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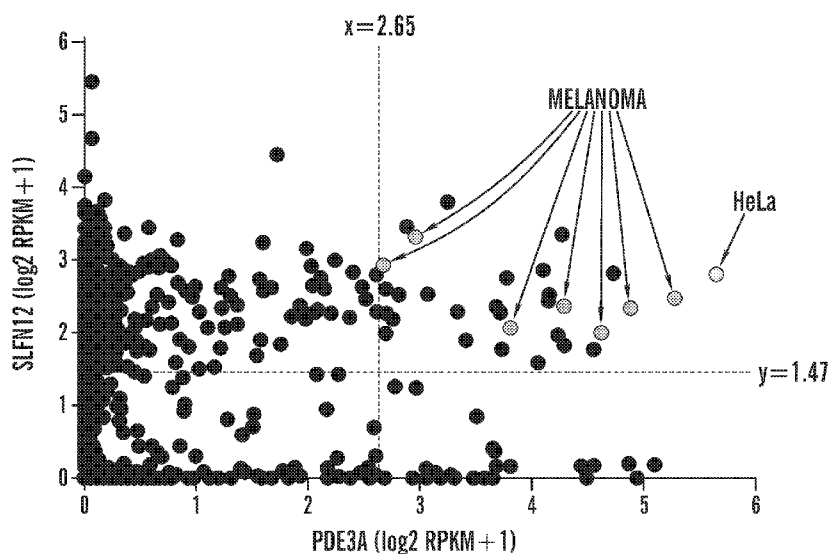


FIG. 1

(57) **Abstract:** The present disclosure features methods for identifying patients having a hyperproliferative disease, disorder, or condition responsive to phosphodiesterase 3 (PDE3) and schlafen family member 12 (SLFN12) complex formation. The hyperproliferative disease, disorder, or condition may be cancer in a patient including glioblastoma, melanoma, ovarian cancer, cervical cancer, sarcoma, or hematopoietic cancers, such as acute myeloid leukemia. Those responsive diseases, disorders, or conditions may be identified using the biomarker AIP and/or TRRAP in combination with those biomarkers pertinent to phosphodiesterase 3 and schlafen family member 12 complexes which may be formed by PDE3 modulation with certain active compounds. Expression of combinations of these biomarkers have been shown to correlate with active compound (e.g., PDE3 modulator, PDE3A modulator, PDE3B modulator) sensitivity.

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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IDENTIFICATION OF PDE3 MODULATOR RESPONSIVE CANCERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority under 35 U.S.C. § 119 to U.S. App. No. 62/901,090, filed on September 16, 2019 and U.S. App. No. 62/754,290, filed November 1, 2018, each of which is hereby incorporated by reference in its entirety.

FIELD OF DISCLOSURE

[0002] The present disclosure relates to the identification of cells (*e.g.*, cancer cells) responsive to complex formation between certain phosphodiesterase 3 proteins (*e.g.*, PDE3A, PDE3B) and schlafen family member 12 (SLFN12) proteins by identifying cells that express certain biomarkers implicated in complex formation. Specifically, cells identified as expressing the aryl hydrocarbon receptor interacting protein (AIP) and/or transformation/transcription domain associated protein (TRRAP) are implicated in the complex formation which results in apoptosis. Methods of treatment or prevention of hyperproliferative diseases, disorders, or conditions associated with these cells are also provided comprising administration of certain chemical agents (*e.g.*, PDE3A modulators) to those cells identified as responsive to complex formation.

BACKGROUND

[0003] Cancer kills over 550,000 people in the United States and over 8 million people world-wide each year. New agents, including small molecules, molecules that impact tissue-specific growth requirements, and immunomodulatory agents, have been shown to benefit a subset of patients whose cancers have unique genomic mutations or other characteristics. Unfortunately, many cancer patients are still left without effective therapeutic options.

[0004] One approach to identify new anti-cancer agents is phenotypic screening to discover novel small molecules displaying strong selectivity between cancer cell lines, followed by predictive chemogenomics to identify the cell features associated with drug response. In the 1990s, Weinstein and colleagues demonstrated that the cytotoxic profile of a compound can be used to identify cellular characteristics, such as gene-expression profiles and DNA copy number,

that correlate with drug sensitivity. The ability to identify the features of cancer cell lines that mediate their response to small molecules has strongly increased in recent years with automated high-throughput chemosensitivity testing of large panels of cell lines coupled with comprehensive genomic and phenotypic characterization of the cell lines. Phenotypic observations of small-molecule sensitivity can be linked to expression patterns or somatic alterations.

[0005] Despite advances in targeted therapies and immunotherapies, certain cancers, such as metastatic melanoma, remain deadly diseases. For example, metastatic melanoma has a 5-year survival rate of only 20%. New therapeutic modalities are therefore needed. These new modalities may be based on new mechanisms of cancer cell killing. For example, some phosphodiesterase 3A (PDE3A) modulators may cause complex formation between PDE3A peptide and schlafen family member 12 (SLFN12) or similar complex formation between PDE3B peptide and schlafen family member 12 (SLFN12) in cancer cells. This complex formation may result in induction of apoptosis. However, inhibition of PDE3 enzymatic activity alone is insufficient to cause cancer cell killing, as neither PDE3 knockout nor treatment with most previously-characterized PDE3A inhibitors kills cancer cells. Thus, in contrast to traditional targeted therapies that leverage cancer cell dependencies created by genomic alteration, these PDE3 modulation therapies cause induced apoptosis via PDE3A-SLFN12 or PDE3B-SLFN12 (“PDE3A/B-SLFN12”) complex formation. Moreover, such apoptotic induction does not occur in all cancer cells expressing PDE3A and SLFN12 indicating an incomplete understanding of the mechanistic underpinnings of this cell death.

[0006] Methods of characterizing malignancies at a molecular level are useful for stratifying patients, thereby quickly directing them to effective therapies. Improved methods for predicting the responsiveness of subjects having cancer are urgently required.

SUMMARY

[0007] In accordance with the foregoing objectives and others, the present disclosure provides methods of identifying cells of a hyperproliferative disease, disorder, or condition, such as cancer cells responsive to PDE3A-SLFN12 complex formation or PDE3B-SLFN12 complex formation, methods for the treatment or prophylaxis of hyperproliferative diseases, disorders, or conditions

identified as being responsive to complex formation, and kits for the determination and treatment of hyperproliferative diseases, disorders, or conditions identified as being responsive to complex formation. Without wishing to be bound by theory, it is believed that cells lacking the aryl hydrocarbon receptor interacting protein (AIP) and/or the transformation/transcription domain associated protein (TRRAP) have decreased or no sensitivity to complex formation or have decreased or no complex formation following contact of the cell with an active compound that typically induces such formation. Certain PDE3 modulatory compounds (*e.g.*, PDE3A modulators, PDE3B modulators, DNMDP, compounds disclosed in WO2019/025562, which is hereby incorporated by reference in its entirety and particularly in relation to compounds of general formula (1), may be able to induce complex formation between PDE3A and SLFN12 or PDE3B and SLFN12 in cancer cells when those cells express AIP and/or TRRAP, which may result apoptosis of the cancer cells.

[0008] Apoptosis may be induced in cells expressing the AIP polypeptide or polynucleotide particularly in relation to cells expressing schlafen family 12 (SLFN12) and phosphodiesterase 3A (PDE3A) or cells expressing schlafen family member 12 (SLFN12) and phosphodiesterase 3B (PDE3B) since AIP is implicated in PDE3A-SLFN12 or PDE3B-SLFN12 complex formation. It has also been found that apoptosis may be induced in cells expressing transformation/transcription domain associated protein (TRRAP) polypeptide or polynucleotide in relation to cells expressing schlafen family member 12 (SLFN12) and phosphodiesterase 3A (PDE3A) or cells expressing schlafen family member 12 (SLFN12) and phosphodiesterase 3B (PDE3B) since TRRAP is implicated in formation of or response to complex formation as well.

[0009] Methods of identifying a subject having a hyperproliferative disease, disorder, or condition, such as a cancer responsive to PDE3A-SLFN12 complex formation or PDE3B-SLFN12 complex formation, are provided comprising detecting:

(i) the expression of aryl hydrocarbon receptor interacting protein (AIP) polypeptides or polynucleotides and/or transformation/transcription domain associated protein (TRRAP) polypeptides or polynucleotides;

(ii) the expression of phosphodiesterase 3A (PDE3A) polypeptides or polynucleotides or the expression of phosphodiesterase 3B (PDE3B) polypeptides or polynucleotides in the cells relative to a reference, and

(iii) the expression of schlafen family member 12 (SLFN12) polypeptides or polynucleotides in the cells relative to a reference;

wherein the hyperproliferative disease, disorder, or condition is characterized as responsive to the complex formation complex formation if:

(i) AIP and/or TRRAP are expressed,

(ii) the expression of PDE3A and/or PDE3B is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference.

In certain implementations, the method may comprise obtaining one or more cells (*e.g.*, cancer cells) of the hyperproliferative disease, disorder, or condition from the subject and detecting:

(i) the expression of aryl hydrocarbon receptor interacting protein (AIP) polypeptides or polynucleotides and/or transformation/transcription domain associated protein (TRRAP) polypeptides or polynucleotides;

(ii) the expression of phosphodiesterase 3A (PDE3A) polypeptides or polynucleotides or the expression of phosphodiesterase 3B (PDE3B) polypeptides or polynucleotides in the cells relative to a reference, and

(iii) the expression of schlafen family member 12 (SLFN12) polypeptides or polynucleotides in the cells relative to a reference;

wherein the hyperproliferative disease, disorder, or condition is characterized as responsive to the complex formation complex formation if:

(i) AIP and/or TRRAP are expressed,

(ii) the expression of PDE3A and/or PDE3B is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference.

