ctgcagttgt ggttgataa tccaaagatt cagctgacat
 ttgaggctac tctccaacaa ttagaagcac cttataacag tgatactgtg cttgtccacc
 gagttcacag ttattttagag cgtcatggtc ttatcaactt cggcatctat aagaggataa
 aacccttacc aactaaaaag acaggaaagg taattattat aggctctggg gtctcaggct
 15 tggcagcagc tcgacagtta caaagttttg gaatggatgt cacacttttg gaagccaggg
 atcgtgtggg tggacgagtt gccacatttc gcaaaggaaa ctatgtagct gatcttgag
 ccatggtggt aacaggtctt ggaggggaatc ctatggctgt ggtcagcaaa caagtaaata
 tggaactggc caagatcaag caaaaatgcc cactttatga agccaacgga caagctgaca
 ctgtcaaggt tcctaaagag aaagatgaaa tggtagagca agagttaaac cggttgctag
 20 aagctacatc ttaccttagt catcaactag acttcaatgt cctcaataat aagcctgtgt
 cccttgcca ggcatggaa gttgtcattc agttacaaga gaagcatgtc aaagatgagc
 agattgaaca ttggaagaag atagtgaaaa ctcaggaaga attgaaagaa cttcttaata
 agatggtaaa tttgaaagag aaaattaaag aactccatca gcaatacaaa gaagcatctg
 aagtaaagcc acccagagat attactgccg agttcttagt gaaaagcaaa cacagggatc
 25 tgaccgccct atgcaaggaa tatgatgaat tagctgaaac acaaggaaa ctagaagaaa
 aacttcagga gttggaagcg aatcccccaa gtgatgtata tctctcatca agagacagac
 aaatacttga ttggcatttt gcaaactctg aatttgctaa tgccacacct ctctcaactc
 tctcccttaa gactgggat caggatgatg actttgagtt cactggcagc cacctgacag
 taaggaatgg ctactcgtgt gtgcctgtgg ctttagcaga aggcctagac attaaactga
 30 atacagcagt gcgacaggtt cgctacacgg cttcaggatg tgaagtata gctgtgaata
 ccgctccac gactcaaac tttatttata aatgcgacgc agttctctgt acccttcccc
 tgggtgtgct gaagcagcag ccaccagccg ttcagtttgt gccacctctc cctgagtgga
 aaacatctgc agtccaaagg atgggatttg gcaaccttaa caagggtgtg ttgtgttttg
 atcgggtgtt ctgggatcca agtgtcaatt tgttcgggca tgttggcagt acgactgcca
 35 gcaggggtga gctcttctc tcttggaacc tctataaagc tccaatactg ttggcactag
 tggcaggaga agctgctggt atcatggaaa acataagtga cgatgtgatt gttggccgat
 gcctggccat tctcaaaggg attttttgta gcagtgcagt acctcagccc aaagaaactg
 tgggtgtctc ttggcgtgct gatccctggg ctcggggctc ttattcctat gttgctgcag
 gatcatctgg aaatgactat gatttaaatg ctcagccaat cactcctggc ccctcgattc
 40 caggtgcccc acagccgatt ccacgactct tctttgcggg agaacatacg atccgtaact
 acccagccac agtgcattgt gctctgctga gtgggctgcg agaagcggga agaattgcag

accagttttt gggggccatg tatacgtgc ctcgccaggc cacaccaggt gttcctgcac
agcagtcctc aagcatgtga

(SEQ ID NO: 1; derived from NCBI Accession Number: NM_001009999.2)

5 Inhibitory nucleic acids useful in the present methods and compositions include those that are designed to inhibit LSD1. SEQ ID NO: 2 is an exemplary shRNA sequence that targets human LSD1:

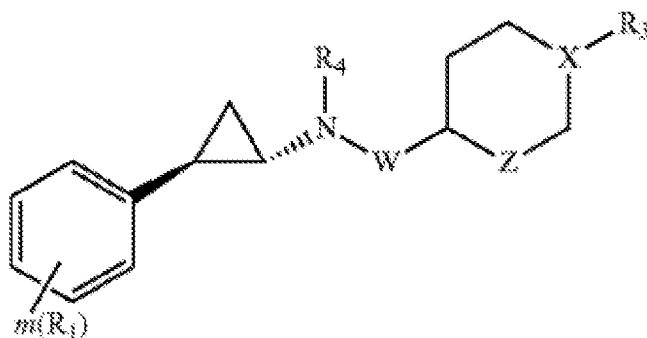
5'- CCGG-GCCTAGACATTAAACTGAATA-CTCGAG-
TATTCAGTTTAATGTCTAGGC-TTTTTG -3' (SEQ ID NO: 2).

10 Bold and underlined portions are targeting/matching sequences in human LSD1 mRNA.

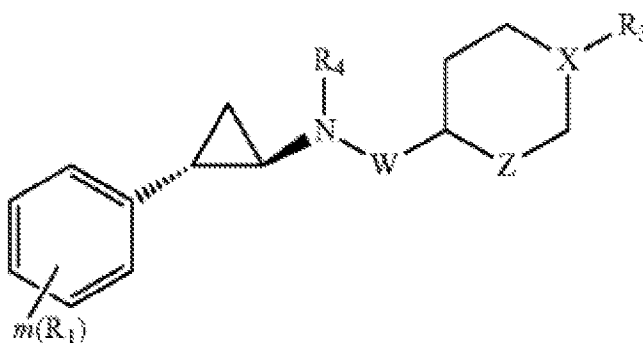
The LSD1 inhibitory nucleic acid can, e.g., comprise SEQ ID NO: 2. For example, the LSD1 inhibitory nucleic acid can be a nucleic acid comprising a sequence that is complementary to a contiguous sequence of at least 5 (e.g., at least 6,
15 at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22) nucleotides present in SEQ ID NO: 2.

The LSD1 inhibitory nucleic acid can be any LSD1 inhibitory nucleic acid that
20 decreases, reduces or silences the expression and/or activity of LSD1. As shown herein, loss of LSD1 increased the expression of HERV-E, HERV-K, HML-2, ERVL, IFN- α , IFN- β , IL-28, ISG15, OASL, RIG-I, TLR3, and MDA-5. In some embodiments, the LSD1 inhibitory nucleic acid is any LSD1 inhibitory nucleic acid that increases or upregulates the expression and/or activity of HERV-E, HERV-K,
25 HML-2, ERVL, IFN- α , IFN- β , IL-28, ISG15, OASL, RIG-I, TLR3, and/or MDA-5.

In some embodiments, the LSD1 inhibitor can be a compound having the structure of Formula I, or Formula II, or a pharmaceutically acceptable salt thereof:



(Formula I)



(Formula II)

5

Wherein R_1 is selected from the group consisting of C_1 - C_6 alkyl, $-\text{NHSO}_2\text{Me}$, $-\text{NHSO}_2\text{Ph}$, arylalkoxy, C_3 - C_7 cycloalkyl, $-\text{NHC(O)}R_a$, 1-methyl-1H-pyrazol-4-yl, hydroxyl, C_1 - C_4 alkoxy, halogen, amino, substituted amino, and $-\text{C(O)}\text{OR}_a$;

R_3 is selected from the group consisting of aryl, heteroaryl $-\text{SO}_2R_a$, $-\text{NHC(O)}R_a$, $-\text{CH}_2\text{C(O)}\text{OR}_a$, $-\text{C(O)}\text{OR}_a$, $-\text{C(O)}R_a$, $-\text{C(O)}\text{NR}_aR_b$, amino, substituted amino, arylalkyl, and heteroarylalkyl;

each R_a is independently hydrogen, phenyl, phenylmethyl, 3,5-dimethylisoxazol-4-yl, 1,2-dimethyl-1H-imidazol-4-yl, C_3 - C_7 cycloalkyl, or C_1 - C_6 alkyl;

R_b is hydrogen or C_1 - C_3 alkyl; or

R_a and R_b together form a 5- or 6-membered heterocycloalkyl ring;

R_4 is H;

W is $-(\text{CH}_2)_{1-4}$ or $-\text{CH}(R_c)(\text{CH}_2)_{0-3}$, in which R_c is $-\text{CN}$ or C_1 - C_4 alkyl;

X is N;

Z is $(CH_2)_q$, wherein q is 0-2, and wherein when q is 0, Z represents a bond; and m is 0-3; or a pharmaceutically acceptable salt thereof. A detailed description regarding these LSD1 inhibitors can be found, e.g., in US Patent No. 9346840, which is incorporated herein by reference in its entirety.

5 In some embodiments, the LSD1 inhibitor is an LSD1 inhibitor known in the art, e.g., in US 20150225401, US 20170129857, US20170281567, US20170281566, US20170183308, US20170283397, US20170209432, US20170044101, US 9493442, US 9346840, WO/2016/007736, WO/2016/161282, US 20160009711, and Fu et al., *Advances toward LSD1 inhibitors for cancer therapy, Future Medicinal Chemistry*,
10 vol. 9, no. 11 (2017); each of which is incorporated herein by reference in its entirety.

Transforming Growth Factor Beta (TGF β)

As used herein, the term “TGF β inhibitor” refers to a therapeutic agent that reduces, decreases, blocks or inhibits the expression or activity of TGF β . The TGF β inhibitor can be, e.g., a selective TGF β inhibitor or a non-selective TGF β inhibitor. In
15 some embodiments of any of the methods described herein, the TGF β inhibitor is a TGF-beta1 inhibitor, a TGF-beta2 inhibitor, or both.

The TGF β inhibitor can be a small molecule, an antibody, an inhibitory nucleic acid or a vaccine. See, e.g., U.S. US 6,509,318; 7,872,020. A non-exhaustive list of TGF β inhibitors is provided in Table 2.

20

Table 2. Exemplary list of TGF β inhibitors

Inhibitor name	Generic name or name used in clinical trials	CAS#
AVID200		
GC1008	Fresolimumab	
LY3200882		1898283-02-7
M7824		
AP12009	Trabedersen	
Lucanix	Belagenpumatucel-L	
Vigil	Gemogenovatucel-T	
LY2157299	Galunisertib	700874-72-2

Numerous anti- TGF β antibodies are known in the art, and are described, e.g., in US 7,527,791; US 5,772,998; US 2018/051075; and US 2018/086764; each of
25 which is incorporated herein by reference in its entirety.

In some instances, the TGF β inhibitor is an inhibitory nucleic acid.

SEQ ID NO: 158 is an exemplary human sequence of TGFβ:

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atgccgccctc cgggctgctg ctgctgccgc tgctgctacc gctgctgtgg ctactggtgc
tgacgcctgg ccggccggcc ggggactat ccacctgcaa gactatcgac atggagctgg
tgaagcggaa gcgcacgag gccatccgcg gccagatcct gtccaagctg cggctcgcca
5 gccccccgag ccagggggag gtgccgcccg gcccgtgcc cgaggccgtg ctgcctgt
acaacagcac ccgcgaccgg gtggccgggg agagtgcaga accggagccc gagcctgagg
ccgactacta cgccaaggag gtcaccgcg tgctaattgt ggaaaccac aacgaaatct
atgacaagtt caagcagagt acacacagca tatatatgtt cttcaacaca tcagagctcc
gagaagcggg acctgaacct gtgttgctct cccgggcaga gctgcgtctg ctgaggctca
10 agttaaaagt ggagcagcac gtggagctgt accagaaata cagcaacaat tcctggcgat
acctcagcaa ccggtgctg gcaccagcg actcgccaga gtggttatct tttgatgtca
ccggagttgt gcggcagtg ttgagccgtg gaggggaaat tgagggcttt cgccttagcg
cccactgctc ctgtgacagc agggataaca cactgcaagt ggacatcaac gggttcacta
ccggccgccc aggtgacctg gccaccattc atggcatgaa ccggcctttc ctgcttctca
15 tggccacccc gctggagagg gcccagcatc tgcaaagctc ccggcaccgc cgagccctgg
acaccaacta ttgcttcagc tccacggaga agaactgctg cgtgcggcag ctgtacattg
acttccgcaa ggacctcggc tggaagtgga tccacgagcc caagggctac catgccaact
tctgcctcgg gccctgcccc tacatttggg gcctggacac gcagtacagc aaggtcctgg
ccctgtacaa ccagcataac ccgggcgcct cggcggcgcc gtgctgcgtg ccgcaggcgc
20 tggagccgct gccatcgtg tactacgtgg gccgcaagcc caaggtggag cagctgtcca
acatgatcgt gcgtcctgc aagtgcagct ga

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(SEQ ID NO: 158; derived from NCBI Accession Number NM_000660.6)

Inhibitory nucleic acids useful in the present methods and compositions include those that are designed to inhibit TGFβ. SEQ ID NO: 159 is an exemplary siRNA sequence that targets human TGFβ: 5'-GCAGAGTACACACAGCATA-3' (SEQ ID NO: 159).

The TGFβ inhibitory nucleic acid can, e.g., comprise SEQ ID NO: 159. For example, the TGFβ inhibitory nucleic acid can be a nucleic acid comprising a sequence that is complementary to a contiguous sequence of at least 5 (e.g., at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22) nucleotides present in SEQ ID NO: 159.

In some embodiments, the TGFβ inhibitor is a TGFβ trap. A TGFβ trap is a polypeptide that can bind to TGFβ and prevent TGFβ from binding to its receptor. In some embodiments, the TGFβ trap is the extracellular domain of human TGFβRII. In some embodiments, the TGFβ trap is AVID200 (O'Connor-McCourt, et al.

"AVID200, a highly potent TGF-beta trap, exhibits optimal isoform selectivity for enhancing anti-tumor T-cell activity, without promoting metastasis or cardiotoxicity." (2018): 1759-1759), a protein having the endoglin (E) domain of TβRIII flanked by the extracellular domain of TβRII (R) (e.g., RER) (Zhu et al. "A Novel TGFβ Trap Blocks Chemotherapeutics-Induced TGFβ1 Signaling and Enhances Their Anticancer Activity in Gynecologic Cancers." Clinical Cancer Research 24.12 (2018): 2780-2793), or a bifunctional anti-PD-L1/TGFβ Trap fusion protein (Knudson, et al. "M7824, a novel bifunctional anti-PD-L1/TGFβ Trap fusion protein, promotes anti-tumor efficacy as monotherapy and in combination with vaccine." Oncoimmunology 7.5 (2018): e1426519).

PD-1 inhibitor

In some instances, the PD-1 inhibitor is a small molecule, an antibody or an inhibitory nucleic acid. In some embodiments, the PD-1 antibody can, e.g., be selected from the group consisting of: nivolumab (Opdivo®) and pembrolizumab (Keytruda®). Numerous anti-PD-1 antibodies are known in the art, and are described, e.g., in US 9771425, US20170240635, US20180030137, US 9914783, US20160362489, US 9084776, US 9102727, US 9492540; each of which is incorporated herein by reference in its entirety.

In some instances, the PD-1 inhibitor is an inhibitory nucleic acid.

SEQ ID NO: 3 is an exemplary human sequence of PD-1:

```

at gcagatccca caggcgccct ggccagtcgt ctggggcggg ctacaactgg
gctggcgggc aggatggttc ttagactccc cagacaggcc ctggaacccc cccaccttct
ccccagccct gctcgtggtg accgaagggg acaacgccac cttcacctgc agcttctcca
acacatcgga gagcttcgtg ctaaactggt accgcatgag cccagcaac cagacggaca
agctggccgc cttccccgag gaccgcagcc agcccgcca ggactgccgc ttcgtgtca
cacaactgcc caacgggcgt gacttcaca tgagcgtggt cagggcccg cgcaatgaca
gcggcaccta cctctgtggg gccatctccc tggccccaa ggcgcagatc aaagagagcc
tgcgggcaga gctcaggggtg acagagagaa gggcagaagt gccacagcc caccacagcc
cctcaccag gccagccggc cagttccaaa ccctgggtggt tgggtgtcgt ggcggcctgc
tgggcagcct ggtgctgcta gtctgggtcc tggcgcgtcat ctgctcccg gccgcagag
ggacaatagg agccaggcgc accggccagc ccctgaagga ggacccctca gccgtgcctg
tggttctctgt ggactatggg gagctggatt tccagtggcg agagaagacc ccggagcccc
ccgtgccctg tgtccctgag cagacggagt atgccacat tgtctttcct agcggaatgg
gcacctcatc ccccgccgc aggggctcag ctgacggccc tcggagtgcc cagccactga
ggcctgagga tggacactgc tcttgcccc tctga

```

(SEQ ID NO: 3; derived from NCBI Accession Number NM_005018.2)

Inhibitory nucleic acids useful in the present methods and compositions include those that are designed to inhibit PD-1. SEQ ID NO: 4 is an exemplary shRNA sequence that targets human PD-1:

5 5'- CCGG-CATTGTCTTTCCTAGCGGAAT-CTCGAG-
ATTCCGCTAGGAAAGACAATG-TTTTTG -3' (SEQ ID NO: 4). Bold and underlined portions are targeting/matching sequences in human PD-1 mRNA.

The PD-1 inhibitory nucleic acid can, e.g., comprise SEQ ID NO: 4. For example, the PD-1 inhibitory nucleic acid can be a nucleic acid comprising a
 10 sequence that is complementary to a contiguous sequence of at least 5 (e.g., at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22) nucleotides present in SEQ ID NO: 4.

15

PD-L1 inhibitor

The PD-L1 inhibitor can be, e.g., a small molecule, an antibody or an inhibitory nucleic acid.

For example, PD-L1 antibodies useful in the presently described methods
 20 include durvalumab (Imfinzi™), atezolizumab (Tecentriq®) and avelumab (Bavencio®). Numerous anti-PD-L1 antibodies are known in the art, and are described, e.g., in US 9789183, US20170319690, US 9624298, US20100086550, US 8617546, US20180079814; each of which is incorporated herein by reference in its entirety.

25 In some instances, the PD-L1 inhibitor is an inhibitory nucleic acid.

SEQ ID NO: 5 is an exemplary human sequence of PD-L1:

at gaggatattt
 gctgtcttta tattcatgac ctactggcat ttgctgaacg catttactgt cacgggtccc
 aaggacctat atgtggtaga gtatggtagc aatatgacaa ttgaatgcaa attcccagta
 30 gaaaaacaat tagacctggc tgcactaatt gtctattggg aaatggagga taagaacatt
 attcaatttg tgcattggaga ggaagacctg aagggttcagc atagtagcta cagacagagg
 gcccggtgtg tgaaggacca gctctccctg ggaaatgctg cacttcagat cacagatgtg
 aaattgcagg atgcaggggt gtaccgctgc atgatcagct atggtggtgc cgactacaag
 cgaattactg tgaaagtcaa tgccccatac aacaaaatca accaaagaat tttggttgtg
 35 gatccagtca cctctgaaca tgaactgaca tgtcaggctg agggctaccc caaggccgaa

gtcatctgga caagcagtga ccatcaagtc ctgagtggta agaccaccac caccaattcc
aagagagagg agaagctttt caatgtgacc agcacactga gaatcaacac aacaactaat
gagattttct actgcacttt taggagatta gatcctgagg aaaaccatac agctgaattg
gtcatcccag aactacctct ggcacatcct ccaaataaaa ggactcactt ggtaattctg
5 ggagccatct tattatgcct tgggtgtagca ctgacattca tcttccgttt aagaaaaggg
agaatgatgg atgtgaaaaa atgtggcatc caagatacaa actcaaagaa gcaaagtgat
acacatttgg aggagacgta a

(SEQ ID NO: 5; derived from NCBI Accession Number NM_014143.3)

10 Inhibitory nucleic acids useful in the present methods and compositions
include those that are designed to inhibit PD-L1.

SEQ ID NO: 6 is an exemplary shRNA sequence that targets human PD-L1:
5'- CCGG-CTGACATTCATCTTCCGTTTA-CTCGAG-
TAAACGGAAGATGAATGTCAG-TTTTTG -3' (SEQ ID NO: 6). Bold and
15 underlined portions are targeting/matching sequences in human PD-L1 mRNA.

In some embodiments, the PD-L1 inhibitory nucleic acid comprises SEQ ID
NO: 6. In some embodiments, the PD-L1 inhibitory nucleic acid is a nucleic acid
comprising a sequence that is complementary to a contiguous sequence of at least 5
(e.g., at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at
20 least, 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least
20, at least 21, e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22)
nucleotides present in SEQ ID NO: 6.

The PD-L1 inhibitory nucleic acid can be any PD-L1 inhibitory nucleic acid
that decreases, reduces or silences the expression and/or activity of PD-L1. For
25 example, the PD-L-1 inhibitory nucleic acid can be any PD-L1 inhibitory nucleic acid
that decreases, reduces or silences the expression and/or activity of PD-L1.

Inhibitory Nucleic Acids

The present disclosure also provides inhibitory nucleic acids for various
targets described herein, e.g., LSD1, TGF β , PD-1, PD-L1, and immune checkpoints.

30 Inhibitory nucleic acids useful in the present methods and compositions
include antisense oligonucleotides, ribozymes, external guide sequence (EGS)
oligonucleotides, siRNA compounds, single- or double-stranded RNA interference
(RNAi) compounds such as siRNA compounds, modified bases/locked nucleic acids
(LNAs), peptide nucleic acids (PNAs), and other oligomeric compounds or

oligonucleotide mimetics which hybridize to at least a portion of the target nucleic acid and modulate its function. In some embodiments, the inhibitory nucleic acids include antisense RNA, antisense DNA, chimeric antisense oligonucleotides, antisense oligonucleotides comprising modified linkages, interference RNA (RNAi), short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof. See, e.g., WO 2010040112.

In some embodiments, the inhibitory nucleic acids are 10 to 50, 10 to 20, 10 to 25, 13 to 50, or 13 to 30 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies inhibitory nucleic acids having complementary portions of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length, or any range therewithin. In some embodiments, the inhibitory nucleic acids are 15 nucleotides in length. In some embodiments, the inhibitory nucleic acids are 12 or 13 to 20, 25, or 30 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies inhibitory nucleic acids having complementary portions of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length, or any range therewithin (complementary portions refers to those portions of the inhibitory nucleic acids that are complementary to the target sequence).

The inhibitory nucleic acids useful in the present methods are sufficiently complementary to the target RNA, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect. "Complementary" refers to the capacity for pairing, through hydrogen bonding, between two sequences comprising naturally or non-naturally occurring bases or analogs thereof. For example, if a base at one position of an inhibitory nucleic acid is capable of hydrogen bonding with a base at the corresponding position of a RNA, then the bases are considered to be complementary to each other at that position. 100% complementarity is not required.

Routine methods can be used to design an inhibitory nucleic acid that binds to the target sequence (e.g., LSD1, PD-1, PD-L1, PD-L2, OX40, TIM3, LAG3, TGF β) with sufficient specificity. In some embodiments, the methods include using bioinformatics methods known in the art to identify regions of secondary structure,

e.g., one, two, or more stem-loop structures, or pseudoknots, and selecting those regions to target with an inhibitory nucleic acid. For example, “gene walk” methods can be used to optimize the inhibitory activity of the nucleic acid; for example, a series of oligonucleotides of 10-30 nucleotides spanning the length of a target RNA can be prepared, followed by testing for activity. Optionally, gaps, e.g., of 5-10 nucleotides or more, can be left between the target sequences to reduce the number of oligonucleotides synthesized and tested. GC content is preferably between about 30-60%. Contiguous runs of three or more Gs or Cs should be avoided where possible (for example, it may not be possible with very short (e.g., about 9-10 nt) oligonucleotides).

In some embodiments, the inhibitory nucleic acid molecules can be designed to target a specific region of the RNA sequence. For example, a specific functional region can be targeted, e.g., a region comprising a known RNA localization motif (i.e., a region complementary to the target nucleic acid on which the RNA acts).

Alternatively or in addition, highly conserved regions can be targeted, e.g., regions identified by aligning sequences from disparate species such as primate (e.g., human) and rodent (e.g., mouse) and looking for regions with high degrees of identity. Percent identity can be determined routinely using basic local alignment search tools (BLAST programs) (Altschul et al., J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656), e.g., using the default parameters.

Pharmaceutical Compositions and Kits

Also provided herein are pharmaceutical compositions that include at least one of any of the LSD1 inhibitors described herein, at least one of any of the TGF β inhibitors described herein, and at least one of any of the immunotherapies (e.g., at least one PD-1 and/or PD-L1 inhibitor) described herein. In some embodiments, the pharmaceutical compositions include at least one of any of the LSD1 inhibitors described herein and at least one of any of the TGF β inhibitors described herein.

The pharmaceutical compositions can be formulated in any matter known in the art. The pharmaceutical compositions are formulated to be compatible with their intended route of administration (e.g., intravenous, subcutaneous, intraperitoneal, rectal or oral). In some embodiments, the pharmaceutical compositions can include a pharmaceutically acceptable carrier (e.g., phosphate buffered saline). The

compositions can include a sterile diluent (e.g., sterile water or saline), a fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvents, antibacterial or antifungal agents, such as benzyl alcohol or methyl parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like, antioxidants, such as ascorbic acid or sodium bisulfite, chelating agents, such as ethylenediaminetetraacetic acid, buffers, such as acetates, citrates, or phosphates, and isotonic agents, such as sugars (e.g., dextrose), polyalcohols (e.g., mannitol or sorbitol), or salts (e.g., sodium chloride), or any combination thereof. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. Preparations of the compositions can be formulated and enclosed in ampules, disposable syringes, or multiple dose vials. Where required (as in, for example, injectable formulations), proper fluidity can be maintained by, for example, the use of a coating, such as lecithin, or a surfactant. Absorption of a therapeutic agent can be prolonged by including an agent that delays absorption (e.g., aluminum monostearate and gelatin). Alternatively, controlled release can be achieved by implants and microencapsulated delivery systems, which can include biodegradable, biocompatible polymers (e.g., ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid).

Compositions containing one or more of agents described herein can be formulated for parenteral (e.g., intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal) administration in dosage unit form (i.e., physically discrete units containing a predetermined quantity of active compound for ease of administration and uniformity of dosage).

Toxicity and therapeutic efficacy of compositions can be determined by standard pharmaceutical procedures in cell cultures or experimental animals (e.g., monkeys). One can, for example, determine the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population): the therapeutic index being the ratio of LD₅₀:ED₅₀. Agents that exhibit high therapeutic indices are preferred. Where an agent exhibits an undesirable side effect, care should be taken to minimize potential damage (i.e., reduce unwanted side effects). Toxicity and therapeutic efficacy can be determined by other standard pharmaceutical procedures.

Single or multiple administrations of formulations can be given depending on for example: the dosage and frequency as required and tolerated by the patient. The

dosage, frequency and timing required to effectively treat a subject may be influenced by the age of the subject, the general health of the subject, the severity of the disease, previous treatments, and the presence of comorbidities (e.g., diabetes). The formulation should provide a sufficient quantity of active agent to effectively treat, prevent or ameliorate conditions, diseases or symptoms. Toxicity and therapeutic efficacy of compositions can be determined using conventional procedures in cell cultures, pre-clinical models (e.g., mice, rats or monkeys), and humans. Data obtained from in vitro assays and pre-clinical studies can be used to formulate the appropriate dosage of any composition described herein (e.g., any of the pharmaceutical compositions described herein).

Efficacy of any of the compositions described herein can be determined using methods known in the art, such as by the observation of the clinical signs of a cancer (e.g., tumor size, presence of metastasis). A therapeutically effective amount of the one or more (e.g., one, two, three, or four) agents described herein will be an amount that treats the disease in a subject (e.g., kills cancer cells) in a subject (e.g., a human subject identified as having cancer), or a subject identified as being at risk of developing the disease (e.g., a subject who has previously developed cancer but now has been cured), decreases the severity, frequency, and/or duration of one or more symptoms of a disease in a subject (e.g., a human). The effectiveness and dosing of any of the composition described herein can be determined by a health care professional or veterinary professional using methods known in the art, as well as by the observation of one or more symptoms of disease in a subject (e.g., a human). Certain factors may influence the dosage and timing required to effectively treat a subject (e.g., the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and the presence of other diseases).

Also provided herein are kits that include at least one of any of the LSD1 inhibitors described herein, at least one of any of the TGF β inhibitors described herein, and/or at least one of any of the immunotherapies, e.g., at least one PD-1 and/or PD-L1 inhibitor, described herein. In some instances, the kits can include instructions for performing any of the methods described herein. In some embodiments, the kits can include at least one dose of any of the pharmaceutical compositions described herein.

The disclosure is further described in the following examples, which do not limit the scope of the disclosure described in the claims.

EXAMPLES

5 **Example 1. Materials and Methods**

Cell lines

MCF-7, T47D, B16, LLC and D4m cells were cultured in normal DMEM supplemented with 10% FBS and 1% penicillin/streptomycin in 5% CO₂ incubator at 37 °C. All cell lines were cultured in 5% CO₂ incubator at 37 °C, and passaged every 10 2-3 days. One day before compound treatment, cells were seeded in 6-well or 12-well plates, and then were treated with 1, 2, or 5 μM GSK-LSD1, or DMSO as mock, in duplicates or triplicates for 5-6 days, during which cells were passaged once and replenished with fresh compound.

15 *Mice*

6–10-wk-old female mice were used for all experiments. WT C57BL/6 mice were purchased from The Jackson Laboratory. Prior to all experiments, purchased mice were allowed one week to acclimate to housing conditions at the Harvard Medical School Animal Facility. For studies using immunodeficient mice, an in- 20 house strain of WT mice was compared to an in-house strain of TCRα^{-/-} mice. The in-house strains of WT and TCRα^{-/-} were originally purchased from The Jackson Laboratory. Colonies for each strain of mice were maintained in the same animal facility at Harvard Medical School. All experimental mice were housed in specific pathogen-free conditions and used in accordance with animal care guidelines from 25 the Harvard Medical School Standing Committee on Animals and the National Institutes of Health. Animal protocols were approved by the Harvard Medical School Standing Committee on Animals.

Gene knockdown by shRNA and rescue assay

30 The shRNA oligos, with sequences for their respective target genes listed in Table 3, were annealed and cloned into pLKO.1-Puromycin⁺ (Puro) or pLKO.1-Blasticidin⁺ (Bsd) lentiviral vector. Lentivirus carrying pLKO.1 plasmid was produced by co-transfecting HEK293T cells with four helper plasmids (pHDM-VSV-

G, pHDM-tat1b, pHDM-HgPM2, and pRC-CMVraII), and by harvesting viral supernatant after 72 h by passing through a 0.45 µm filter. Collected lentivirus was used directly by infecting cells with the addition of 8 µg/ml polybrene (Sigma-Aldrich, cat#H9268), or frozen at -80 °C for later use. Infected cells were selected and expanded with puromycin (Gold Biotechnology, cat#P-600-500) at 1 µg/ml or blasticidin (Sigma-Aldrich, cat#15205) at 5 µg/ml for 5 days before being used for subsequent assays.

For double KD, MCF-7 cells were first transduced with lentiviral pLKO-sh-Scramble-Bsd or pLKO-sh-LSD1-Bsd, and selected with blasticidin for 3 days.

Those cells were then transduced again with lentiviral pLKO-sh-GFP-Puro as control or pLKO-sh-Target-Puro, and selected with both blasticidin and puromycin for 3-5 days to achieve double KD. In this context, pLKO-sh-Scramble-Bsd plus pLKO-sh-GFP-Puro was referred as sh-C, and pLKO-sh-LSD1-Bsd plus pLKO-sh-GFP-Puro was referred as sh-LSD1.

For LSD1 rescue assay, MCF-7 cells were first transduced with lentiviral pLKO-sh-Scramble-Bsd or pLKO-sh-LSD1-Bsd, and selected with blasticidin for 3 days. Those cells were then transduced again with lentiviral pHAGE-CMV-Flag-HA-EV/LSD1/LSD1-K661A (puromycin and sh-LSD1 resistant), and selected with both blasticidin and puromycin for 5 days before subsequent analysis. In this context, pLKO-sh-Scramble-Bsd plus pHAGE-CMV-Flag-HA-EV was referred as sh-C, and pLKO-sh-LSD1-Bsd plus pHAGE-CMV-Flag-HA-EV was referred as sh-LSD1. For alternative rescue method, MCF-7 cells were first transduced with lentiviral pLKO-sh-Scramble-Bsd or pLKO-sh-LSD1-Bsd followed by blasticidin selection for 5 days before subsequent analysis.

Table 3. DNA oligonucleotide sequences

shRNA Target	Sequences	SEQ ID NO:
GFP	GCAAGCTGACCCTGAAGTTCAT	SEQ ID NO: 7
Scramble	CCTAAGGTTAAGTCGCCCTCG	SEQ ID NO: 8
Human LSD1	GCCTAGACATTAACTGAATA	SEQ ID NO: 9
Human TLR3	CCTTACACATACTCAACCT	SEQ ID NO: 10
Human MDA5	CCAACAAAGAAGCAGTGTATA	SEQ ID NO: 11
Human RIG-I	AATTCATCAGAGATAGTCA	SEQ ID NO: 12
Human MAVS #1	GCATCTCTTCAATACCCTT	SEQ ID NO: 13
Human MAVS #2	GGAGAGAATTCAGAGCAAG	SEQ ID NO: 14
Human cGAS	ATCTATTCTCTAGCAACTTAA	SEQ ID NO: 15

Human STING	GCATGGTCATATTACATCG	SEQ ID NO: 16
Human AGO2	GCACAGCCAGTAATCGAGTTT	SEQ ID NO: 17
Human DICER #1	AAGAATCAGCCTCGCAACAAA	SEQ ID NO: 18
Human DICER #4	TCTATTAGCACCTTGATGT	SEQ ID NO: 19
Human TRBP2 #1	GCTGCCTAGTATAGAGCAA	SEQ ID NO: 20
Human TRBP2 #2	TCTACGAAATTCAGTAGGA	SEQ ID NO: 21
Human TRBP2 #4	GGATTCTCTACGAAATTCA	SEQ ID NO: 22
Mouse LSD1 #1	CACAAGGAAAGCTAGAAGA	SEQ ID NO: 23
Mouse LSD1 #2	GGATGGGATTTGGCAACCTTA	SEQ ID NO: 24
Mouse LSD1 #3	AACTCCATGTCATCAGCTACT	SEQ ID NO: 25
Mouse LSD1 #4	CGGCATCTACAAGAGGATAAA	SEQ ID NO: 26
CRISPR gRNA target	Sequences	SEQ ID NO:
Mouse LSD1 #3 (target location exon 2)	ATATTCATCTTCTGAGAGGT	SEQ ID NO: 27
Mouse LSD1 #4 (target location exon 2)	TCTTCCTCAGGTGGGGCTTG	SEQ ID NO: 28
Mouse LSD1 #5 (target location exon 3)	CCTGAGAGGTCATTCGGTCA	SEQ ID NO: 29
Mouse LSD1 #6 (target location exon 3)	CCATGACCGAATGACCTCTC	SEQ ID NO: 30
Mouse MDA5 #4 (target location exon 4)	GGCAGGGATTCAGGCACCAT	SEQ ID NO: 31
RT-qPCR primer target	Sequence	SEQ ID NO:
Human GAPDH forward	AACGGGAAGCTTGTCACTAA	SEQ ID NO: 32
Human GAPDH reverse	TGGACTCCACGACGTACTCA	SEQ ID NO: 33
Human LSD1 forward	GTGGACGAGTTGCCACATTC	SEQ ID NO: 34
Human LSD1 reverse	TGACCACAGCCATAGGATTCC	SEQ ID NO: 35
Human HERV-E forward	GGTGTCACTACTCAATACAC	SEQ ID NO: 36
Human HERV-E reverse	GCAGCCTAGGTCTCTGG	SEQ ID NO: 37
Human HERV-F forward	CCTCCAGTCACAACAACCTC	SEQ ID NO: 38
Human HERV-F reverse	TATTGAAGAAGGCGGCTGG	SEQ ID NO: 39
Human HERV-K forward	ATTGGCAACACCGTATTCTGCT	SEQ ID NO: 40
Human HERV-K reverse	CAGTCAAAATATGGACGGATGGT	SEQ ID NO: 41
Human HML-2 forward	AAAGAACCAGCCACCAGG	SEQ ID NO: 42
Human HML-2 reverse	CAGTCTGAAAACCTTTCTCTA	SEQ ID NO: 43
Human ERVL forward	ATATCCTGCCTGGATGGGGT	SEQ ID NO: 44
Human ERVL reverse	GAGCTTCTTAGTCCTCCTGTGT	SEQ ID NO: 45
Human Line1 forward	GCCAAGATGGCCGAATAGG	SEQ ID NO: 46
Human Line1 reverse	TGGCACTCCCTAGTGAGATGAA	SEQ ID NO: 47
Human AluYA5 forward	ACCATCCCGGCTAAAACGGTGA	SEQ ID NO: 48
Human AluYA5 reverse	GCGATCTCGGCTCACTG	SEQ ID NO: 49
Human IFN- α forward	AATGACAGAATTCATGAAAGCGT	SEQ ID NO: 50
Human IFN- α reverse	GGAGGTTGTCAGAGCAGA	SEQ ID NO: 51
Human IFN- β forward	GCCATCAGTCACTTAAACAGC	SEQ ID NO: 52

Human IFN- β reverse	GAAACTGAAGATCTCCTAGCCT	SEQ ID NO: 53
Human IL-28a/b forward	TCCAGTCACGGTCAGCA	SEQ ID NO: 54
Human IL-28 a/b reverse	CAGCCTCAGAGTGTCTTCT	SEQ ID NO: 55
Human OASL forward	GCAGAAATTTCCAGGACCAC	SEQ ID NO: 56
Human OASL reverse	CCCATCACGGTCACCATTG	SEQ ID NO: 57
Human ISG15 forward	CCTTCAGCTCTGACACC	SEQ ID NO: 58
Human ISG15 reverse	CGAACTCATCTTTGCCAGTACA	SEQ ID NO: 59
Human TLR3 forward	TGGTTGGGCCACCTAGAAGTA	SEQ ID NO: 60
Human TLR3 reverse	TCTCCATTCTGCGCTGTG	SEQ ID NO: 61
Human MDA5 forward	CACTTCCTTCTGCCAACTTG	SEQ ID NO: 62
Human MDA5 reverse	GAGCAACTTCTTTCAACCACAG	SEQ ID NO: 63
Human RIG-I forward	CCAGCATTACTAGTCAGAAGGAA	SEQ ID NO: 64
Human RIG-I reverse	CACAGTGCAATCTTGTCATCC	SEQ ID NO: 65
Human MAVS forward	AGGAGACAGATGGAGACACA	SEQ ID NO: 66
Human MAVS reverse	CAGAACTGGGCAGTACCC	SEQ ID NO: 67
Human cGAS forward	TAACCCTGGCTTTGGAATCAAAA	SEQ ID NO: 68
Human cGAS reverse	TGGGTACAAGGTAAAATGGCTTT	SEQ ID NO: 69
Human STING forward	AGCATTACAACAACCTGCTACG	SEQ ID NO: 70
Human STING reverse	GTTGGGGTCAGCCATACTCAG	SEQ ID NO: 71
Human AGO2 forward	CCGGCCTTCTCTCTGGAAAA	SEQ ID NO: 72
Human AGO2 reverse	GCCTTGTAACGCTGTTGCT	SEQ ID NO: 73
Mouse LSD1 forward	GTGGTGTTATGCTTTGACCGT	SEQ ID NO: 74
Mouse LSD1 reverse	GCTGCCAAAATCCCTTTGAGA	SEQ ID NO: 75
MuERV-L forward	TTTCTCAAGGCCACCAATAGT	SEQ ID NO: 76
MuERV-L reverse	GACACCTTTTTTAAGTATGCGAGCT	SEQ ID NO: 77
Mouse MusD forward	GATTGGTGGAAGTTAGCTAGCAT	SEQ ID NO: 78
Mouse MusD reverse	TAGCATTCTCATAAGCCAATTGCAT	SEQ ID NO: 79
Mouse IAP Pol forward	CTTGCCCTTAAAGGTCTAAAAGCA	SEQ ID NO: 80
Mouse IAP Pol reverse	GCGGTATAAGGTACAATTAAAAGATATGG	SEQ ID NO: 81
Mouse Line1 forward	TTTGGGACACAATGAAAGCA	SEQ ID NO: 82
Mouse Line1 reverse	CTGCCGTCTACTCCTCTTGG	SEQ ID NO: 83
Mouse IFN-a1 forward	CGGTGCTGAGCTACTGGC	SEQ ID NO: 84
Mouse IFN-a1 reverse	TTTGTACCAGGAGTGTCAAGG	SEQ ID NO: 85
Mouse IFN-b forward	GGTGGAATGAGACTATTGTTG	SEQ ID NO: 86
Mouse IFN-b reverse	AGGACATCTCCACGTC	SEQ ID NO: 87
Mouse IL-28b forward	AGCTGCAGGTCCAAGAGCG	SEQ ID NO: 88
Mouse IL-28b reverse	GGTGGTCAGGGCTGAGTCATT	SEQ ID NO: 89
Mouse ISG15 forward	GGTGTCCGTGACTAACTCCAT	SEQ ID NO: 90
Mouse ISG15 reverse	TGGAAAGGGTAAGACCGTCCT	SEQ ID NO: 91
Mouse OASL forward	CAGGAGCTGTACGGCTTCC	SEQ ID NO: 92
Mouse OASL reverse	CCTACCTTGAGTACCTTGAGCAC	SEQ ID NO: 93
Mouse TLR3 forward	GTGAGATACAACGTAGCTGACTG	SEQ ID NO: 94
Mouse TLR3 reverse	TCCTGCATCCAAGATAGCAAGT	SEQ ID NO: 95
Mouse RIG-I forward	AAGAGCCAGAGTGTGAGAATCT	SEQ ID NO: 96

Mouse RIG-I reverse	AGCTCCAGTTGGTAATTTCTTGG	SEQ ID NO: 97
Mouse beta-actin forward	GGCTGTATTCCCCTCCATCG	SEQ ID NO: 98
Mouse beta-actin reverse	CCAGTTGGTAACAATGCCATGT	SEQ ID NO: 99
Mouse GAPDH forward	TGACCTCAACTACATGGTCTACA	SEQ ID NO: 100
Mouse GAPDH reverse	CTTCCCATCTCGGCCTTG	SEQ ID NO: 101
GFP-com forward	GAACGGCATCAAGGTGAACTT	SEQ ID NO: 102
GFPL reverse	TAGCGTAATCTGGAACATCGTATG GGT	SEQ ID NO: 103
GFP-let7 reverse	GACGACCTCGAGTGAGGTAGTAG GTTGTATA	SEQ ID NO: 104
Gene/Strand-specific PCR primer target	Sequence	SEQ ID NO:
Non-human Tag	GCACACGACGACAGACGACGCAC	SEQ ID NO: 105
HERV-E tag-TF	GCACACGACGACAGACGACGCAC CCAGAGTCAGGTGTCACTACTCAA TACAC	SEQ ID NO: 106
HERV-E tag-BR	GCACACGACGACAGACGACGCACT ACTGGAGCAACACGCAGCCTAGGT CTCTGG	SEQ ID NO: 107
HERV-E TF	GGTGTCCTACTCAATACAC	SEQ ID NO: 108
HERV-E BR	GCAGCCTAGGTCTCTGG	SEQ ID NO: 109
HERV-K tag-RF(3)	GCACACGACGACAGACGACGCAC GGGAAGAATGTGTGGCCAATAGT GCGGT	SEQ ID NO: 110
HERV-K tag-BR(3)	GCACACGACGACAGACGACGCAC GGTAGAGATTCTTTTTCTCCCCAT TCCCAG	SEQ ID NO: 111
HERV-K RF(3)	GTGTGGCCAATAGTGCGGT	SEQ ID NO: 112
HERV-K BR(3)	ATTCCTTTTTCTCCCCATTCCCAG	SEQ ID NO: 113
Syn1-tag-TF	GCACACGACGACAGACGACGCAC ATGGAGCCCAAGATGCAGTCCAAG A	SEQ ID NO: 114
Syn1-tag-BR	GCACACGACGACAGACGACGCAC CTAACTGCTTCCTGCTGAATTGGG GCGTA	SEQ ID NO: 115
Syn1-TF	ATGGAGCCCAAGATGCAG	SEQ ID NO: 116
Syn1-BR	CTAACTGCTTCCTGCTGAATTGGG GCGTAG	SEQ ID NO: 117
Human beta-Actin-tag-TF	GCACACGACGACAGACGACGCAC GCTCGTCGTCGACAACGGCTCCGG CAT	SEQ ID NO: 118
Human beta-Actin-tag-BR	GCACACGACGACAGACGACGCAC CAAACATGATCTGGGTCATCTTCTC	SEQ ID NO: 119
Human beta-Actin-TF	GCTCGTCGTCGACAACGGCTCCGG CA	SEQ ID NO: 120

Human beta-Actin-BR	CAAACATGATCTGGGTCATCTTCTC	SEQ ID NO: 121
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Gene deletion by CRISPR/Cas9

The gRNA oligos, with sequences for their respective target genes listed in Table 3, were annealed and cloned into Lenti-CRISPR-v2-Puromycin⁺ vector. To delete target genes, B16 cells were transiently transfected with Lenti-CRISPR-v2 plasmid carrying respective gRNA, and selected with 1 µg/ml puromycin for 2 days. Cells were then transferred into fresh medium without puromycin and seeded at super-low density to allow colony formation from single cell. Colonies were then picked up and expanded for KO validation by immunoblot and by sequencing of target genomic region. For double KO, LSD1 KO B16 cells (clone g5-4) were used for deleting the second target gene as described above.