In some embodiments, the hyperproliferative disease, disorder, or condition is characterized as responsive to complex formation if both AIP and TRRAP are expressed in the cells. In certain implementations, expression of AIP and/or TRRAP may be determined by comparison of expression to the reference and the hyperproliferative disease, disorder, or condition is characterized as responsive to complex formation if:

(i) there is no loss of AIP and/or TRRAP expression relative to the reference (*e.g.*, the expression levels of AIP and/or TRRAP in the cell is more than 50% of the expression level in the reference or more than 90% of the expression levels in the reference or more than 100% of the expression levels in the reference),

(ii) the expression of PDE3A is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference. In some embodiments, the method of identifying a subject having a hyperproliferative disease, disorder, or condition, such as cancer responsive to PDE3A-SLFN12 complex formation, may comprise obtaining one or more cells (*e.g.*, cancer cells) of the hyperproliferative disease, disorder, or condition from the subject and detecting:

(i) the expression of aryl hydrocarbon receptor interacting protein (AIP) polypeptides or polynucleotides and/or transformation/transcription domain associated protein (TRRAP) polypeptides or polynucleotides,

(ii) the expression of phosphodiesterase 3A (PDE3A) polypeptides or polynucleotides relative to a reference, and

(iii) the expression of schlafen family member 12 (SLFN12) polypeptides or polynucleotides relative to a reference;

wherein the hyperproliferative disease, disorder, or condition is characterized as responsive to said complex formation if:

(i) AIP and/or TRRAP are expressed,

(ii) the expression of PDE3A is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference. In some embodiments, the hyperproliferative disease, disorder, or condition is characterized as responsive to complex formation if both AIP and TRRAP are expressed.

[0010] In various implementations, the method of identifying a subject having a hyperproliferative disease, disorder, or condition, such as cancer responsive to PDE3B-SLFN12 complex formation, may comprise obtaining one or more cells (*e.g.*, cancer cells) of the hyperproliferative disease, disorder, or condition from the subject and detecting:

(i) the expression of aryl hydrocarbon receptor interacting protein (AIP) polypeptides or polynucleotides and/or transformation/transcription domain associated protein (TRRAP) polypeptides or polynucleotides,

(ii) the expression of phosphodiesterase 3B (PDE3B) polypeptides or polynucleotides relative to a reference, and

(iii) the expression of schlafen family member 12 (SLFN12) polypeptides or polynucleotides relative to a reference;

wherein the hyperproliferative disease, disorder, or condition is characterized as responsive to said complex formation complex formation if:

(i) AIP and/or TRRAP are expressed,

(ii) the expression of PDE3B is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference. In some embodiments, the hyperproliferative disease, disorder, or condition is characterized as responsive to chemically induced complex formation if both AIP and TRRAP are expressed.

[0011] The cells of the subject may be collected from a tissue sample, a blood sample, or a plasma sample.

[0012] Methods of killing or reducing the survival of a cell (*e.g.*, cancer cell) are also provided wherein the cancer cell is selected as responsive to PDE3A/B-SLFN12 complex formation comprising contacting the cancer cell with a PDE3 modulator (*e.g.*, a PDE3A modulator, a PDE3B modulator), wherein the cell is selected as responsive to the PDE3A/B-SLFN12 complex

formation when the cell expresses AIP and/or TRRAP polypeptides or polynucleotides, has increased expression of SLFN12 polypeptides or polynucleotides relative to a reference, and has increased expression of PDE3A or PDE3B relative to the reference. Typically, the PDE3 modulator (*e.g.*, PDE3A modulator, PDE3B modulator) may be able to induce PDE3A/B-SLFN12 complex formation leading to apoptosis of the cell following contact. In some embodiments, the cancer cell is selected as responsive to PDE3A-SLFN12 complex formation if the cell has increased expression of PDE3A and increased expression of SLFN12 relative to a reference. In some embodiments, the cell is selected as responsive to PDE3B-SLFN12 complex formation if the cell has increased expression of PDE3B and SLFN12 relative to a reference. Many PDE3A modulators also directly bind PDE3B proteins and a PDE3A modulator may be used to induce complexation between SLFN12 and PDE3B.

[0013] In some embodiments, methods for the treatment or prevention of hyperproliferative disease, disorder, or condition (*e.g.* cancer) in a subject are provided comprising administering to the subject a PDE3 modulator (*e.g.*, PDE3A modulators, PDE3B modulators), wherein the subject is identified as having a hyperproliferative disease, disorder, or condition that is responsive to the PDE3 modulator by obtaining one or more cells of the hyperproliferative disease, disorder, or condition (*e.g.* cancer) from the subject (*e.g.*, by obtaining a sample from the subject) and detecting:

(i) the expression of aryl hydrocarbon receptor interacting protein (AIP) polypeptides or polynucleotides and/or transformation/transcription domain associated protein (TRRAP) polypeptides or polynucleotides,

(ii) the expression of phosphodiesterase 3B (PDE3B) polypeptides or polynucleotides relative to a reference, and

(iii) the expression of Schlafen family member 12 (SLFN12) polypeptides or polynucleotides relative to a reference;

wherein the hyperproliferative disease, disorder, or condition is characterized as responsive to said complex formation complex formation if:

(i) AIP and/or TRRAP are expressed,

(ii) the expression of PDE3B is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference. In some embodiments, the hyperproliferative disease, disorder, or condition is characterized as responsive to the PDE3 modulator if both AIP and TRRAP are expressed. The PDE3A modulator may comprise, for example, 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP). In certain implementations, expression of AIP and/or TRRAP may be determined by comparison of expression to the reference and the hyperproliferative disease, disorder, or condition is characterized as responsive to complex formation if:

(i) there is no loss of AIP and/or TRRAP expression relative to the reference (*e.g.*, the expression levels of AIP and/or TRRAP in the cell is more than 50% of the expression levels in the reference or more than 90% of the expression levels in the reference or more than 100% of the expression levels in the reference),

(ii) the expression of PDE3A is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference.

[0014] The expression of any biomarker (*e.g.*, AIP, TRRAP, PDE3A, PDE3B, SLFN12) may be detected by a method selected from the group consisting of immunoblotting, mass spectrometry, immunoprecipitation quantitative PCR, Northern Blot, microarray, enzyme-linked immunosorbent assay (ELISA), in situ hybridization, and combinations thereof. In certain implementations, expression of AIP and/or TRRAP may be determined by comparison to the reference. The cancer cell may be considered to express AIP and/or TRRAP if there is no loss in expression as compared to the reference. In certain implementations, expression of AIP and/or TRRAP may be determined by comparison to the reference and the cell is considered to express AIP and/or TRRAP if there is a small difference (*e.g.*, the cancer cell copy number is within 10% of the copy number of the reference, the cancer cell copy number is within 5% of the reference) between expression in the cancer cell and the reference. Genomics may be used to determine expression and relative expression levels. For example, the cell may be considered to not express AIP and/or TRRAP if the number of copies of the biomarker per cellular genome is less than 1 or less than 2^{-1} or less than 2^{-2} or less than 2^{-3} or less than 2^{-4} or less than 2^{-5} . Conversely, the cell may be considered to

express AIP and/or TRRAP if the number of copies of the biomarker per cellular genome is greater than 1 or greater than 2^{-1} or greater than 2^{-2} or greater than 2^{-3} or greater than 2^{-4} or greater than 2^{-5} .

[0015] Such methods allow for the treatment and/or prevention of hyperproliferative disease, disorders, or conditions caused by the proliferation of cells responsive to complex formation and, in particular, complex formation induced by PDE3 modulation. In some embodiments, the cell is a cancer cell. For example, the hyperproliferative disease, disorder, or condition may be selected from bladder, brain, breast, cervical, colorectal, endometrial, esophageal, gallbladder, gastric, glioblastoma, kidney, leukemia (*e.g.*, acute myelogenous leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia), liver (*e.g.*, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, angiosarcoma, hemangiosarcoma, hepatoblastoma), lung (*e.g.*, non-small cell lung cancer, small cell lung cancer, mesothelioma), melanoma, ovarian, pancreatic, prostate, multiple myeloma, sarcoma (*e.g.*, osteosarcoma, soft-tissue sarcoma), thyroid, urinary tract, or uterine cancer. In certain implementations the cancer may be a hematopoietic cancer, such as acute lymphoblastic leukemia, acute myelogenous leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute monocytic leukemia, Hodgkin's lymphoma, or non-Hodgkin's lymphoma.

[0016] Various routes of administration are useful for treatment modalities. In some embodiments, the PDE3 modulator (*e.g.*, PDE3A modulator, PDE3B modulator) is administered orally. In other embodiments, the PDE3 modulator is administered by intravenous injection.

[0017] Kits for identifying a subject having cancer as responsive to complex formation including chemically induced complex formation (*e.g.*, cells responsive to PDE3 modulators, cells responsive to PDE3A modulators, cells responsive to PDE3B modulators) are also provided, wherein the kit comprises a first capture reagent that binds AIP polypeptide and/or a second capture reagent that binds TRRAP polypeptide. In some embodiments, the kit comprises a third capture reagent that binds PDE3A polypeptide and/or a fourth capture reagent that binds SLFN12 polypeptide and/or a fifth capture reagent that binds PDE3B. In some embodiments, the kit further comprises a PDE3 modulator (*e.g.*, PDE3A modulator, PDE3B modulator), such as DNMDP or a compound of WO2019/025562. It will be understood that the numeric identifiers for the capture

reagents (*e.g.*, first, second, third, fourth, fifth) do not indicate the total quantity of capture reagents in each kit. The PDE3 modulator may be present in a pharmaceutical formulation sufficient to deliver a therapeutically effective amount to a subject in need thereof.

[0018] In one embodiment, a cancer expressing AIP, which is required for SLFN12/PDE3A complex formation, is identified as responsive to treatment with compound X ((6S)-5-[4'-fluoro-2-(trifluoromethyl)biphenyl-4-yl]-6-methyl-3,6-dihydro-2H-1,3,4-oxadiazin-2-one).

BRIEF DESCRIPTION OF FIGURES

[0019] FIG. 1 illustrates the reads per kilobase of transcript per million mapped reads (RPKM) for the 49 cell lines measured for PDE3A sensitivity and identifies the biomarker positive cancer cell lines in addition to the HeLa cells. A high proportion of biomarker-positive cell lines are from melanoma patients.

[0020] FIG. 2A shows DNMDP 72 h Cell Titer-Glo assay dose response curves for HeLa cells and the 7 melanoma cell lines showing DNMDP sensitivity. As can be seen, C32 and RVH421 are only partially sensitive. FIG. 2B shows DNMDP 72 h Cell Titer-Glo assay dose response curves for several cell lines sensitive to DNMDP treatment. FIG. 2C shows DNMDP 72 h Cell Titer-Glo assay dose response curves for several cell lines partially sensitive or insensitive to DNMDP treatment.

[0021] FIG. 3A shows the survival of cells as compared to cells with CRISPR KO of SLFN12 following at various DNMDP concentrations. A2058 and SKMEL3 are representative melanoma cell lines. Survival is measured with a 72 h Cell Titer-Glo assay and CRISPR was performed with sg4, SLFN12 CRISPR guide RNA #4. FIG. 3B shows the survival of HeLa cells as compared to PDE3A knockout cells (with and without ectopic PDE3B expression).

[0022] FIG. 4 identifies those CRISPR gene targets resulting in decreased sensitivity for DNMDP cancer cell killing in HeLa cells. AIP, SLFN12, and PDE3A knockout cause the greatest increase in cell survival in the presence of 25 nM DNMDP. The results are plotted as log fold change (LFC) of gene CRISPR guide RNA representation among all genes compared to -log p-values, indicating the likelihood of significance. TRRAP also exhibited a significant increase in cell survival in the presence of 25 nM DNMDP.

[0023] FIG. 5 compares the gene copy number and expression of AIP in the measured cell lines and identifies the UACC257 cell line as lacking AIP expression. As shown in FIG. 2A, UACC257 does not have sensitivity to DNMDP.

[0024] FIG. 6A illustrates the results of the 72-hour Cell Titer-Glo assay with independent AIP CRISPR gRNAs (sg) in HeLa cells. FIG. 6B shows the results of the 72-hour Cell Titer-Glo assay with independent AIP CRISPR gRNAs (sg) in A2058 melanoma cells. FIG. 6C is an immunoblot revealing that AIP knockout decreases PDE3A protein levels in DNMDP-sensitive cell lines.

[0025] FIG. 7 is an immunoblot illustrating that AIP knockout prevents DNMDP induced complex formation. PDE3A immunoprecipitates from HeLa cells transiently transfected with V5-tagged SLFN12 and treated with 10 μ M DNMDP.

[0026] FIG. 8A is a graph and FIG. 8B is an image of an immunoblot. The two figures show that that AIP is necessary for Compound X induced cancer cell killing and induction of the PDE3A-SLFN12 complex. FIG. 8A shows that cervical cancer cell viability is reduced at increasing concentration of Compound X. This effect is not observed when AIP is knocked out using CRISPR (KOsg2, KOsg3). FIG. 8B is an image of an immunoblot. An anti-PDE3A antibody was used to pull down a PDE3A-SLFN12 complex. Complex formation between PDE3A and FLAG-tagged SLFN12 was induced in the presence of Compound X and in the presence of DNMDP. Complex formation was not observed when AIP was knocked out using Crispr (KO sg2, KO sg3). HeLa cells were treated with 10 μ M DNMDP or 10 μ M Compound X.

DETAILED DESCRIPTION

[0027] Definitions

[0028] Detailed embodiments of the present disclosure are disclosed herein; however, it is to be understood that the disclosed embodiments are merely illustrative of the disclosure that may be embodied in various forms. In addition, each of the examples given in connection with the various embodiments of the disclosure is intended to be illustrative, and not restrictive.

[0029] All terms used herein are intended to have their ordinary meaning in the art unless otherwise provided. All concentrations are in terms of percentage by weight of the specified component relative to the entire weight of the topical composition, unless otherwise defined.

[0030] As used herein, “a” or “an” shall mean one or more. As used herein when used in conjunction with the word “comprising,” the words “a” or “an” mean one or more than one. As used herein “another” means at least a second or more.

[0031] As used herein, all ranges of numeric values include the endpoints and all possible values disclosed between the disclosed values. The exact values of all half integral numeric values are also contemplated as specifically disclosed and as limits for all subsets of the disclosed range. For example, a range of from 0.1% to 3% specifically discloses a percentage of 0.1%, 1%, 1.5%, 2.0%, 2.5%, and 3%. Additionally, a range of 0.1 to 3% includes subsets of the original range including from 0.5% to 2.5%, from 1% to 3%, and from 0.1% to 2.5%. It will be understood that the sum of all weight % of individual components will not exceed 100%.

[0032] By “PDE3A polynucleotide” is meant any nucleic acid molecule encoding a PDE3A polypeptide or fragment thereof. An exemplary PDE3A nucleic acid sequence is provided at NCBI Ref: NM_000921.4:

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1  gggggccact  gggaattcag  tgaagagggc  accctatacc  atggcagtg  cggcgacgc
61  tgcacgagtc  agggacaagc  ccgtccacag  tggggtgagt  caagccccc  cggcgggccc
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1681 tccagaatct gctgacacaa ctgccaaaca aagcctaggt tctcacaggg ccttaactta
1741 cactcagagt gccccagacc tatccctca aatcctgact ccacctgtta tatgtagcag
1801 ctgtggcaga ccatattccc aagggaatcc tgctgatgag cccttgagga gaagtggggt
1861 agccactcgg acaccaagta gaacagatga cactgctcaa gttacctctg attatgaaac
1921 caataacaac agtgacagca gtgacattgt acagaatgaa gatgaaacag agtgcctgag
1981 agagcctctg aggaaagcat cggcttgtag cacctatgct cctgagacca tgatgtttct
2041 ggacaaacca attcttgctc ccgaacctct tgtcatggat aacctggact caattatgga
2101 gcagctaaat acttggaatt ttccaatttt tgatttagtg gaaaatatag gaagaaaatg
2161 tggccgtatt cttagtcagg tatcttacag actttttgaa gacatgggcc tctttgaagc
2221 ttttaaaatt ccaattaggg aatttatgaa ttattttcat gctttggaga ttggatatag
2281 ggatatcctt tatcataaca gaatccatgc cactgatgtt ttacatgctg ttgggtatct
2341 tactacacag cctattccag gcctctcaac tgtgattaat gatcatgggt caaccagtga
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2461 taatgtgaca gatgataaat acggatgtct gtctgggaat atccctgcct tggagttgat
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2581 cctggttgca actagtgtct ctcaggcggt gctatataac gatcgttcag ttttgagaaa
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2701 taaccttgac catgtggaat ttaagcattt ccgtttcctt gtcattgaag caattttggc
2761 cactgacctg aagaaacact ttgacttcgt agccaaattt aatggcaagg taaatgatga
2821 tgttggaata gattggacca atgaaaatga tcgtctactg gtttgtcaaa tgtgtataaa
2881 gttggctgat atcaatggtc cagctaaatg taaagaactc catcttcagt ggacagatgg
2941 tattgtcaat gaattttatg aacagggtga tgaagaggcc agccttggtat taccataag
3001 ccccttcatg gatcgttctg ctccctcagct ggccaacctt caggaatcct tcatctctca
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3121 ggaagacagc gatgagtcag gagatactga tgaccagaaa gaagaggagg aagaagcacc
3181 agcaccaaat gaagaggaaa cctgtgaaaa taatgaatct ccaaaaaaga agactttcaa
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3301 gaaagtcatt gaagaggagc aacggttggc aggcatagaa aatcaatccc tggaccagac
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3421 gaaaccaaga ggcgaggaga tacciaacca aaagccagac cagtgaacaat ggaatagaatg
3481 ggcgtgtttt ccaaacagat tgacttgtca aagactctct tcaagccagc acaacattta
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3721 gtatataagc tcccacatag atacatgtaa aacatattca caccatgca cgcacacaca
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4501 cacctaagag ctgaagtcag gtcttttaat cagggttagaa ttctaaatga tgccagagaa
4561 ggcttgggaa attgtacttc agcgtgatag cctgtgtctt cttaatttgc tgcaaaatat

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4621 gtggtagaga aagaaaagga aacagaaaaa tcaactctggg ttatatagca agagatgaag
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4741 gttgtagttt gtctttcagg ggtgaaggtc ccactgacaa cccctgttgt ggtgttccac
4801 acgctgtttg ttggggtagc ttccatcggc agtctggccc attgtcagtc atgcttcttc
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5581 gatattgtac tccccctttt caaagcatta ttttacaata attcatggga ttttaaaaaa
5641 taaggcaaag ataatacgac aaaaaatata catggtttca aggcaaattc tccaataagt
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5761 cagtatatatt ctgaattaac accaggtctt cattatttag aacttactaa attgttttca
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5941 cttctcatat cccttgcatc taagtactta gcaaagtcaa tattttccca ttttccaaat
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6301 ataaattgct attacagcta gacgtcaatt gggataaata aagggatgaa gatccactaa
6361 gtttgtgact ttcatacaca ccagtagcat ctcaaaggat gctaagggac attttctgcc
6421 agtagagttc tccccctttt tggtagacagc aatattatta tgttcacatc taactccaga
6481 gcttacttcc tgtggtgcca atgtatttgt tgcaatttac tacattttta tatgagccta
6541 tttataggtg ccattaaact caggtctttc aaatgaaaga gtttctagcc cacttaggga
6601 aaaagataat tgttttagaaa accataaaat caatggtagg aaaagttgga actggttacc
6661 tggtgccat ggttctctgt taaaataaagt aagagaccag gtgtattctg agtgtcatca
6721 gtgttatttt cagcatgcta ataaatgtct ttccggttat atatctatct aaattaacct
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6901 cagggatata tctatattag tcttcatctg atgaatgaag aaattttctc atattatggt
6961 caagaaagta tttacttcct aaaaatagaa ttcccgattc tgtctatttt ggttgaatac
7021 cagaacaaat ctttccgttg caatcccagt aaaacgaaag aaaaggaata tcttacagac
7081 tgttcatatt agatgtatgt agactgttaa tttgcaattt ccccatattt cctgcctatc
7141 ttaccagat aactttcttt gaaggtaaaa gctgtgcaaa aggcattgaga ctcaggccta
7201 ctctttgttt aaatgatgga aaaaatataa ttattttcta agtaataaaa gtataaaaat
7261 tatcattata aataaagtct aaagtttgaa attattaatt taaaaaaaaa aaaaaaaaaa

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(SEQ ID NO:1)

[0033] By “PDE3A polypeptide” is meant a protein or fragment thereof having at least 85% amino acid sequence identity to the sequence provided at NCBI Ref No. NP_000912.3 that

catalyzes the hydrolysis of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). An exemplary human full-length PDE3A amino acid sequence is:

MAVPGDAARVRDKPVHSGVVSQAPTAGRDCCHRADPASPRDSGCRGCWGDVLQPLRSSRKLSS
ALCAGSLSFLLALLVRLVRGEVGCLEQCKEAAAAEEEEEAAPGAEGGVFPGPRGGAPGGGARLS
PWLQPSALLFSLLCAFFWMGLYLLRAGVRLPLAVALLAACCGGEALVQIGLVGEDHLLSLPAA
GVVLSCLAAATWLVLRLRLGVLMIALTSAVRTVSLISLERFKVAWRPYLAYLAGVLGILLARYV
EQILPQSAEAAAPREHLGSQLIAGTKEDIPVFKRRRRSSSVVSAEMSGCSSKSHRRTSLPCIPREQLM
GHSEWDHKRGPRGSQSSGTSITVDIAVMGEAHGLITDLLADPSLPPNVCTSLRAVSNLLSTQLTF
QAIHKPRVNPVTSLSENYTCSDSEESSEKDKLAIPKRLRRSLPPGLLRVSSTWTTTTSATGLPTLE
PAPVRRDRSTSIKLQEAPSSSPDSWNNPVMMLTKSRSFTSSYAISAANHVKAKKQSRPGALAKI
SPLSSPCSSPLQGTPASSLVSKISAVQFPESADTTAKQSLGSHRALTYTQSAPDLSPQILTPPVICSS
CGRPYSQGNPADEPLERSGVATRTPSRTDDTAQVTSDYETNNNSDSSDIVQNEDETECLREPLRK
ASACSTYAPETMMFLDKPILAPEPLVMDNLDSEIMEQLNTWNFPFDLVENIGRKCGRILS
QVSYRLFEDMGLFEAFKIPIREFMNYFHALEIGYRDIPYHNRIHATDVLHAVWYLTTPQPI
PGLSTVINDHGSTSDSDSDSGFTHGHMGYVFSKTYNVTDDKYGCLSGNIPALELMALYV
AAAMHDYDHPGRITNAFLVATSAPQAVLYNDRSVLENHHAAAAWNLFMSRPEYNFLIN
LDHVEFKHFRFLVIEAILATDLKKHFDVAKFNGKVNDVGDWNTNENDRLLVCQMCIK
LADINGPAKCKELHLQWTDGIVNEFYEQGDEEASLGLPISPFMDRSAPQLANLQESFISHI
VGPLCNSYDSAGLMPGKWVEDSDESGDTDDPEEEEEEEAPAPNEEETCENNESPKKKTFK
RRKIYCQITQHLLQNHKMWKKVIEEEQRLAGIENQSLDQTPQSHSSEIQAIKEEEEEKKG
KPRGEEIPTQKPDQ

(SEQ ID NO:2)

[0034] Several PDE3A isoforms are known including PDE3A1, PDE3A2, and PDE3A3. PDE3A1 comprises amino acids 146-1141, PDE3A2 isoform 2 comprises amino acids 299-1141, and PDE3A3 comprises amino acids 483-1141 of the full-length PDE3A amino acid sequence. Additionally, spliced transcript variants encoding multiple isoforms have been observed for PDE3A. One such transcript variant has NCBI Ref No. NM_001244683 which has an associated protein sequence (NP_001231612.1):

MVTIFSKSWSFYWEKSSGTSITVDIAVMGEAHGLITDLLADPSLPPNVCTSLRAVSNLLS
 TQLTFQAIHKPRVNPVTSLSENYTCSDSEESSEKDKLAIPKRLRRSLPPGLLRVSSTWTT
 TTSATGLPTLEPAPVRRDRSTSIKLQEAPSSSPDSWNNPVMMTLTKSRSFTSSYAISAANH
 VKAKKQSRPGALAKISPLSSPCSSPLQGTPASSLVSKISAVQFPESADTTAKQSLGSHRAL
 TYTQSAPDLSPQILTPPVICSSCGRPYSQGNPADEPLERSGVATRTPSRTDQTAQVTSDYE
 TNNNSDSSDIVQNEDETECLREPLRKASACSTYAPETMMFLDKPILAPEPLVMDNLD SIM
 EQLNTWNFPIDLVENIGRKCGRILSQVSYRLFEDMGLFEAFKIPIREFMNYFHALEIGYR
 DIPYHNRIHATDVLHAVWYLTQPIPGSLTVINDHGSTS DSDSDSGFTHGHMGYVFSKT
 YNVTDDKYGCLSGNIPALELMALYVAAAMHDYDHPGRTN AFLVATSAPQAVLYNDRS
 VLENHHA AAAWNLFMSRPEYNFLINLDHVEFKHFRFLVIEAILATDLKKHFDFVAKFNG
 KVNDDVGIDWTNENDRLLVCQMCIKLADINGPAKCKELHLQWTDGIVNEFYEQGD EEA
 SLGLPISPFMDRSAPQLANLQESFISHIVGPLCNSYDSAGLMPGKWVEDSDESGDTDDPE
 EEEEEAPAPNEEETCENNESPKKKTFRRKIYCQITQHLLQNHKMWKKVIEEEQRLAGIE
 NQSLDQTPQSHSSEIQAIKEEEEEK GKPRGEEIPTQKPDQ

(SEQ ID NO:3). In some embodiments, the expression of isoforms of PDE3A in the cell may be measured.

[0035] By “PDE3B polynucleotide” is meant any nucleic acid molecule encoding a PDE3B polypeptide or fragment thereof. An exemplary PDE3B nucleic acid sequence is provided at NCBI Ref: NM_000922.3:

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1  gctcgcgcgc ccaacggacc aggctggggc cgtgaggtaa ctggtgcagc cagcggaggt
61  gggaggcgac actgagtctc cagtcccag aggtgcccga gggaaaagga ggcggcagct
121 aaactggtcc tggagagaag ccccttcgc cctctcctc agccagcatg tcccggactc
181 cgccgctcct cagtccgcgc ggtggggacc ccgggccgtg gcggccggcg cagccctgac
241 gggttgcgaa ccagggggcg cccgaacgc gggggttggg gtctgggagc gcgagcggcc
301 gctacggtac gagcggggtg tgctgagtc cgtggccacc cccggcccca gccatgagga
361 gggacgagcg agacgcaaaa gccatgcggt ccctgcagcc gccggatggg gccggctcgc
421 cccccgagag tctgaggaac ggctacgtga agagctgctg gagcccttg cggcaggacc
481 ctccgcgcgg cttcttcttc cacctctgcc gcttctgcaa cgtggagctg cggccgccgc
541 cggcctctcc ccagcagccg cggcgtctgt ccccttctg ccgggcgcgc ctctcgctgg
601 gcgccctggc tgcctttgtc ctgcacctgc tgcggggcgc ggaaccgag agctgggctg
661 ccggggccgc ctggtctgcg acgtctgtga gcgtgtgttc gcacagcttg agccccctct
721 tcagcatcgc ctgtgccttc ttcttcctca cctgcttct caccggacc aagcggggac
781 ccggcccggg ccggagctgc ggctcctggt ggctgctggc gctgcccgc tgctgttacc
841 tgggggactt cttggtgtgg cagtgggtgt cttggccttg ggggatggc gacgcagggt
901 ccgcggcccc gcacacgccc ccggaggcgg cagcgggcag gttgctgctg gtgctgagct
961 gcgtagggct gctgctgacg ctgcgcaccc cgtgcggct ccggcactgc gttctgggtg
1021 tgctcctggc cagcttcgtc tgggtgggtc ccttcaccag cctcgggtgc ctgccctccg

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1081 ccctcaggcc gctgctctcc ggcctgggtgg ggggcgctgg ctgcctgctg gccctgggggt
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1201 aagaaaaagt gcctgtgatc cgaccccgga ggaggtccag ctgcgtgtcg ttaggagaaa
1261 ctgcagccag ttactatggc agttgcaaaa tattcaggag accgtcgttg ccttgatattt
1321 ccagagaaca gatgattctt tgggattggg acttaaaaca atgggtataag cctcattatc
1381 aaaattctgg aggtggaaat ggagttgatc tttcagtgtc aaatgaggct cgcaatatgg
1441 tgtcagatct tctgactgat ccaagccttc caccacaagt catttcctct ctacggagta
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1801 caaatctact gactatcccg aagcaaaggc catcttctgt atcactgact caccatgtag
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1921 cagtgtctac tggctctcta actaatcgat caccataga atttctgtat actgctgatt
2081 ttcttaataa gccaagcgtt atcttgcaga gatctctggg caatgcacct aatactccag
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2221 aggaaacaga gaagaaagac agcagaaaaa tatttcagga aggtgataag tggctaacag
2281 aagaggcaca gagtgaacag caaacaataa ttgaacagga agtatcactg gacctgattt
2341 tagtagaaga gtatgactca ttaatagaaa agatgagcaa ctggaatttt ccaatttttg
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5341 acacactatg tgtaaacacag tcccaccact tattactaat aaaattttga ctgataattt
5401 atatttgcac ttacaatata tatatcctgt ccttatattt ctctagagta cattttccat
5461 catgtttaag tgtatttctg ctattatttc ctctcctgca gaatacatatc aagtgtatgt
5521 gtataaagtc atacatgtac aagcatgcat attgagattg aatcacattt ccatactgtc
5581 tgttattttta ttgggtttta tattgggttt ctttagttta tgttgttttc tcaaaagcag
5641 catttttaaat tacgaatact ggacttattg gattttaatta taaatccaat tactactgga
5701 aactcatttt tacataatat agtccttaaa ttattttaacc cttgctaagt aattgacata
5761 tgtaacaata actagcctaa agaaacccaa aaaagtatct ctcccagact gaaacttaaa
5821 aattcgttaag tgtaagaaag aatgtgagaa tatattaaat gcacactgta ccattagatg
5881 aaatcttact tgagaaattg ccataagcca tattacagat cttactttgt tactgaatca
5941 gattaatttc ttgttataat aattttcatc ataaattttc tattttttaa gccgctggta
6001 ctagaaatat tcttttaatg ctatatctat gtacctactg acacattttt ctccataaaa
6061 gtacttttaa aaattacttc atgatttgaa a

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(SEQ ID NO:4)