Generation of ERV expression construct and transduction

Primers with sequences listed in table S1 were used to get 2kb fragment of HERV-K and 2kb fragment of HERV-E through PCR amplification with insertion of stop codons at 5' ends and additional 30bp elongation primers at 3' ends. HERV-K and HERV-E fragments with reverse complementary elongation primers at their 3' ends were mixed, denatured, annealed and elongated, followed by PCR amplification to generate 4kb HERV-(K+E) fusion fragment, which was further cloned into pHAGE-CMV-Flag-HA lentiviral vector thus expressing sense transcript of HERV-K and antisense transcript of HERV-E. Viral package and transduction were performed at described above. MCF-7 cells transduced with HERV-(K+E) were cultured for 48 hours without drug selection before subsequent analysis.

RNA extraction and RT-qPCR

All reagents, buffers and containers used for RNA work were RNase-free grade or treated with 0.1% v/v DEPC (Sigma-Aldrich cat#D5758) if applicable, to eliminate RNase contaminants in this section and other relevant sections. For total RNA extraction, cells in culture were directly lysed in TRIzol (Life Technologies, cat#15596018) after medium removal. RNA extraction was performed according to the manufacture's instructions. The extracted RNA was reversely transcribed into cDNA using PrimeScriptTM RT Reagent Kit (Clontech cat#RR037B) according to the

manufacturer's instructions, with following modifications: 2 µg of RNA samples with the addition of primers were first denatured at 70 °C for 5 min and cooled down on ice before the addition of buffer and reverse transcriptase; incubation time (at 37 °C) was increased up to 30 min. The obtained cDNA samples were diluted and used for
 5 real-time quantitative PCR (RT-qPCR). SYBR green (Roche, cat#06649416001) and gene specific primers with sequences listed in Table 3 were used for PCR amplification and detection on a LightCycler 480 system (Roche).

Strand-specific PCR for detection of sense and antisense ERV transcripts

10 The strand-specific PCR method was adapted from (Chiappinelli et al., 2015) and performed with PrimeScript™ RT Reagent Kit (Clontech cat#RR037B) with modifications. In brief, gene- and strand-specific primers (GSP) were synthesized with an extra Tag sequence (listed in Table 3, which does not exist in human genome) at 5'-end to generate Tag-GSP (for example, HERV-E Tag-BR for sense strand, Tag-
 15 TF for antisense strand), and were used for reverse transcription, following these steps: 1 µg total RNA in 6 µl H₂O was mixed with 1 µl Tag-GSP (10 µM stock), pre-heated at 65 °C for 5 min and cooled down on ice; then added 2 µl buffer (5X), 0.5 µl reverse-transcriptase and 120 ng actinomycin D (Sigma-Aldrich, cat#A9415) in 0.5 µl H₂O to a total volume of 10 µl; incubated at 50 °C for 50 min for only first strand
 20 cDNA synthesis and deactivated at 85 °C for 5 min; finally added 1 U RNase H (New England Biolabs, cat#M0297S) and incubated at 37 °C for 20 min, followed by ethanol precipitation for cDNA purification. The obtained cDNA was then used for PCR amplification with paired primers: Tag-primer in pair with TF-primer for amplifying sense strand and Tag-primer in pair with BR-primer for amplifying
 25 antisense strand. The amplicons were visualized on 1.5% agarose gels.

DsRNA analysis by RNase digestion and RT-qPCR

For dsRNA analysis by RNase A digestion, 5 µg total RNA extracted from MCF-7 or B16 cells was dissolved in 46 µl H₂O and mixed well with 3.5 µl NaCl (5
 30 M stock). Then 0.5 µl RNase A (10 mg/ml stock, Thermo Fisher Scientific, cat#EN0531) or H₂O as mock was added to a total volume of 50 µl and mixed well, followed by incubation at room temperature for 10 min. Afterwards, 1 ml TRIzol was directly added to the mixture to terminate digestion, followed by RNA extraction.

The RNA transcripts of selected retrotransposons were measured by RT-qPCR with GAPDH (Actb for B16 cells) as an internal control. The ratios of (retrotransposon/GAPDH)_{RNase-A}/(retrotransposon/GAPDH)_{mock} were calculated as enrichment fold.

5 For dsRNA analysis by RNase T1 digestion, 2 µg total RNA extracted from MCF-7 was dissolved in 16 µl H₂O and mixed well with 2 µl buffer (10X). Then 2 µl RNase T1 (1U/µl stock, Thermo Fisher Scientific, cat#AM2283) or H₂O as mock was added to a total volume of 20 µl and mixed well, followed by incubation at 37 °C for 30 min. Afterwards, 1 ml TRIzol was directly added to the mixture to terminate
10 digestion, followed by RNA extraction and analysis as described above.

DsRNA analysis by J2 pulldown

Purified total RNA from control or LSD1 KD MCF-7 cells was used for J2 pulldown assay. J2 antibody (Scicons, cat#10010200) and mouse IgG control (Santa
15 Cruz Biotechnology, cat#sc-2025) were first conjugated (1 µg per pulldown) to Protein G dynabeads (Life Technologies, cat#10008D), respectively. For each pulldown, 30 µg RNA was mixed with 500 µl immunoprecipitation (IP) buffer (350 mM NaCl, 25 mM Tris pH7.4, 5 mM DTT and 0.5% NP-40), followed by the addition of 0.5 µl RNase A (10 mg/ml stock, Thermo Fisher Scientific, cat#EN0531)
20 and thorough mixing. The addition of RNase A was to reduce the overwhelming single-stranded RNA (ssRNA) and enrich dsRNA for J2 capture. Then, the whole mixture was mixed with washed beads and rotated at 4 °C for 2h. Afterwards, the beads were washed with IP buffer and incubated in 50 µl Proteinase K digestion solution (1xTE, 100 mM NaCl, 1% SDS, and 1 µl Proteinase K (20 mg/ml stock,
25 Thermo Fisher Scientific, cat#AM2546)) at 45 °C for 20 min. The elutes were directly added to 1 ml TRIzol for RNA purification and RT-qPCR analysis as described above.

DsRNA analysis by J2 immunoblot

30 Purified total RNA from B16 cells was subjected to digestion with mock, RNase T1 (Thermo Fisher Scientific, cat#AM2283) and RNase III (Thermo Fisher Scientific, cat#AM2290) in their respective buffers and according to the manufacturer's instructions, or RNase A (Thermo Fisher Scientific, cat#EN0531)

under high salt condition (350 mM NaCl). The digestion was deactivated by the addition of TRIzol and RNA was subsequently purified. Equal volume (2.5 µl) of purified RNA was dotted on Hybond N+ membrane (GE Healthcare, cat#RPN119B), dried and autocrosslinked in a UV stratalinker 2400 (Stratagene) two times. The membrane was then blocked in 5% milk in PBS-T (0.1% Tween-20) for 30 min and probed with J2 antibody at 4 °C overnight. On the next day, the membrane was washed with PBS-T three times and probed with secondary goat-anti-mouse HRP antibody (Millipore cat#AP124P) in 5% milk at room temperature for 1 h. The membrane was washed again with PBS-T three times and ECL was applied for film development. Afterwards, the membrane was stained with methylene blue solution (0.3% w/v methylene blue + 30% v/v ethanol + 70% v/v H₂O) to visualize RNA presence.

Protein extraction and immunoblot analysis

Cells in culture were washed with ice-cold PBS twice to completely remove residual medium. RIPA lysis buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 50 mM Tris pH8) supplemented with protease inhibitor (Roche, cat#0469313001) and phosphatase inhibitor (Roche, cat# 04906837001) was directly added to cell layers and scraped on ice. Cell lysates were transferred to small tubes and lysed on ice for 10 min before being cleared by top-speed centrifugation at 4 °C. Protein concentrations in lysates were measured by Bio-Rad protein assay (Bio-Rad, #5000006) and adjusted equally between samples, followed by the addition of SDS loading dye (5X) and boiled at 95 °C for 5 min. Equal volume and equal quantity of protein samples were subjected to SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad, cat#162-0097). The membrane was blocked in 5% milk at room temperature for 1 h and incubated with appropriate antibodies at 4 °C overnight. On the next day, the membrane was washed with PBS-T three times and incubated with appropriate secondary HRP antibodies in 5% milk at room temperature for 1 h. The membrane was washed again with PBS-T three times and ECL was applied for film development.

ELISA

The ELISA assay was performed with a Human IFN Beta ELISA Kit (pbl assay science, cat#41415-1) according to the manufacturer's instructions.

LSD1 demethylase assay

5 LSD1 demethylase assays were carried out with proteins immunoprecipitated by anti-HA magnetic beads from MCF-7 cells stably expressing FH-LSD1, FH-LSD1-K661A, FH-AGO2 and FH-AGO2-K726R. In order to increase basal methylation level on purified AGO2, MCF-7 cells stably expressing FH-AGO2 and FH-AGO2-K726R were treated with 2 μ M GSK-LSD1 for 24 hours before being used
10 for IP purification. In a reaction of 50 μ l volume, immunoprecipitated LSD1 (~2 μ g, estimated by SDS-PAGE and coomassie blue staining) and AGO2 (~1 μ g) were incubated in demethylation buffer (50 mM Tris pH 8.5, 50 mM KCl, 5 mM MgCl₂, 0.5% BSA, 5% glycerol, and complete EDTA-free protease inhibitors) on a thermoshaker at 37 °C for 4 hours. The reaction was terminated by adding SDS
15 loading dye (5X) and boiling at 95 °C for 5 min, followed by SDS-PAGE and immunoblot analysis. In each experiment, calf thymus histones were used as LSD1 demethylase substrate in parallel to check the activity of immunoprecipitated LSD1 by immunoblot analysis of H3K4me2.

20 *GFPL/GFP-let-7 dual reporter assay*

The reporter assay for miRISC activity was performed as previously described (Qi et al., 2008). In brief, U2OS cell line stably expressing dual reporters, GFPL and GFP-let-7, was transduced with lentiviral shRNA against scramble, LSD1 or AGO2. Cells were selected with puromycin at 1 μ g/ml and G-418 (Research Products
25 International, cat#G64500) at 200 μ g/ml for 4 days before subsequent analysis. The expression of GFPL and GFP was measured by immunoblot and RT-qPCR as described in the above sections. Protein signals in immunoblot were quantified by ImageJ software according to the user manual. The ratios of GFPL over GFP protein signals in different samples were calculated and the ratio in control shRNA sample
30 was considered as 100% miRISC activity.

Cell colony formation assay

B16 cells growing at 80% confluence were trypsinized and transferred into fresh medium in single cell suspension. Cell numbers were counted and diluted appropriately for seeding to 6-well plates (500 cells per well) or 12-well plates (200 cells per well). Cells were allowed to grow for 6 days, with fresh medium addition at day 3 without absorbing old medium, before staining with crystal violet solution (0.5% w/v crystal violet powder, 80% v/v H₂O and 20% v/v methanol).

Mouse tumor models

Mice were anesthetized with Avertin (2.5%), shaved at the injection site, and then injected in the flank subcutaneously with 250,000-500,000 B16-F10 tumor cells. Tumors were measured every 2–3 days once palpable (long diameter and short diameter) with a caliper. Tumor volume was determined using the volume formula for an ellipsoid: $1/2 \times D \times d^2$ where D is the longer diameter and d is the shorter diameter. Mice were sacrificed when tumors reached 2 cm³ or upon ulceration/bleeding.

For antibody treatments, mice were given 100 µg antibody i.p. at days 14, 16, 18, and 20 post tumor injection using the following antibodies: anti-PD-1 (clone 29F.1A12) (Dana Farber Cancer Institute, Boston, MA). Rat IgG2a isotype control antibody was purchased from BioXCell (cat#BE0089). Prior to treatments mice were randomized such that treatment groups had similar average tumor volumes prior to treatment initiation.

B16 metastasis assay

200K B16.F10 (scramble or LSD1 KO) were transferred intravenously via tail vein injection. Lungs were removed 14 days post injection and fixed overnight in Fekete's solution. Visible metastases were counted in blinded fashion by two investigators.

Tumor infiltrating leukocyte flow cytometry

Tumors were excised day 14 post injection and cut into 2 mm sized pieces in collagenase and DNase. Samples were dissociated with a Gentle MACS, incubated for 20 minutes at 37°C, dissociated with a Gentle MACS again, and passed through a 70 µm filter. To enrich for leukocytes samples were spun through a Percoll gradient.

Leukocytes were isolated from the interface of the 40 and 70% Percoll gradient, stained, and analyzed for fluorescent markers. For intracellular staining the Ebioscience Foxp3 Fixation Permeabilization Kit was used. All antibodies were purchased from Biolegend (CD45.2, CD11b, CD3, CD4, CD8b, Foxp3, Granzyme-B, 5 Ki67, CD44, PD-L1, MHC-1).

Tumor infiltrating lymphocyte TCR sequencing

T cells were enriched from tumors as above followed by sorting for CD8b⁺ T cells. Genomic DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen, 10 cat#69506) and submitted to Adaptive Biotechnologies for mouse TCRB CDR3 survey sequencing. Data was analyzed using Adaptive Biotechnologies' online analysis platform.

Directional RNA-Seq of MCF-7 cells

15 Purified total RNA was quantified by Qubit (Invitrogen) and analyzed by Agilent Bioanalyzer to assess RNA integrity. 1 µg RNA (RIN>9) was used to generate rRNA-depleted RNA with NEBNext® rRNA Depletion Kit (New England Biolabs, cat# E6310S) according to the manufacturer's instructions. The rRNA-depleted RNA was subjected to Agilent Bioanalyzer to ensure the complete removal 20 of rRNA, and then used to generate directional RNA library with NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (New England Biolabs, cat# E7760L) and NEBNext® Multiplex Oligos for Illumina® (New England Biolabs, cat# E7335L) according to the manufacturer's instructions. Library concentrations were quantified by Qubit (Invitrogen) and mixed equally for sequencing at HiSeq 25 2500 (Illumina) to generate 50 bp reads from paired-ends. The raw data are deposited at the Gene Expression Omnibus (GEO) under the subseries entry GSE105001.

ChIP-Seq of MCF-7 cells

Cell nuclei were obtained by lysing whole cells in hypotonic buffer (10 mM 30 HEPES pH 7.9, 10 mM KCl, 1.5 mM MgCl₂, 0.34 M sucrose, 10% v/v glycerol, 1 mM DTT, and 0.1% v/v Triton X-100) supplemented with protease inhibitor. After washing with PBS, nuclei were fixed in 1% formaldehyde for 10 min at room temperature, followed by quenching in 125 mM glycine. Nuclei were then washed

twice with ice-cold PBS, lysed in ChIP sonication buffer (50 mM HEPES pH7.9, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.2% SDS) supplemented with protease inhibitor, and were subjected to sonication to obtain DNA fragments of 300-800 bp. Subsequent procedures were carried out by following the Epigenesys protocol (<https://www.epigenesys.eu/en/>). The following antibodies were used to ChIP: anti-LSD1 (Abcam, cat# ab17721), anti-H3K4me1 (Abcam, cat#ab8895), anti-H3K4me2 (EMDMillipore, cat#07-030), and mouse IgG (Santa Cruz Biotechnology, cat# sc-2025).

ChIP-Seq libraries were prepared using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, cat#E7370L) and NEBNext® Multiplex Oligos for Illumina® (New England Biolabs, cat# E7335L) according to the manufacture's instructions. Library concentrations were quantified by Qubit (Invitrogen) and mixed equally for sequencing at HiSeq 2500 (Illumina) to generate 50 bp reads from single-end. The raw data are deposited at the Gene Expression Omnibus (GEO) under the subseries entry GSE105001.

Statistic analysis

Statistical analyses were performed using GraphPad Prism 6 software. For RT-qPCR and protein quantification, data were shown as mean $\pm$ s.d., and considered statistically significant with p values < 0.05 by unpaired Student's t test. For tumor growth, data were presented as mean $\pm$ s.e.m., and considered statistically significant with p values < 0.05 by unpaired Student's t test for comparing two groups or by two-way ANOVA for multiple comparisons. For comparing mouse survival curves, Log-rank (Mantel-Cox) test was used.

ChIP-Seq & RNA-Seq analysis

For ChIP-seq and RNA-seq data, all statistical analysis and visualization was performed with R (version 3.4.0) unless otherwise specified. Student's t-test was used to determine whether significant shift in mean occurs for all comparisons unless otherwise specified.

ChIP-seq analysis

Raw reads were aligned to hg19 or mm9 using bwa (version 0.7.2-r351) (PMID: 22569178). The resulted sam files were converted to bam with samtools (version 0.1.18 (r982:295) (PMID: 21245279). MACS2 (version 2.0.10.20131216) (PMID: 18798982) was used to call peak on the bam files. BedGraph files containing
5 signal per million reads produced from MACS2 were converted to bigwig files with ucsc tool kit (315). ChIP-seq signals were first extracted with bwtool (version 1.0) (PMID: 24489365) from bigwig files and then visualized in R.

Repeat annotation was downloaded from UCSC for hg19 and mm9, only ERVs were used for downstream analysis. To select ERVs, ERV families originated
10 in Homo Sapien or Mus Musculus were downloaded from Repbase (<http://www.girinst.org/repbase/>). A peak catalogue consisting all possible peak intervals in ChIP-seq (histones and LSD1) was produced and ERVs were filtered with this catalogue. ChIP-seq signals were extracted with bwtool (version 1.0) (PMID: 24489365) from bigwig files and then visualized in R.

RNA-seq analysis

Raw reads were aligned to hg19 or mm9 using STAR (v2.4.2a) with the parameter “quantMode” set as “GeneCounts” to produce count table for each gene. Differential gene analysis was performed on gene raw counts in R with edgeR
20 package (v3.18.1) (PMID: 22287627) from bioconductor. Read count table was filtered so that genes with at least one count across conditions were kept. The negative binomial generalized log-linear model was used in differential analysis. A FDR cut-off of 0.05 was used to determine significantly differentially expressed genes. The R package gProfileR (v0.6.4, PMID: 27098042) was used to perform
25 gene enrichment analysis on differential genes. Geneset enrichment analysis (GSEA) was performed with R package clusterProfiler (v3.4.4, PMID: 22455463).

The function analyzeRepeats.pl from Homer (PMID: 20513432) software was used to get raw counts for repeats from RNA-seq data. Differential expression for repeats was performed with edgeR the same way as for genes.

TCGA data analysis

For the association of LSD1 expression with survival, patient vital status (dead and alive) was used as surrogate end-point and patient dichotomized by LSD1

expression. The proportional hazards (PH) assumption was tested using the `cox.zph` function in the R Survival package (v2.41-3) with 0.1 as cutoff. A log-rank test was used instead if the PH assumption failed.

5 For analyzing LSD1 expression versus T cell infiltration in each tumor, the total expression of CD8A (log2 counts per million) was used to assess the infiltration of cytotoxic T-lymphocytes, and correlations were computed versus LSD1 expression.

10 For human SKCM data, patients were divided into tertiles based on LSD1 expression, and then the second and third tertiles were combined into one group (named LSD1-int/high) due to a lack of observable difference in survival curves between them.

For gene ontology, the online DAVID (<https://david.ncifcrf.gov/summary.jsp>) was used to analyze the differentially expressed genes (>2 fold) between LSD1 KD and WT control under the category of GOTERM_BP_DIRECT. For identification of enriched gene sets, the two expression data sets were further utilized and Gene Set Enrichment Analysis (GSEA) was performed based on these normalized data using GSEA tool (<http://www.broad.mit.edu/gsea/>) with C2. The raw and processed data of ChIP-Seq and RNA-Seq are deposited at the Gene Expression Omnibus (GEO) under the subseries entry GSE105001.

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Example 2. Lysine-specific demethylase 1A (LSD1) represses ERV expression and antiviral mimicry in human cancer cells

To identify chromatin regulators that control tumor responses to host immunity, a curated screening with compounds targeting chromatin factors was initiated. The screen was designed with two readouts: up-regulation of ERV transcripts and interferon activation, based on the following rationale: (1) ERVs are known to be transcriptionally silenced by epigenetic mechanisms; (2) interferons regulate tumor responses to host immunity (Parker et al. (2016) Nature Reviews Cancer 16:131-144); (3) a potential correlation between ERV activity and tumor immunity has been suggested (Rooney et al. (2015) Cell 160:48-61; Kassiotis and Stoye (2016) Nat Rev Immunology 16(4):207-219) and these two events may be linked by interferon activation (Chiappinelli et al. (2015) Cell 162(5):974-986).

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In this screen, an LSD1 catalytic inhibitor, GSK-LSD1, was shown to significantly induce the up-regulation of a few randomly selected ERVs, type I and type III interferons, as well as interferon-stimulated genes (ISGs) in MCF-7 cells (FIG. 1A). Of note, the PCR primers detected overall transcript levels of the corresponding ERV subfamilies, which may be transcribed from multiple genomic loci. To ascertain that this was caused by a GSK-LSD1 on-target effect, shRNA-mediated LSD1 knockdown (KD) was performed (FIG. 1B), which yielded essentially the same results (FIGs. 1C-D). Furthermore, re-introduction of wild type (WT) LSD1 but not catalytic inactive LSD1 (LSD1-K661A) back into LSD1 KD cells fully restored repression of four tested ERVs, as well as IFN- β and IL-28 activation (FIGs. 1E-G). These results demonstrated that demethylase activity of LSD1 is necessary for ERV repression, which is consistent with the LSD1 inhibitor result (FIG. 1A). Neither DNMT protein expression nor global DNA methylation was affected by LSD1 inhibition (FIGs. 1H and 1I), suggesting a DNA methylation-independent pathway. In addition, the induction of ISGs such as ISG15 was also suppressed by LSD1 rescue (FIG. 1J). These observations were recapitulated in T47D, another breast cancer cell line (FIG. 1K), and 293T cells, a kidney cell line (FIG. 1L) suggesting that these effects were not limited to MCF-7 cells and may be of broad significance in human cancer cells. Together, these results suggest that LSD1 may repress the expression of a group of ERVs and regulate interferon activation in human cancer cells.

Next, transcriptomic analysis was carried out to comprehensively explore how LSD1 regulates ERV expression and interferon activation. A significant impact of LSD1 inhibition on gene expression in MCF-7 cells (FIG. 1M). Gene ontology (GO) enrichment analysis of these differentially expressed genes revealed that the up-regulated genes were significantly enriched in GO terms related to type I interferon response and antiviral response (FIG. 1N), whereas the down-regulated genes seemed to be enriched in GO terms related to neuronal development (FIG. 1O). GSEA analysis confirmed the remarkable enrichment in type I interferon and antiviral responsive pathways in LSD1 KD cells compared to WT control (FIG. 2A). However, almost none of the up-regulated interferon/antiviral responsive genes ($\text{FDR} < 0.05$ and $\log_2(\text{FC}) > 0$, 125 in total) appeared to be direct targets of LSD1, as ChIP-seq analysis failed to identify LSD1 at their promoters (Table 4 and FIG. 2B)

An example of such genes indirectly regulated by LSD1 at their promoters and an example of LSD1 direct target genes were shown in FIGs. 2C and 2D.

Table 4. List of up-regulated interferon/antiviral responsive genes in LSD1 KD cells compared to WT control, related to FIG. 2.

No LSD1-bound	LSD1-bound
CD274, IFIT3, IFI6, HERC5, XAF1, OASL, IFIH1, IFI35, DHX58, GBP4, TMEM106A, IFI44, RSAD2, APOBEC3G, APOL1, PARP12, SAMD9L, MB21D1, TRIM22, IRF7, CXCL10, CXCL11, IFI27, PSMB9, IFIT2, TLR3, BST2, SP110, STX11, UBE2L6, GBP2, GBP1, OAS1, GMPR, TRIM21, MX2, SP100, PSMB8, NMI, ISG15, HLA-F, TAP1, TYMP	DDX60, IFIT1, OAS2, OAS3, IFI44L, EPSTI1, APOL6, IFIT5, MX1, EIF2AK2, TNFSF10, LAMP3, TDRD7, RARRES3, STAT1, KIF5C, SAMHD1, PLSCR1, PDZD2, ISG20, IL15

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Nevertheless, those genes are, by and large, downstream ISGs and therefore are unlikely to be directly responsible for the up-regulation of no-LSD1-bound genes and interferon pathway activation. A main mode of interferon/antiviral responses by LSD1 inhibition is speculated to be through activation of an upstream event, such as ERV transcript expression. The expression of repetitive elements in RNA-seq data was then analyzed. Many of the repetitive elements were up-regulated by LSD1 inhibition (FIG. 2E), including a number of ERVs (either sense or anti-sense transcripts) were significantly increased in LSD1 KD cells (FIG. 2F). Furthermore, many ERVs appeared to be direct targets of LSD1 as they were bound by LSD1 and showed elevated H3K4me2 levels upon LSD1 KD (FIG. 2G). (*HERV-E* is shown as an example in FIG. 2H). Importantly, a number of up-regulated retrotransposons, including ERVs, LTRs and LINEs, were expressed in both sense and antisense directions with overlapping sequences potentially allowing for the formation of double stranded RNAs (FIG. 2J). This observation was confirmed by analyzing a number of selected ERVs using strand-specific PCR (FIG. 2K). Thus, these findings demonstrated that LSD1 is important for transcriptionally silencing ERVs, consistent with a previous report suggesting that LSD1 regulates the expression of repetitive

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elements in mouse embryonic stem cells (mESCs) (Macfarlan et al. (2011) *Genes Dev* 25(6): 94-607).

To determine whether ERV transcript up-regulated caused by LSD1 inhibition was a causal factor for the induction of IFN/antiviral responsive genes, an engineered
5 4kb ERV fragment was ectopically expressed without protein coding capacity, derived from HERV-K and HERV-E. Its RNA overexpression readily caused the induction of IFNs and ISGs in MCF7 cells (FIGs. 2L and 2M), which demonstrated the sufficiency of ERV up-regulation in triggering IFN activation.

10 **Example 3. TLR3 and MDA5 sense dsRNA accumulation caused by LSD1 abrogation, which triggers interferon activation**

To determine whether ERV up-regulation as well as other retrotransposons in both sense and antisense directions in LSD1 KD cells contribute to the generation of dsRNAs, which may then trigger interferon activation, RNases and a dsRNA-specific
15 antibody (J2) were used (White et al. (2014) *Nat Struct Mol Biol* 21(6): 552-559) to measure the presence of dsRNA. RNase A (under high salt condition) cleaves single stranded (ss) RNA and preserves dsRNA (Roulois (2015) *Cell* 162: 961-973). By digesting total RNA isolated from control and LSD1 KD cells, and by normalizing to undigested RNA, the relative dsRNA enrichment was calculated in the presence and
20 absence of LSD1. dsRNA enrichment for a number of ERVs as well as a few other retrotransposons was much higher in LSD1 KD samples as compared to control samples (FIG. 2M). Using the dsRNA-specific J2 antibody, more transcripts of selected retrotransposons were captured in LSD1 KD samples (FIG. 2N). These results provide evidence for the elevation of intracellular dsRNA levels, which are
25 elevated as a result of LSD1 inhibition. Intracellular dsRNAs are recognized by pattern recognition receptors, TLR3, MDA5 and RIG-I, which are involved in subsequent activation of the interferon pathways (Takeuchi and Akira (201) *Cell* 140:805-820). In LSD1 KD cells, all three dsRNA sensors were among the up-regulated genes identified by RNA-seq (FIG. 2O). Furthermore, all three sensors
30 were induced considerably at the protein level in LSD1 KD cells as well (FIG. 2P), implying that those sensors might be responsible for detecting intracellular dsRNA accumulation in the absence of LSD1. To identify which sensor was essential for recognizing dsRNAs to elicit cellular responses, expression of individual sensors were

inhibited by shRNA-mediated knockdown in LSD1 KD cells, and the impact of knockdown on interferon activation was assessed. Each shRNA efficiently knocked down the expression of its target sensor (FIG. 3A). Importantly, abrogation of TLR3 and MDA5, but not RIG-I, significantly diminished the induction of interferon- β , IL-28 as well as ISGs without altering ERV expression level (FIGs. 3B-D and FIG. 4D). In addition, the abrogation of MAVS, which is a downstream adaptor of the MDA5 pathway, also blocked interferon activation in LSD1 KD cells (FIGs. 3E and 4E). As a further control, two key molecules, cGAS and STING, were knocked down in the cytoplasmic DNA sensing pathway (Chen et al. (2016) Nat Immunol 17(10): 1142-1149) and showed that cytoplasmic DNA is unlikely the trigger of interferon responses in LSD1 KD cells (FIGs. 3F, 3G and 3H). Therefore, dsRNA recognition by TLR3 and MDA5 was essential for IFN activation upon LSD1 inhibition, consistent with the observation that the up-regulated IFN/antiviral responsive genes were indirect targets of LSD1 (FIG. 2B).

Previous studies of these sensors (Takeuchi and Akira (201) Cell 140:805-820) suggest that TLR3 and MDA5 recognize dsRNAs that are at least 40bp in length or longer, respectively (see, e.g., Liu et al. (2008) Science 320(5874):379-381; Kato et al. (2006) Nature 441(7089):101-105, and Kato et al. (2008) J Exp Med 205(7): 1601-1610), whereas RIG-I prefers ssRNA or short dsRNA with 5' triphosphate ends (see, e.g., Pichlmair et al. (2006) Science 314(5801): 997-1001, Hornung et al. (2006) Science 314: 994-997; and Kato et al. (2008) J Exp Med 205(7): 1601-1610). The involvement of TLR3 and MDA5 in response to dsRNA stress is consistent with the directional RNA-seq analysis suggesting that those enriched dsRNAs are sufficiently long to be recognized by the dsRNA sensors TLR3 and MDA5.

Example 4. Decreased RISC activity due to loss of LSD1 reinforces intracellular dsRNA stress and promotes IFN activation

Double stranded RNAs derived from ERV transcripts can go on to trigger interferon responses or be processed by the RISC complex to generate endogenous small interfering RNA (endo-siRNA) and RNA interference (see, e.g., Watanabe et al. (2008) Nature 453(7194): 539-543; Tam et al. (2008) Nature 453(7194): 534-538; and Okamura and Lai (2008) Nat Rev Mol Cell Biol 9(9): 673-678). Thus the steady state of dsRNA pool is determined not only by ERV transcription but also the action of the

RISC complex. Next, it was determined whether LSD1 might also regulate the RISC complex to influence the steady state of dsRNA pool. LSD1 KD led to reduced protein expression of key components (DICER, AGO2 and TRBP2) of RISC (FIG. 4A). The regulation of RISC is dependent on LSD1 catalytic activity, as re-introduction of WT LSD1 but not LSD1-K661A back into LSD1 KD cells restored the protein expression of DICER, AGO2 and TRBP2 (FIG. 4A). In contrast, an obvious impact of LSD1 on the expression of Drosha, a crucial enzyme for miRNA biogenesis was not observed (FIG. 4B). Consistent with the above expression analysis, LSD1 KD also resulted in an elevated expression of a GFP reporter (FIGS. 4C, 4F and 4G), whose expression was under the control of *let-7* miRISC activity (Qi et al. (2008) Nature 455(7211): 421-424). This finding suggests that RISC may also be involved in dsRNA stress and interferon responses. Indeed, when AGO2 expression was inhibited by shRNA, an increase was observed in dsRNA abundance derived from a few retrotransposons tested (FIG. 4H), leading to the induction of interferon- β and IL-28 as well as ISGs (FIGS. 4I and 4J). Similarly, inhibition of either DICER or TRBP2 also activated the interferon pathway (FIGS. 4K-4N). Therefore, disruption of the RISC complex, which perturbs intracellular dsRNA homeostasis, is sufficient to elicit IFN activation. To confirm that RISC complex is necessary for LSD1 inhibition-stimulated IFN activation, the reduction of RISC was compensated by overexpressing AGO2 in LSD1 KD cells. AGO2 overexpression significantly diminished dsRNA accumulation caused by LSD1 inhibition, leading to a reduction in IFN activation (FIGS. 4O-4Q). Collectively, these findings suggest that, in addition to regulating ERV transcription, LSD1 also regulates the expression of RISC components and consequently RISC activity. Both actions of LSD1 contribute to its suppression of dsRNA accumulation.

Example 5. LSD1 regulates AGO2 protein demethylation and stability

In order to understand the mechanism by which LSD1 regulates the expression of RISC components, it was determined whether LSD1 inhibition-induced dsRNA stress, which was previously reported to decrease DICER protein expression (Wiesen and Tomasi, 2009), was involved. To this end, dsRNA stress was released by blocking its recognition by dsRNA sensors; TLR3 KD fully restored the protein level of DICER but not AGO2 or TRBP2 in LSD1 KD cells (FIG. 5A). This result

confirmed that the regulation of DICER expression by LSD1 was indirect, through dsRNA stress, while it also suggested that the regulation of AGO2 and TRBP2 expression was likely independent of dsRNA stress. Given that AGO2 is the central component responsible for RNA cleavage, the role of AGO2 was investigated in greater detail. No alterations in AGO2 RNA levels were detected upon LSD1 KD (FIG. 5B), suggesting the regulation occurs at post-transcriptional level. Indeed, in a cyclohexamide (CHX) chase assay, a substantial decrease in AGO2 protein half-life was detected when LSD1 was inhibited (FIGs. 5C and 5D), implicating a regulatory role of LSD1 in AGO2 protein stability. Interestingly, LSD1 was found to physically interacted with RISC complex as shown by co-immunoprecipitation assays using whole cell lysate (WCL) of MCF-7 cells stably expressing FH-AGO2 or FH-TRBP2 (FIGs. 5E and 5F). In addition, this physical interaction likely occurred in the nucleus, because a portion of RISC components was detected in the nuclear fraction and LSD1 is exclusively localized in the nucleus (FIG. 5G). As further evidence, reciprocal co-immunoprecipitation with WCL or nuclear extract (NE) of MCF-7 cells stably expressing FH-LSD1 was performed, and a much stronger interaction between LSD1 and AGO2 in NE was detected compared with that in WCL (FIG. 5H).

To investigate whether LSD1 regulates AGO2 stability by controlling AGO2 methylation, overexpressed FH-AGO2 from MCF-7 cells with or without LSD1 inhibition were purified and used for mass spectrometry analysis. A lysine residue at position 726 (K726) was consistently mono-methylated when LSD1 was inhibited either by shRNA-mediated KD or by GSK-LSD1 (FIG. 5I). To validate this finding, an antibody was raised that preferentially recognized AGO2 peptides mono-methylated at K726 (K726me1) compared with un-methylated or di-methylated peptides (FIG. 5J). Furthermore, this antibody detected increased K726me1 on ectopically expressed AGO2 when LSD1 was inhibited, which can be abrogated by substituting K726 with arginine (K726R) or alanine (K726A) (FIGs. 5K and 5L). Importantly, this methyl specific antibody also detected more mono-methylation at K726 on endogenous AGO2 upon LSD1 inhibition (FIG. 5M), suggesting that LSD1 regulates AGO2 demethylation *in vivo*. To gain more insights into this regulation, an *in vitro* demethylation assay was performed using immunoprecipitated FH-LSD1, FH-LSD1-K661A (catalytically compromised LSD1), FH-AGO2 and FH-AGO2-K726R proteins purified from mammalian cells. FH-LSD1 but not FH-LSD1-K661A

decreased K726me1 level on FH-AGO2, but had no observable effects on FH-AGO2-K726R (FIGs. 5N and 5O). Together, these results show that LSD1 regulates AGO2 demethylation at K726 *in vivo*, most likely through its catalytic activity against AGO2.

To ascertain that K726 demethylation is responsible for sustaining AGO2 stability, its methylation was blocked by a substitution of lysine for arginine, and observed an increased stability for AGO2-K726R compared to wild-type AGO2 under physiological condition as well as in response to LSD1 inhibition (FIG. 5P). Taken together, LSD1 modulates AGO2 stability by regulating AGO2 demethylation at K726, which is required for basal RISC activity that critically controls intracellular dsRNA homeostasis. Inhibition of LSD1 disrupts the above pathway, in addition to causing ERV up-regulation, leading to dsRNA stress and IFN activation in human cancer cells.

Example 6. LSD1 abrogation-induced dsRNA stress suppresses tumor cell growth *in vitro*

To address the biological consequence of LSD1 inhibition-induced dsRNA stress, and in particular, whether dsRNA stress-triggered cellular responses can be harnessed for anti-tumor immunity, C57BL/6 syngeneic mouse models were used. First, it was determined whether the previous observations made in human cells could be recapitulated in mouse cells. Lewis lung carcinoma (LLC), D4.m3A cells and B16 melanoma cells are all mouse tumor cell lines on the C57BL/6 genetic background with poor immunogenicity (Lechner et al. (2013) J Immunother 36(9): 477-489). LSD1 inhibition by Clustered Regularly Short Palindromic Repeats (CRISPR)/Cas9-mediated gene deletion resulted in upregulation of retrotransposons and activation of IFN pathways in those lines (FIGs. 6A-6D and 7A-7F), which recapitulated the findings in human cells described in Examples 2-5. In addition, dsRNA accumulation was observed in response to LSD1 loss (FIGs. 6E-6G). Taken together, the present results showed that LSD1 restrained intracellular dsRNA stress and interferon activation in both human and mouse cancer cells. LSD1 inhibition either by shRNA-mediated KD or by CRISPR/Cas9-mediated KO resulted in compromised growth of B16 cells *in vitro* (FIGs. 6H-6K), consistent with what has been reported previously for LSD1 in other cell lines (see, e.g., Zhang et al. (2013) Cell Rep 5(2): 445-457;

Harris et al. (2012) Cancer Cell 21(4): 473-487; and Mohammad et al. (2015) Cancer Cell 28(1): 57-69). To determine whether the growth phenotype is due to LSD1 abrogation-induced dsRNA stress, the dsRNA sensor MDA5 or TLR3 was deleted in LSD1 KO B16 cells (FIGs. 6L and 6M). The deletion of MDA5 significantly, albeit partially, rescued the growth defect of LSD1 KO B16 cells (FIGs. 6N and 6O), suggesting that the growth defect was in part due to the dsRNA stress induced by LSD1 deletion. At the molecular level, the induction of interferons and ISGs, but not dsRNA abundance, was significantly diminished in the LSD1/MDA5 double knockout (DKO) cells (FIGs. 6P and 6Q). As a control, deletion of MDA5 alone had minimal effects on B16 cell growth and interferon activation (FIGs. 8A-C). No apparent rescue was observed by TLR3 genetic deletion (FIG. 8D), which could be explained by the observation that TLR3 was not or minimally expressed in B16 cells (*data shown*). Therefore, similar to human cells, removal of dsRNA sensors blocks downstream interferon activation triggered by dsRNA stress caused by LSD1 loss.

A similar rescue effect on cell growth by blocking IFN pathway in LSD1 KO B16 cells. Indeed, the deletion of IFNAR1, a crucial subunit for type 1 IFN receptor, also diminished IFN activation and partially restored cell growth (FIGs. 8E-H), in line with the suppressive effect of type 1 IFN on cell growth. In addition, IFN- β deletion also displayed a similar, albeit milder, rescue effect (FIGs. 8I-L). LSD1-abrogation in mouse cancer cells causes dsRNA stress and subsequent IFN activation, leading to cell growth inhibition *in vitro*.

Example 7. LSD1 abrogation-induced dsRNA stress triggers anti-tumor T cell immunity *in vivo*

The role of LSD1 in basic cancer biology has been previously reported, which includes sustaining cancer stem cell self-renewal and suppressing differentiation, promoting cell proliferation, enhancing an epithelial-to-mesenchymal transition (EMT) as well as modulating metastasis (reviewed in Hosseini and Minucci, 2017). However, those studies used either *in vitro* cell culture systems or transplanted human cancer cells into immuno-deficient, in which the role of LSD1 in regulating tumor response to host immunity was not possibly to be explored.

To determine whether LSD1 deletion-induced dsRNA stress and interferon activation might trigger anti-tumor immunity *in vivo*, C57BL/6 WT mice were

subcutaneously inoculated with B16 cells. The deletion of LSD1 in B16 cells significantly inhibited tumor growth *in vivo* (FIGs. 9A and 9B), in agreement with the previous *in vitro* observations (FIGs. 6H-K). To distinguish the role of LSD1 in regulating tumor autonomous growth versus host anti-tumor immunity, both immunocompetent (WT) and immunodeficient, T-cell receptor α (TCR α) KO, mice were used for subcutaneous tumor growth assays. Although LSD1 deletion inhibited B16 tumor growth in WT mice, there was no growth difference between LSD1 KO and control B16 tumors in the TCR α -deficient mice (FIGs. 9C and 9D). This result indicated that LSD1 inhibition in tumor cells elicits potent anti-tumor T cell immunity *in vivo*, rather than affecting tumor autonomous growth, to restrain tumor burden. The reason for the loss of autonomous growth defects of LSD1 KO cells *in vivo* is not known at the present time, however, this could be due to the possibility that host somatic cells, such as stromal cells in the tumor microenvironment, foster cell proliferation and tumor growth by secreting growth-promoting factors that may substitute the need for LSD1 in cell proliferation.

To confirm that host anti-tumor T cell immunity was boosted by tumor-intrinsic dsRNA stress as elucidated above, tumor growth of LSD1 KO and LSD1/MDA5 DKO B16 cells was compared in immunocompetent mice. Deletion of MDA5 was sufficient to diminish LSD1 inhibition-elicited anti-tumor immunity, evidenced by the finding that LSD1/MDA5 DKO tumors displayed similar growth ability as control tumors targeted with scrambled gRNA or MDA5 single KO tumors (FIGs. 9E and 9F). To further examine whether MDA5-associated type 1 IFN response is essential for LSD1 inhibition-elicited anti-tumor immunity, IFN- β production was abrogated in LSD1 KO B16 tumors (FIGs. 6L and 8L), which completely reverse growth inhibition to a level comparable to that of the control of IFN- β single KO tumors in the immunocompetent mice (FIG. 9G). In addition to controlling tumor growth, LSD1 inhibition resulted in a marked reduction in B16 tumor metastasis (FIGs. 9H and 9I). Thus, LSD1 inhibition-caused dsRNA stress and resultant IFN response sensitize tumors to T cell immunity, likely by increasing tumor immunogenicity.

This finding is consistent with previous reports demonstrating that LSD1 promotes cell proliferation in cell culture and in mouse xenograft models (Zhang et al. (2013) Cell Rep 5(2): 445-457; and Mohammad et al. (2015) Cancer Cell 28(1): 57-

69). However, since these studies transplanted human tumor cells into immunodeficient mice, the potential impact from tumor microenvironments, in particular immune cells, was not taken into consideration. When syngeneic immunodeficient and immunocompetent mice were used in parallel, LSD1 was found not to be required for B16 tumor autonomous growth in vivo, revealing that LSD1 inhibition mainly elicits a potent anti-tumor adaptive immunity, which drastically reduces tumor growth.

Example 8. LSD1 inhibition manifests tumor immunogenicity and increases T cell infiltration

To further elucidate the mechanism connecting LSD1 inhibition to enhanced anti-tumor T cell immunity, the impact of tumor cell-intrinsic LSD1 on T cell activity in the tumor microenvironment was determined. By analyzing tumor-infiltrating lymphocytes (TILs) in transplanted B16 tumors, LSD1 ablation in tumor cells resulted in a significant increase in CD4⁺ and CD8⁺ T cell infiltration, indicating a stronger ability to induce T cell immunity (FIG. 10A). Importantly, the increase in T cell infiltration was diminished when MDA5 was concurrently ablated (FIG. 10A). In contrast, no significant alteration in T cell populations was detected in draining lymph nodes (dLNs) of B16 tumor-bearing mice (FIG. 10B), suggesting the impact on T cells by tumor cell LSD1 ablation is restricted to tumor sites. To assess the functional activity of CD8⁺ TILs, the expression of a proliferation marker, Ki-67, and a cytotoxic factor, Granzyme-B (GzmB) were detected, but neither showed a noticeable alteration when LSD1 was deleted in B16 tumor cells (FIG. 10C). Thus, in consideration of the aforementioned tumor growth inhibition (FIGs. 9A-I), these results suggest that increased T cell infiltration mediated by dsRNA recognition pathway imparts potent anti-tumor immunity to LSD1-null tumors.

To investigate whether the increased T cell infiltration is associated with increased TCR repertoire diversity of CD8⁺ TILs in LSD1 KO B16 tumors, the clonality and entropy of these T cells was analyzed by T cell receptor (TCR) sequencing, but no significant changes were found compared to their counterparts in WT tumors (FIG. 10D). Thus, the increased T cell infiltration in LSD1 KO tumors is not due to an unlikely alteration in tumor antigenicity.

To investigate tumor cell characteristics, which are associated with tumor response to T cell immunity and critically regulated by LSD1 inhibition-induced dsRNA stress, GFP-labeled B16 lines were created to facilitate the accurate isolation of tumor cells from *in vivo* transplanted tumors, and then these *ex vivo* tumor cells were used for transcriptomic analysis. LSD1 deletion significantly altered gene expression profile in B16 tumor cells *in vivo*, which appeared to be suppressed when MDA5 was simultaneously deleted (FIGs. 10E and 10F). Consistent with the *in vitro* results with human cancer cells, LSD1 ablation also led to upregulation of ERVs by regulating their transcription in B16 tumor cells *in vivo* (FIGs. 10G-I), suggesting the aforementioned mechanism is conserved *in vivo*.

Genes whose expression was selectively up-regulated ($\text{FDR} < 0.05$ and $\log_2(\text{FC}) > 1$) in LSD1 KO tumor cells compared with control tumor cells were filtered out for GO analysis. This analysis showed that immune response-related biological processes, including innate immune response, response to IFN- β , defense response to virus and MHC protein complex, were ranked among the top 10 GO terms in LSD1 KO tumor cells (FIG. 10J), providing evidence for the increased tumor immunogenicity. The GO term response to IFN- γ was also significantly enriched (FIG. 10J), implying an increased response of LSD1 KO tumor cells to T cell killing. In addition, genes associated with inflammatory response were also enriched in LSD1 KO tumor cells as analyzed by GSEA (FIG. 10K). Importantly, the induced expression of genes associated with the top 10 GO terms was significantly diminished by simultaneous MDA5 deletion in LSD1 KO cells (FIG. 10L), suggesting a critical role of dsRNA recognition pathway in mediating tumor immunogenicity. Of note, there was no apparent alteration by LSD1 deletion of cell proliferation pathways by GSEA (FIG. 10M), which further supported the notion that autonomous growth of B16 tumor cells in syngeneic mice is likely independent of LSD1 status.

In order to validate the findings from RNA-seq, it was determined whether antigen presentation on tumor cell surface restricted by MHC-1, whose alteration enables immune escape and is commonly found in solid tumors, is affected by LSD1. In the RNA-seq analysis, most MHC-1 coding genes were upregulated in LSD1 KO B16 cells, among which the induction of H2-D1 and H2-K1, encoding classical class 1 antigens, was largely dependent on MDA5 pathway (FIG. 10N). Consistently, in flow cytometric analysis of GFP-labeled B16 cells isolated from *in vivo* transplanted

tumors, LSD1 deletion caused a marked induction of MHC-1 expression on tumor cell surface, which was completely abrogated by concurrent deletion of MDA5 (FIG. 10O). Altogether, these results show that LSD1 inhibition through the dsRNA recognition pathway manifests tumor immunogenicity, associated with increased T cell infiltration.

To examine whether the enhanced tumor immunogenicity by LSD1 inhibition is a generalizable mechanism, another “cold” tumor model, D4m melanoma, in which LSD1 ablation also caused increased dsRNA levels and IFN activation, was used (FIGs. 6C and 6G). In syngeneic immunocompetent mice, LSD1 KO D4m tumors displayed slower growth than wild type control tumors (FIGs. 10P and 10Q). Consistently and critically, increased T cell infiltration in LSD1 KO tumors and elevated MHC-1 expression on the surface of LSD1 KO tumor cells was found compared with control tumors (FIGs. 10R and 10S), indicating enhanced T cell immunity. Thus, these results suggested that the enhanced tumor immunogenicity by LSD1 inhibition was not limited to B16 tumor model and are of broader significance.

Notably, RNA-seq and flow cytometry identified up-regulation of PD-L1 expression in the B16 tumor cells *in vivo*, which is independent of MDA5 (FIGs. 10T and 10U). It is possible that PD-L1 induction may suppress the functional activity of CD8⁺ TILs (Juneja et al., 2017), thus compromising the anti-tumor effect of increased TILs caused by LSD1 inhibition. In summary, our results reveal a critical impact of tumor cell-intrinsic LSD1 on modulating tumor response to T cell immunity.

Example 9. LSD1 inhibition overcomes tumor resistance to PD-1 blockade

In cancer patients, the presence of CD8⁺ TILs that are suppressed by PD-L1 predicts the responsiveness to PD-(L)1 blockade (see, e.g., Herbst et al. (2014) Nature 515(7528): 563-567; and Tumeh et al. (2014) Nature 515(7528): 568-571). B16 tumors have high expression of PD-L1 expression but poor immunogenicity, and are known to be non-responsive to PD-1/PD-L1 blockade in the absence of vaccination (see, e.g., Chen et al. (2015) Cancer Immunol Res 3(2): 149-160; Kleffel et al. (2015) Cell 162(6): 1242-1256; and Juneja et al. (2017) J Exp Med 214(4): 895-904). Given that LSD1 inhibition elicits anti-tumor immunity, it was determined whether LSD1 inhibition would sensitize B16 tumors to PD-1/PD-L1 blockade. Consistent with previous reports (see, e.g., Chen et al. (2015) Cancer Immunol Res

3(2): 149-160; Kleffel et al. (2015) Cell 162(6): 1242-1256; and Juneja et al. (2017) J Exp Med 214(4): 895-904), PD-1 blockade alone had no overt effects on wild type B16 tumor growth (FIGs. 11A and 11B). Strikingly, PD-1 blockade showed a dramatic effect on controlling LSD1 KO B16 tumors (FIGs. 11A and 11B).

5 Furthermore, this responsiveness to PD-1 blockade doesn't rely on tumor size, because re-scheduled anti-PD-1 administration when tumor sizes reached a set volume also had a profound effect on controlling growth of LSD1 KO tumors but not WT tumors (FIGs. 11C and 11D). Moreover, this profound delay in tumor growth was achieved with a late initiation of PD-1 blockade as well as a low dose of blocking antibody. These results demonstrated a strong synergy between LSD1 inhibition and PD-1 blockade in controlling tumor growth and suggest that targeting LSD1 bypasses the need for vaccination to obtain PD-1 blockade responsiveness in the B16 tumor model. Importantly, these results also implicate increased T cell infiltration caused by LSD1 inhibition as a likely mechanism underlying the synergism between LSD1 inhibition and PD-1 blockade. Taken together, the combination of LSD1 inhibition and PD-1 blockade may work through simultaneously eliciting anti-tumor adaptive immunity and reinvigorating dysfunctional T cells to achieve a synergistic effect for tumor treatment. Given the general role of LSD1 in regulating dsRNA and interferon responses, targeting LSD1 in combination with anti-PD-(L)1 may prove to be a broadly applicable new strategy in cancer immunotherapy. Furthermore, LSD1 inhibition may overcome the resistance of B16 tumors to PD-1 blockade by increasing immunogenicity.

Example 10. Anti-PD-1 treatment of B16 tumors

25 To determine whether the synergistic effect between LSD1 inhibition and PD-1 blockade relies on the dsRNA sensor MDA5, immunocompetent C57BL/6 mice are anesthetized with Avertin (2.5%), shaved at the injection site, and then these mice are injected in the flank subcutaneously with 250,000-500,000 B16-F10 tumor cells of scramble, LSD1 KO or LSD1/MDA5 DKO (20 mice per genetically modified tumors, 20X3=60 mice in total). Tumors are measured every 2-3 days once palpable (long diameter and short diameter) with a caliper. Tumor volume is determined using the volume formula for an ellipsoid: $1/2 \times D \times d^2$ where D is the longer diameter and d is

the shorter diameter. Mice are sacrificed when tumors reached 2 cm³ or upon ulceration/bleeding.

For antibody treatments, mice are given 100 µg antibody intra-peritoneally when tumor size reaches 200 mm³ or at day 8-10. Antibody treatment is repeated every other day for a total of four injections. The following antibodies are used: half mice receiving anti-PD-1 (clone 29F.1A12) (Dana Farber Cancer Institute, Boston, MA) and the other half mice receiving rat IgG2a isotype control antibody are purchased from BioXCell (cat#BE0089). Prior to treatments mice are randomized such that treatment groups have similar average tumor volumes prior to treatment initiation.

Example 11. LSD1 chemical inhibitor in combination with anti-PD-1 treatment for B16 tumors

To assess the efficacy of LSD1 chemical inhibition in combination with anti-PD-1 treatment in controlling B16 tumor growth, WT B16 tumor cells are inoculated as described in Example 10 (40 mice in total: 10 mice for vehicle + isotype; 10 mice for GSK2879552 + isotype; 10 mice for vehicle + anti-PD-1; 10 mice for GSK2879552 + anti-PD-1). For inhibitor treatment, GSK2879552 is orally administrated or intra-peritoneally injected 1.5 mg/kg daily or every other day starting from the second day after tumor inoculation. For antibody treatment, anti-PD-1 or isotype is intra-peritoneally injected every other day starting from day 8 after tumor inoculation for a total of four injections. Tumor volume is recorded and is determined using the volume formula for an ellipsoid: $1/2 \times D \times d^2$ where D is the longer diameter and d is the shorter diameter. Mice are sacrificed when tumors reached 2 cm³ or upon ulceration/bleeding.

Example 12. Syngeneic tumor models with LLC, D4M and Renca cells

To determine whether these findings can be generalized to other tumor models, LSD1 is deleted by CRISPR/Cas9 LLC cells, D4M cells and Renca cells. LLC LSD1 KO cells, D4M LSD1 KO cells and Renca LSD1 KO cells (250,000-500,000 per mouse) are injected into their syngeneic immunocompetent mice (LLC to B6 mice, D4M to B6 mice and Renca to Balb/c mice). Tumors are measured every 2-3 days once palpable (long diameter and short diameter) with a caliper. Tumor

volume is determined using the volume formula for an ellipsoid: $1/2 \times D \times d^2$ where D is the longer diameter and d is the shorter diameter. Mice are sacrificed when tumors reached 2 cm³ or upon ulceration/bleeding.

5 **Example 13. WT versus LSD1 KO B16 tumor growth in B6 mice for tumor-infiltrating lymphocyte (TIL) analysis**

To analyze the tumor immunogenicity and anti-tumor immunity caused by LSD1 deletion, B16 tumor cells are inoculated into immunocompetent mice as described in Example 10 (5 mice for WT control and 5 mice for LSD1 KO B16 cells).

10 At day 12, mice are sacrificed and tumors are collected. Isolated tumors are then excised into small pieces and are digested by collagenase to obtain single cell suspension. Some of the cells are directly stained with appropriate antibodies for profiling various immune cells, including CD4 T cells, CD8 T cells, macrophages, DCs and NK cells. Some of the cells are fixed, are permeabilized and are stained with
15 anti-TCR β , anti-CD4, anti-CD25 and anti-Foxp3 for Treg cells, and anti-TCR β , anti-CD8 and anti-GzmB for effector CD8 T cells. In addition, some of the cells are re-stimulated for intracellular cytokine staining, such as IFN- γ and IL-2. Stained cells are subjected to flow cytometry for analysis. Alternatively, some tumor samples are subjected to IHC analysis.

20

Example 14. WT versus LSD1 KO B16 tumor growth in B6 mice for tumor-infiltrating lymphocyte (TIL) analysis

To analyze the tumor immunogenicity and anti-tumor immunity in the setting of LSD1 ablation plus anti-PD-1 treatment, B16 tumor cells are inoculated into
25 immunocompetent mice, which previously would have received anti-PD-1 or isotype antibody injection at day 8 and day 10 (5 mice for scramble B16 + isotype; 5 mice for LSD1 KO B16 + isotype; 5 mice for scramble B16 + anti-PD-1; 5 mice for LSD1 KO B16 + anti-PD-1). At day 12, TIL are analyzed as described in Example 13.

30 **Example 15. B16 tumor growth in WT or IFNAR1 KO mice**

To determine if B16-derived IFN- β is important for LSD1 deletion-induced anti-tumor immunity and what types of cells are the crucial targets of IFN- β , B16 tumor cells are inoculated into immunocompetent WT or IFNAR1 KO mice, which

would receive anti-PD-1 or isotype antibody injection at day 8, 10, 12 and 14 (5 mice for scramble B16 + isotype; 5 mice for LSD1 KO B16 + isotype; 5 mice for scramble B16 + anti-PD-1; 5 mice for LSD1 KO B16 + anti-PD-1; 5 mice for LSD1/IFN- β DKO B16 + isotype; 5 mice for LSD1/ IFN- β DKO B16 + anti-PD-1, 5 mice for LSD1/ IFNAR1 DKO B16 + isotype; 5 mice for LSD1/ IFNAR1 DKO B16 + anti-PD-1). Tumors are measured every 2–3 days once palpable (long diameter and short diameter) with a caliper. Tumor volume is determined using the volume formula for an ellipsoid: $1/2 \times D \times d^2$ where D is the longer diameter and d is the shorter diameter. Mice are sacrificed when tumors reached 2 cm³ or upon ulceration/bleeding.

Example 16. Translational Significance

To demonstrate that these findings have translational significance, the public datasets on human cancer were explored. *LSD1* was infrequently mutated, amplified or deleted in a majority of cancer types examined (FIG. 12A), but LSD1 was found to be overexpressed in cancerous tissues compared with normal tissues in a variety of cancer types (FIG. 12B). To determine whether LSD1 expression level in tumors correlated with clinical outcome, patients of each cancer type were divided by LSD1 expression median, and overall survival between the two groups was compared. This analysis showed that LSD1-high group had a significantly shorter overall survival time than LSD1-low group for a number of cancer types (FIG. 12C), suggesting LSD1 overexpression is a poor prognostic factor. In line with the finding that LSD1 inhibition caused IFN/antiviral response in *in vitro* MCF-7 cells and *ex vivo* B16 cells (FIGs. 1N and 10J), LSD1 expression level was found to be inversely correlated with IFN/antiviral response in a variety of cancer types in TCGA cancer patient dataset (FIG. 12D). LSD1 expression level was also inversely correlated with CD8⁺ T cell infiltration in most cancer types (FIG. 12E), consistent with the finding of increased T cell infiltration by LSD1 inhibition in mouse models (FIGs. 10A and 10P).

Further analysis on the TCGA skin cutaneous melanoma (SKCM) cohort showed that patient group with low LSD1 expression (LSD1-low) had better survival probability than that with intermediate or high LSD1 expression (LSD1-int/high) (FIG. 12F), and consistently, LSD1-low group was associated with increased expression of genes enriched in immune responses (FIG. 12G). Specifically, both

CD8a and GzmB were expressed higher in the LSD1-low group than in the LSD1-int/high group, indicating increased CD8⁺ T cell infiltration (FIGs. 12H and 12I).

Example 17. Lsd1 ablation induces Tgfb expression through histone modifications in cancer cells

To identify tumor cell-derived factors that may compromise the cytotoxicity of CD8⁺ TILs, *ex vivo* RNA-seq data was studied to determine immune suppressive molecules or pathways including cytokines, metabolites, nutrient consumption that are well documented. Candidate genes were sorted based on two criteria: transcripts that were considerably expressed in B16.F10 tumor cells and whose expression was significantly induced by LSD1 ablation. This analysis uncovered two hits, TGF- β subfamily and M-CSF, which exerts potent suppression on T cells and dendritic cells (DCs) in tumor microenvironment [P. Seefer 2015; David (2018) Nat Rev Mol Biol 19(7): 419-435; Neubert (2018) Sci Transl Med 10(436): pii:eaan3311].

In both *in vitro* MCF-7 cells (a human breast cell line) and *ex vivo* B16.F10 cells (a mouse melanoma cell line), the expression of Tgfb1 and Tgfb3 was significantly induced by Lsd1 deletion as identified by RNA-seq (FIGs. 13A and 13B). To validate the effect of Lsd1 ablation on Tgfb induction, qPCR analysis was performed on *in vitro* cultured B16 cells, and Tgfb subfamily genes were consistently upregulated by Lsd1 deletion, which were rescued upon re-introduction of LSD1 (FIG. 13C). In addition, IFN pathway was not involved in Lsd1 ablation-induced Tgfb expression (FIG. 13C). The inducible effect of Lsd1 ablation on Tgfb expression was confirmed in two additional mouse cell lines, D4m.3A (a melanoma line with *Braf*^{V600E} and *Pten* loss) and LLC (Lewis lung carcinoma) (FIGs. 13E and 13F). These results suggest that LSD1 inhibition induces the expression of Tgfb subfamily in various cancer cells.

To gain insight into the regulatory mechanism of Tgfb expression by LSD1, ChIP-PCR assay was performed. LSD1 occupancy at the promoter regions of all three *Tgfb* genes was found (FIG. 14A), and the deletion of Lsd1 led to the significant accumulation of H3K4me2 and H3K27ac at the promoter of Tgfb1 (FIGs. 14B and 14C), implicating the transcriptional activation of *Tgfb1*. Thus, the induction of Tgfb expression by Lsd1 deletion was likely due to the increased gene transcription.

Example 18. TGF- β pathway does not influence the immune stimulatory effect of Lsd1 ablation in cancer cells

Given three TGF- β s bind the same receptors and they may have functional redundancy [David (2018) Nat Rev Mol Biol 19(7): 419-435; Morikawa (2016) Cold Spring Harb Perspect Biol 8(5):pii: a021873], all three TGF- β s were genetically
 5 deleted to completely abrogate the activity of tumor cell-derived TGF- β s, and then the biological consequences in the context of tumor immunity and immunotherapy were examined. The ablation of three *Tgfb* genes in Lsd1 knockout (KO) or wild-type (WT) B16.F10 cells was achieved by CRISPR/Cas9 technology and confirmed by the
 10 sequencing of gene loci, which all showed the out-of-frame shifts. The qPCR analysis of *Tgfb* transcripts showed their decreased abundance in Lsd1, *Tgfb1*, *Tgfb2* and *Tgfb3* quadruple knockout (Lsd1/*Tgfb* QKO) cells (FIG. 15A). In addition, the diminished induction of *Serpine1*, a reporter gene for TGF- β pathway activation, further supported the abrogation of TGF- β s in Lsd1/*Tgfb* QKO cells (FIG. 15B).
 15 Next, it was determined whether inducible TGF- β s affected the immune stimulatory effect of Lsd1 ablation. A panel of interferon genes (IFNs) and interferon-stimulated genes (ISGs) mostly showed comparable expression between Lsd1 single KO and Lsd1/*Tgfb* QKO cells *in vitro* (FIGs. 15C and 15D), suggesting inducible TGF- β s had no overt effect on tumor cell-intrinsic immunogenic properties resulted from Lsd1
 20 ablation.

Example 19. Abrogation of inducible TGF- β s in Lsd1 null tumors potentiates antitumor T cell immunity

Four groups of B16.F10 tumor cells (Scramble, Lsd1 KO, *Tgfb* triple KO and Lsd1/*Tgfb* QKO) were inoculated into immunocompetent syngeneic mice to examine
 25 the *in vivo* biological consequences. While *Tgfb* deletion alone had no observable effect on tumor growth, the abrogation of inducible TGF- β s in Lsd1 null tumors showed additional effect on controlling tumor growth, evidenced by the significantly delayed tumor growth of Lsd1/*Tgfb* QKO tumors compared with Lsd1 KO tumors (FIGs. 16A and 16B). To examine if the antitumor effect of TGF- β abrogation relies
 30 on T cell immunity, tumor growth was compared in immunocompetent (WT) and immune deficient T cell receptor α (TCR α) KO mice. Although Lsd1/*Tgfb* QKO inhibited B16.F10 tumor growth in WT mice, there was no growth difference between Lsd1/*Tgfb* QKO and scramble tumors in TCR α KO mice (FIGs. 16C and 16D).

Together, these results indicate that the abrogation of inducible TGF- β s in Lsd1 null tumors potentiates antitumor T cell immunity.

Considering TGF- β s are critically involved in tumor invasion and metastasis (Massague (2008) Cell 134(2): 215-230), the effect of co-targeting LSD1 and TGF- β s in tumor metastasis were assessed. In a B16 lung metastasis model, Lsd1/Tgfb QKO tumor cells showed significantly reduced lung metastasis compared with either Lsd1 KO or Tgfb TKO cells (FIGs. 17A and 17B). This result suggested a cooperative effect of co-targeting LSD1 and TGF- β s on suppressing tumor metastasis.

Example 20. Tumor cell-derived TGF- β s induced by Lsd1 ablation suppress the cytotoxic molecules of CD8⁺ TILs

To elucidate the immunologic and molecular mechanism of inducible TGF- β -mediated retardation of antitumor immunity elicited by Lsd1 ablation, the activity of TILs in tumor microenvironment were analyzed. Consistent with the *in vitro* observation that TGF- β s have no overt effect on Lsd1 ablation-stimulated tumor cell-intrinsic immune responses, T cell infiltration in Lsd1 KO and Lsd1/Tgfb QKO tumors had no observable difference, albeit both were elevated compared with scramble control (FIGs. 18A and 18B). In Lsd1 KO tumors, similar as was reported in Sheng (2018) Cell Death Differ 25(5): 918-934, the activation of CD8⁺ TILs remains unaltered, evidenced by the constant expression of GzmB and Ki-67 (FIGs. 18C-E). Intriguingly, the expression of GzmB by CD8⁺ TILs in Lsd1/Tgfb QKO tumors was significantly increased (FIG. 18C). The expression of Ki-67 seemed to be also induced by abrogation of TGF- β s, even though the increase was not interpreted as statistically significant (FIG. 18D). These results suggest that the abrogation of inducible TGF- β s unleashes the cytotoxicity of increased CD8⁺ TILs in Lsd1 null tumors, which may potentiate antitumor immunity.

In summary, potent immune suppressor TGF- β s were identified that when induced by Lsd1 ablation detrimentally dampened the effector function of CD8⁺ TILs and thus the antitumor T cell immunity of Lsd1 inhibition, implying a strategy of combining LSD1 inhibition, TGF- β blockade and PD-(L)1 blockade for treating certain refractory tumors.

Example 21. PD-1 blockade eradicates refractory tumors when LSD1 and TGF- β are ablated

The co-targeting of LSD1 and TGF- β in tumor cells created an immune privileged microenvironment, leading to increased T cell infiltration and enhanced T cell cytotoxicity, which may render refractory tumors greatly responsive to anti-PD-1 therapy. To address this, Lsd1/Tgfb QKO and scramble B16 tumor cells were transplanted into immunocompetent mice subcutaneously and grew 10 days to form solid tumors before anti-PD-1 or isotype control treatment. Scramble tumors with or without anti-PD-1 treatment as well as Lsd1/Tgfb QKO tumors without anti-PD-1 treatment showed continuous growth in volume, whereas 6 of 10 Lsd1/Tgfb QKO tumors receiving anti-PD-1 treatment disappeared and additionally one tumor showed reduced tumor volume compared to that before anti-PD-1 treatment over a period of 22 days (FIGs. 19A-D). These results suggest that co-targeting LSD1 and TGF- β in tumor cells may enable the anti-PD-1-mediated eradication of refractory tumors.

Example 22. TGF- β s Secreted by LSD1 Null Tumors Act on CD8 $^{+}$ T Cells and Suppress Their Cytotoxicity

To determine if cytotoxic CD8 $^{+}$ TILs were the direct and primary targets of tumor cell-derived TGF- β s in LSD1 null tumors, the TGF- β pathway in T cells was specially blocked using CD4-dnT β RII transgenic mice, which express a dominant negative TGF- β receptor on T cells that attenuates T cell response to TGF- β signals. It was speculated that if LSD1 ablation-induced TGF- β s directly act on T cells, the growth difference between Lsd1 KO and Lsd1/Tgfb QKO tumors observed in WT mice should be diminished when they were implanted in the CD4-dnT β RII transgenic mice. To test this, an in vivo tumor growth assay was performed by implanting Lsd1 KO and Lsd1/Tgfb QKO B16 tumors in CD4-dnT β RII transgenic and littermate WT mice. In line with the limited expression of TGF- β s in Lsd1/Tgfb QKO tumors (FIG. 20A), those tumors grew similarly in WT and CD4-dnT β RII transgenic mice, both slower than Lsd1 KO tumors implanted in WT mice as expected (FIG. 20B). These data thus support a prominent role of tumor cell-derived TGF- β s in suppressing T cell immunity in LSD1 null B16 tumors, where the host cell-derived TGF- β s may have limited contributions. When Lsd1 KO tumors were implanted in CD4-dnT β RII transgenic mice, their growth was significantly suppressed to a level even slower than

Lsd1/Tgfb QKO tumors that were implanted in either WT or transgenic mice (FIG. 20B). In line with the tumor growth phenotype, the expression of GzmB by CD8+ TILs in Lsd1 KO tumors was increased by the presence of dnTβRII to a similar level as those in Lsd1/Tgfb QKO tumors (FIG. 20C). Thus, TGF-β upregulation in B16 tumor cells resulted from LSD1 loss directly acts on CD8+ TILs and suppresses the expression of cytotoxic molecules.

The slower growth of the Lsd1 KO tumors compared with Lsd1/Tgfb QKO tumors in CD4-dnTβRII transgenic mice, where TGF-βs' effect on T cells are blocked, could be due to a growth inhibitory effect of TGF-βs on B16 tumor cells, since TGF-β is known to play a tumor cell growth inhibitory role by suppressing cell cycle progression, promoting differentiation and inducing apoptosis. To confirm the growth inhibitory effect of TGF-βs on Lsd1 KO B16 tumor cells in vivo, a TGF-β dominant negative receptor (TβRII-DN) was ectopically expressed on the membrane of Lsd1 KO B16 cells, which did not interfere with TGF-β expression but rendered B16 cells unresponsive to TGF-β signals (FIG. 20D). When implanted in the WT mice, the Lsd1 KO tumors expressing TβRII-DN grew significantly faster than those responsive to TGF-β signals (FIG. 20E). Thus, TGF-βs induced by LSD1 ablation in B16 tumors play at least two opposing roles: primarily, paracrine TGF-βs play a pro-tumor role through acting on CD8+ TILs and suppressing their cytotoxicity, which compromises the antitumor effect of LSD1 loss; secondarily, autocrine TGF-βs play an antitumor role through acting on tumor cells and inhibiting cell survival/proliferation.

To directly evaluate the suppressive effect of LSD1 ablation-induced TGF-βs on CD8+ T cell cytotoxicity, an in vitro cytotoxicity assay reported previously was used. Splenic CD8+ T cells from OT-I mice expressing TCR specific for Ova257-264 peptide (or OT-I cells) were first activated by in vitro stimulation with anti-CD3/anti-CD28, and then these cells were incubated with genetically modified B16 cells that were pulsed with an Ova257-264 peptide. B16 cells without the peptide pulse were included as controls for antigen-dependent killing effects. Indeed, OT-I cells exhibited stronger Ova-dependent killing effect towards Lsd1/Tgfb QKO than Lsd1 KO B16 cells (FIG. 20F).

Example 23. Anti-PD-1 Treatment Eradicates LSD1 Null Tumors When TGF- β is Concurrently Blocked, Resulting in Immunity Against Tumor Re-challenge

Experiments were designed to determine if TGF- β is a critical inhibitor of tumor response to anti-PD-1 therapy mainly through expelling T cell infiltration.

Without being bound by theory, in the poorly immunogenic B16 tumor model, tumor cell-derived TGF- β s may be unlikely to have a major effect on T cell infiltration, because neither Tgfb deletion alone nor their deletion on top of Lsd1 loss changed T cell numbers within TME. Without being bound by theory, data showed TGF- β s in B16 tumor model play a critical role in suppressing CD8⁺ T cell cytotoxicity. The response of Lsd1/Tgfb QKO tumors to anti-PD-1 treatment was tested. Lsd1/Tgfb QKO tumors were implanted in WT mice and established up to an average volume of ~250 mm³ (ranging from 100 to 400 mm³) before the initiation of anti-PD-1 treatment. Surprisingly, ~44% of Lsd1/Tgfb QKO tumors were rejected by PD-1 blockade without recurrence up to 60 days (FIG. 21A). This is in stark contrast to previous studies that LSD1 null B16 tumors could not be completely rejected by anti-PD-1 treatment. In another experiment, anti-PD-1 treatment was initiated when Lsd1/Tgfb QKO tumors grew up to an average volume of ~50 mm³, in which tumor rejection rate was raised further to ~60% (FIG. 21B), suggesting that the rejection rate of Lsd1/Tgfb QKO tumors by PD-1 blockade is influenced by tumor burdens.

Next, experiments designed to substitute genetic perturbation of Tgfb with systemic delivery of a TGF- β blocking antibody were performed. While the combination of anti-TGF- β and anti-PD-1 showed a modest effect on controlling scramble B16 tumors, it significantly extended the survival of mice carrying LSD1 null tumors where ~33% tumor rejection rate was observed (FIG. 21C). These results demonstrate an inhibitory role of TGF- β s that impede the response of LSD1 null tumors to anti-PD-1 treatment, highlighting the importance of blocking TGF- β s when LSD1 is targeted in this combination immunotherapy.

To determine whether mice which rejected Lsd1/Tgfb QKO tumors upon anti-PD-1 treatment had developed immunity, surviving mice were re-challenged with WT B16 tumors on the left hind flank 60 days after clearance of the primary tumors. While naïve, age-matched control mice all grew tumors and reached the end-point within 30 days, 5 out of 6 mice cured by Lsd1/Tgfb ablation plus anti-PD-1 treatment rejected secondary B16 tumors (FIG. 22A and FIG. 22B). Mice survived from B16 re-

challenge were subsequently challenged with the irrelevant MC38 colorectal adenocarcinoma on the right front flank 60 days after secondary tumor clearance. In contrast to the re-challenge of B16 tumors, MC38 tumors grew similarly in those mice compared with their counterparts in the naïve age-matched mice (FIG. 22C and FIG. 22D). These results suggest that tumor bearing-mice cured by Lsd1/Tgfb ablation plus anti-PD-1 treatment may have developed immunologic memory

OTHER EMBODIMENTS

It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating cancer in a patient, the method comprising:
administering to a patient in need of cancer treatment a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor, a transforming growth factor beta (TGF β) inhibitor, and at least one of a programmed-cell death 1 (PD-1) inhibitor and a programmed-cell death ligand 1 (PD-L1) inhibitor, to thereby treat cancer in the patient.
2. A method of treating cancer in a patient, the method comprising:
administering to a patient in need of cancer treatment a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor, a transforming growth factor beta (TGF β) inhibitor, and at least one immunotherapy, to thereby treat cancer in the patient.
3. A method of inducing or increasing an immunological response to a cancer or tumor, the method comprising:
administering to a patient in need thereof a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor, and a transforming growth factor beta (TGF β) inhibitor, to thereby induce or increase the immunological response to the cancer or tumor in the patient.
4. A method of reducing the likelihood of recurrence of a cancer or tumor in a patient, the method comprising:
treating cancer in the patient by surgery, chemotherapy, or radiation therapy; and
administering to the patient in need thereof a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor, and a transforming growth factor beta (TGF β) inhibitor, to thereby reduce or prevent recurrence of the cancer or tumor in the patient.

5. The method of claim 3 or 4, wherein the method further comprises administering an immunotherapy to the patient.
6. The method of any one of claims 1-5, wherein the method further comprises identifying the patient as having cancer prior to administering.
7. The method of any one of claims 1-6, wherein the method comprises administering a LSD1 inhibitor and a PD-1 inhibitor.
8. The method of any one of claims 1-7, wherein the method comprises administering a LSD1 inhibitor, a PD-1 inhibitor, and a PD-L1 inhibitor.
9. The method of any one of claims 1-7, wherein the method comprises administering a LSD1 inhibitor and a PD-L1 inhibitor.
10. The method of any one of claims 1-8, wherein the method comprises administering an TGF β inhibitor.
11. The method of any one of claims 2-10, wherein the at least one immunotherapy is selected from the group consisting of: an antibody, an adoptive cellular therapy, an antibody-drug conjugate, a toxin, a cytokine therapy, a cancer vaccine, and a checkpoint inhibitor.
12. The method of claim 11, wherein the checkpoint inhibitor is a CTLA-4 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, an OX40 inhibitor, a TIM3 inhibitor, or a LAG3 inhibitor.
13. The method of any one of claims 1-12, wherein the LSD1 inhibitor is selected from the group consisting of: a small molecule, an antibody, and an inhibitory nucleic acid.

14. The method of any one of claims 1-13, wherein the LSD1 inhibitor is an inhibitory nucleic acid, and wherein the inhibitory nucleic acid is a small interfering RNA or a short hairpin RNA.
15. The method of claim 14, wherein the inhibitory nucleic acid is a short hairpin RNA and the short hairpin RNA comprises SEQ ID NO: 2.
16. The method of any one of claims 1-13, wherein the LSD1 inhibitor is a small molecule selected from the group consisting of: tranilcypromine, RN 1 dihydrochloride, GSK-LSD1, GSK2879552, ORY1001, GSK690, namoline, Cpd 2d, S2101, OG-L002, SP2509, CBB2007 and IMG-7289.
17. The method of any one of claims 1 and 6-15, wherein the PD-1 inhibitor is selected from the group consisting of: a small molecule, an antibody, and an inhibitory nucleic acid.
18. The method of any one of claims 1 and 6-17, wherein the PD-1 inhibitor is an inhibitory nucleic acid, and the inhibitory nucleic acid is a small interfering RNA or a short hairpin RNA.
19. The method of claim 18, wherein the inhibitory nucleic acid is a short hairpin RNA, and the short hairpin RNA comprises SEQ ID NO: 4.
20. The method of any one of claims 1 and 6-17, wherein the PD-1 inhibitor is an anti-PD-1 antibody (e.g., nivolumab or pembrolizumab).
21. The method of any one of claims 1 and 6-19, wherein the PD-L1 inhibitor is selected from the group consisting of: a small molecule, an antibody, and an inhibitory nucleic acid.

22. The method of claim 21, wherein the PD-L1 inhibitor is an inhibitory nucleic acid, and wherein the inhibitory nucleic acid is a small interfering RNA or a short hairpin RNA.
23. The method of claim 22, wherein the inhibitory nucleic acid is a short hairpin RNA, and the short hairpin RNA comprises SEQ ID NO: 6.
24. The method of claim 21, wherein the PD-L1 inhibitor is an anti-PD-L1 antibody (e.g., durvalumab, atezolizumab or avelumab).
25. The method of any one of claims 1-24, wherein the TGF β inhibitor is selected from the group consisting of: a small molecule, an antibody, an inhibitory nucleic acid, and a vaccine.
26. The method of claim 25, wherein the TGF β inhibitor is a small molecule (e.g., galunisertib).
27. The method of claim 25, wherein the TGF β inhibitor is a vaccine (e.g., gemogenovatucl-T).
28. The method of claim 25, wherein the TGF β inhibitor is an inhibitory nucleic acid, and wherein the inhibitory nucleic acid is a small interfering RNA, a short hairpin RNA or an antisense oligonucleotide.
29. The method of claim 28, wherein the inhibitory nucleic acid is a siRNA, and the siRNA comprises SEQ ID NO: 159.
30. The method of claim 28, wherein the inhibitory nucleic acid is an antisense oligonucleotide (e.g., belagenpumatucl-L, trabedersen)

31. The method of claim 25, wherein the TGF β inhibitor is an anti-TGF β antibody (e.g., fresolimumab).
32. The method of any one of claims 1-31, wherein the cancer is a primary tumor.
33. The method of any one of claims 1-31, wherein the cancer is a metastatic tumor.
34. The method of any one of claims 1-32, wherein the cancer is selected from the group consisting of: melanoma, acute myeloid leukemia (AML), squamous cell carcinoma, renal cell carcinoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), gastric cancer, bladder cancer, kidney cancer, head and neck cancer, Ewing sarcoma, Hodgkin's lymphoma, Merkel cell carcinoma, breast cancer and prostate cancer.
35. The method of any one of claims 1-34, wherein the cancer is a non-T-cell-infiltrating cancer.
36. The method of any one of claims 1-35, wherein the cancer is a PD-1 and/or PD-L1 refractory cancer.
37. The method of any one of claims 1-35, wherein the cancer is a PD-1 and/or PD-L1 resistant cancer.
38. The method of any one of claims 1-36, wherein the patient has previously received a cancer treatment.
39. The method of any one of claims 1-38, wherein administering occurs at least once a week.
40. The method of any one of claims 1-39, wherein administering is via intravenous, subcutaneous, intraperitoneal, rectal, and/or oral administration.