[0036] By “PDE3B polypeptide” is meant a protein or fragment thereof having at least 85% amino acid sequence identity to the sequence provided at NCBI Ref No. NP_000913.2. An exemplary human full-length PDE3A amino acid sequence is:

```

MRRDERDAKAMRSLQPPDGAGSPPESLRNGYVKSCVSPLRQDPPRGFFHLCRFCNVEL
RPPASPQQPRRCSPFCRARLSLGALAAFVLALLLGAEPESWAAGAAWLRTLLSVCSHS
LSPLFSIACAFFFLTCLFTRTKRGPGPGRSCGSWLLALPACCYLGDFLVWQWWSWPW
GDGDAGSAAPHTPPEAAAGRLLLVLSCVGLLLTLAHPLRLRHCVLVLLASFVWWVSF
TSLGSLPSALRPLLSGLVGGAGCLLALGLDHFFQIREAPLHPRLSSAAEEKVPVIRPRRS
SCVSLGETAASYYGSKIFRRPSLPCISREQMILWDWDLKQWYKPHYQNSGGGNGVDL
SVLNEARNMVSDLLTDPSLPPQVISSLRSISLMGAFSGSCRPKINPLTPFPGFYPCSEIEDP
AEKGDRKLNKGLNRNSLPTPQLRRSSGTSGLLPVEQSSRWDRNNGKRPHQEFGISSQGC
YLNGPFNSNLLTIPKQRSSSVSLTHHVGLRRAGVLSSLSPVNSSNHGPVSTGSLTNRSPIE

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FPDTADFLNKPSVILQRS LGNAPNTPDFYQQLRNSDSNLCNSCGHQMLKYVSTSESDGT
 DCCSGKSGEEENIFSKESFKLMETQQEEETEEKDSRKLFQEGDKWLTEEAQSEQQTNIEQ
 EVSLDLILVEEYDSLIEKMSNWNFPIFELVEKMGEKSGRILSQVMYTLFQDTGLLEIFKIP
 TQQFMNYFRALENGYRDIPYHNRIHATDVLHAVWYLTTRPVPGLQQIHNGCGTGNETD
 SDGRINHGRIAYISSKSCSNPDESYGCLSSNIPALELMALYVAAAMHDYDHPGRTNAFLV
 ATNAPQAVLYNDRSVLENHHAASAWNLYLSRPEYNFLLHLDHVEFKRFRFLVIEAILAT
 DLKKHFDFLAEFNAKANDVNSNGIEWSNENDRLLVCQVCIKLADINGPAKVRDLHLKW
 TEGIVNEFYEQGDEEANLGLPISPFMDRSPQLAKLQESFITHIVGPLCNSYDAAGLLPGQ
 WLEAEEDNDTESGDDEDGEELDEDEEMENNLNPKPPRRKSRRRIFCQLMHHLTENHKI
 WKEIVEEEEKCKADGNKLQVENSSLPQADEIQVIEEADEEE

(SEQ ID NO:5)

[0037] By “SLFN12 polynucleotide” is meant any nucleic acid molecule encoding a SLFN12 polypeptide or fragment thereof. An exemplary SLFN12 nucleic acid sequence is provided at NCBI Ref: NM_018042.4:

```

1  tttgtaactt cacttcagcc tccattgat cgctttctgc aaccattcag actgatctcg
61 ggctcctatt tcatttacat tgtgtgcaca ccaagtaacc agtgggaaaa ctttagaggg
121 tacttaaacc ccagaaaatt ctgaaaccgg gctcttgagc cgctatcctc gggcctgctc
181 ccaccctgtg gagtgcactt tcgttttcaa taaatctctg cttttgttgc ttcattcttt
241 ccttgctttg tttgtgtgtt tgtccagttc tttgttcaac acgccaagaa cctggacact
301 cttcactggg aacatatatt ggcaagccaa ccaggagaaa agaatttctg cttggacact
361 gcatagctgc tgggaaaatg aacatcagtg ttgatttggg aacgaattat gccgagttgg
421 ttctagatgt ggggaagagtc actcttgagg agaacagtag gaaaaaatg aaggattgta
481 aactgagaaa aaagcagaat gaaagtgtct cagcagctat gtgtgctctg ctcaattctg
541 gagggggagt gatcaaggct gaaattgaga atgaagacta tagttataca aaagatggaa
601 taggactaga tttggaaaat tcttttagta acattctgtt atttgttccg gagtacttag
661 acttcatgca gaatggtaac tactttctga tttttgtgaa gtcattggagc ttgaacacct
721 ctggtctgcg gattaccacc ttgagctcca atttgtacaa aagagatata acatctgcaa
781 aagtcatgaa tgccactgct gcactggagt tcctcaaaga catgaaaaag actagaggga
841 gattgtatatt aagaccagaa ttgctggcaa agaggccctg tgttgatata caagaagaaa
901 ataacatgaa ggccttgggc ggggtttttt ttgatagaac agaacttgat cggaaagaaa
961 aattgacctt tactgaatcc acacatgttg aaattaaaaa cttctcgaca gaaaagttgt
1021 tacaacgaat taaagagatt ctccctcaat atgtttctgc atttgcaaat actgatggag
1081 gatatttggt cattggttta aatgaagata aagaaataat tggcttttaa gcagagatga
1141 gtgacctcga tgacttagaa agagaaatcg aaaagtccat taggaagatg cctgtgcatc
1201 acttctgtat ggagaagaag aagataaatt attcatgcaa attccttgga gtatatgata
1261 aaggaagtct ttgtggatat gtctgtgcac tcagagtggg gcgcttctgc tgtgcagtgt
1321 ttgctaaaga gcctgattcc tggcatgtga aagataaccg tgtgatgcag ttgaccagga
1381 aggaatggat ccagttcatg gtggaggctg aacccaaatt ttccagttca tatgaagagg
1441 tgatctctca aataaatacg tcattacctg ctccccacag ttggcctctt ttggaatggc
1501 aacggcagag acatcactgt ccagggctat caggaaggat aacgtatact ccagaaaacc
1561 tttgcagaaa actgttctta caacatgaag gacttaagca attaatatgt gaagaaatgg

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1621 actctgtcag aaagggctca ctgatcttct ctaggagctg gtctgtggat ctgggcttgc
1681 aagagaacca caaagtcctc tgtgatgctc ttctgatttc ccaggacagt cctccagtcc
1741 tatacacctt ccacatggta caggatgagg agtttaaagg ctattctaca caaactgccc
1801 taaccttaaa gcagaagctg gcaaaaattg gtggttacac taaaaaagtg tgtgtcatga
1861 caaagatctt ctacttgagc cctgaaggca tgacaagctg ccagtatgat ttaaggtcgc
1921 aagtaattta ccctgaatcc tactatttta caagaaggaa atacttgctg aaagcccttt
1981 ttaaagcctt aaagagactc aagtctctga gagaccagtt ttcccttgca gaaaatctat
2041 accagataat cggatatagat tgctttcaga agaatagataa aaagatgttt aaatcttgtc
2101 gaaggctcac ctgatggaaa atggactggg ctactgagat atttttcatt atataattga
2161 taacattctc taattctgtg aaaatatctt tttgaaaact ttgcaagtta agcaacttaa
2221 tgtgatgttg gataattggg ttttgtctat tttcacttct ccctaaataa tcttcacaga
2281 tattgtttga gggatattag gaaaattaat ttgttaactc gtctgtgcac agtattatth
2341 actctgtctg tagttcctga ataaattttc ttccatgctt gaactgggaa aattgcaaca
2401 cttttattct taatgacaac agtgaaaatc tcccagcata tacctagaaa acaattataa
2461 cttacaaaag attatccttg atgaaactca gaatttccac agtggggaatg aataagaagg
2521 caaaactcat

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(SEQ ID NO:6)