41. The method of any one of claims 1 and 3-40, wherein the LSD1 inhibitor and the at least one PD-1 inhibitor or PD-L1 inhibitor are administered simultaneously to the patient.
42. The method of any one of claims 1-36, wherein the LSD1 inhibitor is administered to the patient prior to administration of the PD-1 inhibitor or PD-L1 inhibitor.
43. The method of claim 41, wherein the administration of the LSD1 inhibitor is stopped before the administration of the PD-1 inhibitor or the PD-L1 inhibitor.
44. The method of any one of claims 1-42, wherein the LSD1 inhibitor and the TGF β inhibitor are administered simultaneously to the patient.
45. The method of any one of claims 1-42, wherein the LSD1 inhibitor is administered to the patient prior to the administration of the TGF β inhibitor.
46. The method of any one of claims 1-45, wherein the administration of the LSD1 inhibitor is stopped before the administration of the TGF β inhibitor.
47. The method of anyone of claims 1-42, wherein the TGF β inhibitor is administered to the patient prior to the administration of the LSD1 inhibitor or the at least one PD-1 inhibitor or PD-L1 inhibitor.
48. The method of anyone of claims 1 and 6-44, wherein the LSD1 inhibitor, the TGF β inhibitor and the at least one PD-1 inhibitor or PD-L1 inhibitor are administered simultaneously to the patient.
49. The method of any one of claims 1-48, wherein the method further comprises administering a chemotherapeutic agent.

50. The method of any one of claims 1-49, wherein treating comprises reducing the volume of a primary tumor in the patient.
51. The method of any one of claims 1-50, wherein treating comprises delaying cancer progression in the patient.
52. The method of any one of claims 1-51, wherein treating comprises modifying the tumor microenvironment of a cancer in the patient.
53. The method of any one of claims 1-52, wherein treating comprises sensitizing a cancer to a checkpoint inhibitor therapy.
54. The method of any one of claims 1-53, wherein treating comprises decreasing the risk of developing at least one metastatic tumor in the patient.
55. The method of any one of claims 1-54, wherein treating comprises decreasing the rate of tumor growth in the patient.
56. The method of any one of claims 1-55, wherein treating comprises eliciting tumor-intrinsic double-stranded RNA stress in a cancer cell in the patient.
57. A method of treating a patient at risk for developing cancer, the method comprising: administering to the patient in need of cancer treatment a therapeutically effective amount of a lysine-specific demethylase 1A (LSD1) inhibitor, and a transforming growth factor beta (TGF β) inhibitor, to thereby treat cancer in the patient.
58. The method of claim 57, wherein the TGF β inhibitor is selected from the group consisting of: a small molecule, an antibody, an inhibitory nucleic acid, and a vaccine.

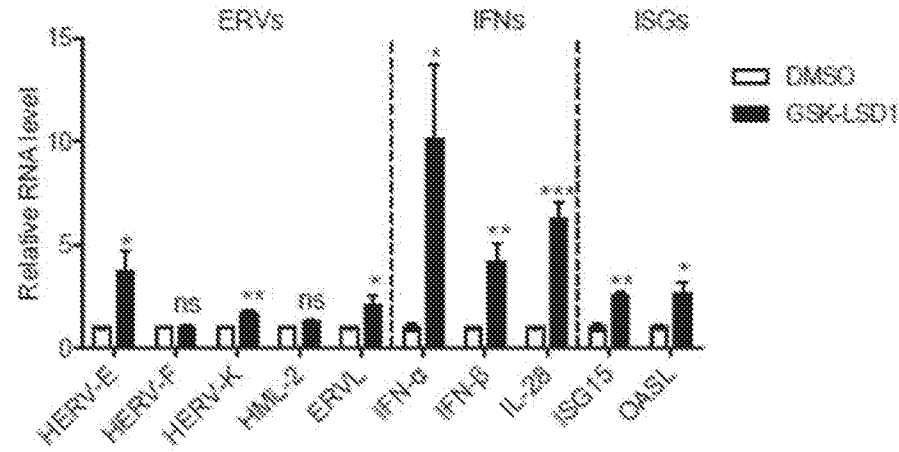


FIG. 1A

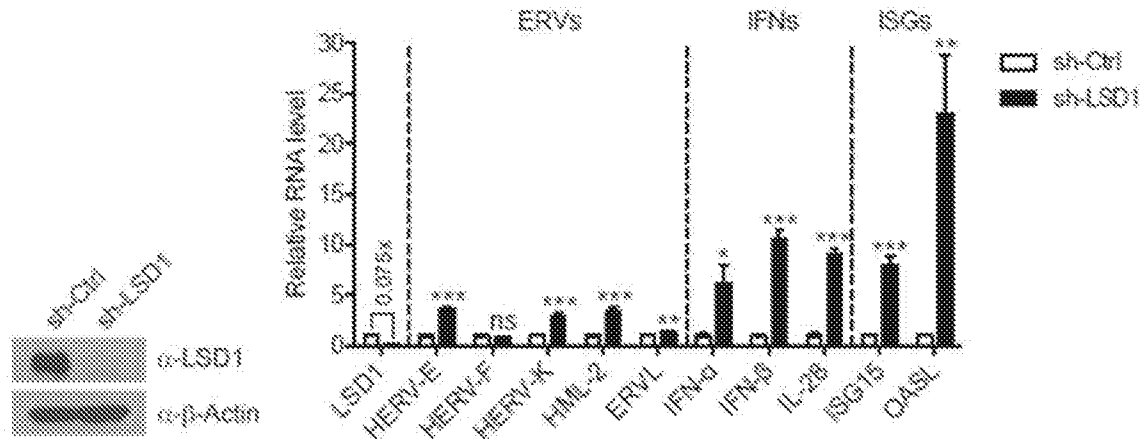


FIG. 1B

FIG. 1C

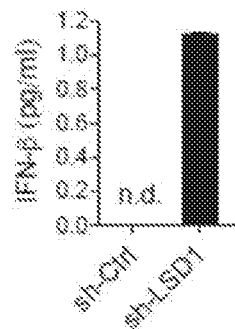


FIG. 1D

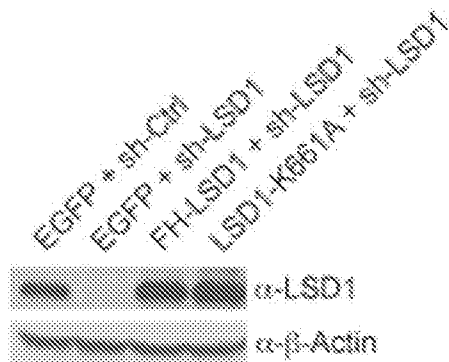


FIG. 1E

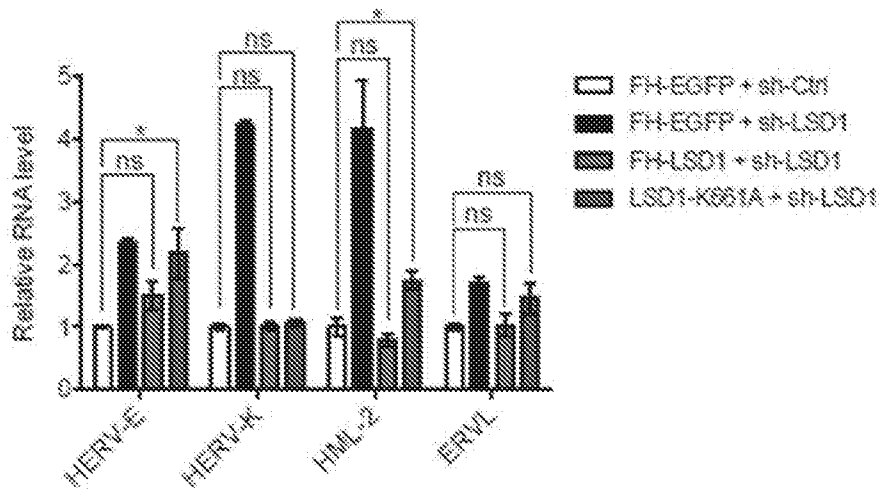


FIG. 1F

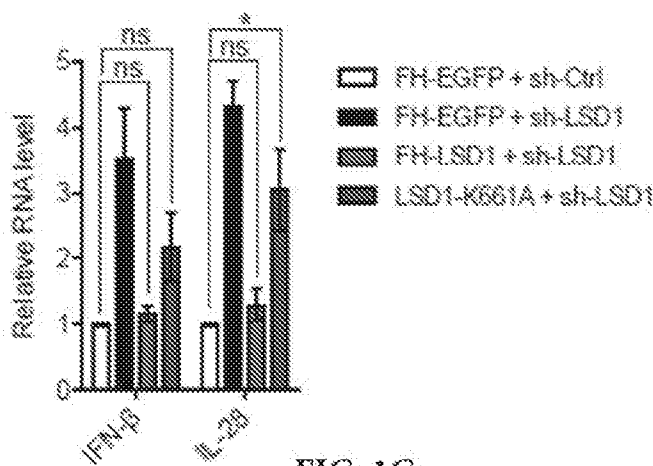


FIG. 1G

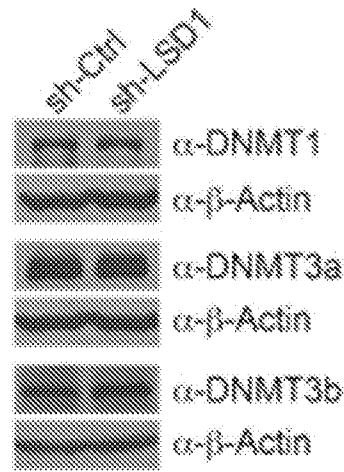


FIG. 1H

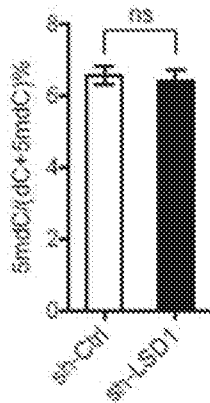


FIG. 1I

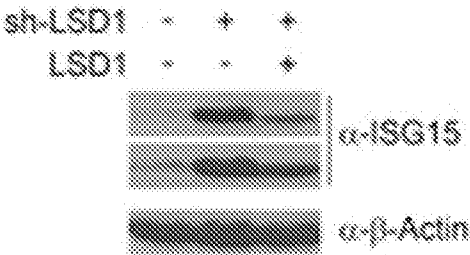


FIG. 1J

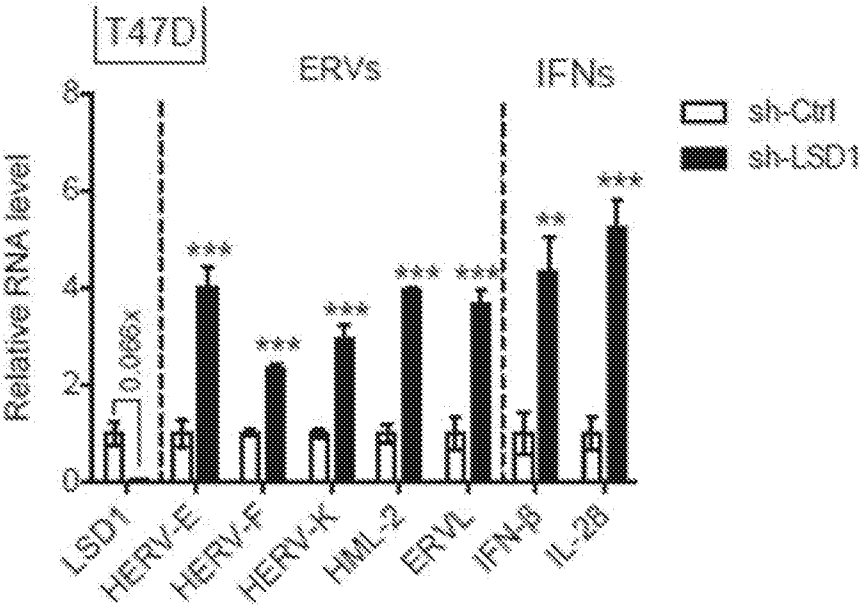


FIG. 1K

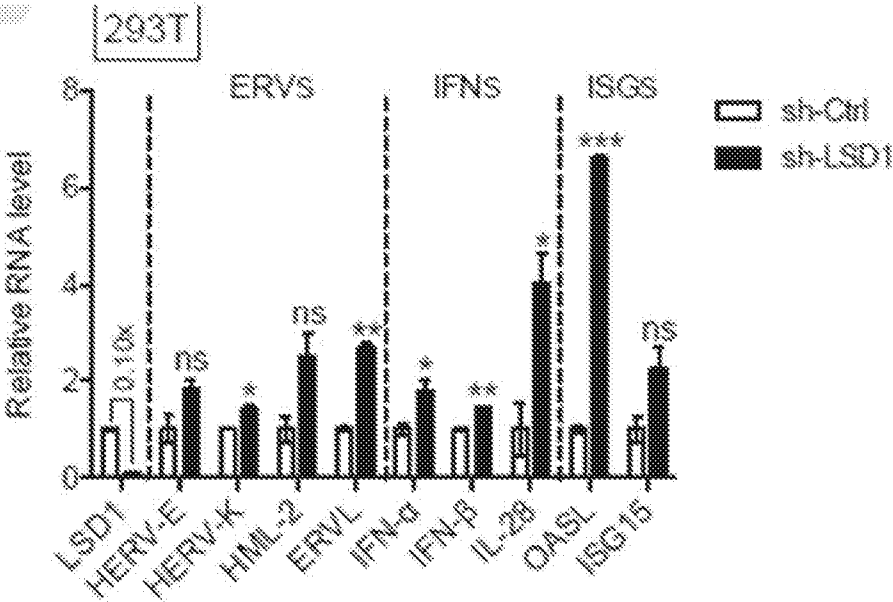


FIG. 1L

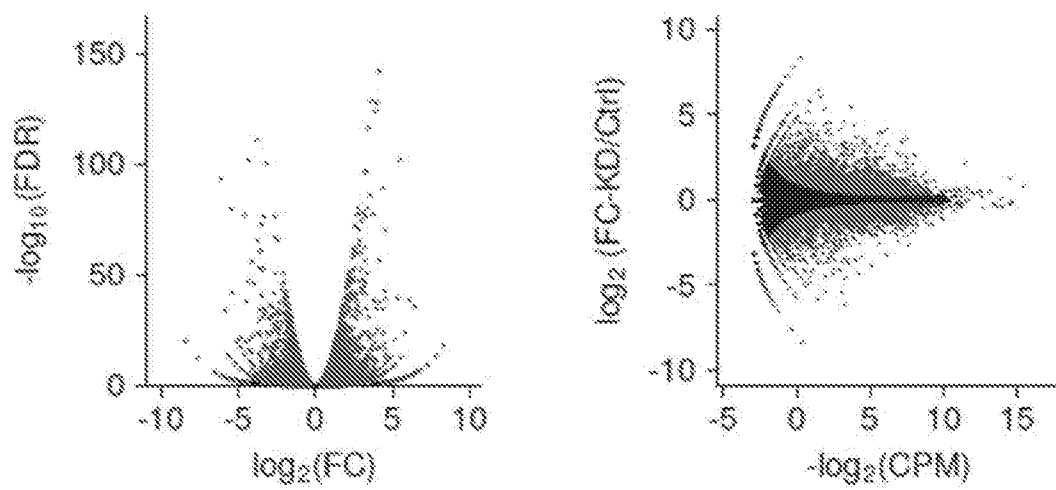


FIG. 1M

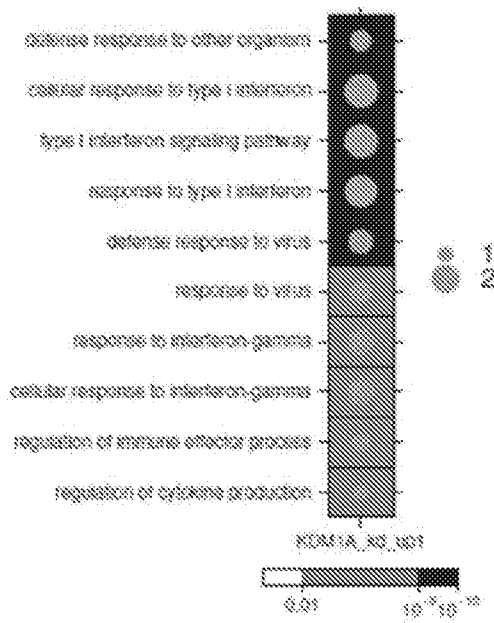


FIG. 1N

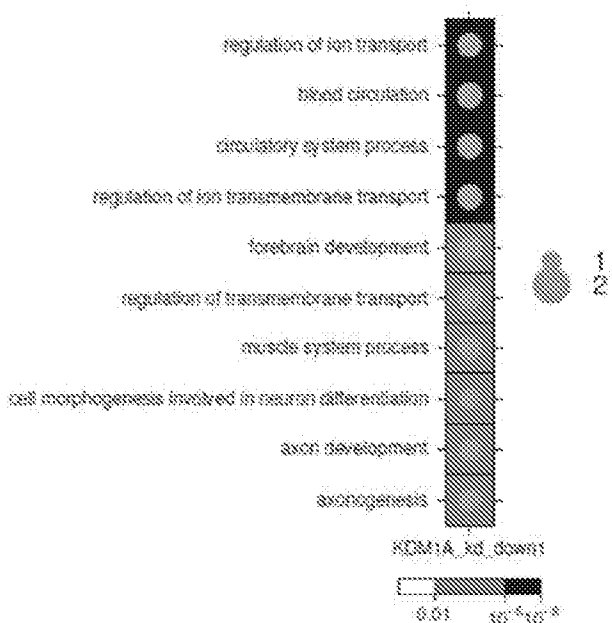


FIG. 1O

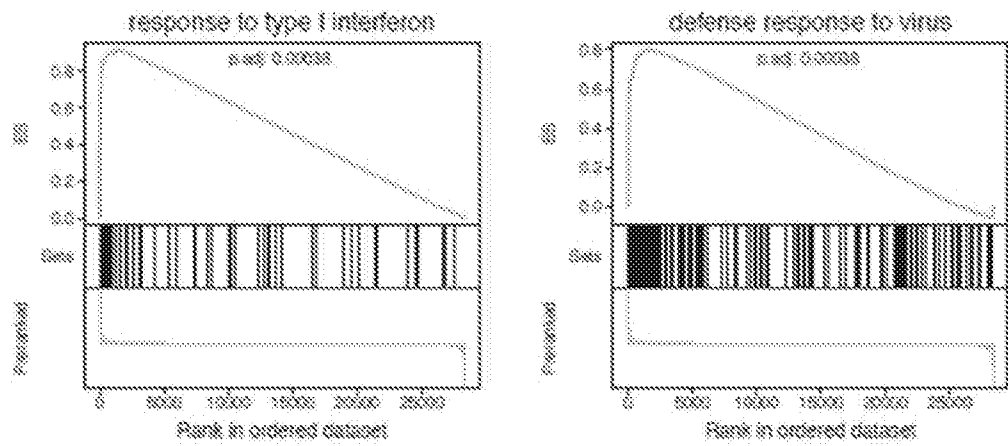


FIG. 2A

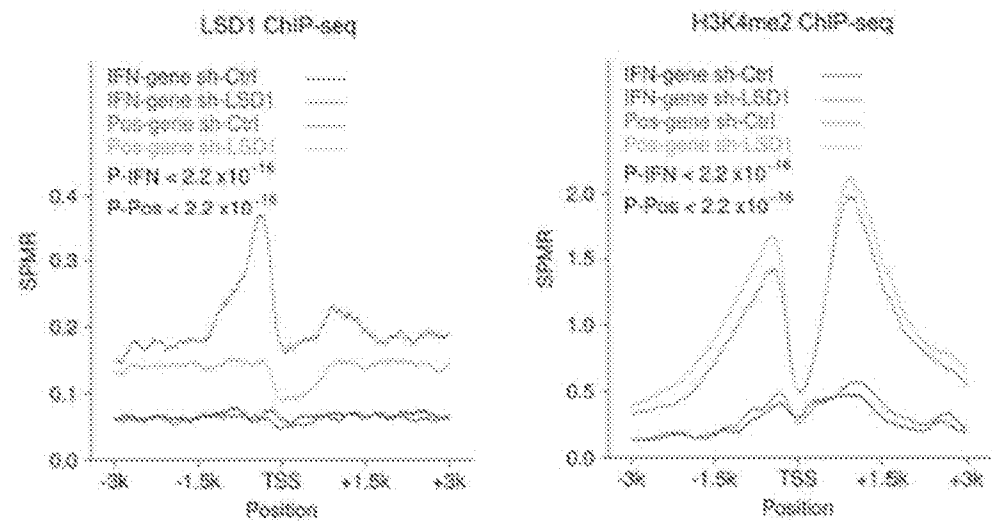


FIG. 2B

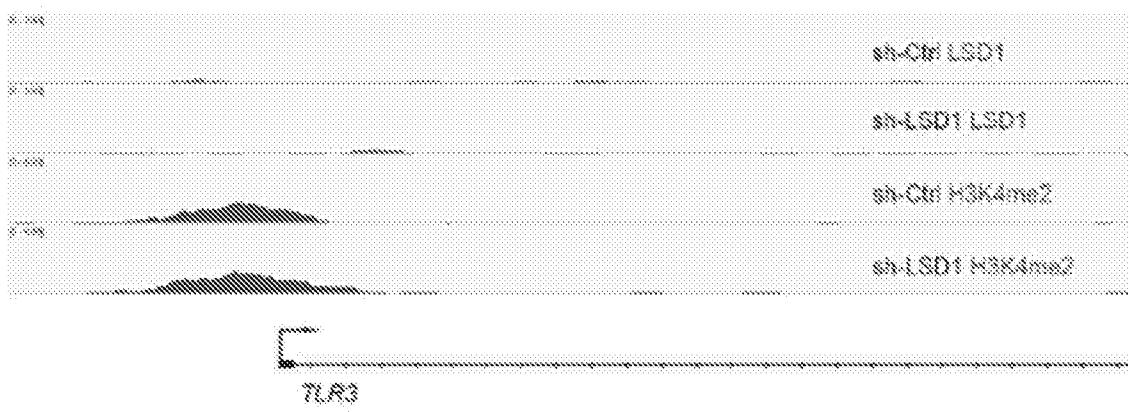


FIG. 2C

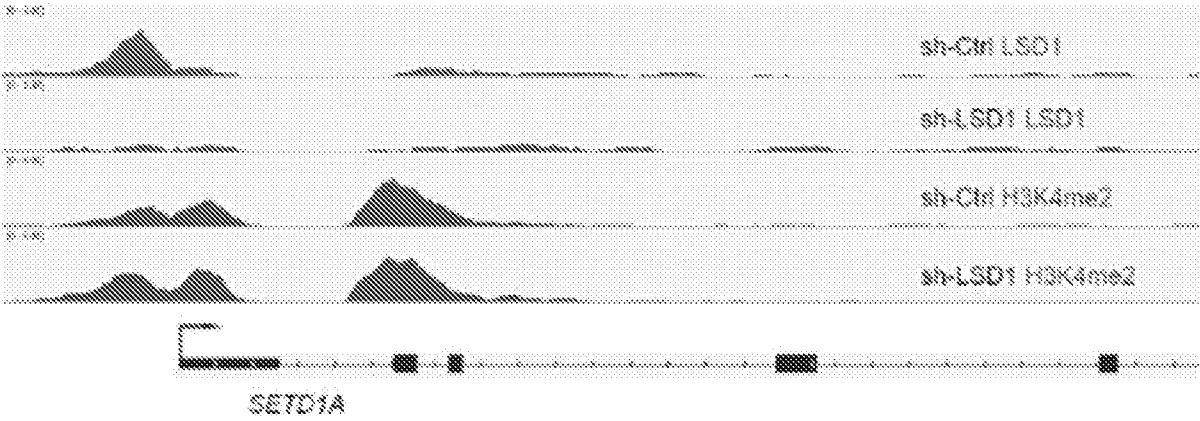


FIG. 2D

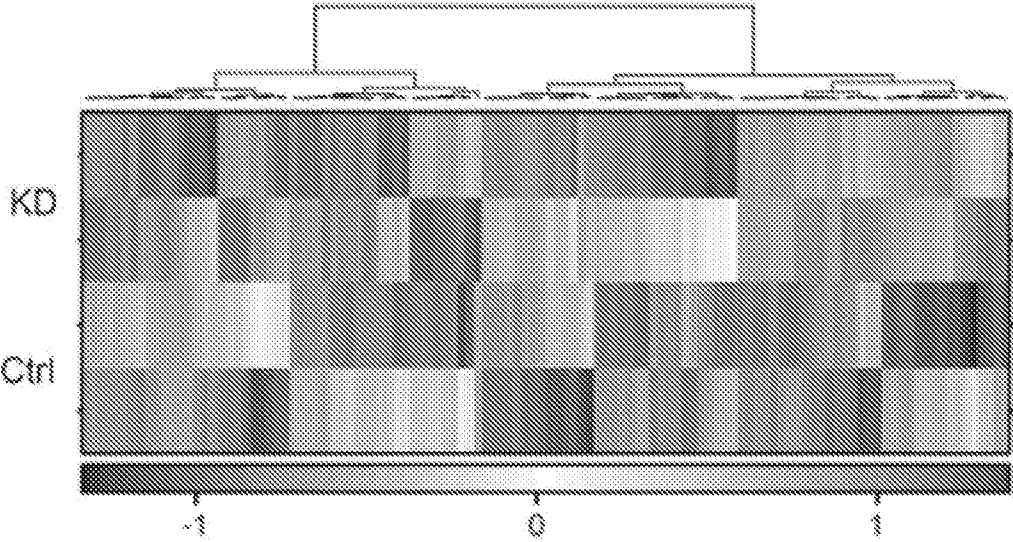


FIG. 2E

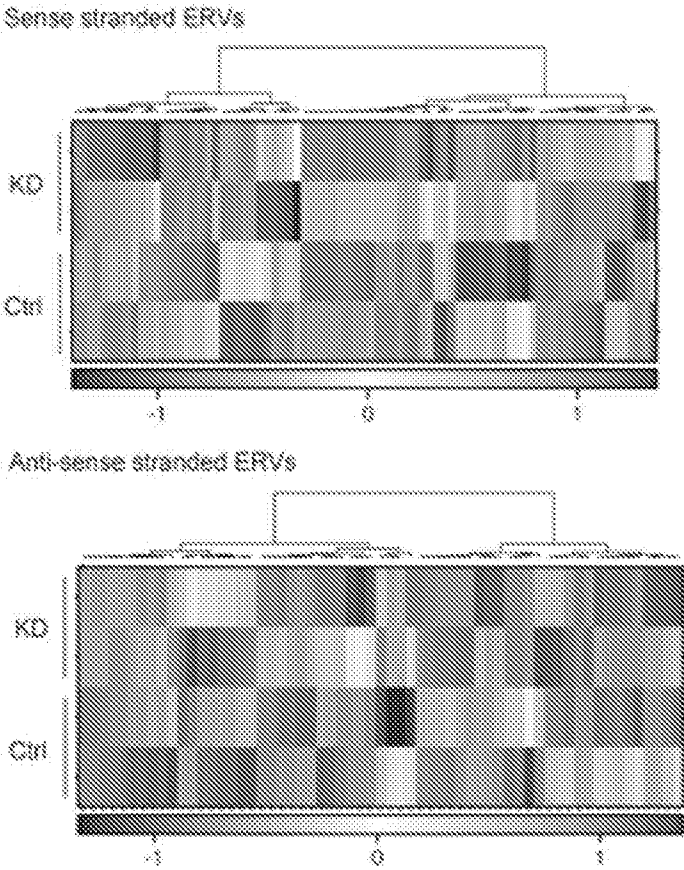


FIG. 2F

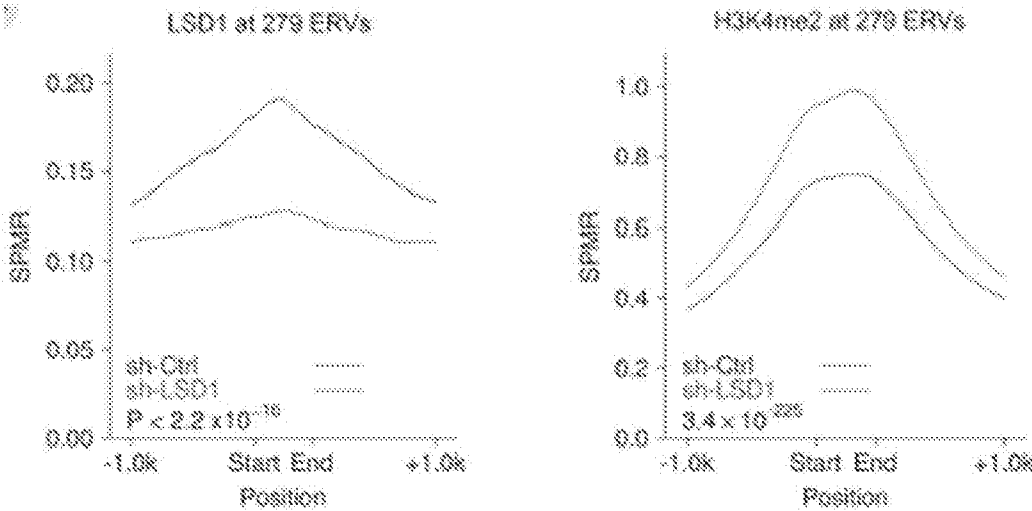


FIG. 2G

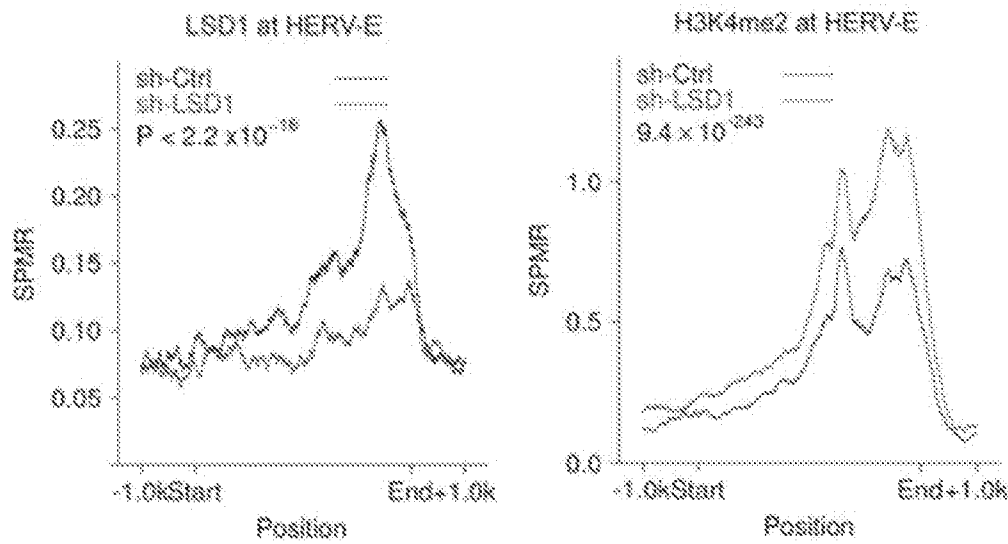


FIG. 2H

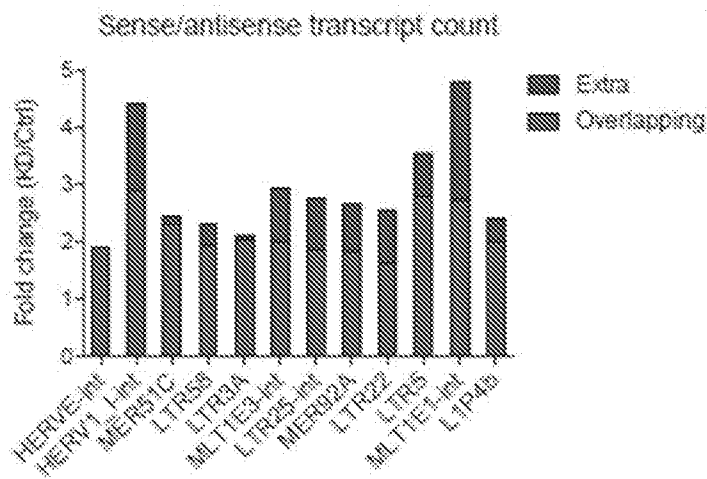


FIG. 2I

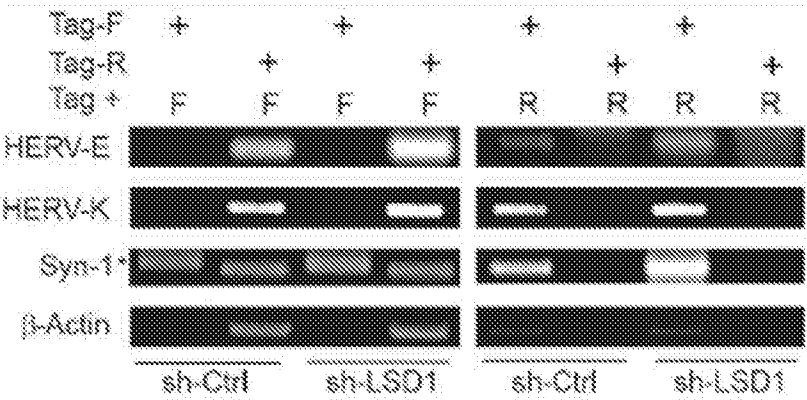


FIG. 2J

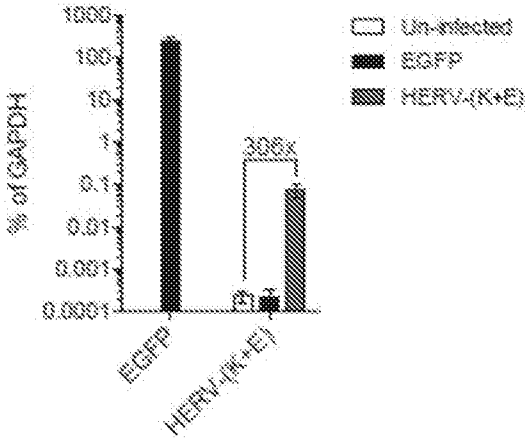


FIG. 2K

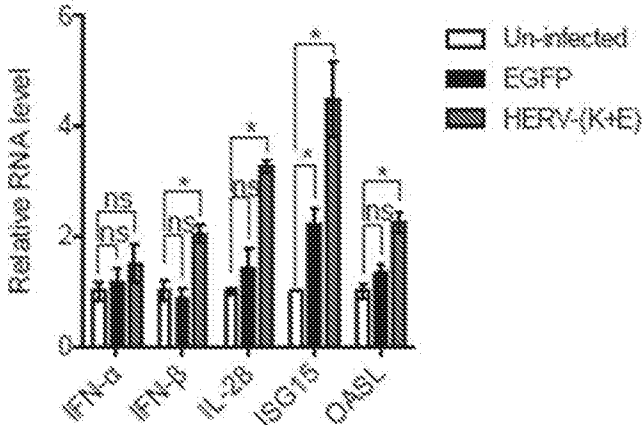


FIG. 2L

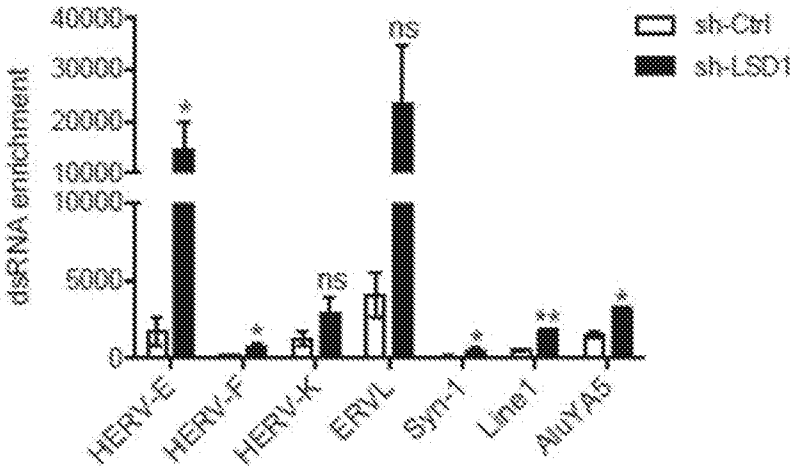


FIG. 2M

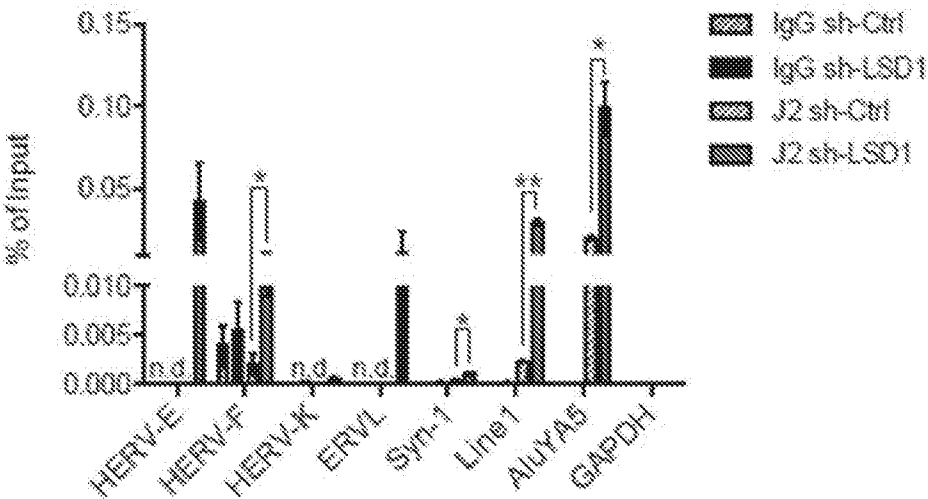


FIG. 2N

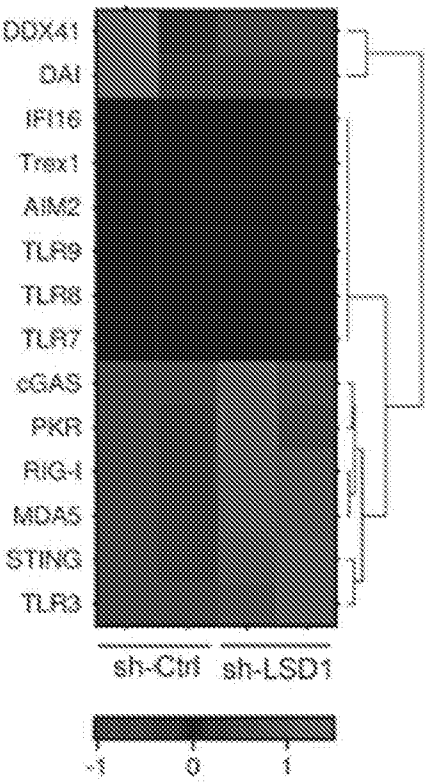


FIG. 2O

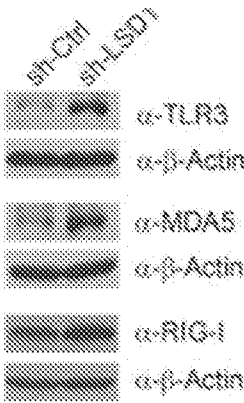


FIG. 2P

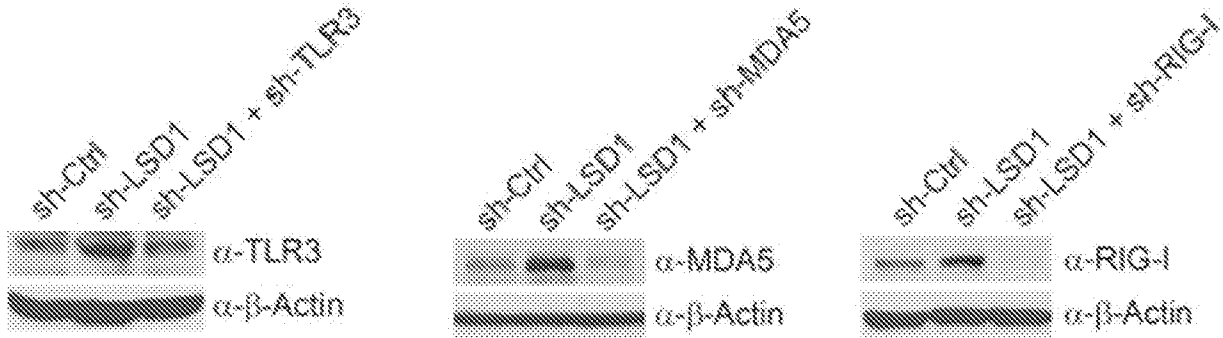


FIG. 3A

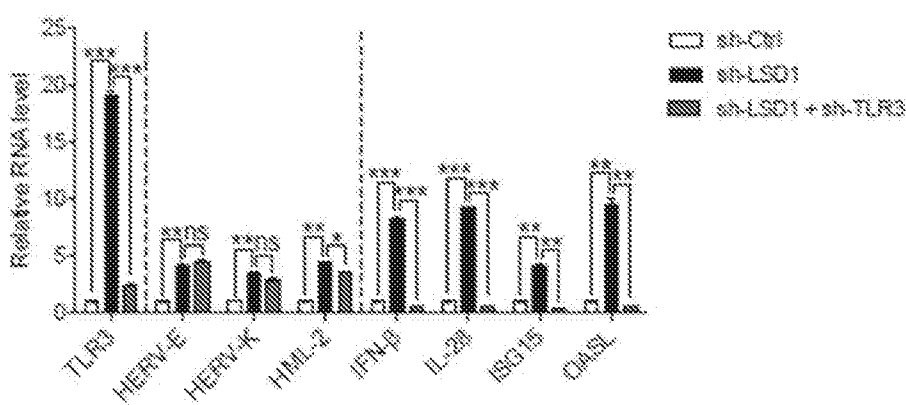


FIG. 3B

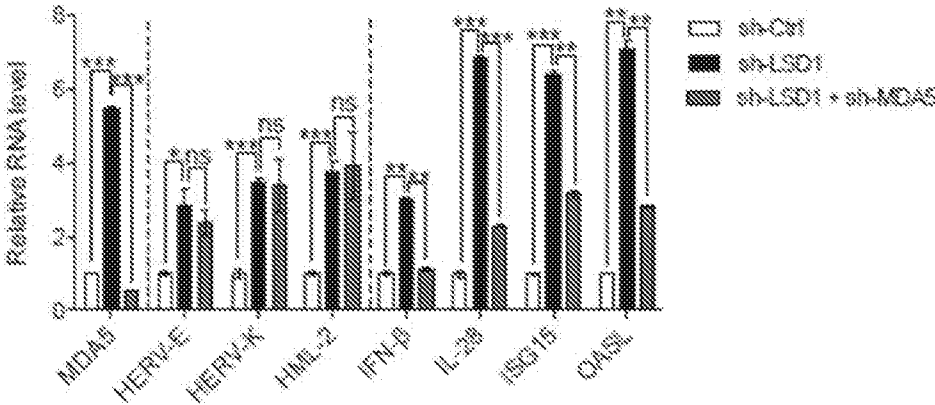


FIG. 3C

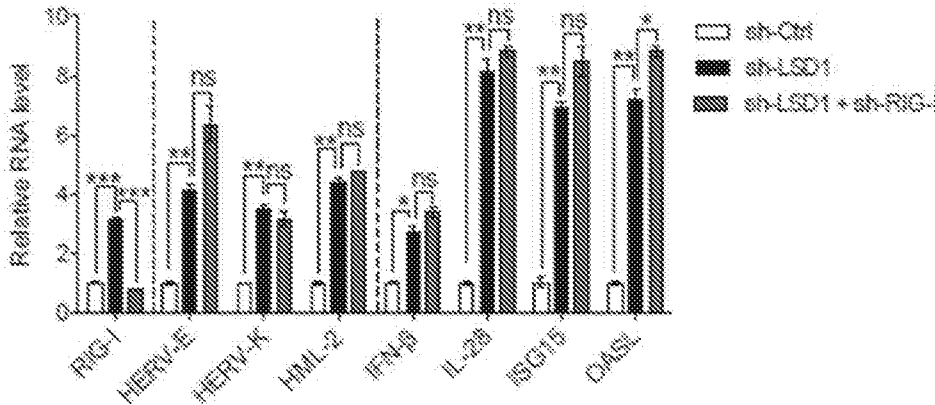


FIG. 3D

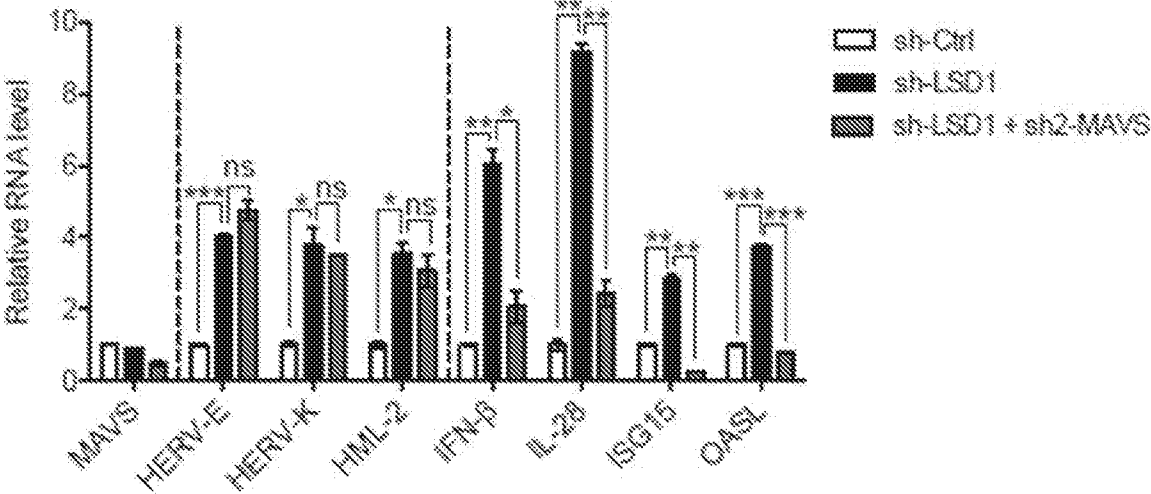


FIG. 3E

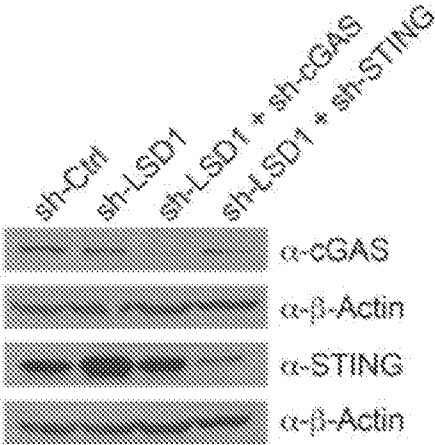


FIG. 3F

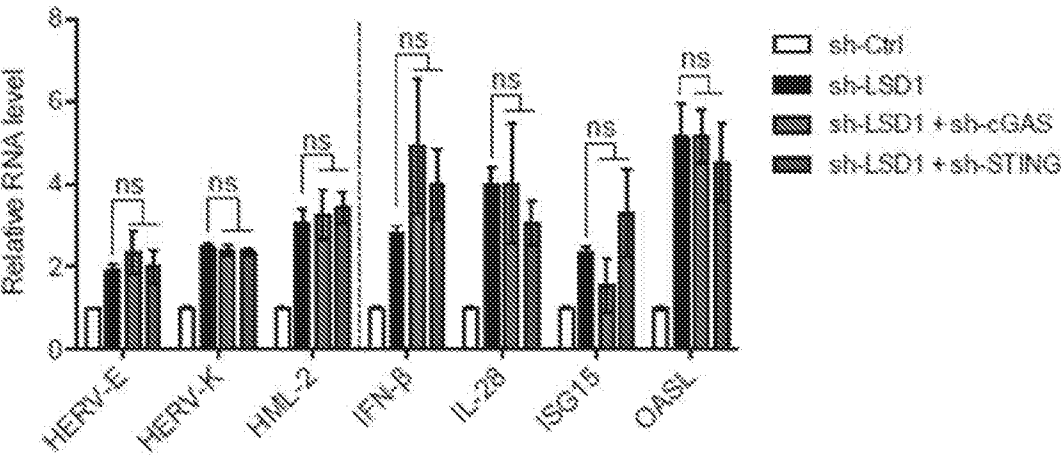


FIG. 3G

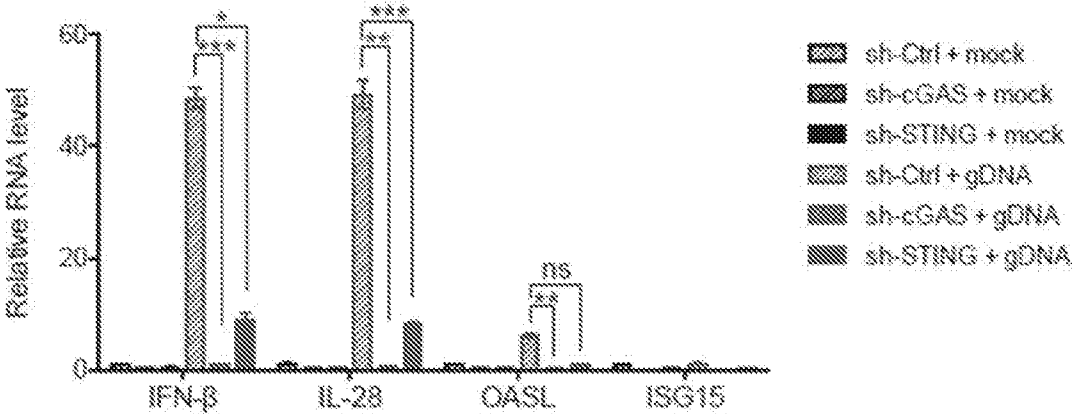


FIG. 3H

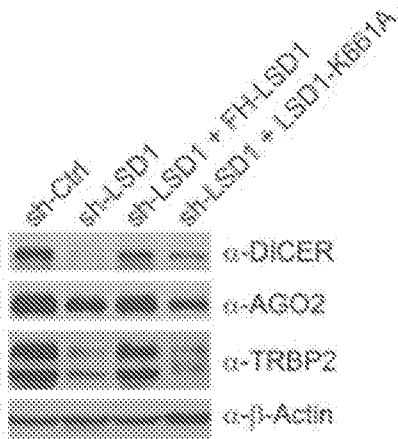


FIG. 4A

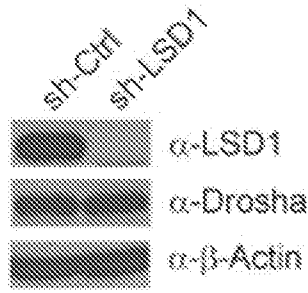


FIG. 4B

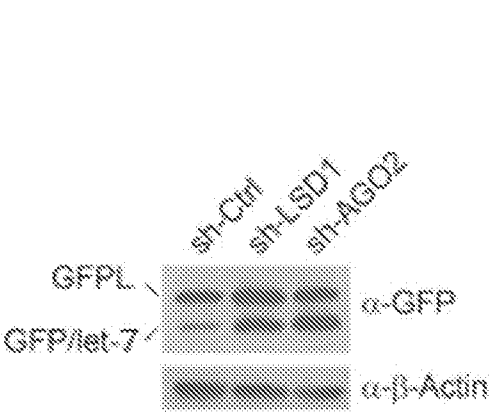


FIG. 4C

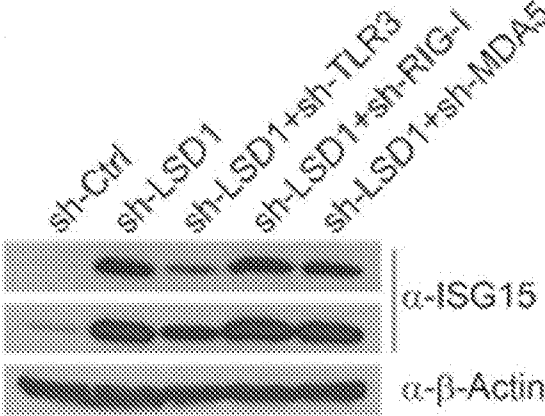


FIG. 4D

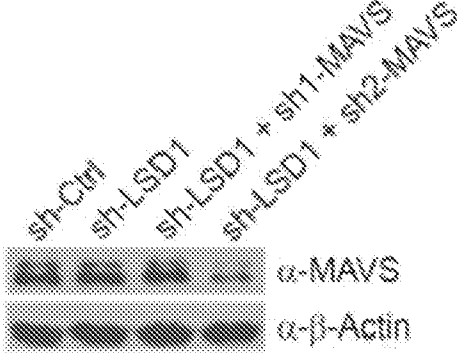