[0038] By “SLFN12 polypeptide” is meant a protein or fragment thereof having at least 85% amino acid sequence identity to the sequence provided at NCBI Ref No. NP_060512.3 that interacts with PDE3A when bound to DNMDP and other complex inducing compounds. An exemplary human SLFN12 amino acid sequence is:

```

MNISVDLETNYAELVLDVGRVTLGENSRKKMKDCKLRKKQNESVSRAMCALLNSGGG
VIKAEIENEDYSYTKDGIGLDLENSFSNILLFVPEYLDFMQNGNYFLIFVKSWSLNTSGLR
ITTLSSNLYKRDITSAKVMNATAALEFLKDMKKTRGRLYLRPELLAKRPCVDIQEENNM
KALAGVFFDRTELDRKEKLTFTESTHVEIKNFSTEKLLQRIKEILPQYVSFAFANTDGGYLF
IGLNEDKEIIGFKAEMSDLDDLEREIEKSIRKMPVHHFCMEKKKINYSCKFLGVYDKGSL
CGYVCALRVERFCCAVFAKEPDSWHVKDNRVMQLTRKEWIQFMVEAEPKFSSSYEEVI
SQINTSLPAPHSWPLLEWQRQRHHCPGLSGRITYTPENLCRKLFLQHEGLKQLICEEMDS
VRKGSLIFSRWSVDLGLQENHKVLC DALLISQDSPPVLYTFH MVQDEEFKGYSTQTAL
TLKQKLAKIGGYTKKVCVMTKIFYLSPEGMTSCQYDLRSQVIYPESYYFTRRKYLLKAL
FKALKRLKSLRDQFSFAENLYQIIGIDCFQKNDKKMFKSCRRLT.

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(SEQ ID NO:7)

[0039] By “AIP polynucleotide” is meant any nucleic acid molecule encoding an AIP polypeptide or fragment thereof. An exemplary AIP nucleic acid sequence is provided at NCBI Ref: NM_003977.2:

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1  ttttggcttc tgccctcaac caaaatggcg ctagctcgga agctgccgag gtgctaggag
61  ttgccgaagc aagtccgga gctaccgagc gagtccgga gttgccgaaa gggagcagcg
121 gggaaggagg atggcgata tcatcgcaag actccgggag gacgggatcc aaaaacgtgt
181 gatacaggaa ggccgaggag agctcccga ctttcaagat gggaccaagg ttcgtgtcta
241 ccctaccctt cccccctct gcggcgtggt ggcgatgca ggcgggagga ggccttaggc
301 gagaggttgc gcatgcccag agggcagcgt cactgcccc taccgctcac atgcagaact
361 cgacgctgat tgggctgaat ttaagtaggg ggtgaattcg ggcctgtctg ccccgcccc
421 tggctcggcc ttgtagcagc attggtgggg gaggcgctca gtcatcacia gcgggttggg
481 gttgggggtt gatctcagtg cttgggcaga cccacgctg gaggaaccc agggccggga
541 gtggtcctcg ggtatctggg tttcaaggct catgatcctt tgtagatgga agggccttct
601 gaaaacactt agaccaactg ccgctgttta gagtggaaaa ccaagacctt gggacgtgca
661 aagccggaga acgggcccag aggtcaggtc tcccagacag ggactcttta gcagccttcc
721 tgctgacta ggggcttgtt gggacagatg agggttggga agtaaagaac cccccacttt
781 tctccttttt gccaggcccc cagatccagc cctctgccc gcttctcccc caacctacia
841 ctccaggctt cctgcttct cctgtagtgt cctcctccc gagtgtttt cccagctgcc
901 acttgtttgc agagtaggga acctcccagg ggcagcccc gtgccagca gagcagtcag
961 gcaggacatg cacattgagc aaatgagcac atgccccctg gccagcaccg tgccgaatcg
1021 ggcagctaag catcctagcc cagtgcagta taagtgcct gagagcagag gggagctgca
1081 tggctggagt gatccgctgt atgaaaagat atcttctcta agaagagaca ggtgtgtggt
1141 tgtgggttca tgcccccag tgctgggggg ttggtggcgt tggagaagg ggtggcaag
1201 ggggatcctg gatggaacag acatcagaag gagagatgtg aacaatggca cccaagatc
1261 agaaacaggt ggtgttaaat aaccaatcgc cagcactgat tgagtgtca ctattcgaac
1321 attgtgttac atgcttcaca cgtttatttc ctacaatgtg agataggtag tgttgttgat
1381 tccgttttac cgatgtggaa actgacttca gagatgcagc atggtgcggc agttaagagc
1441 gtgggctcct ctaaccatat cctgtcgaga gttcaatctc caaacctctt ttctctgcac
1501 ccacccccag tgttatctct aaaaactctc cctgcccgga ttactcccag atgcagctct
1561 ccagtcatta actgtctctt aaacctgata tatagctccc tactcaccat atccacctgg
1621 aagcctggtt ggcaactcac acttaacctg ctccacctga ggcttctccg tgtcagggga
1681 accaacaacc ttcccgttgt tcagggcaaa aaccttagca tctctgtggt cctcccagtc
1741 tcacatccaa catcacatcc tcaatatcca gccaggatct gagttctcac cacttctgcc
1801 atcactgctt gggctccaggc catcctcatc tccagcctgg gttactgcag cgacctctaa
1861 ctctcctgcc tcttttgtcc ctctgtggtc tgttctcgtc ccagcagccg agcccatgcc
1921 agattcaatt ctttttttgc tcggagccac tcagtggctt ccatcacaga gtgaaaaaca
1981 gaggcctcac catagcctac aggcctgtgt aggtccacc ctactgacct gggtagctc
2041 cctgtctgac cctgtggtgt accccacccc ctcttccact ctgctctgcc acactggcat
2101 tgctgctctt gaacacatca tgcatttgaa acgggaagtt ccttgtctc cctcgaggg
2161 cgtgcgatgg gggagtggct cgcttcttca gtgcccgt gctcagacct ctgggggagc
2221 atacagatgg gcaggctgtg ggctccgacc tcatggcagt gtctaggggt gaatatttac
2281 agctccgtgt gttctagggt gctcttttag tttgtctatg ggaggcttgt gttaccagc
2341 tcaattagac ccccttctt atcacaagga cagagggtt tctgtagtct ggggttttct
2401 tgccttgatg tactggagta ctggagaatt agatcacttg tgggcttggga gaatgattgc
2461 aaattttttt ttatttttta ttttattttt ttttcttgag atggagtctt actctgtgtg
2521 cccaggctgg agtgcaatgg cacaatctct gcctcccagg ttcaagcaat tctcctgcct
2581 cagcctccca agtagctgag attacatgtg cctgacacca ggcccggcta atttttaaaa
2641 atgttttttag tagagatggg cttttaccat gttggccaag ctggtttcaa acgccttttt
2701 tttttttttt ttttttgaga cggagtcttg ctctattgcc caagctggag tgcagtggca
2761 tgatctcggt tcaactgcaac ctccaccttc tgggttcaag tgattctcct gcctcagcct
2821 cccaagtagc tgggattaca ggcaccgccc atcatggcca gctaattttt gtatttttag
2881 tagagacggg gttttgccac attggccagg ctggtcttga actcctgacc tcaggtgatc
2941 caccgcctt ggagatggtc ttcccctggg gttgggccac ttggtggccc cacctctcct
3001 ctgactgccc cagccaaact ccgcctcttc ctgccagttg atgacctgcc agcgtgcagg
3061 tgctgtcag tgtgatcttc tgcttcttgc tcccctgaca tctctcaat gaccaggagc
3121 tgcgtctctg ctgatgggct cctctgacat ctggtgcct gtgggtctac cccctagggg
3181 tgttgggttt ttataggcac aggatagggg tgtggcaggc cagggtgggtc ttgggaaatg

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3241 caacatttgg gcaggaaatg cctgttctca cctaggtctg tgggggtgga accctaccca
 3301 gggaccacgc cctcctctac ccagcacttc ccttctcccc ttccaaatta tttaacagga
 3361 ccatgctcct cccttcccag cacttccata tcacattgtc ccactgcaag gcttttttac
 3421 acatgctgtt cttttggcct agaaagtccc tatcccaggg tccacttggc ttgctttctt
 3481 ctttactccc caacccccca ctctgtttaa tccagcccca accctcttgc cctgctgttt
 3541 cccaagcacg tggcttcacc tgccatgaca tattgttttg tttgatgccc atctcctccc
 3601 tctagaagcg ccatgtgagc tccagggggg cagggaacttt tttgtgtttt gcttgctgcc
 3661 atgttctggg gtctagcaca gagcttgggc acatagtagg tgcttagtaa atatctgttg
 3721 aggaatgact ggagtcagac tgcttggaact cttgttccca ctacagccacc cactagccgt
 3781 gtggcttggg cctattcctc ccctccttgt ggctttgttt tctcaccagc gtgggaggat
 3841 gaagccaggt gtaaggtcag gtgggtgtccc cggggaagcc ccgtccctta tgccgtctgc
 3901 aggcggggga ctggacttct ccttgggggt cagggtgagg gtttgtgcct ttgcctgacc
 3961 tcgcatgtgg cccacaggcc acgttccact accggacgct gcacagtga gacgagggca
 4021 ccgtgctgga cgacagccgg gctcgtggca agcccatgga gctcatcatt ggcaagaagt
 4081 tcaagctgcc tgtgtgggag accatcgtgt gcacatgcg agaaggggag attgccagt
 4141 tcctctgtga catcaaggtg tctgtcctgt acctgtctgc ggtggctgtc cagccaagcc
 4201 ccttctctat tccctatccc cagggtcctc cctccctcca ccctctgcta gactgccacc
 4261 cgctttcttt ttttttttga gatggagtct tgctctgtcg ccaggctgg agtgagtggy
 4321 tacgatctca gctcactgca ccctccacct cctgggttca agcgattctt ctgcctcagc
 4381 ctcccagata gctgggatta caaacacccg ccatgatgcc tggctaattt ttgtattttt
 4441 agtagagaca gggtttcacc atgttggcca ggctggctct gaacacctga cctcaggtga
 4501 tccaccgccc ttggcctccc aaagtgtgg gattataggc gtgtgccacc gcgcccggcc
 4561 caccactctt ttccagacca ccacaccagc ctgctgatgg cgtcctggcc tccattccgc
 4621 cttcccctat tagccagact gaggccaggg gactcgttct caaatgcaaa tgacctgtac
 4681 atccctttgt ttcaaacctc tatgactcct ggtcactgta aggatagagc acagggggtc
 4741 ctacttcat gttgctgata cattcttggg aactgtgact aagagaaaaa acatacatca
 4801 ggttttttct cagccaccgt catttctctc agcaaaattt tgttagaaca ttgatgagaa
 4861 gaaaaatttg tttcgttatg tattgtttcg cctacagtca cagtttccaa gaacctactt
 4921 aggaggttaa gtgaggactt aaaccgtata agctatagct gctcacatag ctttttgggg
 4981 gctggccctt gccgtctcac cctcttactc aaacctccctg cttttccttt ccattcccct
 5041 tcttagccaa gatcttccct cttccttcaa agcttattcc tgggtcacca cctctaggaa
 5101 gccctccctg actgctagtg gttggctcaa ctcccatgtt tgggtcctcc aacctcatg
 5161 ccctgcatgg ccaggatctg ctcttctgcc ttgtcctagg ctattgcaga gcagggatct
 5221 ggctgtttta cttctagctt tgggatgccc agcgcgagcc agtccagagc caagactcag
 5281 gaaatgcccg ctgatggcag cccggcagtc agcccctgtc cagacaacag ggcagtggga