FIG. 4E

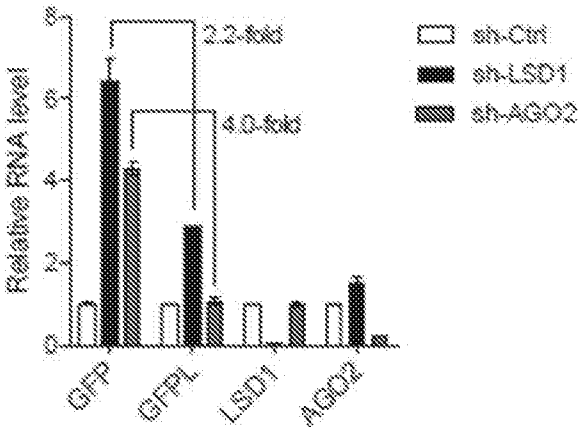


FIG. 4F

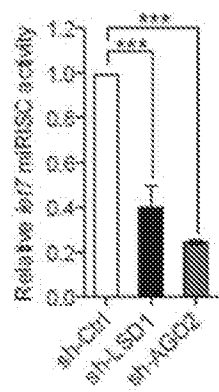


FIG. 4G

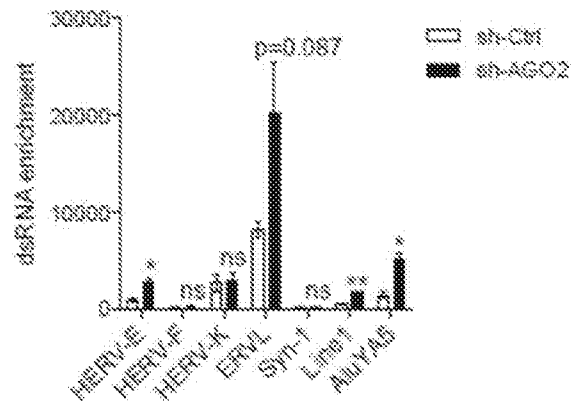


FIG. 4H

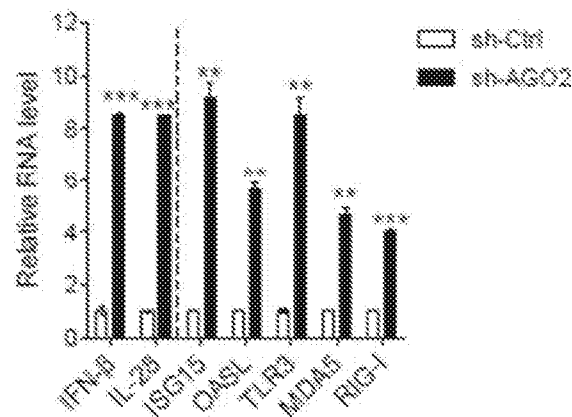


FIG. 4I



FIG. 4J

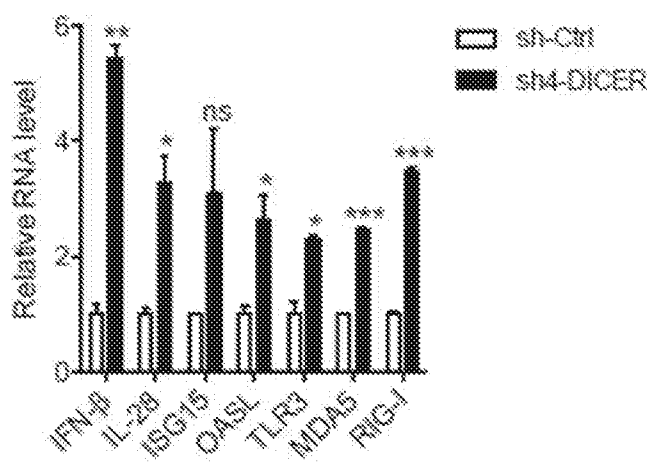


FIG. 4K



FIG. 4L

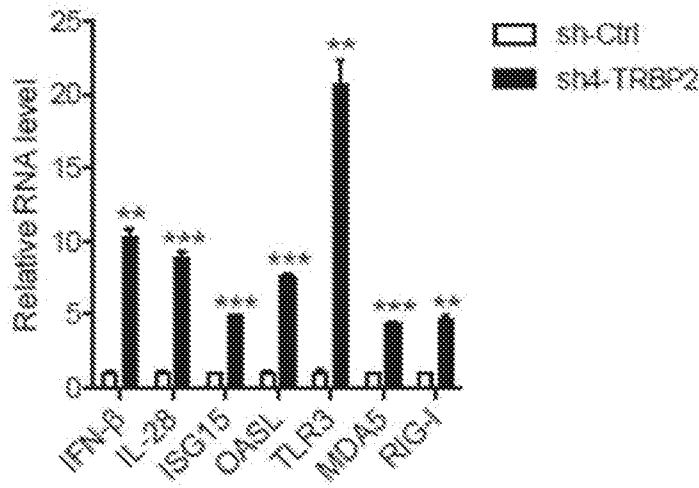


FIG. 4M

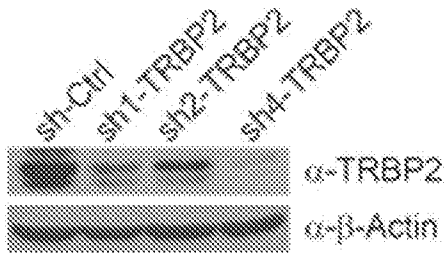


FIG. 4N

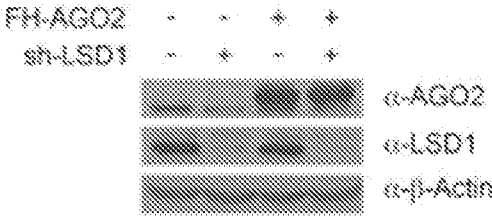


FIG. 4O

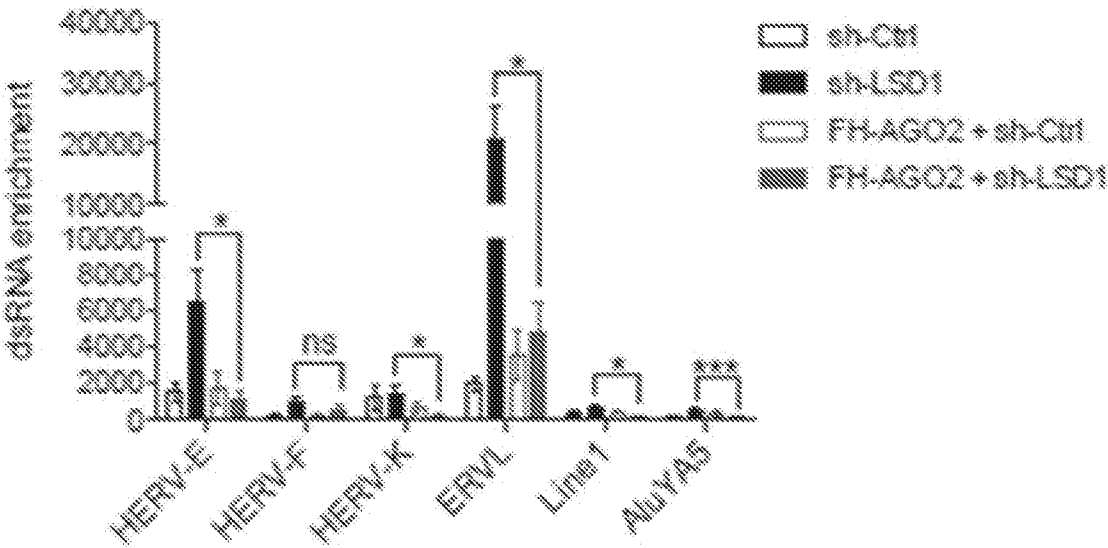


FIG. 4P

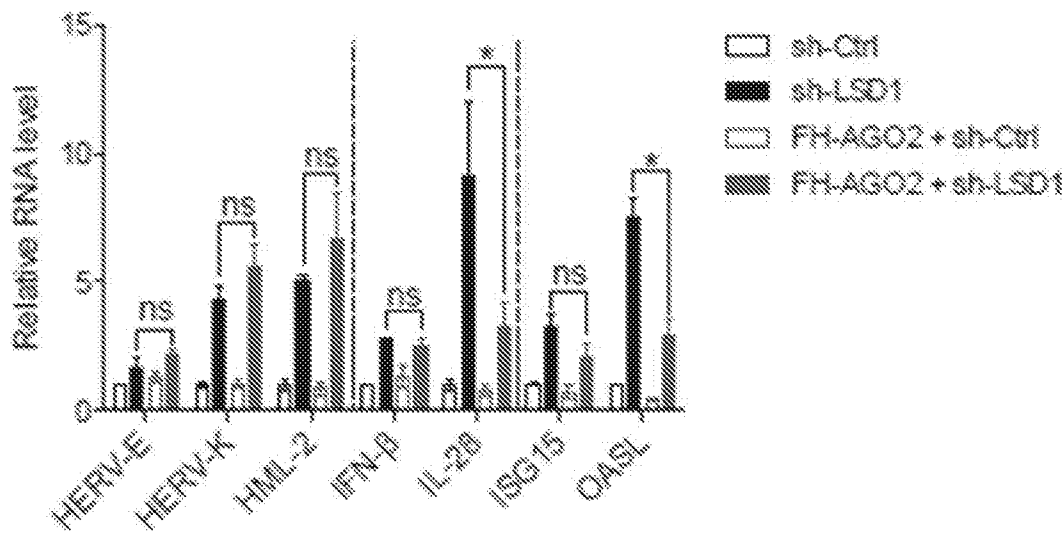


FIG. 4Q

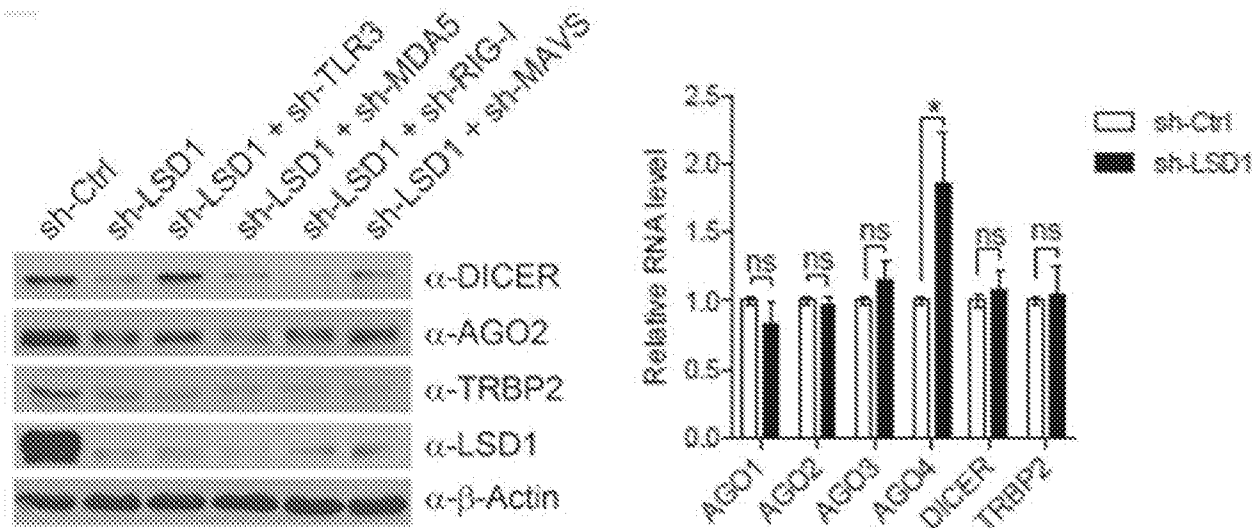


FIG. 5A

FIG. 5B

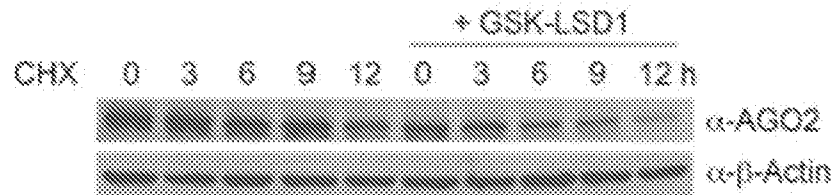


FIG. 5C

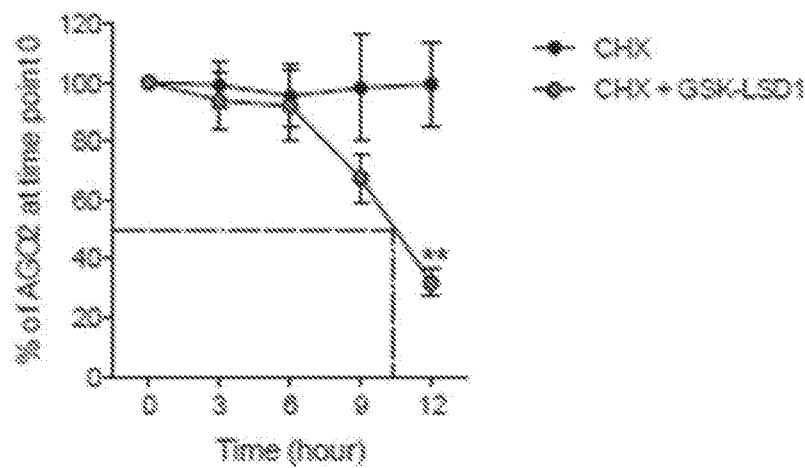


FIG. 5D

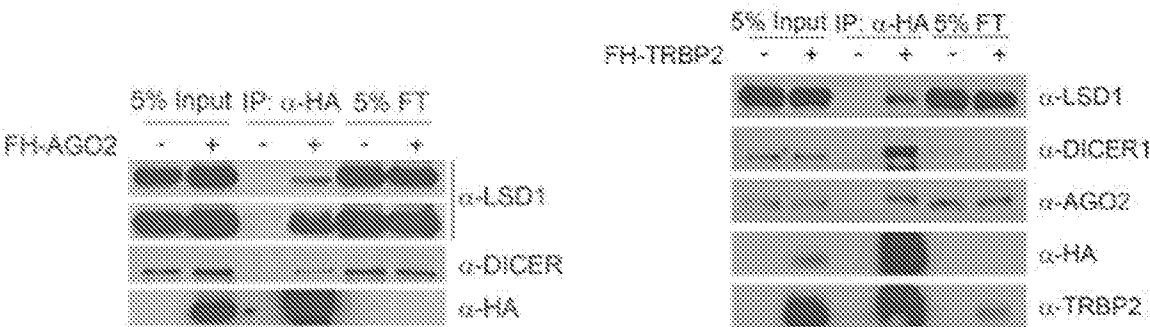


FIG. 5E

FIG. 5F

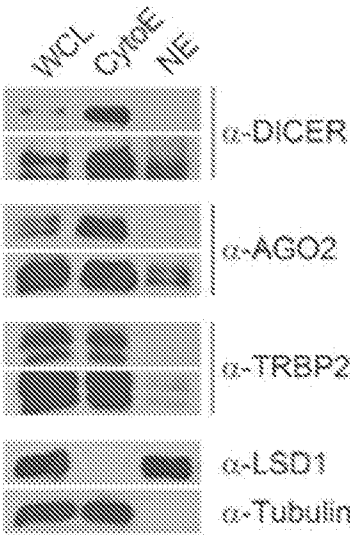


FIG. 5G

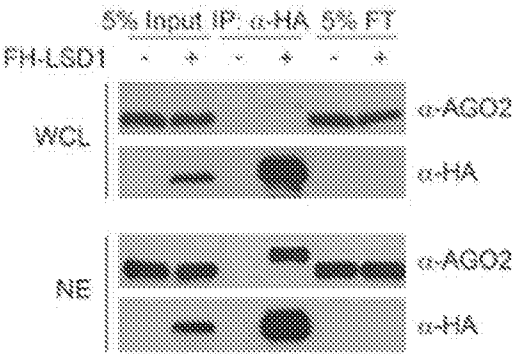


FIG. 5H

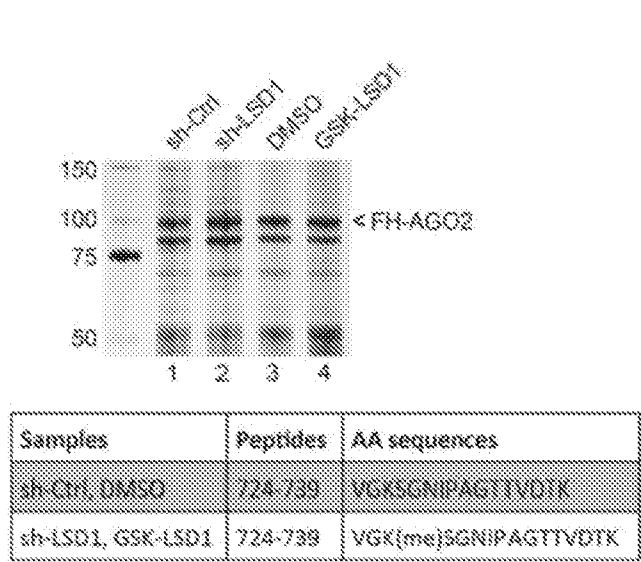


FIG. 5I

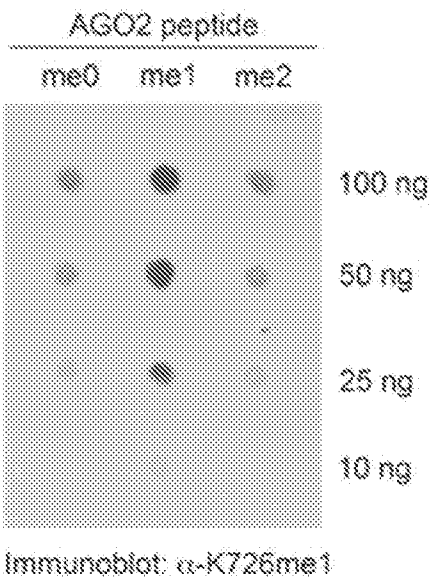


FIG. 5J

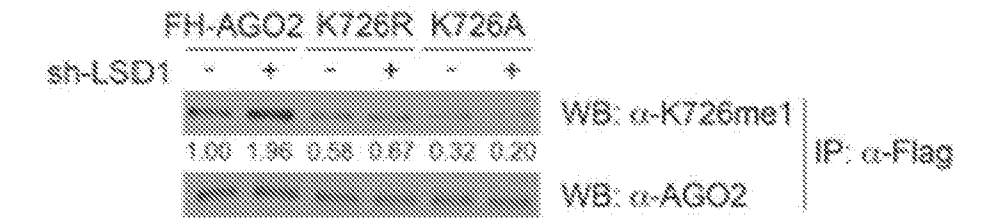


FIG. 5K

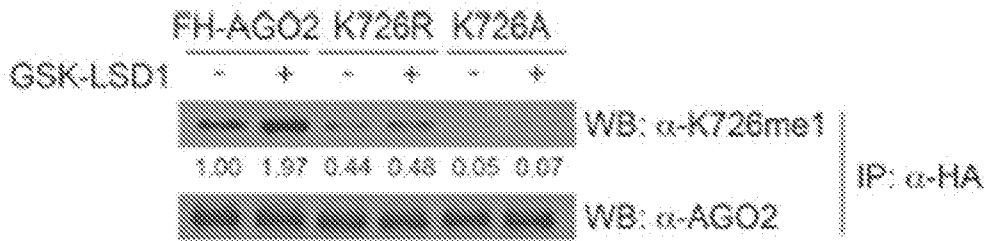


FIG. 5L

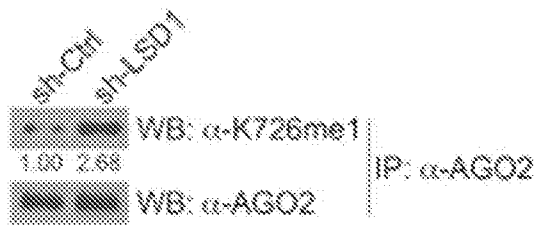


FIG. 5M

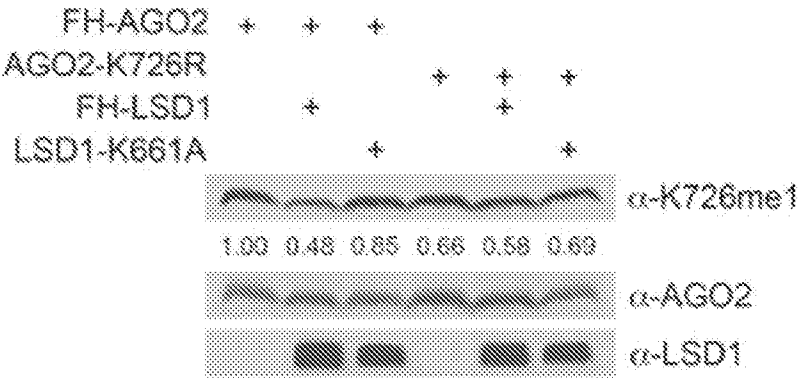


FIG. 5N

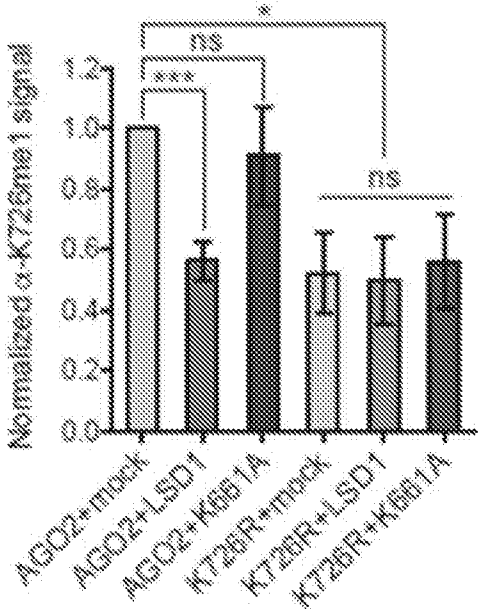


FIG. 5O

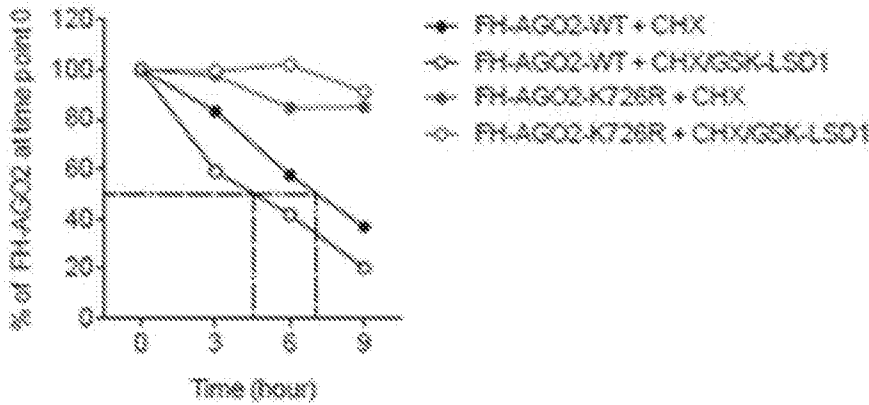


FIG. 5P

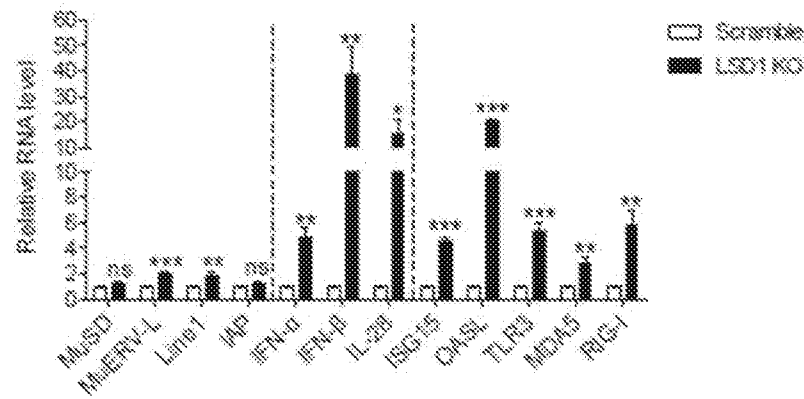


FIG. 6A

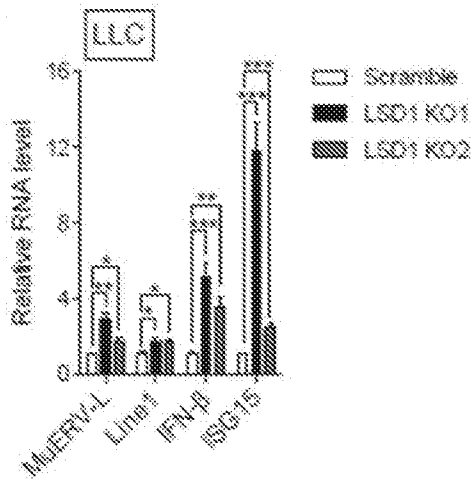


FIG. 6B

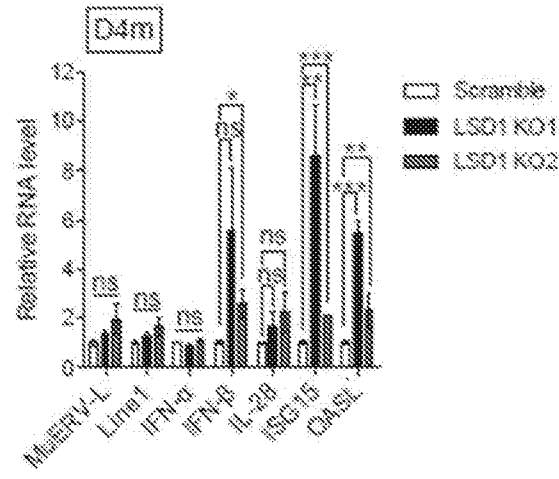


FIG. 6C

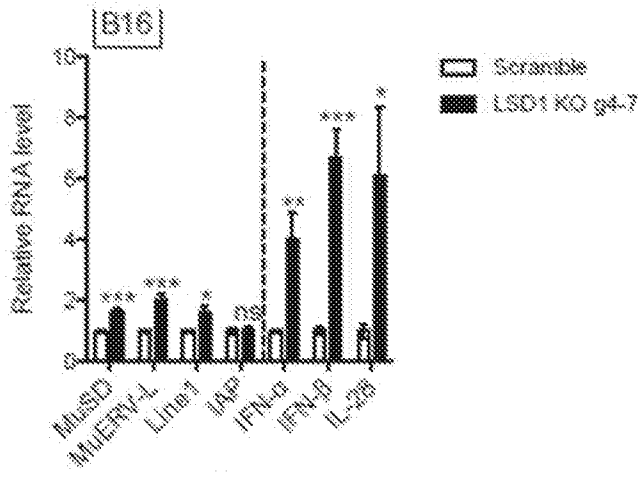


FIG. 6D

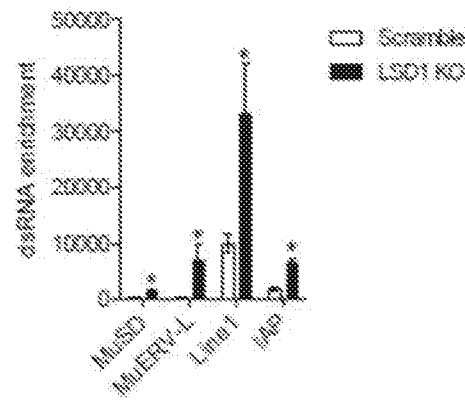


FIG. 6E

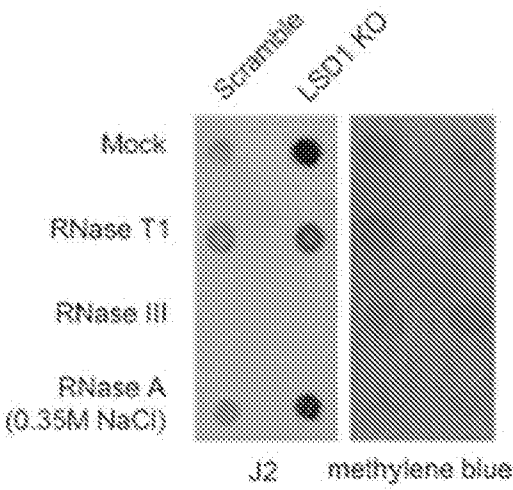


FIG. 6F

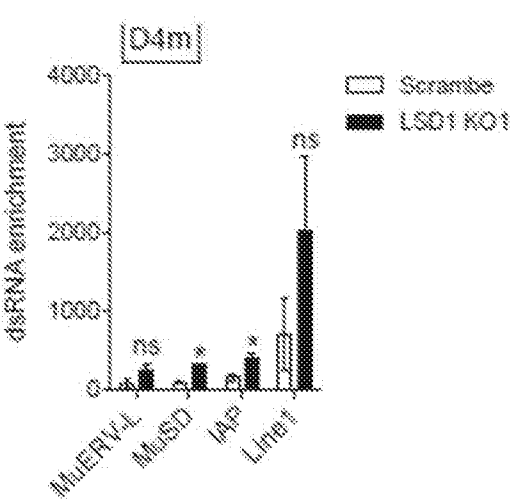


FIG. 6G

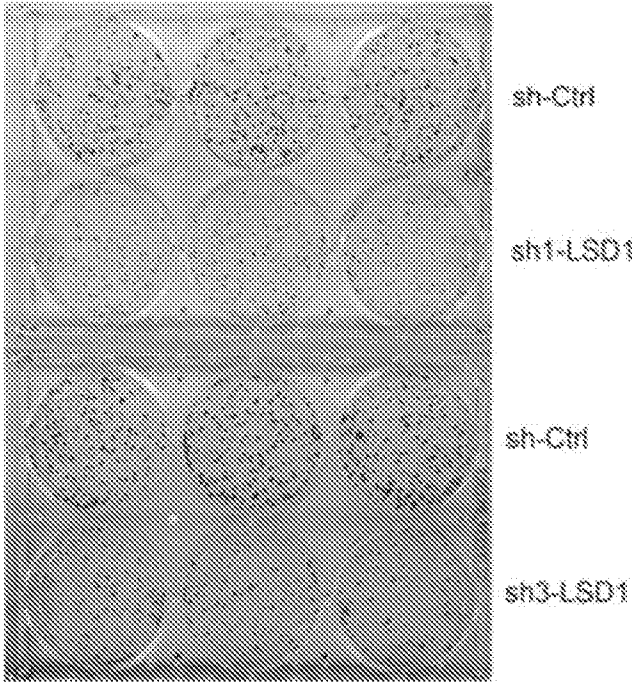


FIG. 6H

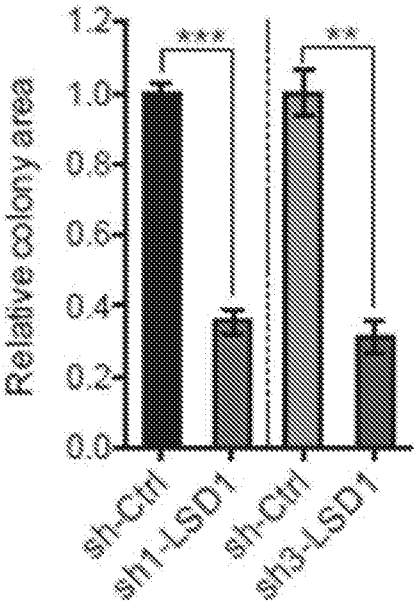


FIG. 6I

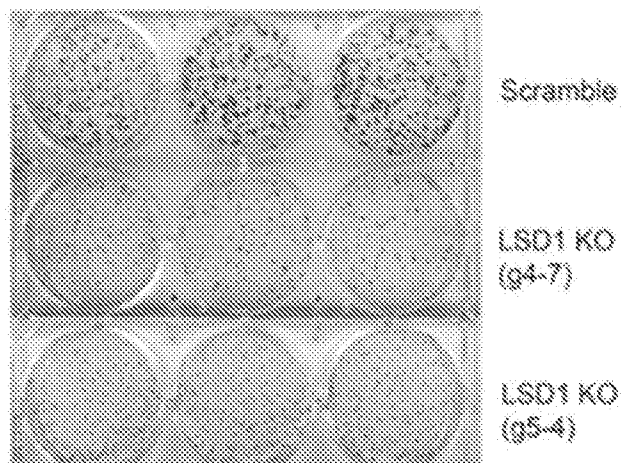


FIG. 6J

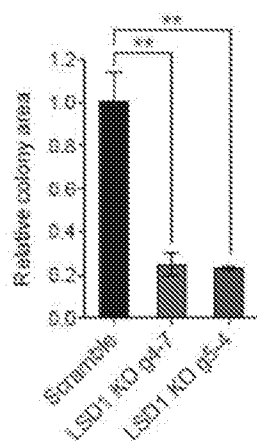


FIG. 6K

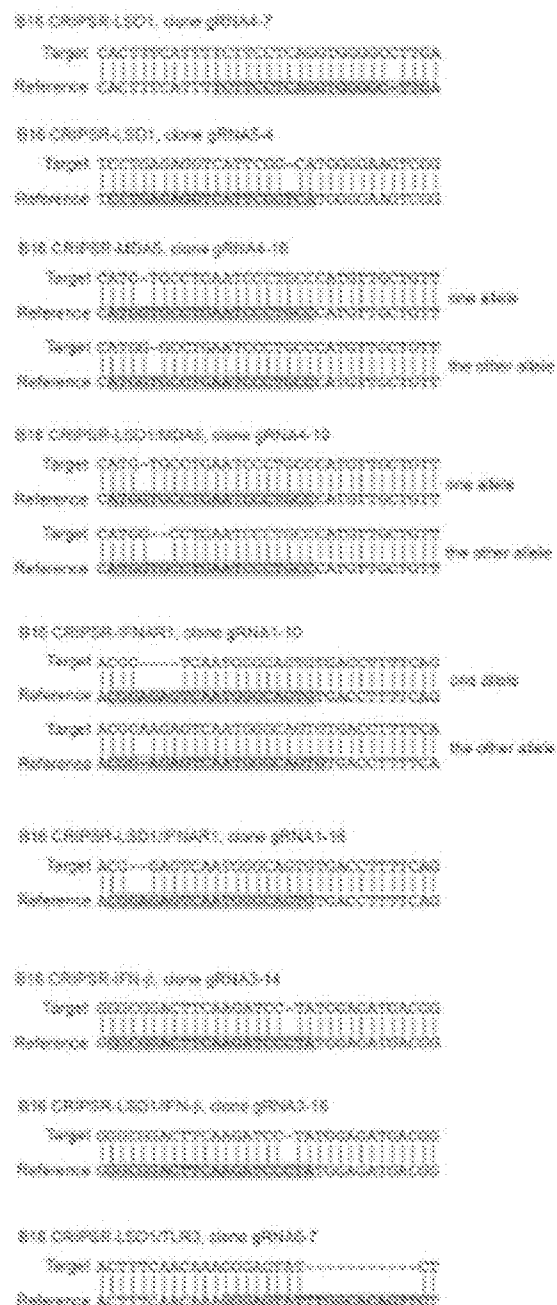


FIG. 6L

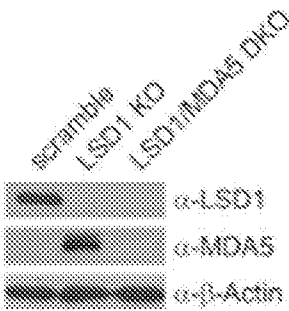


FIG. 6M

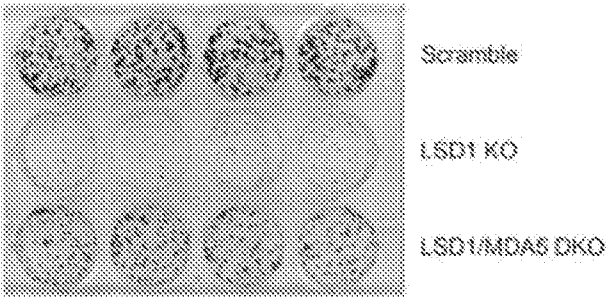


FIG. 6N

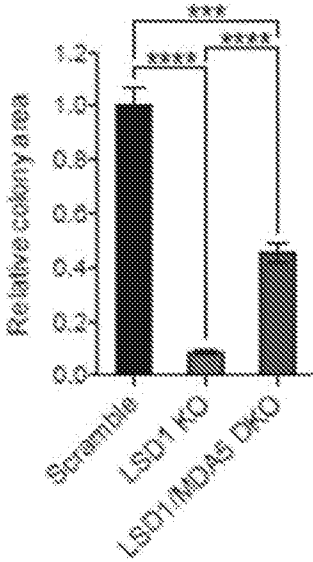


FIG. 6O

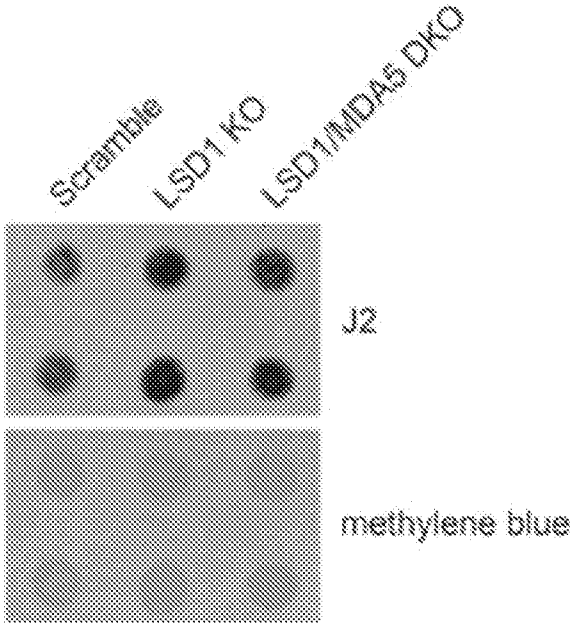


FIG. 6Q

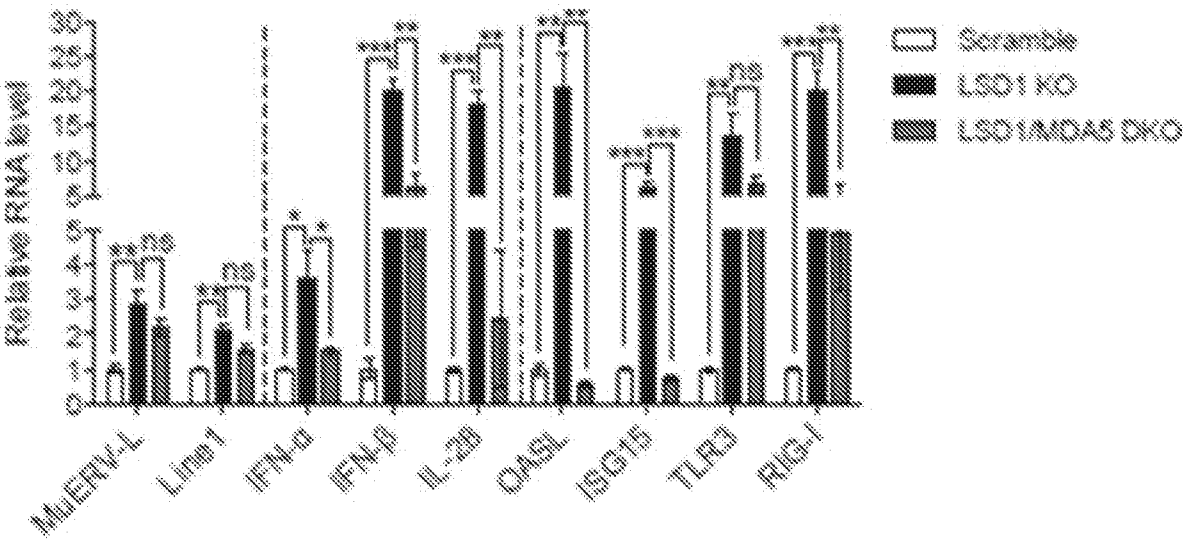
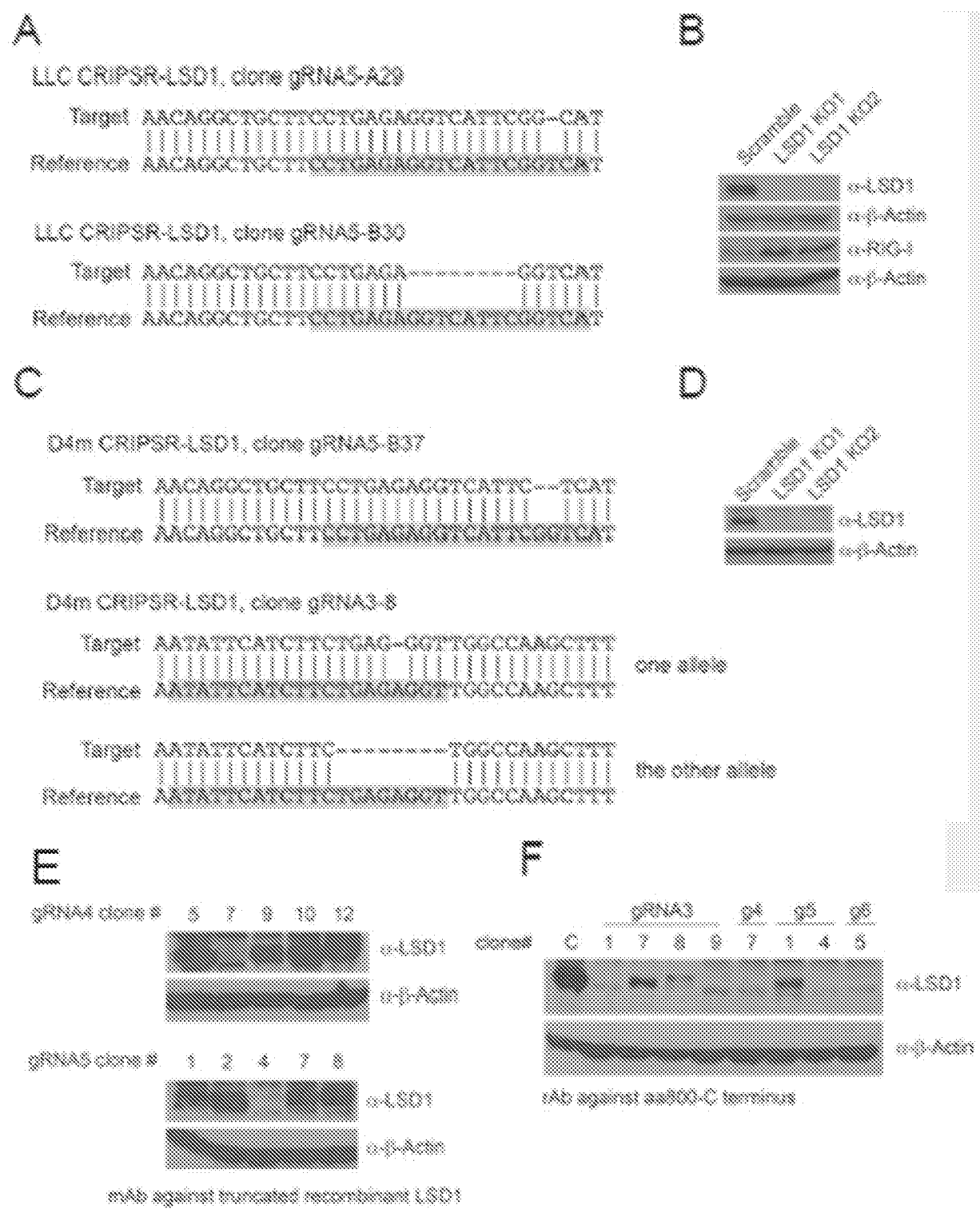


FIG. 6P



FIGs. 7A-F

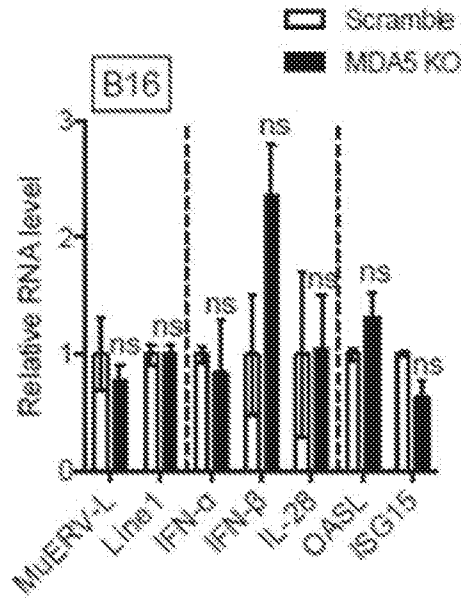


FIG. 8A

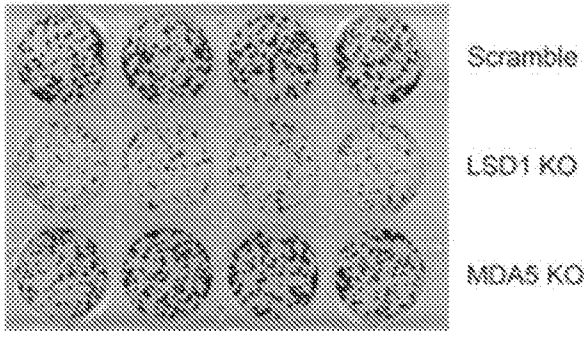


FIG. 8B

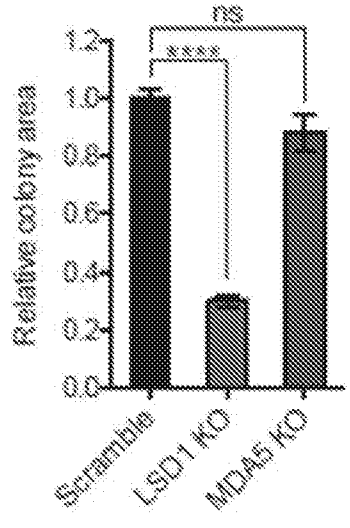


FIG. 8C

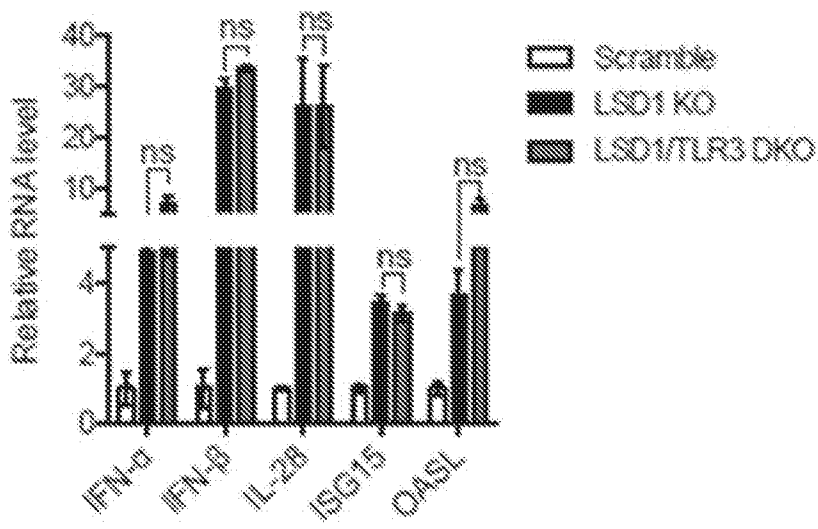


FIG. 8D

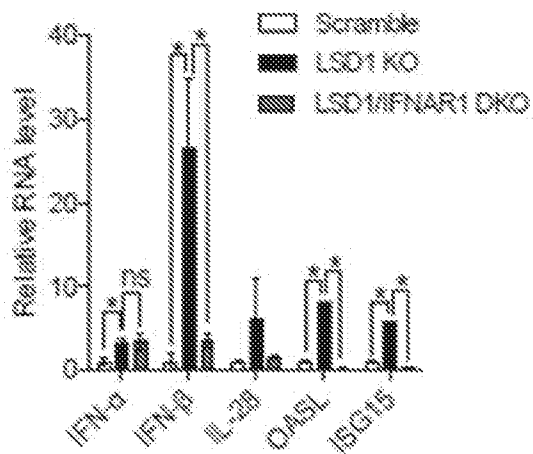


FIG. 8E

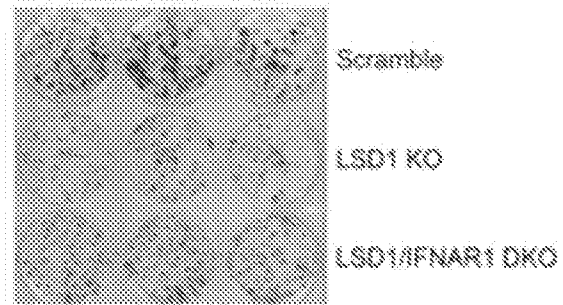


FIG. 8F

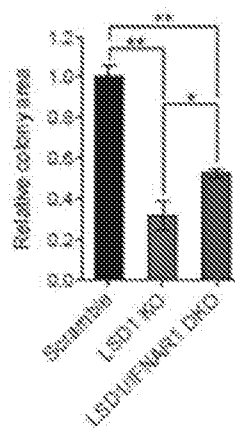


FIG. 8G

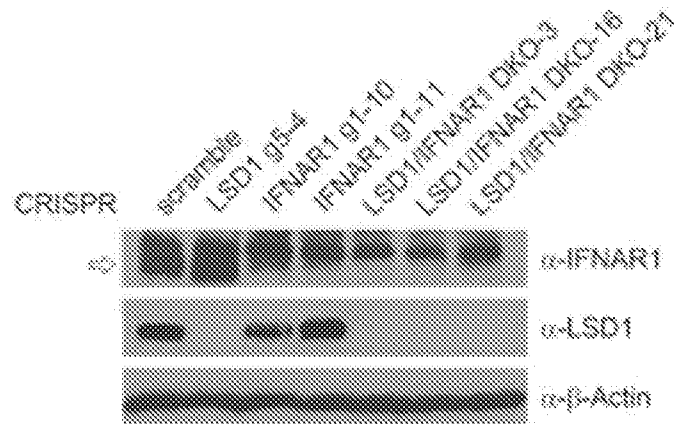


FIG. 8H

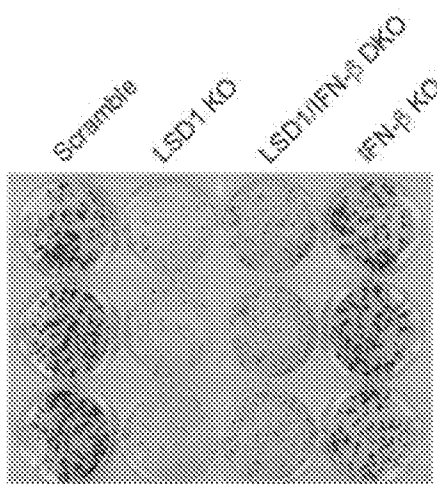


FIG. 8I

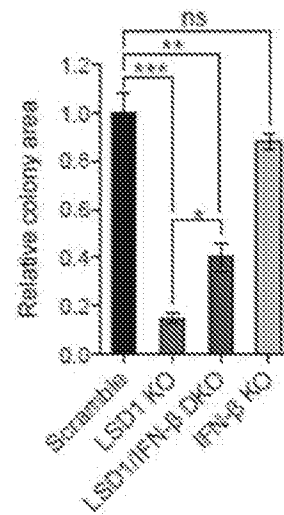


FIG. 8J

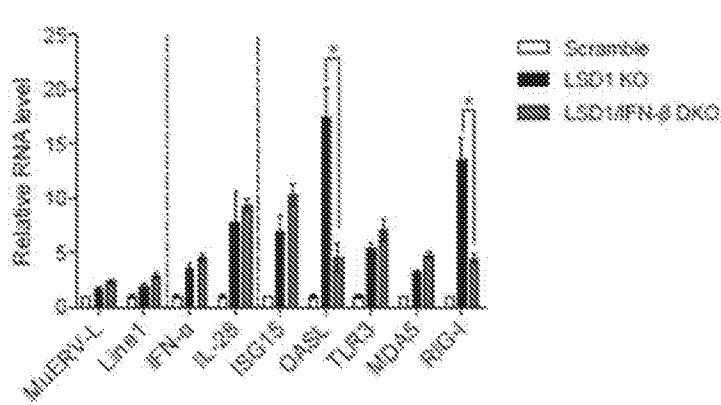


FIG. 8K

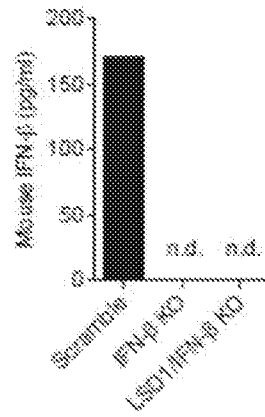


FIG. 8L

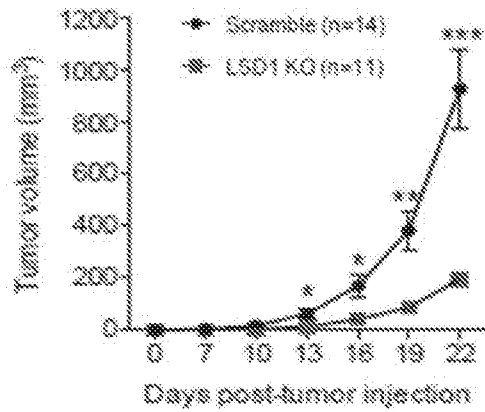


FIG. 9A

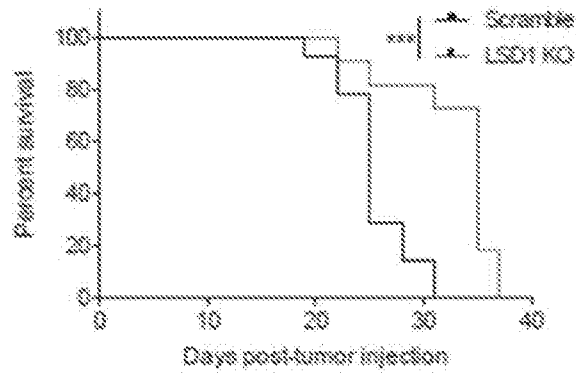


FIG. 9B

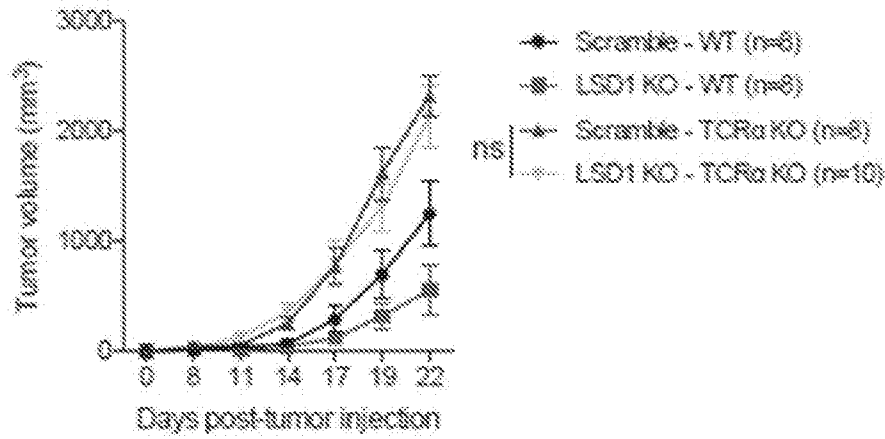


FIG. 9C

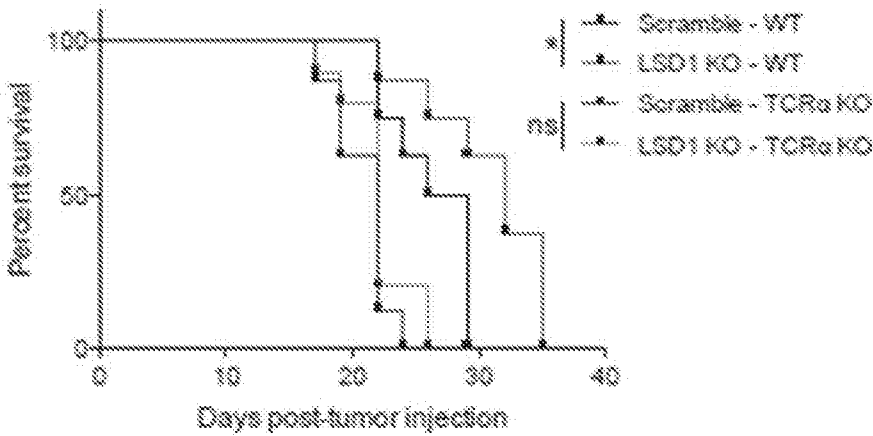


FIG. 9D

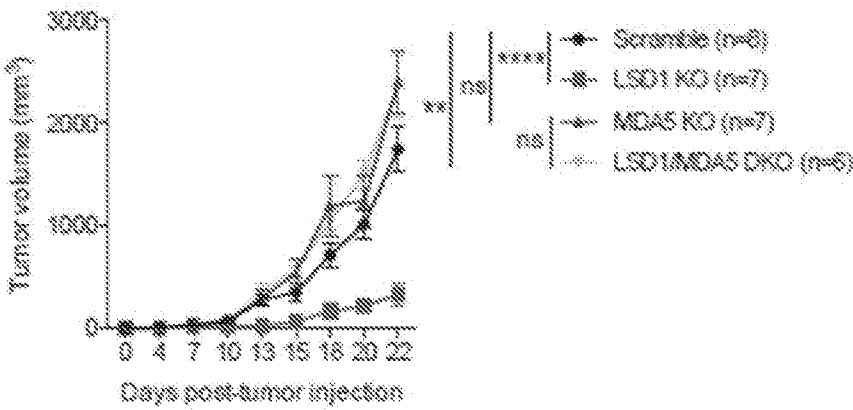


FIG. 9E

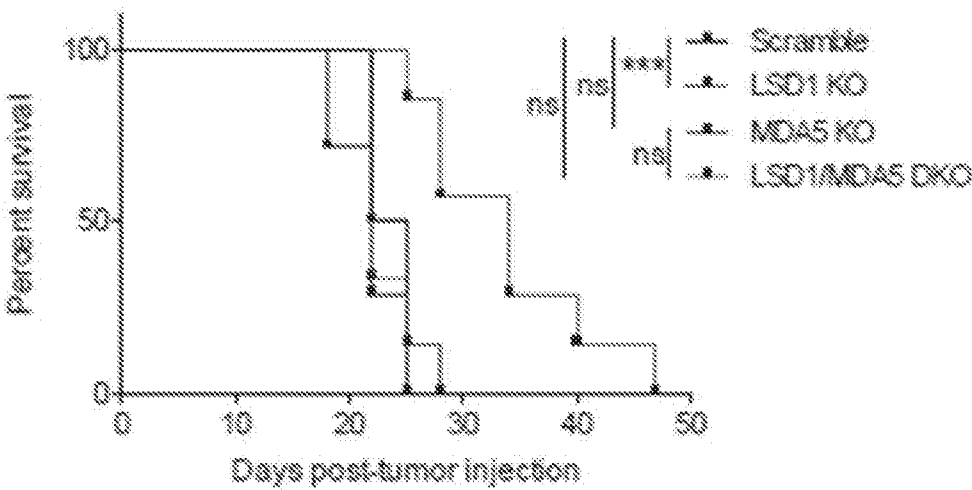


FIG. 9F

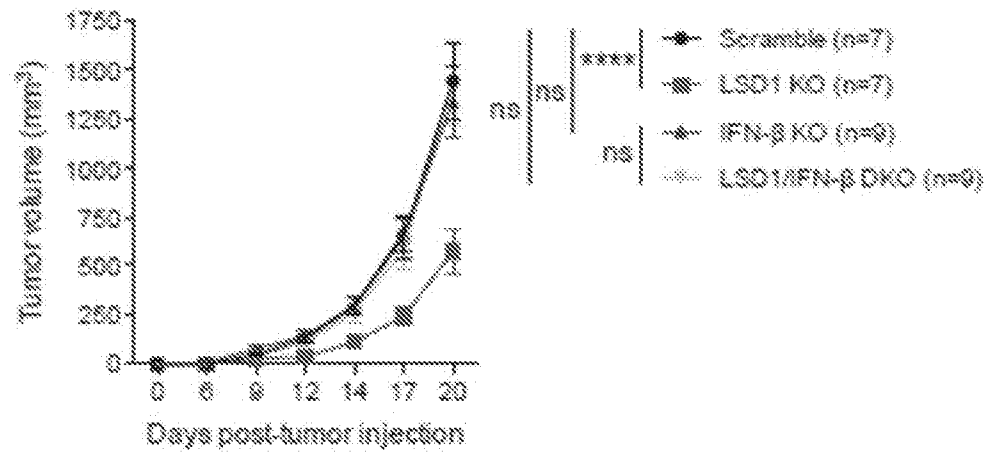


FIG. 9G

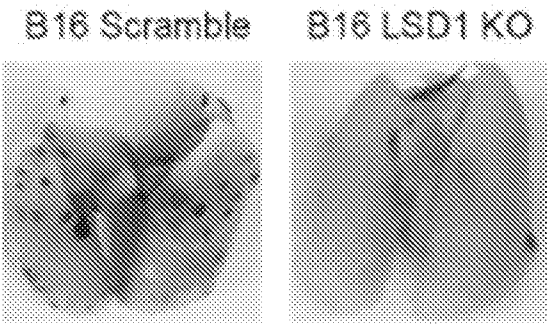


FIG. 9H

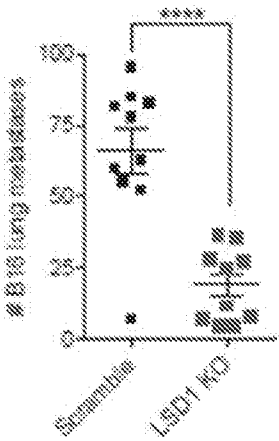


FIG. 9I

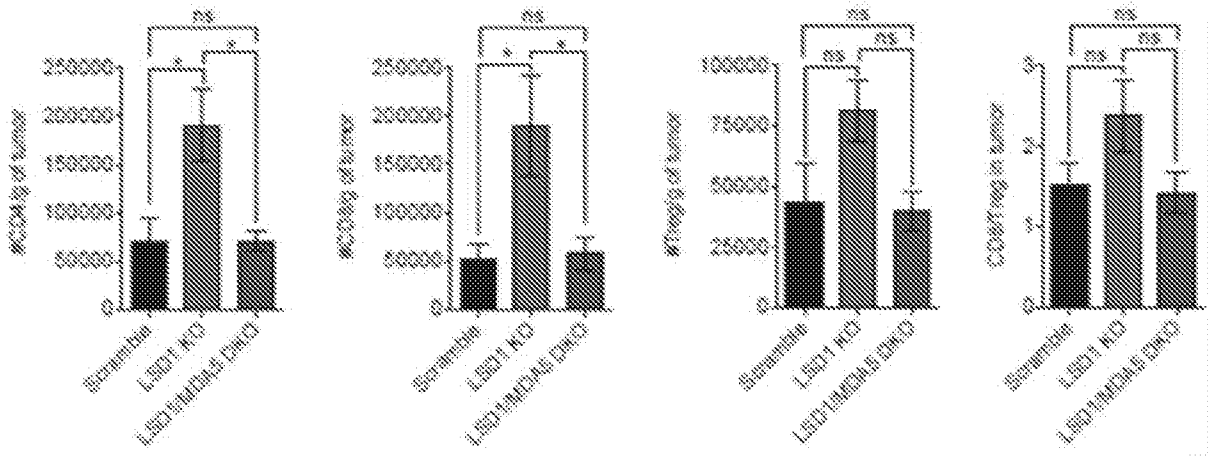


FIG. 10A

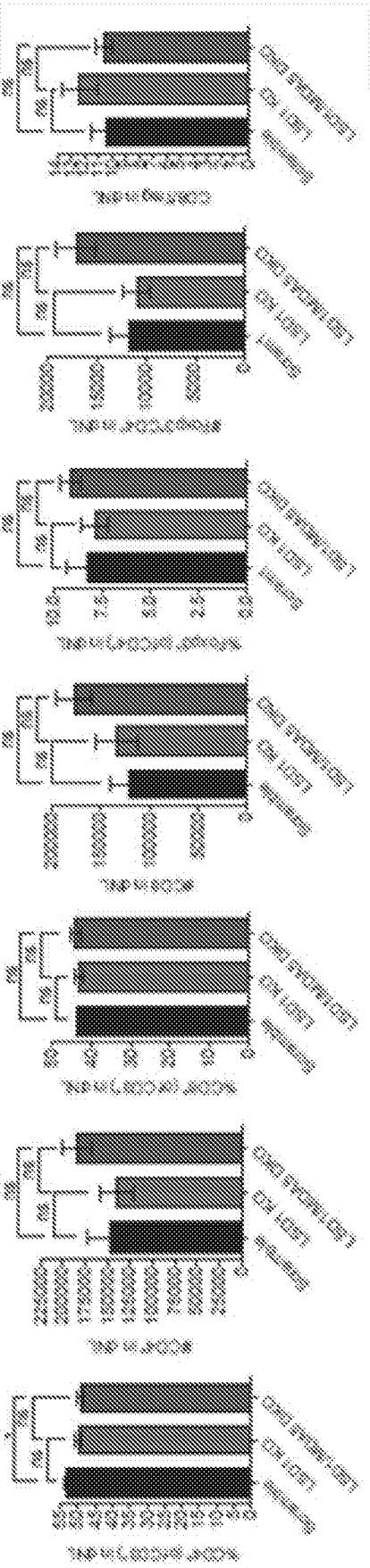


FIG. 10B

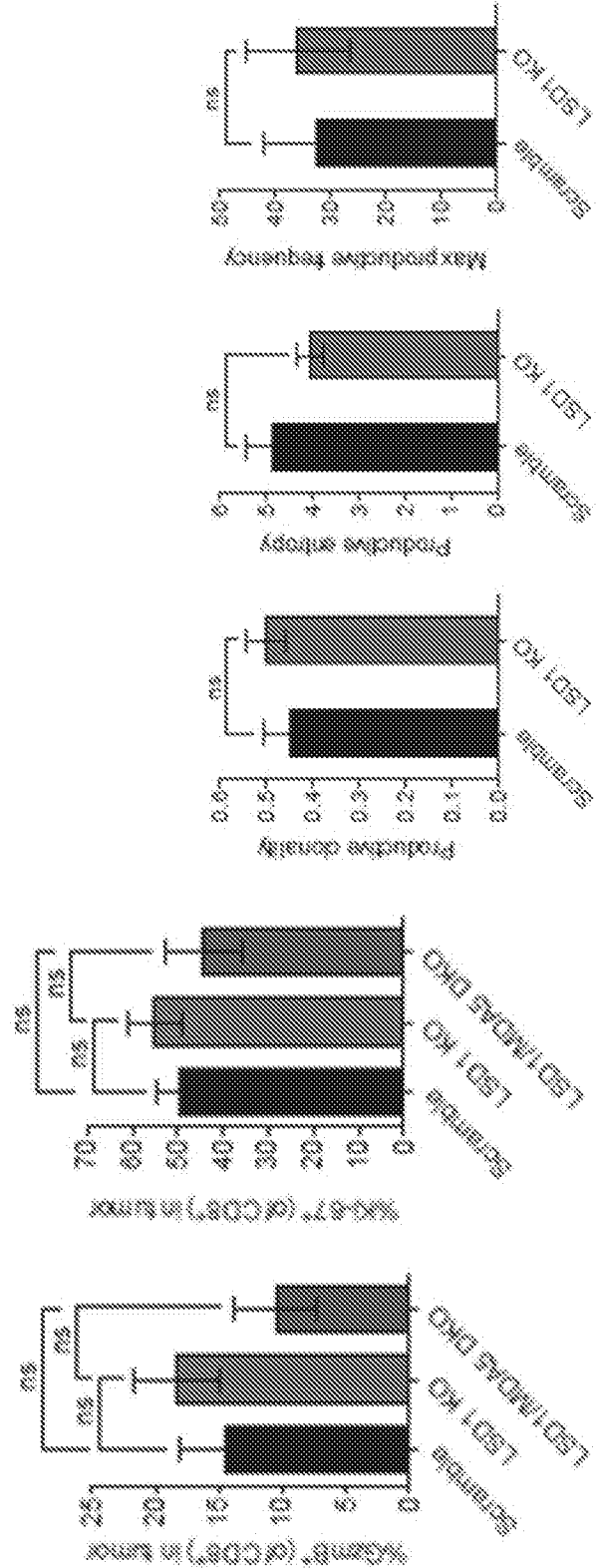


FIG. 10C

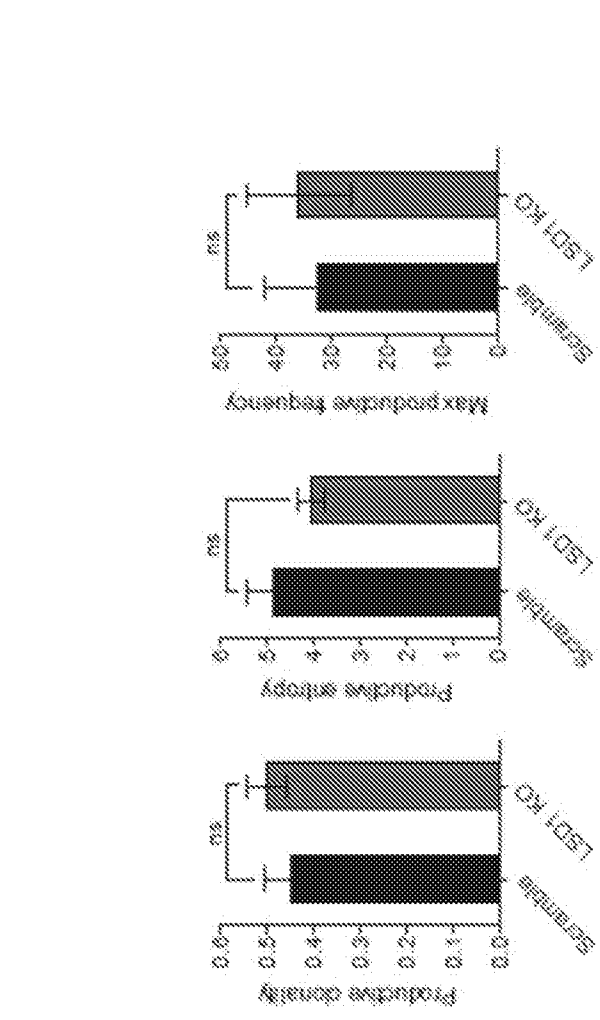


FIG. 10D

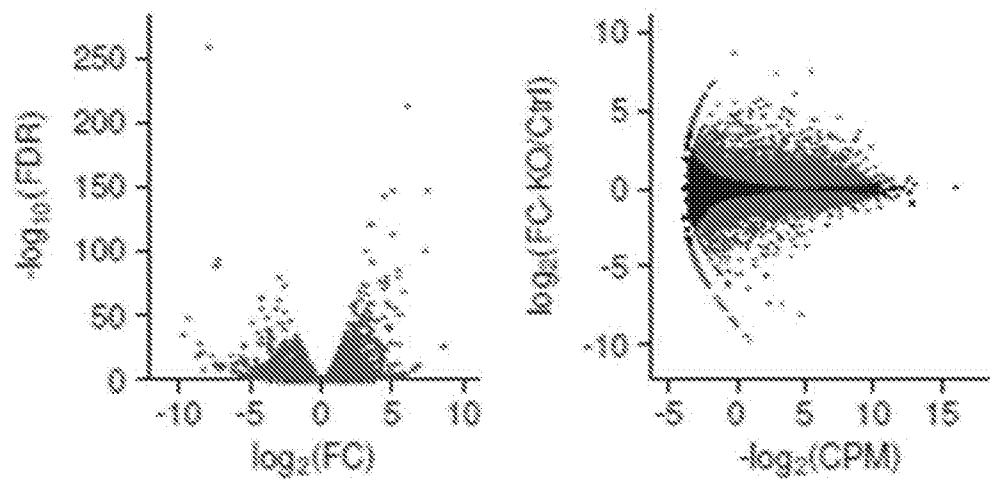


FIG. 10E

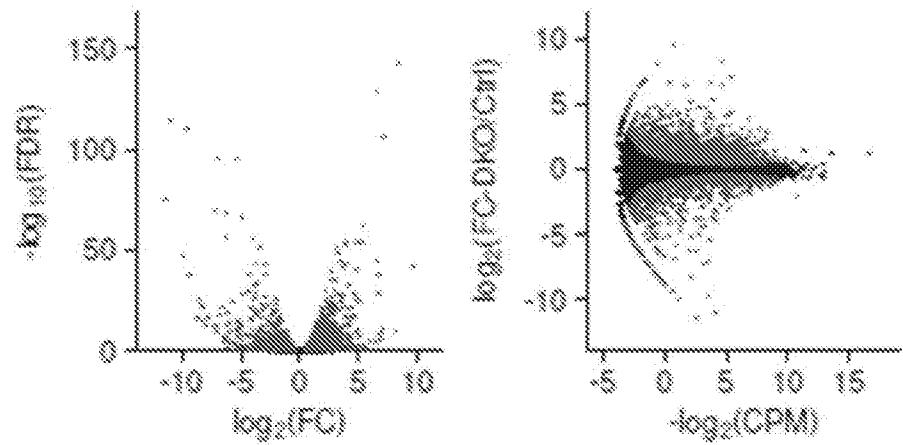


FIG. 10F

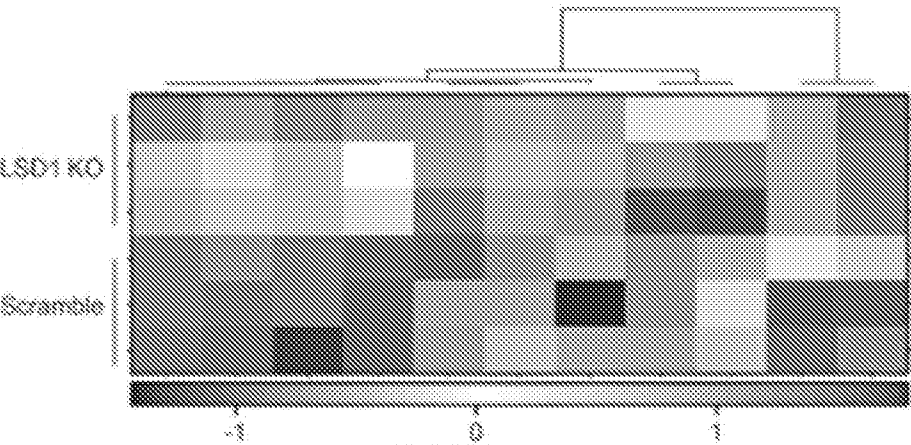


FIG. 10G

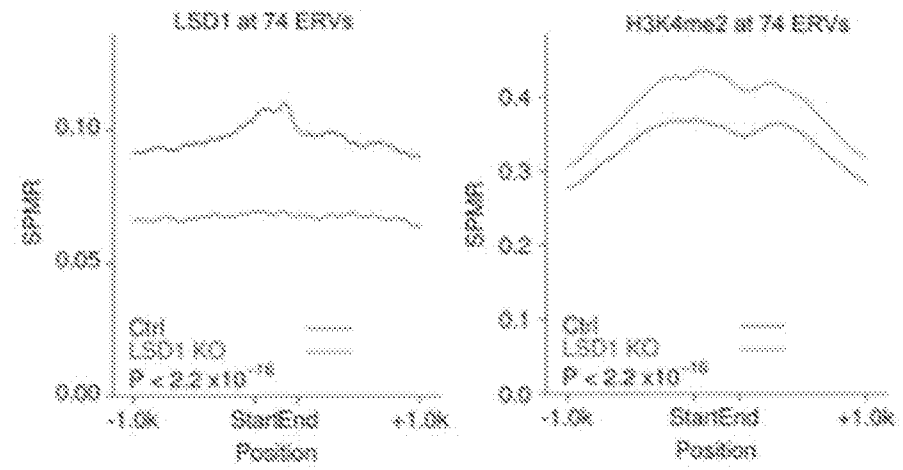


FIG. 10H

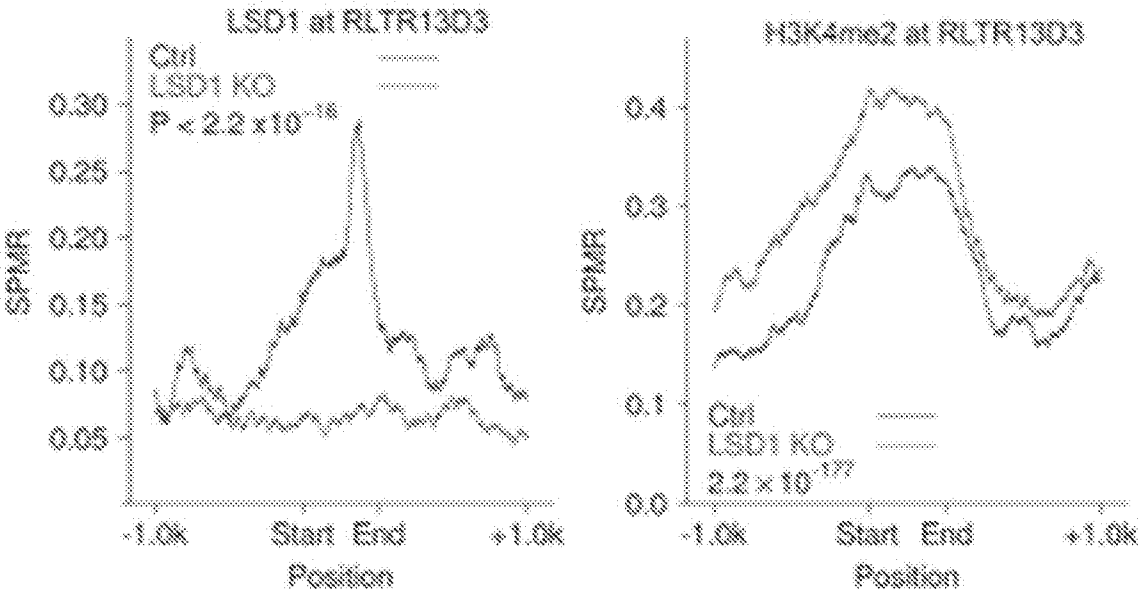


FIG. 10I

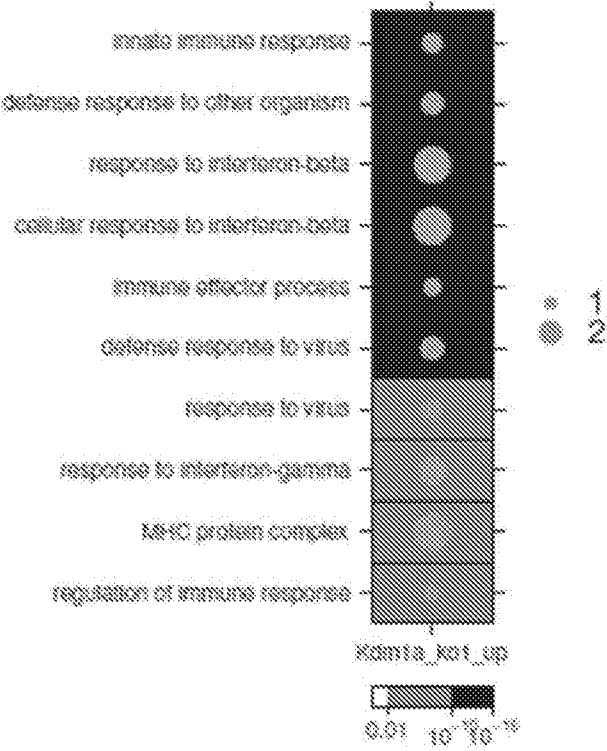


FIG 10J

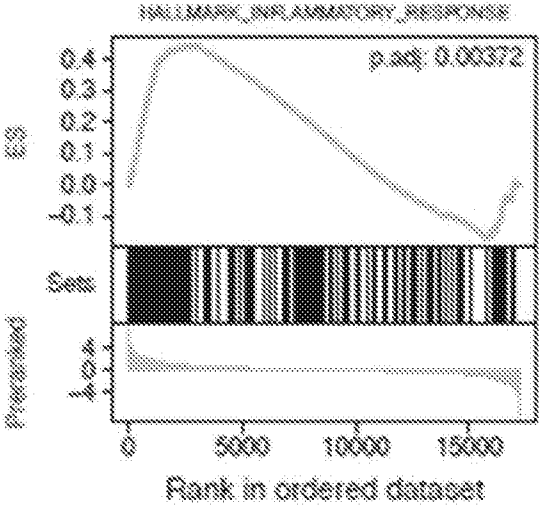


FIG. 10K

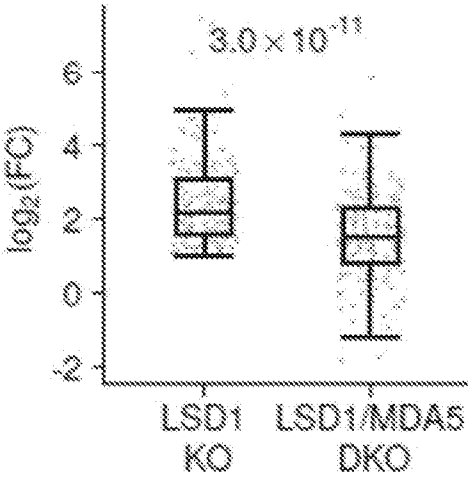
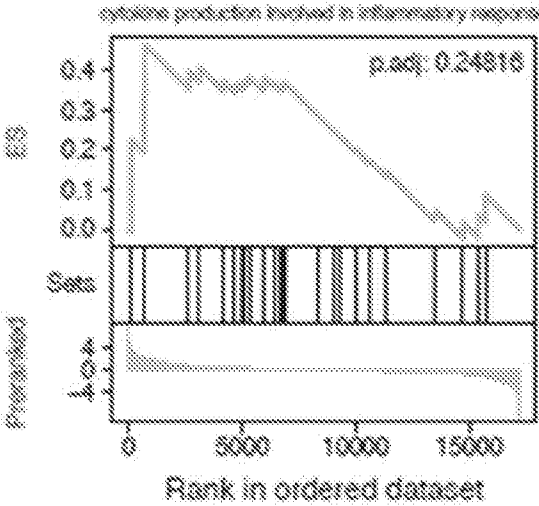


FIG. 10L

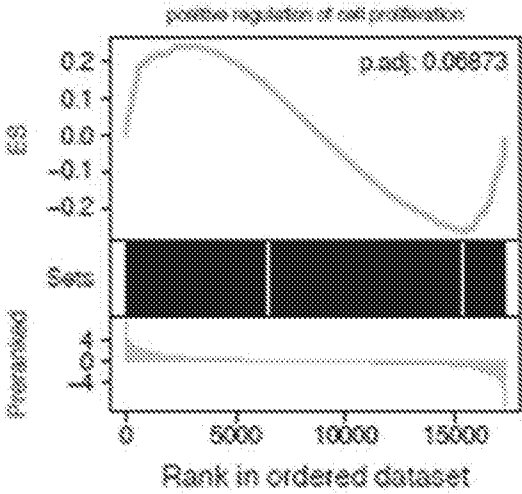


FIG. 10M

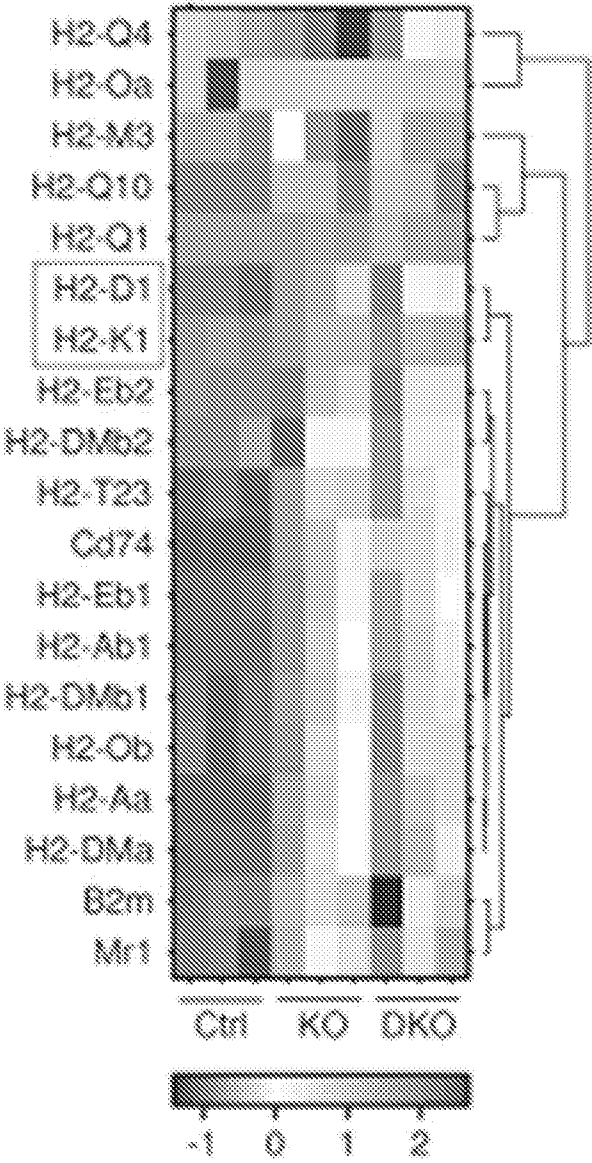


FIG. 10N

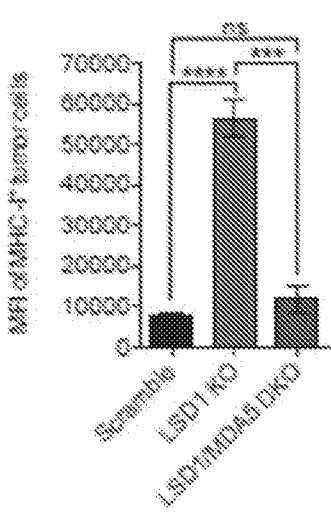


FIG. 10O

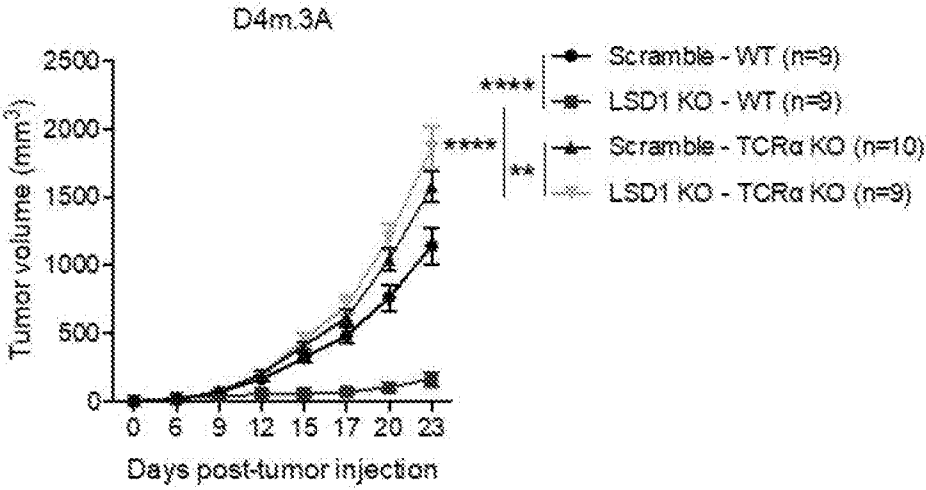


FIG. 10P

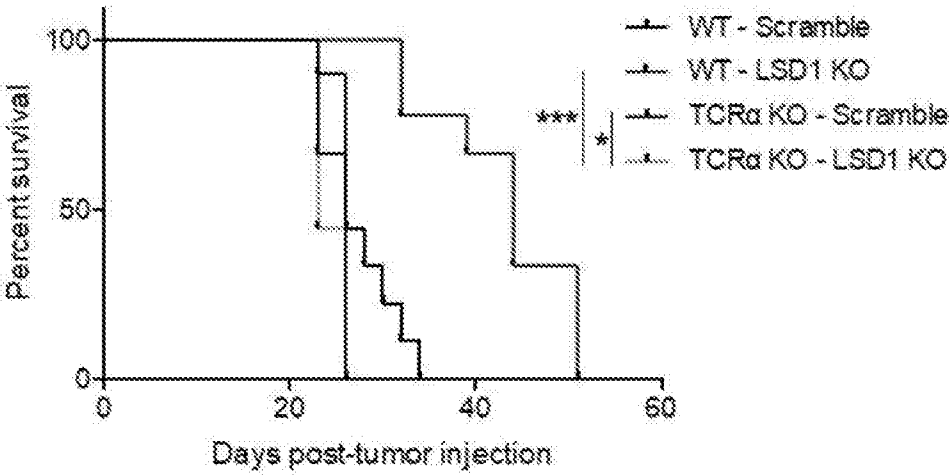


FIG. 10Q

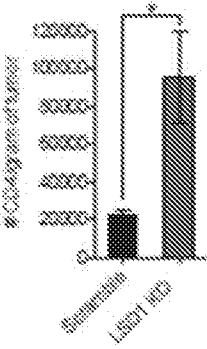


FIG. 10R

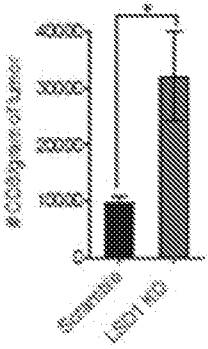
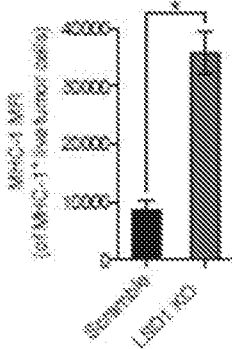


FIG. 10S



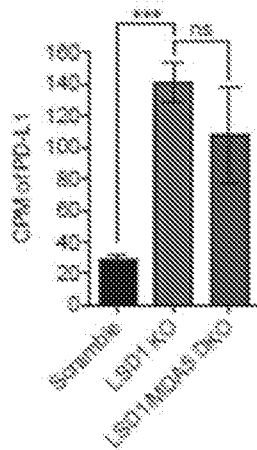


FIG. 10T

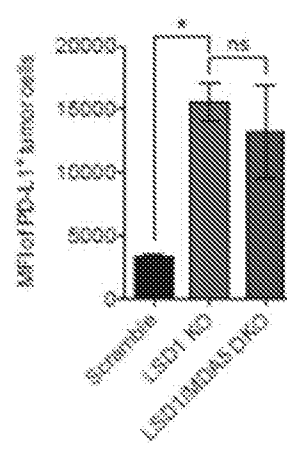


FIG. 10U

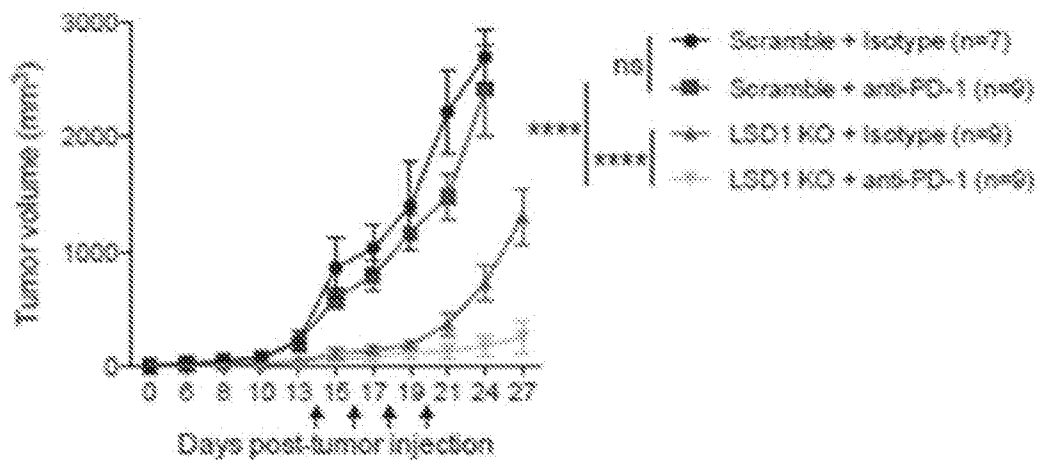


FIG. 11A

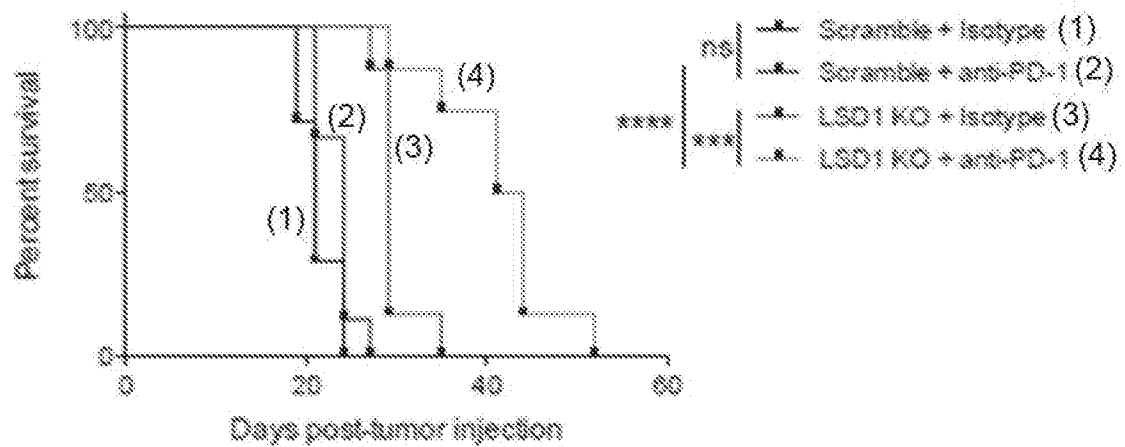


FIG. 11B

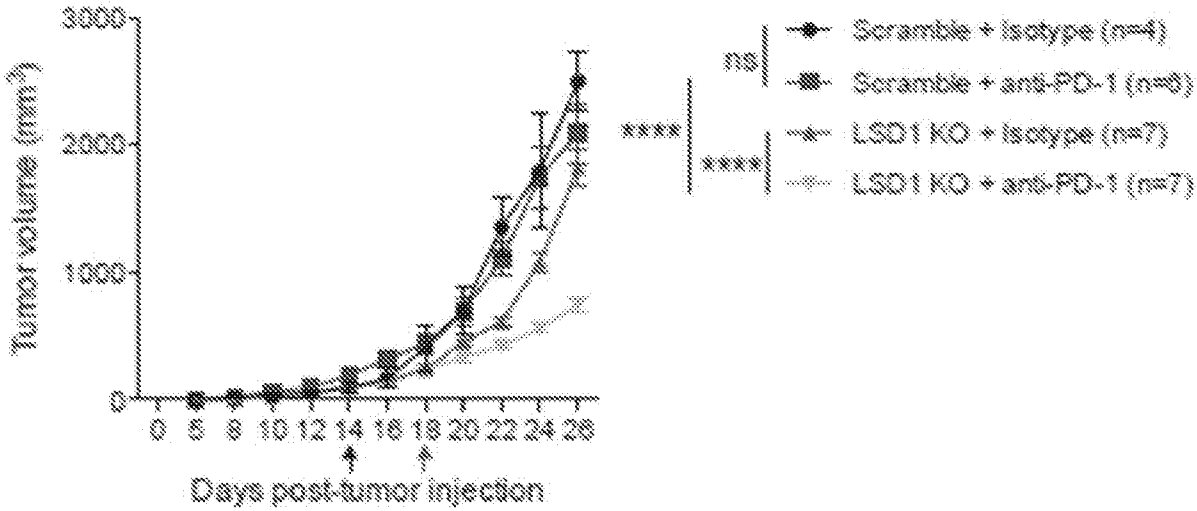


FIG. 11C

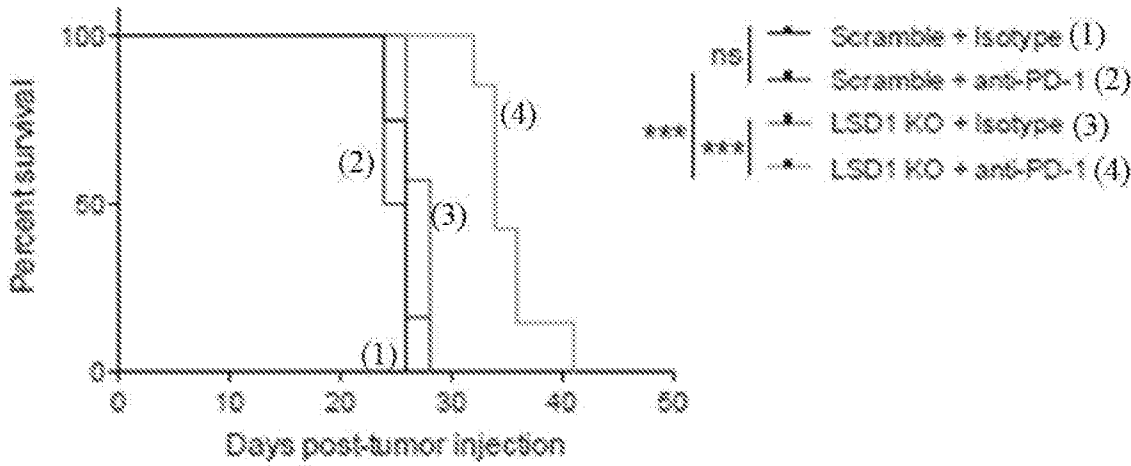
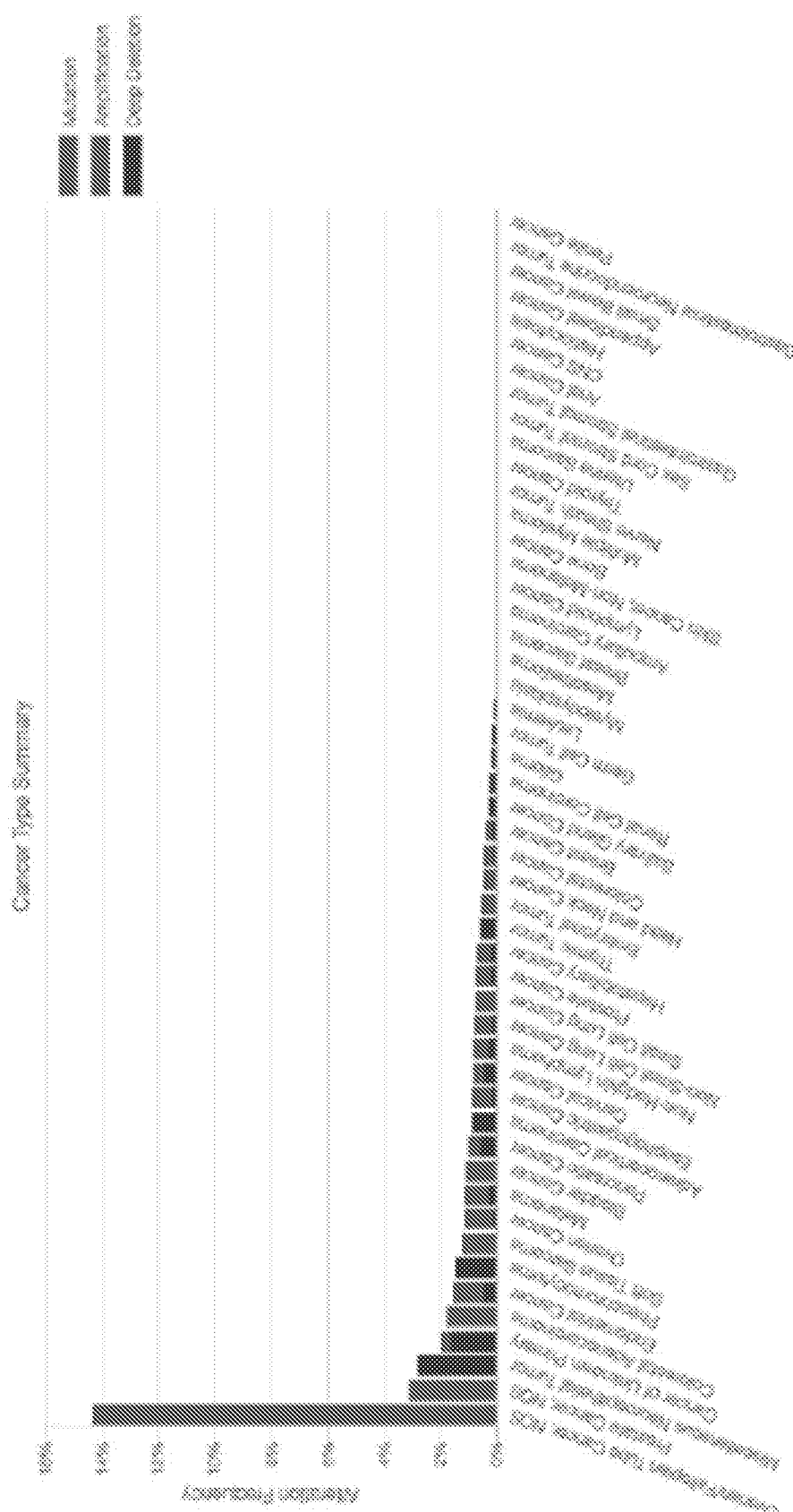