 5341 ggagtgggga ggacccgggt aggaggaatc tggttatctg gttcccacca gcttagcagc
 5401 tttgccaagc aagagattag aggctaggtc ccctatgcct gtctccctgt ggggtttttt
 5461 tttttttgac taagtctcac tctgttgccc aggctggagt gcagtggcgt gatcttggct
 5521 cactgcaacc accatctcct gggttcagct gattctctgc cttagctgcc tgagtagctg
 5581 ggattacagg cacctgccat cgtgcccggc tcatttttgt attttagcag agacgcggtt
 5641 tcaccatggt ggtcaggctg gtcttgaact cctgacctca ggtgatccgc ccgccttggc
 5701 ctcccgaagt gctaggatta caggcgtgag ccaccacatc cggcctccct gagggttttg
 5761 aagtggctgg cctgggcca gctctgaggt aggcctcag tggggtgtgg gtggggcaga
 5821 aggaggagct gctgggaaca gaatgtgggg ggcccagtt ctttgcatag tccagcaaag
 5881 ggccttatcc tctggaggga gaggaggtaa gaattctact gggcctgtaa ggaccaggga
 5941 gacaggggtt gatggtaggc atgtgtctgt ggtgggggtg aggaggggt taggtgctct
 6001 gtttgggtgg cagagaatgt ggcagaagct ggggcttcac caggagagag ggctgagcga
 6061 ctggaggagt cctgaattaa aagcctcctg tgcttaaacg gagtagggtc ccagttgtca
 6121 ctctctgggc cttgggtgtt gttctcagat ggtgggtggg aagggggctg ggccttgtgg
 6181 acccggtgac cagccagccc acggtgacag agccccggc gcccttgcct tcccgcagca
 6241 tgtgttctg tacccgctgg tggccaagag tctccgcaac atcgcggtgg gcaaggaccc
 6301 cctggagggc cagcggcact gctgcggtgt tgcacagatg cgtgaacaca gctccctggg
 6361 catgctgac ctggacgcc tgacagcaga ccccagccc ctcatcttc acatggagat
 6421 gctgaagggt agggggccacc gcgcctggtc tcaccaggcc cccactgccc agcctcaggg

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6481 cggcgctggc ctgtccaccc aggggtggtg ggatccgcag gtggactgct gggggagcgg
6541 acagagacaa gaaaacctgt gcaggacctt tggcagtacc ctgggtctcc ttctctctc
6601 cttcacatct caaatgtcac ctctccagg aaaccggccc tgcccacccg gtctctcat
6661 tctctgtctc gcagcagctc atttccttta tagcctctgc cgcacctga agtcccttg
6721 aattcatgga ttctctgtc catttaggga acctgccatg cagcatgatc tctgcgagg
6781 cagggtcttt caccgtcttg ttactgttc tattcttagc acttggcaca gtgtgggca
6841 cacaggagat gtgacatcga tgtttgatgc tttttgagtg acaagtagct ctgtgtctg
6901 tgtgtgatgt ctggggggccc agccagccca gatgtgggtc aggtctgctg ctgacggacg
6961 cagctgtggt gtccccgagc cccgctgtga tatgcccac gccctgcagg tggagagccc
7021 tggcacgtac cagcaggacc catgggccat gacagacgaa gagaaggcaa aggcagtgcc
7081 acttatccac caggagggca accggttgta ccgcgagggg catgtgaagg aggtctgtc
7141 caagtactac gatgccattg cctgcctcaa gaacctgcag atgaaggtag tgcctggagg
7201 ctgaggggga ggatggatgg aggggggtgt ggagccaggg ggcccagggt tacagcttct
7261 ccccgctccc tgccccata ctcccaggaa cagcctgggt cccctgaatg gatccagctg
7321 gaccagcaga tcacgccgct gctgctcaac tactgccagt gcaagctggt ggtcgaggag
7381 tactacgagg tgctggacca ctgctcttcc atctcaaca agtacgacgg tgagcaccgg
7441 gccctgggct gccgggggct gcgagtggtc agagagtggc cttctctctg tctactgtg
7501 ggtcaagacc tagcctttca caaccccat tctgagctcc caggggggct tgactaaatg
7561 cctctactcg gcagggctgt gggcccatg gtgccaatga agcatgaatg gtgtattggg
7621 ggtgggggtg catcctcagg tcaggagggt ctctctctcc cctgtgggct catggtgcca
7681 ggagacatga gggcaggcag ctggccagga tccccctca tgcccttgca tgccactgc
7741 cactggcct cccctgcaga caacgtcaag gcctacttca agcggggcaa ggccacgcg
7801 gccgtgtgga atgcccagga ggcccaggct gactttgcca aagtgtgga gctggacca
7861 gccctggcgc ctgtggtgag ccgagagctg caggccctgg aggcacggat ccggcagaag
7921 gacgaagagg acaaagccc gttccggggg atcttctccc attgacagga gcacttggcc
7981 ctgccttacc tgccaagccc actgctgcag ctgccagccc cctgcccgt gctgcgtcat
8041 gcttctgtgt atataaaggc ctttatttat ctctctctga

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(SEQ ID NO:8)

[0040] By “AIP polypeptide” is meant a protein or fragment thereof having at least 85% amino acid sequence identity to the sequence provided at NCBI Reference Sequence: NP_003968.2 that can bind the aryl hydrocarbon receptor. AIP polypeptides may regulate expression of many xenobiotic metabolizing enzymes and bind specifically to and inhibit the activity of hepatitis B virus. Three transcript variants encoding different isoforms have been found for this gene. An exemplary human AIP amino acid sequence is:

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MADIARLREDGIQKRVIQEGRGELPDFQDGTKATFHRYRTLHSDDEGTVLDDSRARGKP
MELIIGKKFKLPVWETIVCTMREGEIAQFLCDIKHVVLVPLVAKSLRNIAVGKDPLEGQR
HCCGVAQMREHSSLGHADLDALQQNPQPLIFHMEMLKVESPGTYQQDPWAMTDEEKA
KAVPLIHQEGNRLYREGHVKEAAAKYYDAIACLNLMKEQPGSPEWIQLDQQITPLLL
NYCQCKLVVEEYYEVLHDCSSILNKYDDNVKAYFKRGKAHAAVWNAQEAQADFAKV
LELDPALAPVVSRELQALEARIRQKDEEDKARFRGIFSH.

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(SEQ ID NO: 9)

[0041] By “TRRAP polypeptide” is meant a protein or fragment thereof having at least 85% amino acid sequence identity to the sequence provided at NCBI Reference Sequence: NP_001231509.1 having histone acetyltransferase complex recruiting activity. An exemplary human TRRAP amino acid sequence is (which encodes the longer isoform):

MAFVATQGATVVDQTTLMKKYLQFVAALTDVNTPDETKLKMMQEVSENFENVTSPPQ
YSTFLEHIIPRFLTFLQDGEVQFLQEKPAQQLRKLVLLEIIHRIPTNEHLRPHTKNVLSVMFR
FLETENEENVLICLRIIIHLHKQFRPPITQEIIHFLDFVKQIYKELPKVVNRYFENPQVIPEN
TVPPPEMVGMITTIAVKVNPEREDSETRTHSIIIPRGSLSLKVLAEPLIIVVLMYQLYKLNIIH
NVVAEFVPLIMNTIAIQVSAQARQHKLYNKELYADFIAAQIKTSLFLAYIIRIYQELVTKY
SQQMVKGMLQLLSNCPAETAHLRKELLIAAKHILTTLELRNQFIPCMDKLFDESILIGSGY
TARETLRPLAYSTLADLVHHVRQHLPLSDLSLAVQLFAKNIDDESLPSSIQTMSCKLLLN
LVDCIRSKSEQESGNGRDVLMRMLEVFVLKFHTIARYQLSAIFKKCKPQSELGAVEAAL
PGVPTAPAAPGPAPSPAPVPAPPPPPPPPPATPVTPAPVPPFEKQGEKDKEDKQTFQVTD
CRSLVKTLVCGVKTTITWGITSCKAPGEAQFIPNKQLQPKETQIYIKLVKYAMQALDIYQV
QIAGNGQTYIRVANCQTVRMKEEKEVLEHFAGVFTMMNPLTFKEIFQTTVPYMERISK
NYALQIVANSFLANPTTSALFATILVEYLLDRLPEMGSNVELSNLYLKLFLKLVFGSVSLF
AAENEQMLKPHLHKIVNSSMELAQTAKPEYNYFLLLRALFRSIGGGSHDLLYQEFPLLP
NLLQGLNMLQSGLHKQHMKDLFVELCLTPVRLSSLLPYLPMLMDPLVSALNGSQTLV
SQGLRTLELCVDNLQPDFLYDHIQPVRAELMQALWRTLRLNPADSISHVAYRVLGKFGGS
NRKMLKESQKLHYVVTEVQGPSITVEFSDCKASLQLPMEKAIETALDCLKSANTEPYYR
RQAWEVKICFLVAMMSLEDNKHALYQLLAHPNFTEKTIPNVIISHRYKAQDTPARKTFE
QALTGAFMSAVIKDLRPSALPFVASLIRHYTMVAVAQQCGPFLPCYQVGSQPSTAMFH
SEENGSKGMDPLVLIDAIACMAYEEKELCKIGEVALAVIFDVASIIIGSKERACQLPLFS
YIVERLCACCYEQAWYAKLGGVVSIFLMERLPLTWVLQNQQTFLKALLFVMMDLTG
EVSNGAVAMAKTTLEQLLMRCATPLKDEERAEIIVAAQEKSFHHVTHDLVREVTSPNS
TVRKQAMHSLQVLAQVTGKSVTVIMEPHKEVLQDMVPPKKHLLRHQPANAQIGLMEG
NTFCTTLQPRLFTMDLNVVEHKVFYTELLNLCEAEDSALTKLPCYKSLPSLVPLRIAALN
ALAACNYLPQSREKIIAALFKALNSTNSELQEAGEACMRKFLEGATIEVDQIHTHMRPLL
MMLGDYRSLTLNVVNRLTSVTRLFPNSFNDKFCQMMQHLLRKWMEVVVITHKGGQRS

DGNESISECGRCPLSPFCQFEEMKICSAIINLFHLIPAAPQTLVKPLLEVVMKTERAMLEIA
GSPFREPLIKFLTRHPSQTVELFMMEATLNDPQWSRMFMSFLKHKDARPLRDVLAANPN
RFITLLLPGGAQTAVRPGSPSTSTMRLDLQFQAIKIISIIVKNDDSWLASQHSLSVQLRRV
WVSENFQERHRKENMAATNWKEPKLLAYCLLNYCKRNYGDIELLFQLLRAFTGRFLCN
MTFLKEYMEEEEIPKNYSIAQKRALFFRFVDFNDPNFGDELKAKVLQHILNPAFLYSFEKG
EGEQLLGPPNPEGDNPESITSVFITKVLDPEKQADMLDSLRIYLLQYATLLVEHAPHHHID
NNKNRNSKLRRMLMTFAWPCLLSKACVDPACKYSGHLLLAHIIAKFAIHKKIVLQVFHSL
LKAHAMEARAIVRQAMAILTPAVPARMEDGHQMLTHWTRKIIVEEGHTVPQLVHILHLI
VQHFKVYYPPVRHHLVQHMMVSAMQRLGFTPSVTIEQRRLAVDLSEVVIKWELQRIKDQQ
PDSMDPNSSGEGVNSVSSSIKRGLSVDSAQEVKRFRTATGAISAVFGRSQSLPGADSL
AKPIDKQHTDTVVNFLIRVACQVNDNTNTAGSPGEVLSRRCVNLLKTALRPDMWPKSE
LKLQWFDKLLMTVEQPNQVNYGNICTGLEVL SFLTTLVQSPAILSSFKPLQRGIAACMTC
GNTKVLRAVHSLLSRLMSIFPTEPSTSSVASKYEELECLYAAVGKVIYEGLTNYEKATNA
NPSQLFGTLMILKSACSNNPSYIDRLISVFMRS LQKMVREHLNPQAASGSTEATSGTSEL
VMLSLELVKTRLAVMSMEMRKNFIQAILTSLIEKSPDAKILRAVVKIVEEWVKNNSPMA
ANQTPTLREKSILLVKMMTYIEKRFPEDLELNAQFLDLVNYVYRDETLSGSELTAKLEPA
FLSGLRCAQPLIRAKFFEVDNSMKRRVYERLLYVTCSQNWEAMGNHFWIKQCIELLLA
VCEKSTPIGTSCQGAMLPSITNVINLADSHDRAAFAMVTHVKQEPRERENSESKEEDVEI
DIELAPGDQTSTPKTKELSEKDIGNQLHMLTNRHDKFLDTLREVKTGALLSAFVQLCHIS
TTLAECTWVQLFPRLWKILSDRQQHALAGEISPFLCSGSHQVQRDCQPSALNCFVEAMS
QCVPPPIRPCVLKYLKGKTHNLWFRSTLMLEHQAFEEKGLSLQIKPKQTTEFYEQESITPPQ
QEILDSLAE LYSLLQEEDMWAGLWQKRCKYSETATAIAYEQHGFFEQAQESYEKAMDK