FIG. 11D



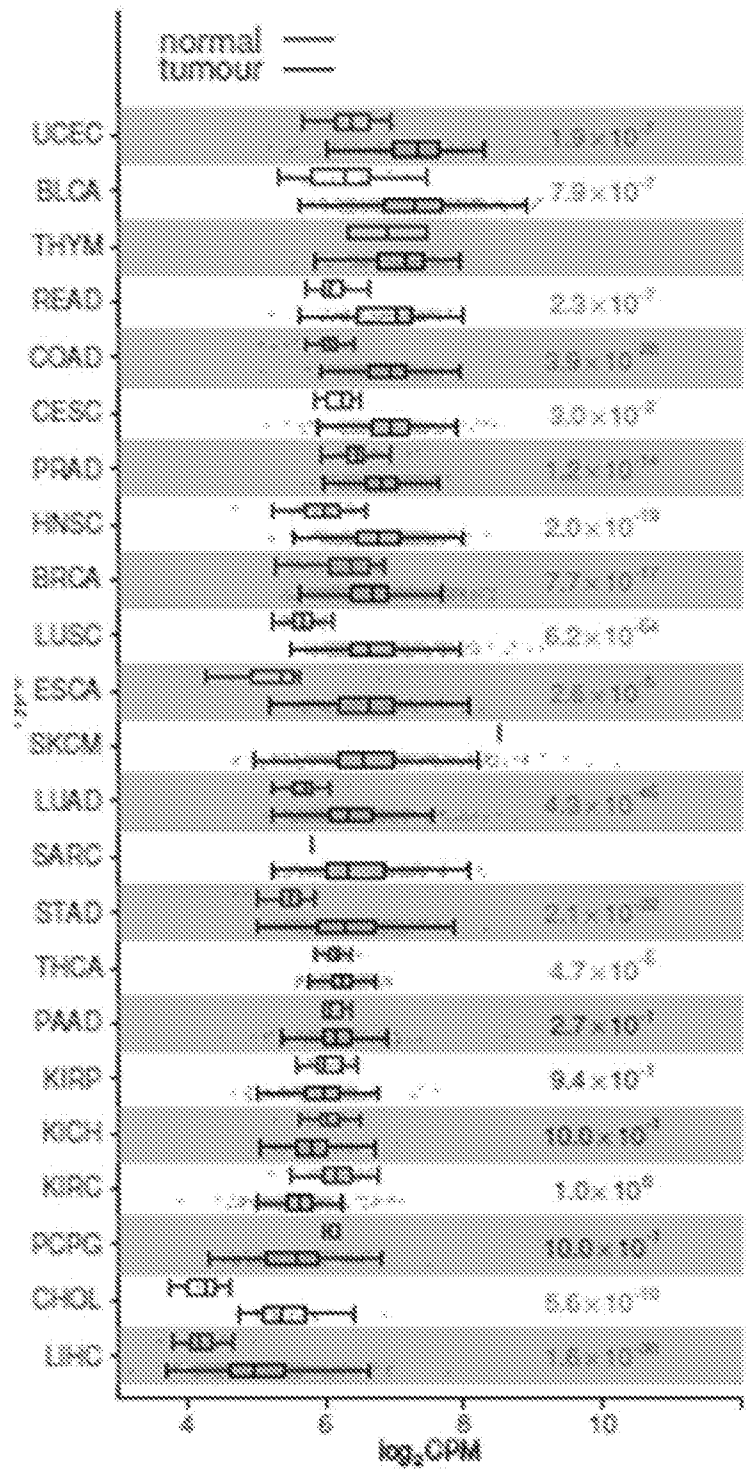


FIG. 12B

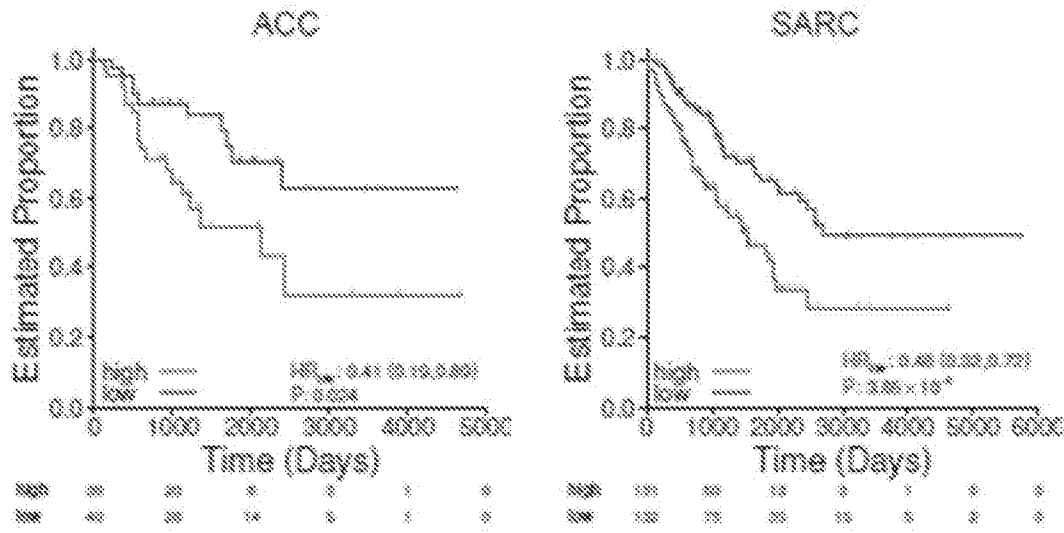


FIG. 12C

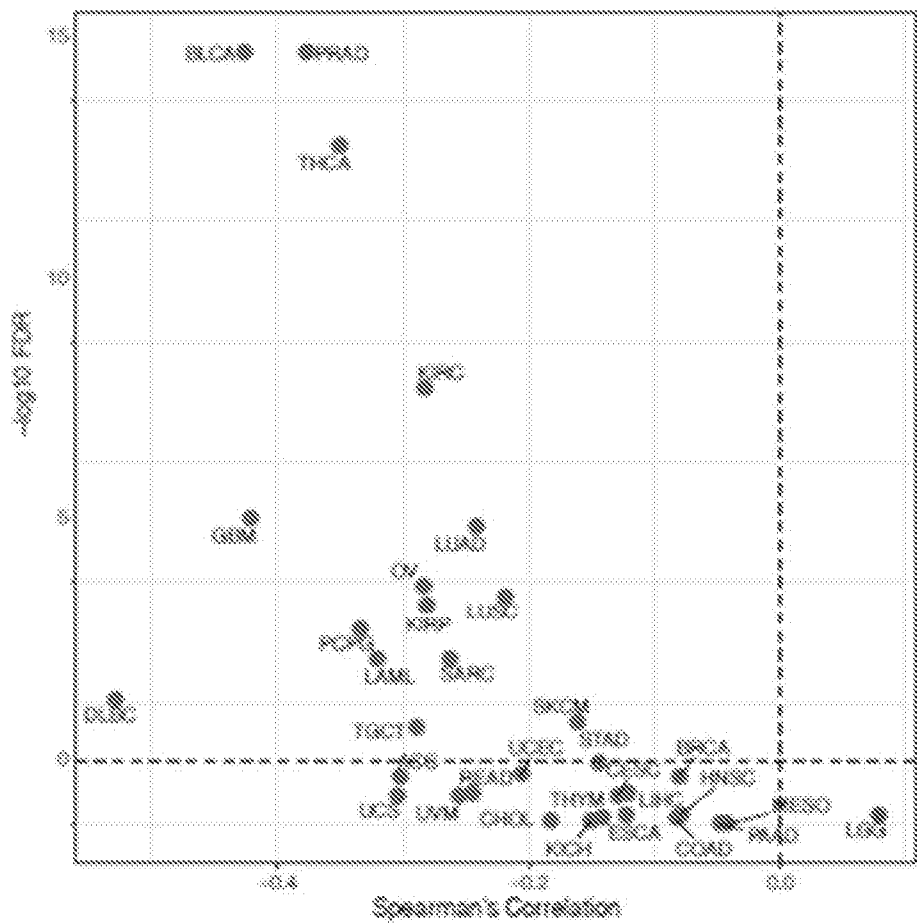


FIG. 12D

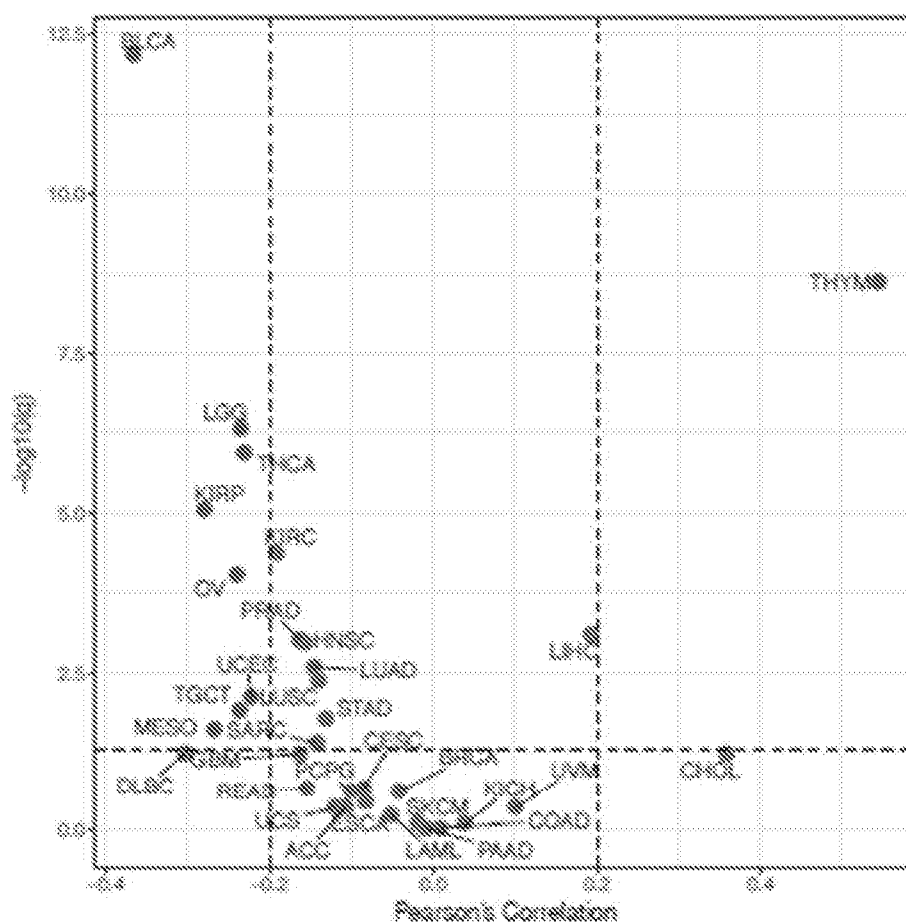


FIG. 12E

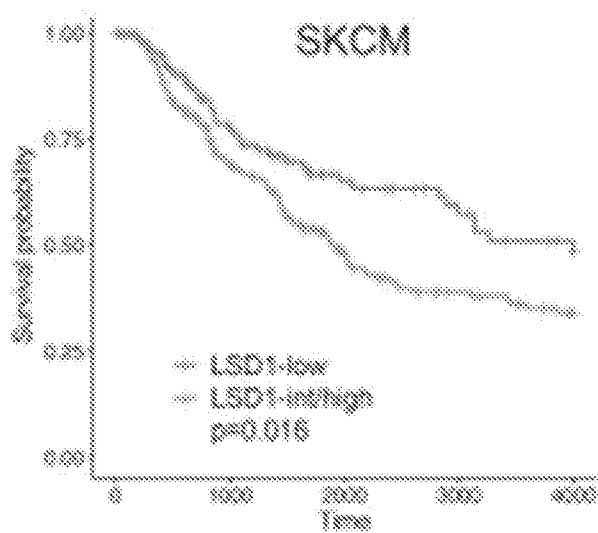


FIG. 12F

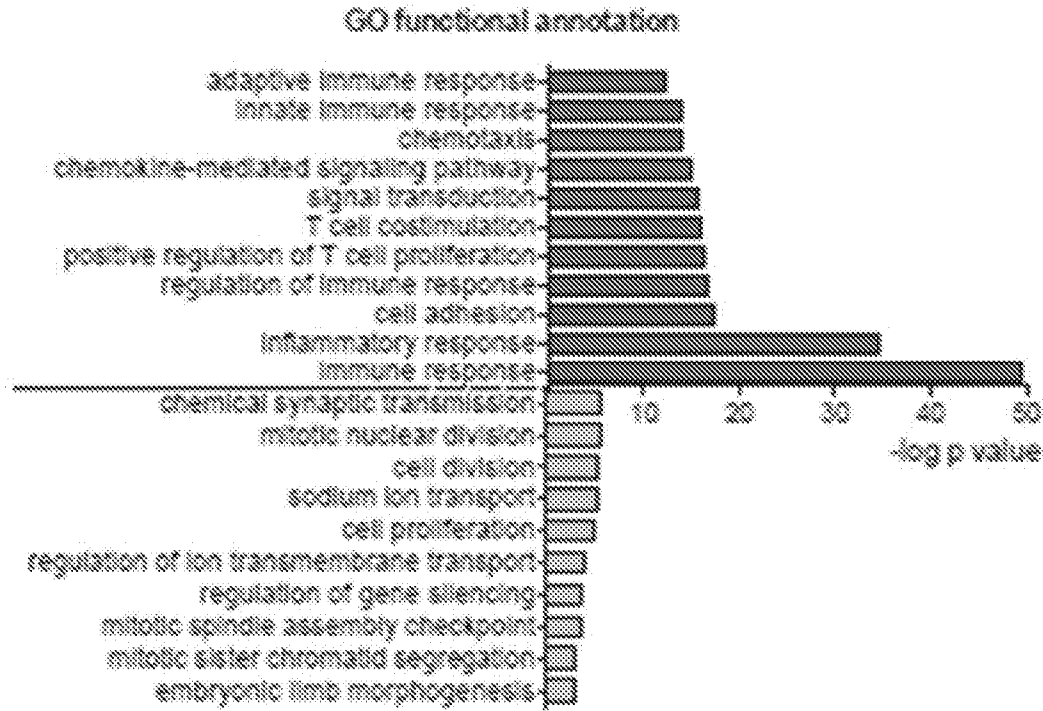


FIG. 12G

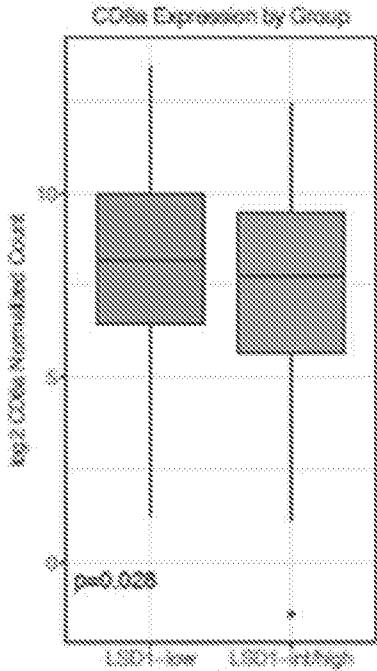


FIG. 12H

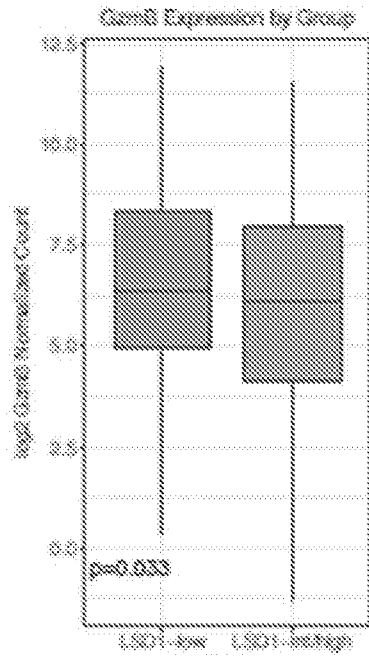
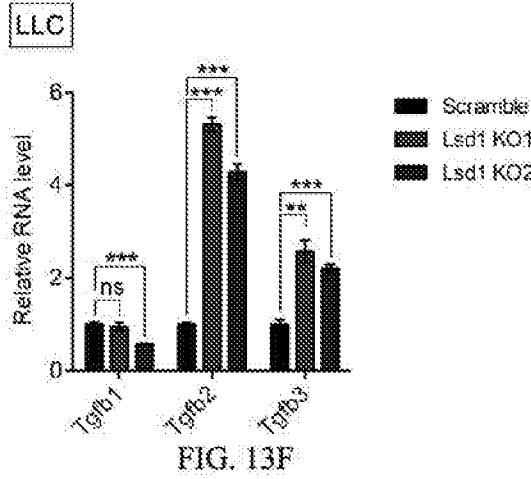
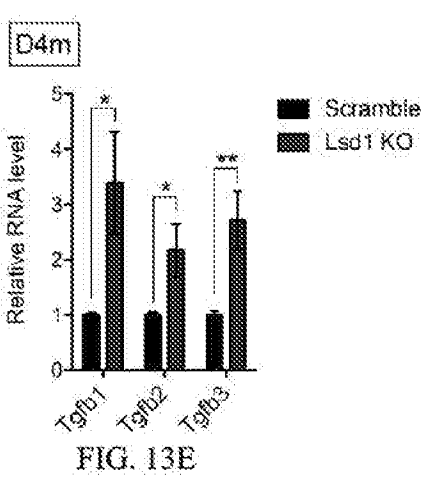
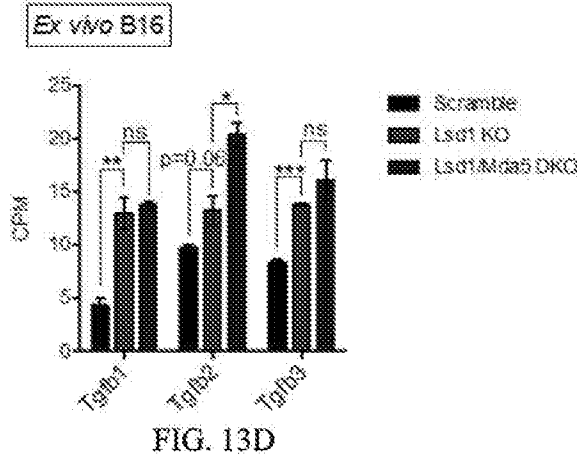
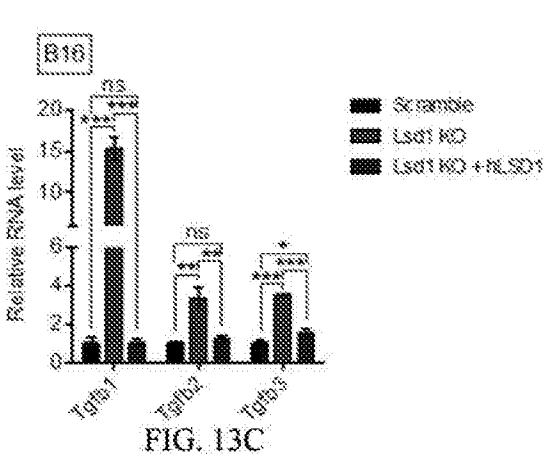
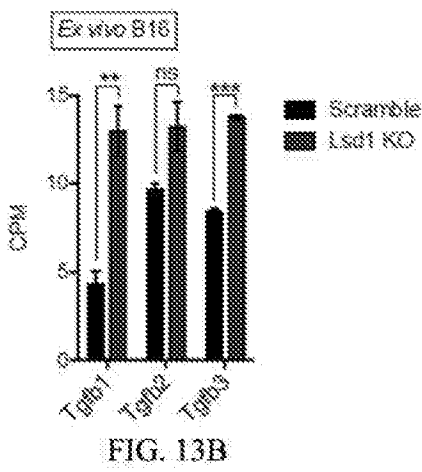
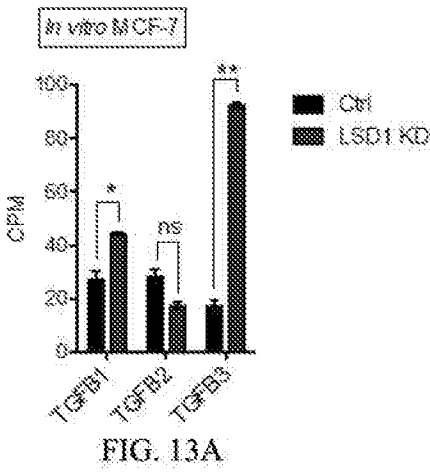
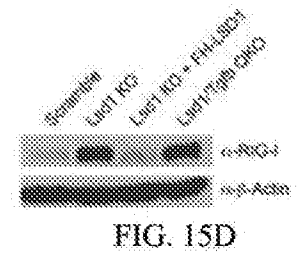
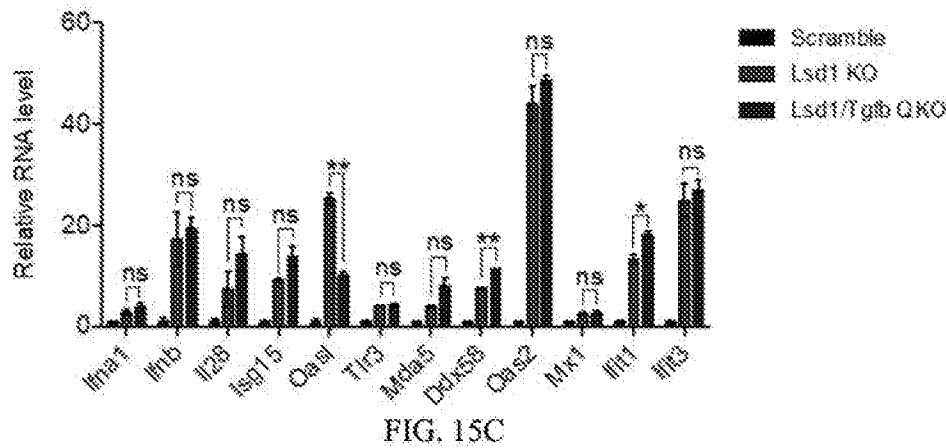
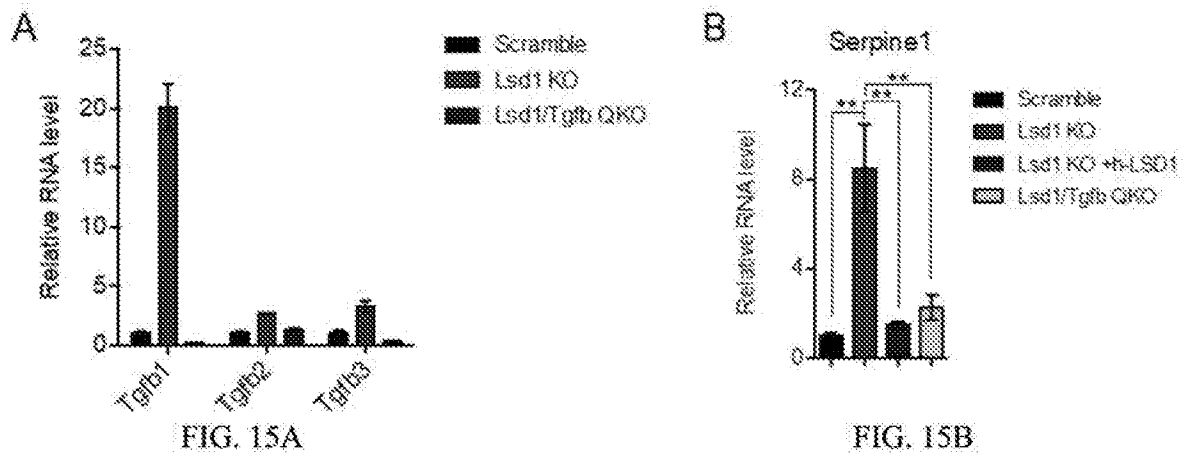
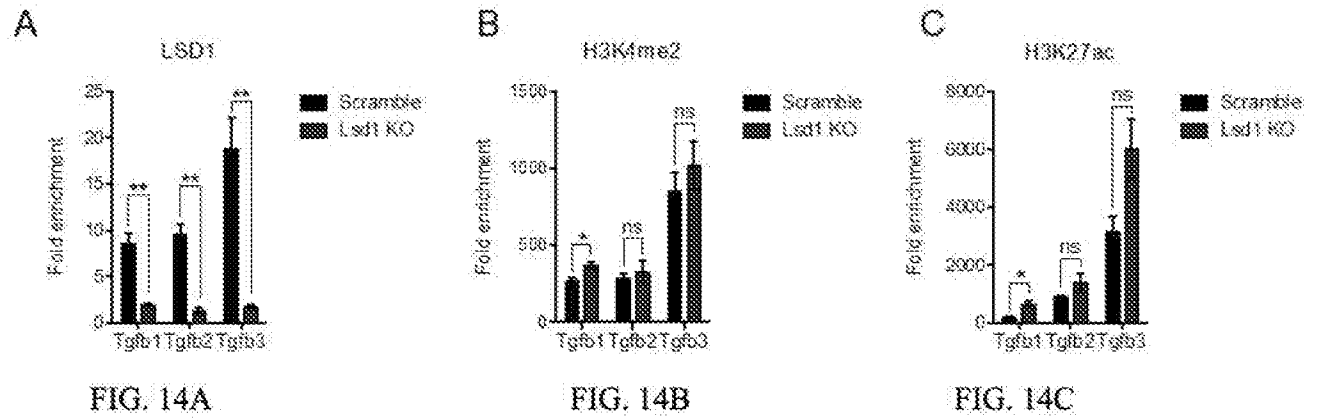


FIG. 12I





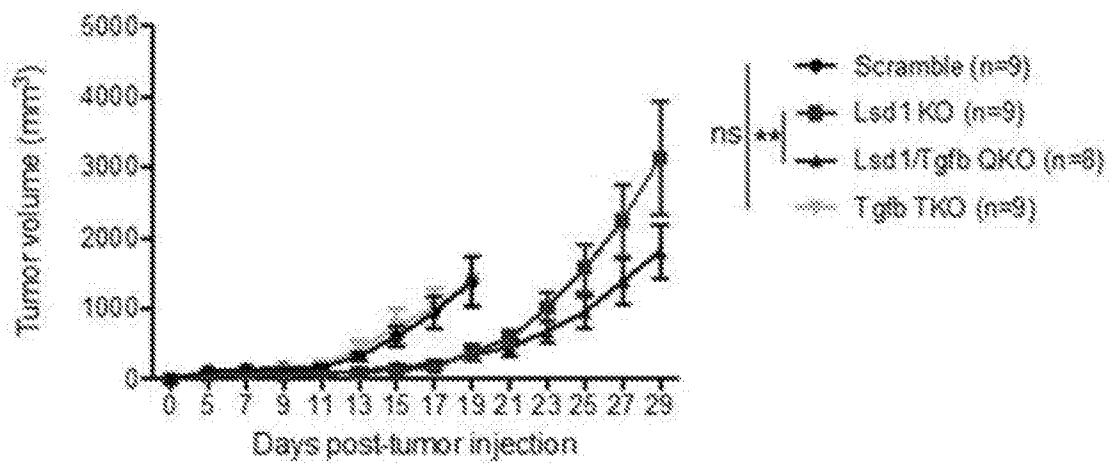


FIG. 16A

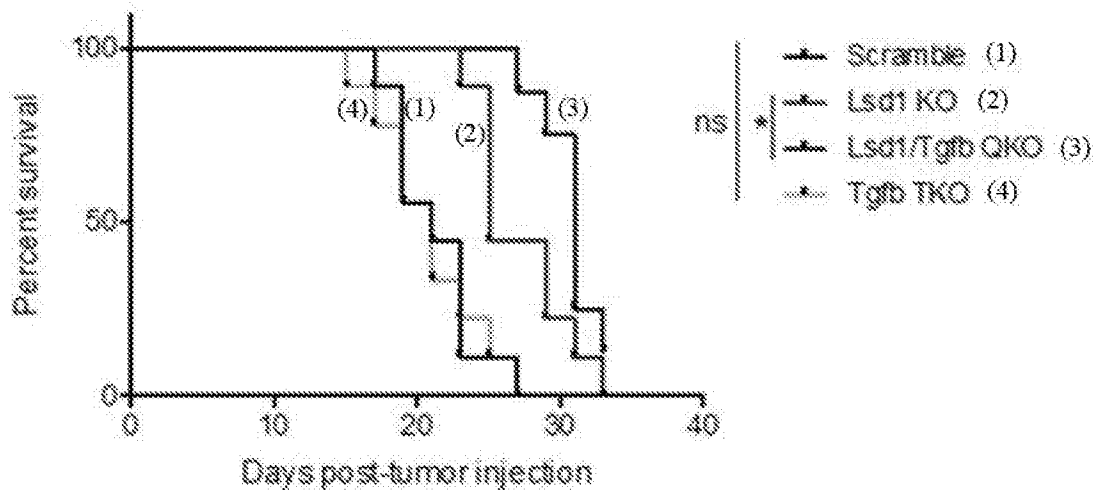


FIG. 16B

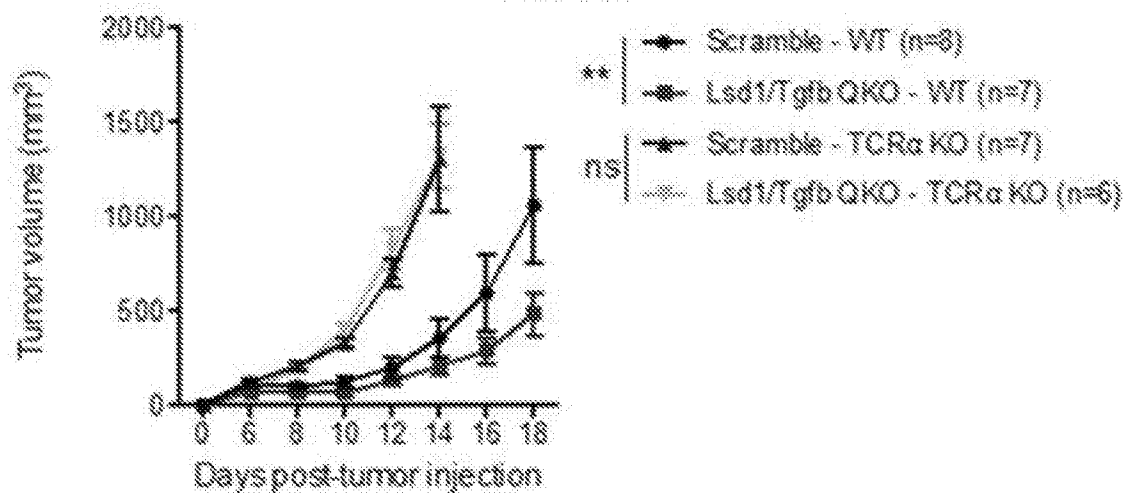


FIG. 16C

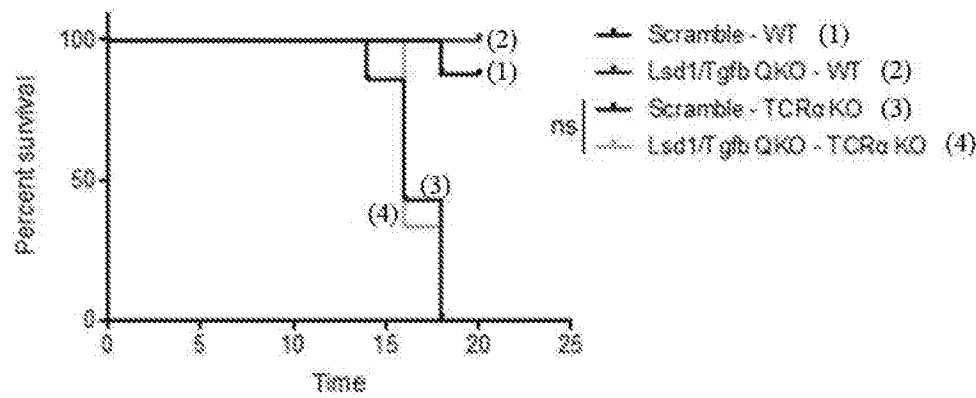


FIG. 16D

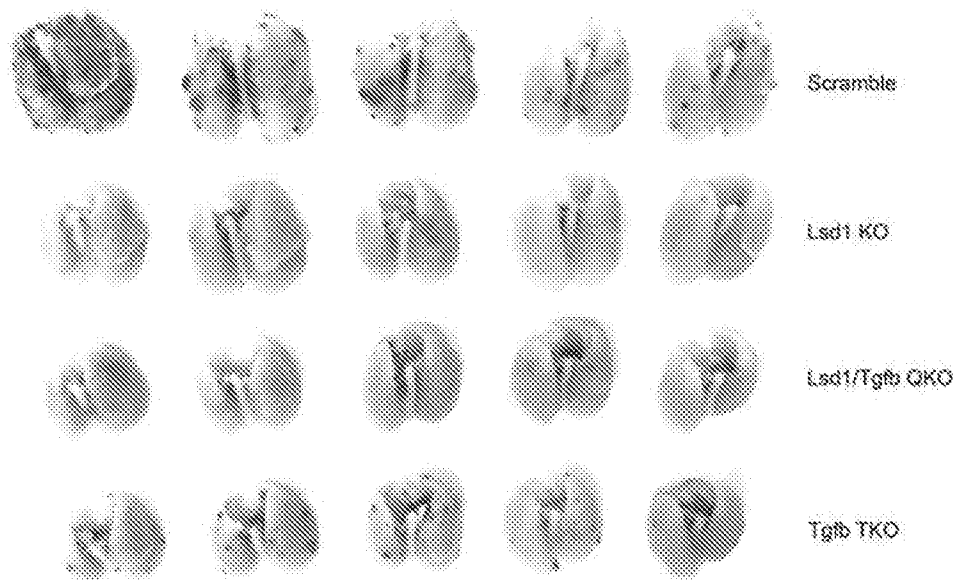


FIG. 17A

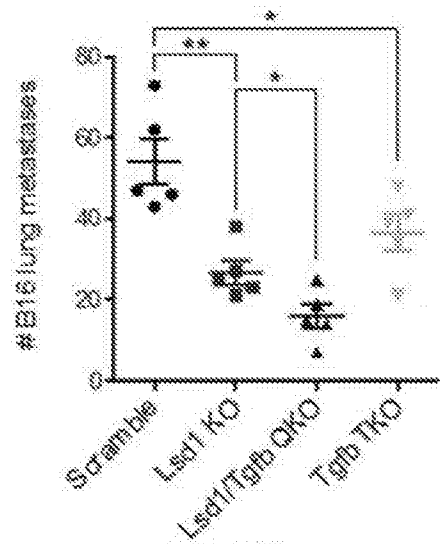


FIG. 17B

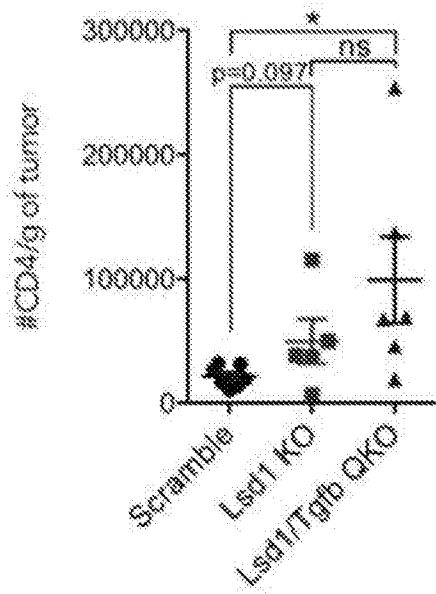


FIG. 18A

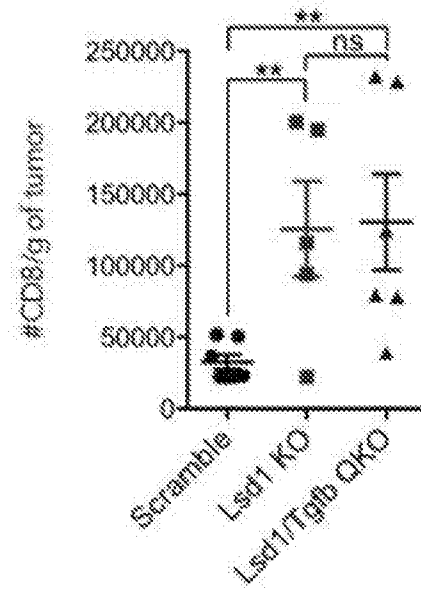


FIG. 18B

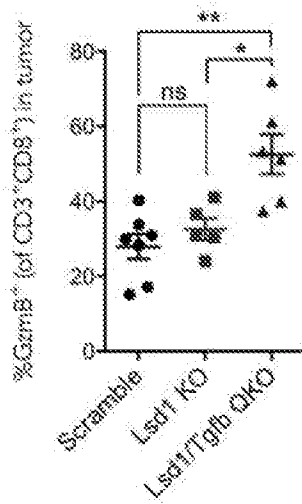


FIG. 18C

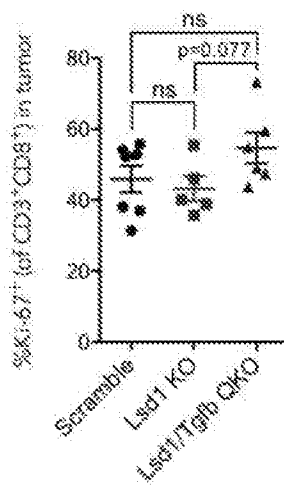


FIG. 18D

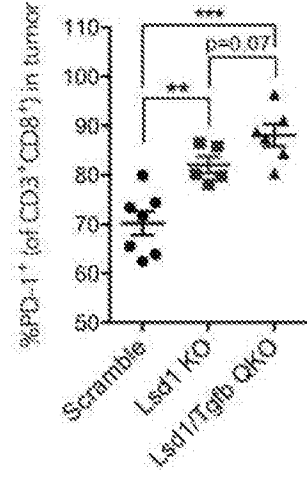


FIG. 18E

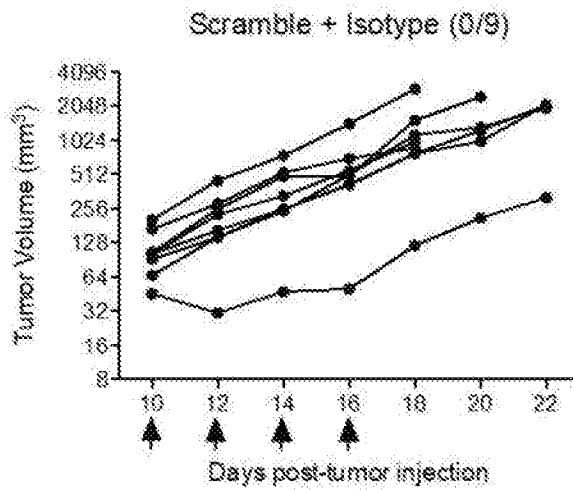


FIG. 19A

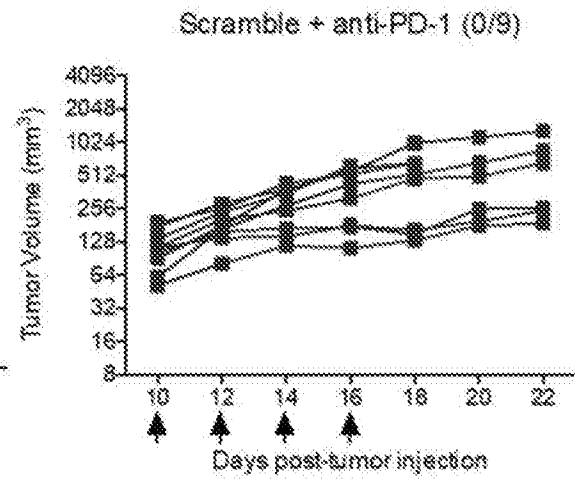


FIG. 19B

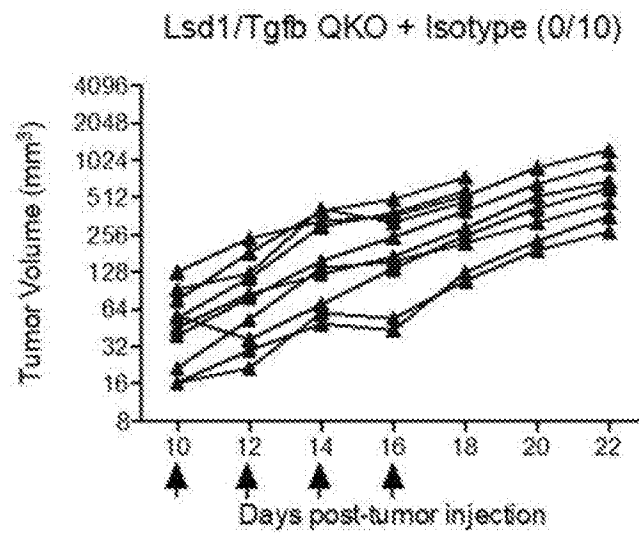


FIG. 19C

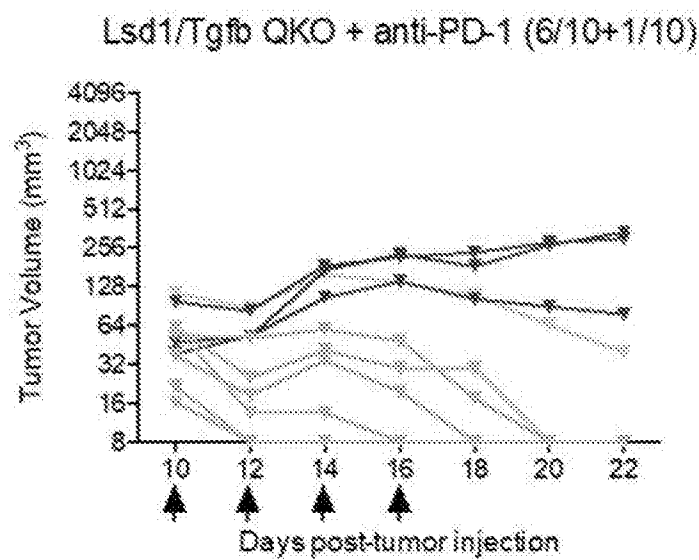
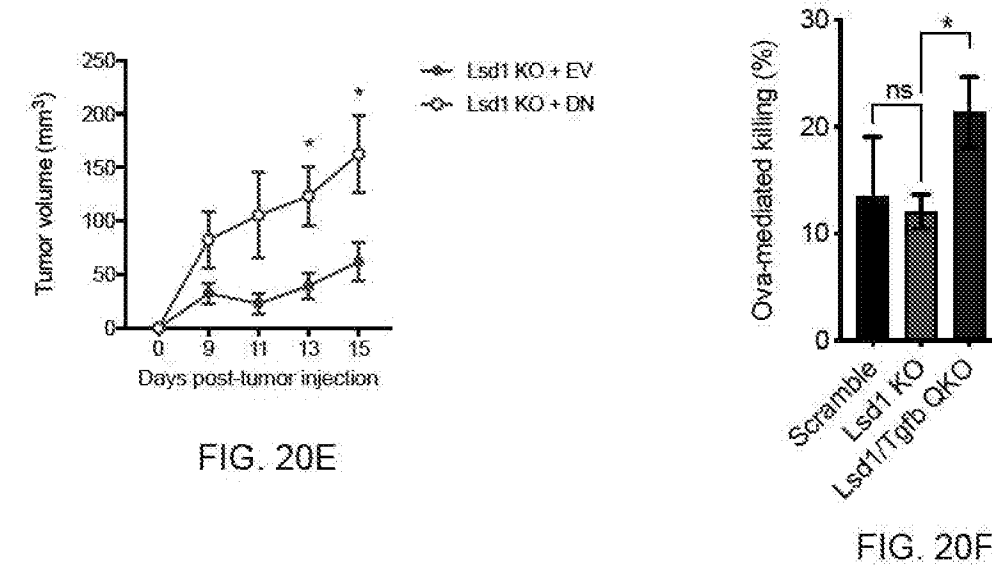
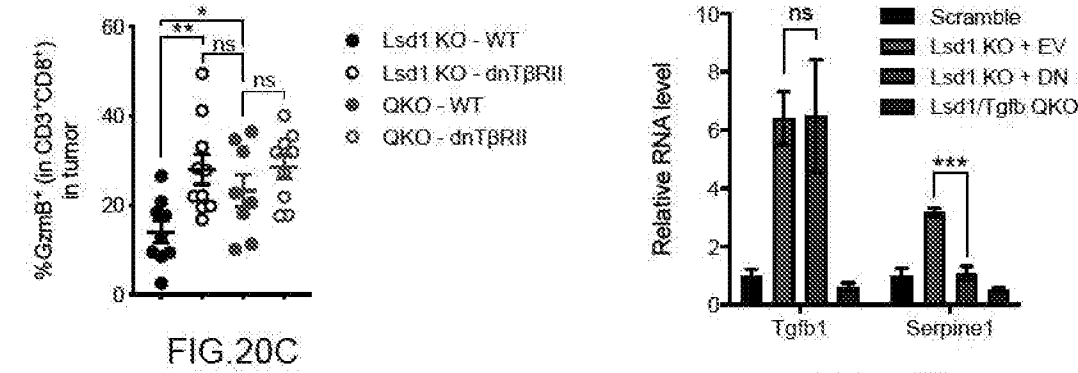
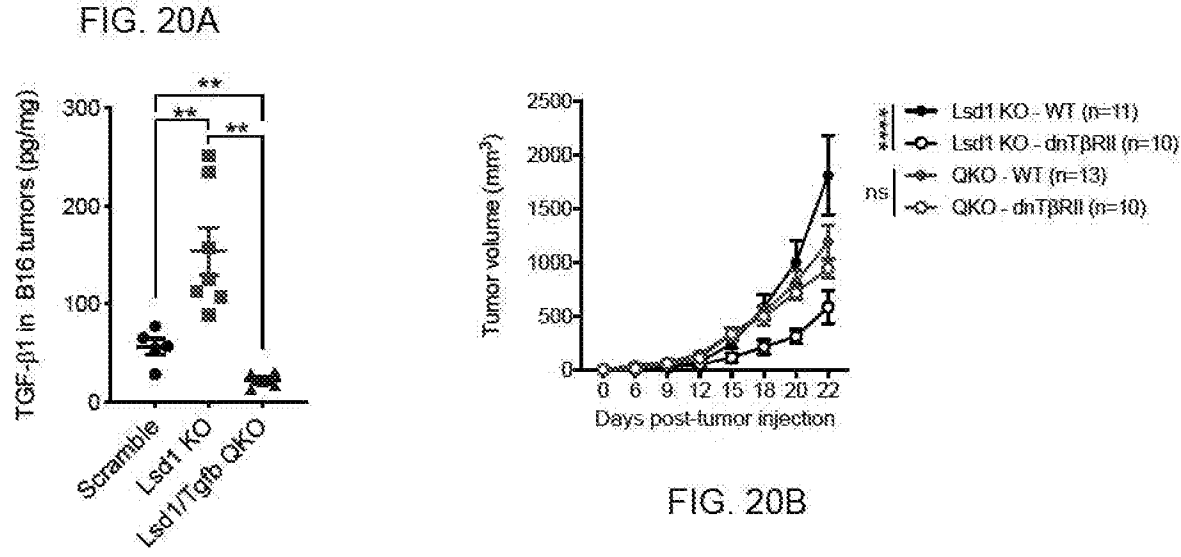


FIG. 19D



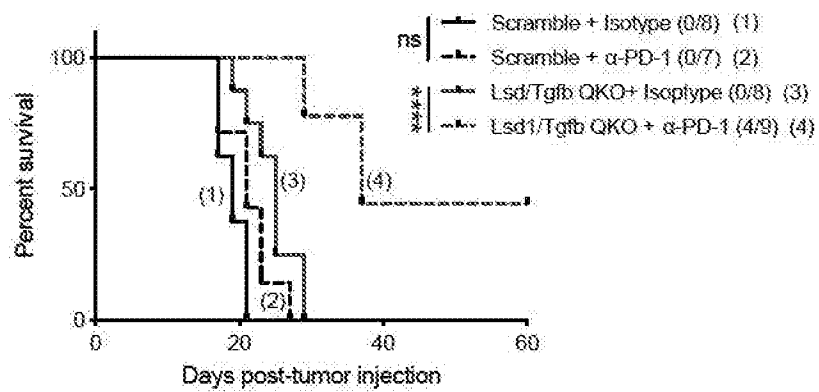


FIG. 21A

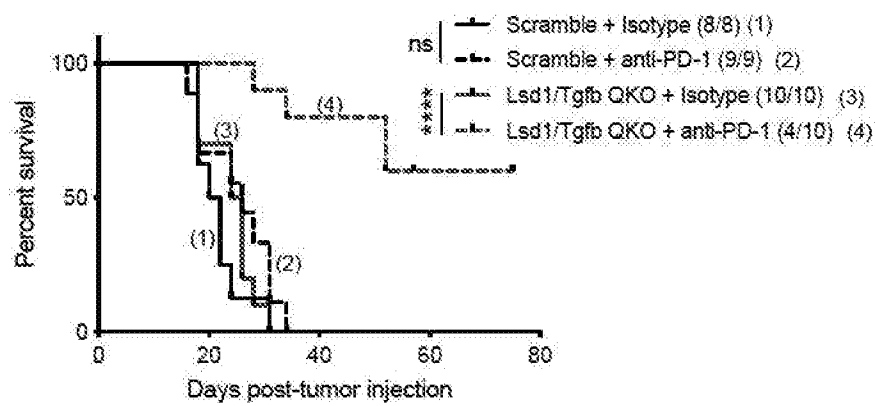


FIG. 21B

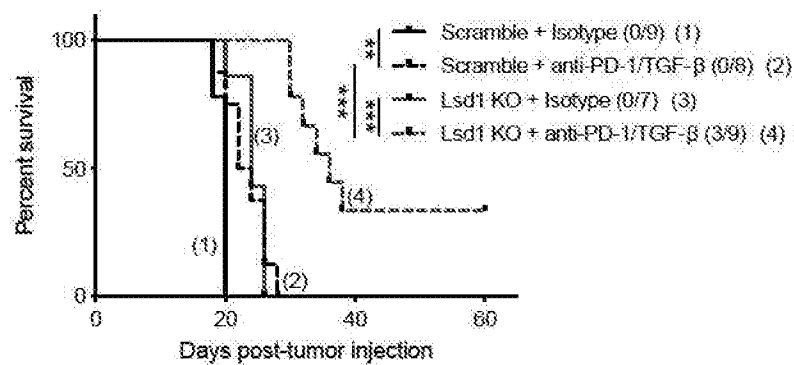


FIG. 21C

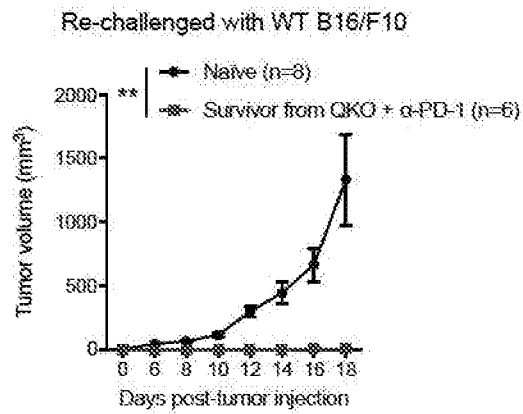


FIG. 22A

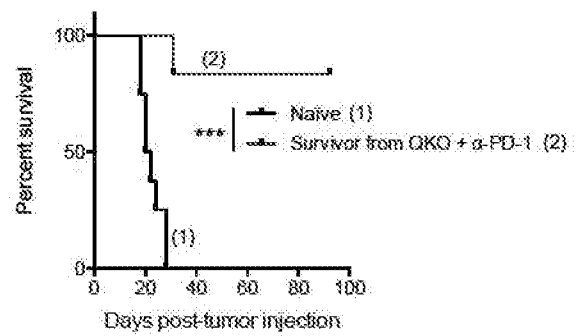


FIG. 22B

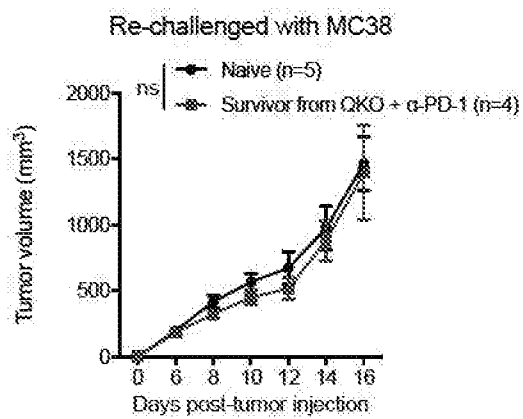


FIG. 22C

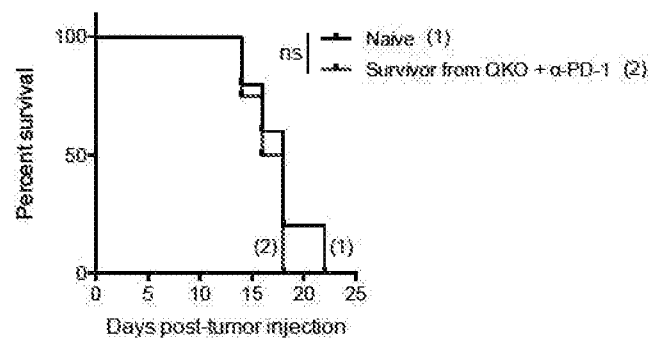


FIG. 22D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/12530

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/395; A61P 35/04, 37/04; C07K 16/28; A61K 31/505, 31/506, 39/39 (2020.01)

CPC - A61K 39/395; A61P 35/04, 37/04; C07K 16/28; A61K 31/505, 31/506, 39/39, 39/3955, 45/06; G01N 39/001134, 33/50; C07D 213/81, 239/14, 263/57, 401/04, 413/06, 413/14; A61P 35/02; C07K 14/47, 16/2827; C12N 15/11, 15/113

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0289315 A1 (XOMA TECHNOLOGY LTD.) 06 October 2016; paragraphs [0016]-[0018], [0033], [0046], [0053], [0062], [0244]	1-4, 5/3-4, 57-58
Y	WO 2017/114497 A1 (NOVARTIS AG) 06 July 2017; page 1, lines 25-26; page 3, line 5; page 4, line 23	1-4, 5/3-4, 57-58
A	US 2018/0256711 A1 (GENENTECH, INC.) 13 September 2018; entire document	1-4, 5/3-4, 57-58
A	WO 2017/190009 A1 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM) 02 November 2017; entire document	1-4, 5/3-4, 57-58
A	(WANG, Y et al.) LSD1 Is a Subunit of the NuRD Complex and Targets the Metastasis Programs in Breast Cancer. Cell. 21 August 2009, Vol. 138, pages 660-672; DOI 10.1016/j.cell.2009.05.050	1-4, 5/3-4, 57-58
E, X	WO 2020/014332 A1 (NIKANG THERAPEUTICS, INC.) 16 January 2020; entire document	1-4, 5/3-4, 57-58

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

08 May 2020 (08.05.2020)

Date of mailing of the international search report

05 JUN 2020

Name and mailing address of the ISA/US

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Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US20/12530

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-56
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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