AKKEHERSNASPAIFPEYQLWEDHWIRCSKELNQWEALTEYGQSKGHINPYLVLECAW
RVSNWTAMKEALVQVEVSCPKEMAWKVNMYRGYLAICHPEEQQLSFIERLVEMASSL
AIREWRRLPHVVS HVHTPLLQAAQQIIELQEAAQINAGLQPTNLGRNNSLHDMKTVVKT
WRNRLPIVSDDL SHWSSIFMWRQH HYQGKPTWSGMHSSSIVTAYENSSQHDPSSNNAM
LGVHASASAI IQYGKIARKQGLVNALDILSRIHTIPTVPIVDCFQKIRQQVKCYLQLAGVM
GKNECMQGLEVIESTNLKYFTKEMTAEFYALKGMFLAQINKSEEANKAFSAAVQMHDV
LVKAWAMWGDYLENIFVKERQLHLGVSAITCYLHACRHQNESKSRKYLA KVLWLLSF
DDDKNTLADAVDKYCIGVPPIQWLAWIPQLLTCLVGSEGKLLLNLSQVGRVYPQAVYF

PIRTL YLTLKIEQRERYKSDPGPIRATAPMWRC SRIMHMQRELHPTLLSSLEGIVDQMVW
 FRENWHEEVLRQLQQGLAKCYSVAFEKSGAVSDAKITPHTLNFVKKLVSTFGVGLENV
 SNVSTMFSSAASESLARRAQATAQDPVFQKLKGQFTTDFDFSVP GSMKLHNLISKLLKKW
 IKILEAKTKQLPKFFLIEEKCRFLSNFSAQTA EVEIPGEFLMPKPTHYYIKIARFM PRVEIV
 QKHNTAARRLYIRGHNGKIYPYLV MNDA CLTESRREERVLQLLRLLNPCLEKRKETT KR
 HLFFTVPRVVAVSPQMRLVEDNPSSL SLVEIYKQRC AKKGIEHDNPISRY YDRLATVQA
 RGTQASHQVLRDILKEVQSNMVPR SMLKEWALHTFPNATDYWTFRKMFTIQLALIGFA
 EFVLHLNRLNPEMLQIAQDTGKLN VAYFRFDINDATGDLDANRPVPFRLTPNISEFLT TI
 GVSGPLTASMIAVARCFAQPNFKVDGILKTVLRDEIIA WHKKTQEDTSSPLSAAGQPEN
 MDSQQLVSLVQKAVTAIMTRLHNLAQFEGGESK VNTLVAAANSLDNL CRMDPAWHP
 WL

(SEQ ID NO: 11)

[0042] By “TRRAP polynucleotide” is meant any nucleic acid molecule encoding an TRRAP polypeptide or fragment thereof. An exemplary TRRAP nucleic acid sequence is provided at NCBI Ref: NM_001244580.1:

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1  cgcgccgggg cctggtgctc ggtcggcggg tgctgccgct ttaagcgggg gcgggactgc
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121 ctgcaggagc cgggccgccg aggtcggggc tgggtgaact catggacctg atacttttct
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9061 caggcttaca gccaaccaac ctgggaagga acaacagcct gcacgacatg aagacggtgg
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9481 gcgtcatggg caaaaacgag tgcattgcag gccttgaagt tattgaatct acaaatttaa
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9781 acgagagcaa atcgaggaaa tacttagcca aggtgctgtg gcttttgagt tttgatgatg
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12421 ctctgagagt ctgcaggcgg ctctgtgctt ttttatctc ggctcttcgg atgtcttcta
12481 gacatttact atcactgcac ctgaagaaaa aatcactttt accttcctaa tttaaaaaga
12541 caaacagaa atgtacgttc cttcgctagc tttagtctt ctgttcccat ttttataaat
12601 ctgagcattg ataatgttct atctaaattt gtacagtgtg attttttttt ttagaataaa
12661 tattttataa aagggttaaa aaaaaaaaaa aaaaa

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(SEQ ID NO: 10)

[0043] In certain implementations, the marker that detects polynucleotide may be the polynucleic region that encodes the protein.

[0044] By “alteration” is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods, such as those described herein. As used herein, an alteration includes at least a 10% change in expression levels, for example a 25% change, a 40% change, and a 50% or greater change in expression levels.

[0045] A “chemically induced” complex (*e.g.*, chemically induced PDE3A-SLFN12 complex, chemically induced PDE3B-SLFN12 complex) is the complex formed by indicated agents following contact with an active compound, such as a PDE3A modulator or a PDE3B modulator. Typically, the active compounds described herein are a chemical compound inducing PDE3A-SLFN12 or PDE3B-SLFN12 complexes, such as *e.g.* DNMDP or a compound of WO2019/025562.

[0046] By “modulator” is meant any agent that binds to a polypeptide and alters a biological function or activity of the polypeptide. A modulator includes, without limitation, agents that increase binding of a polypeptide to another agent. For example, a modulator may promote binding of a polypeptide to another polypeptide. In some embodiments a modulator of PDE3A or PDE3B promotes binding of these proteins to SLFN12. In some embodiments, a modulator of PDE3A polypeptide is DNMDP. In other embodiments, a modulator of PDE3A is an exemplified compound of WO2019/025562.

[0047] The term “capture reagent” refers to a reagent, for example an antibody or antigen binding protein, capable of binding a target molecule or analyte to be detected in a biological sample. The capture reagent may be immobilized, for example on an assay surface, such as a solid substrate or reaction vessel. The capture reagents described herein may bind to one or more of PDE3A, PDE3B, SLFN12, AIP, and TRRAP.

[0048] “Detect” refers to identifying the presence, absence or amount of the analyte to be detected. In particular embodiments, the analyte is an AIP, TRRAP, PDE3A, PDE3B, or SLFN12 polypeptide.

[0049] By “effective amount” or “therapeutically effective amount” is meant the amount of a compound described herein required to ameliorate the symptoms (*e.g.*, treat, prevent) of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present disclosure for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. In some embodiments, the compound is DNMDP or a compound of WO2019/025562.

[0050] The terms “healthy”, “normal” and “non-neoplastic” are used interchangeably herein to refer to a subject or particular cell or tissue that is devoid (at least to the limit of detection) of a disease condition, such as a neoplasia. In some embodiments, the reference may be a healthy cell.

[0051] The disclosure provides a number of targets that are useful for the development of highly specific drugs to treat or a disorder characterized by the methods delineated herein. In addition, the methods of the disclosure provide a route for analyzing virtually any number of compounds

for effects on a disease described herein with high-volume throughput, high sensitivity, and low complexity.

[0052] By “fragment” is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

[0053] By “marker” or “biomarker” is meant any protein or polynucleotide having an alteration in expression level or activity relative to a reference that is associated with a disease or disorder, such as cancer. In particular embodiments, a marker of the disclosure is AIP (*e.g.*, AIP polypeptide, AIP polynucleotide), TRRAP (*e.g.*, TRRAP polypeptide, TRRAP polynucleotide), PDE3A (*e.g.*, PDE3A polypeptide, PDE3A polynucleotide), PDE3B (*e.g.*, PDE3B polypeptide, PDE3B polynucleotide), or SLFN12 (*e.g.*, SLFN12 polypeptide, SLFN12 polynucleotide). In certain implementations, the marker may comprise portions of a polynucleotides sequence (*e.g.*, SEQ ID NOS: 1, 4, 6, 8, 10) which encode the polypeptide (*e.g.*, AIP polypeptide, TRRAP polypeptide, PDE3A polypeptide, PDE3B polypeptide, SLFN12 polypeptide). In some embodiments, the marker may have any one of SEQ ID NOS: 1-11. In some embodiments, a marker may comprise at least 75% or at least 80% or at least 85% sequence identity to SEQ ID NOS 2, 3, 5, 7, 9, or 11. In certain embodiments, the presence of a marker in a cancer cell identifies that cell as responsive to therapy.

[0054] Nucleic acid molecules (*e.g.*, polynucleotides) useful in the methods of the disclosure include any nucleic acid molecule that encodes a polypeptide of the disclosure or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having substantial identity to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the disclosure include any nucleic acid molecule that encodes a polypeptide of the disclosure or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Typically, when

polynucleotides hybridize and at least one strand of a nucleic acid molecule hybridizes, they are able to pair to form a double-stranded molecule between complementary polynucleotide sequences (*e.g.*, a gene described herein), or portions thereof, under various conditions of stringency.

[0055] For example, “stringent” salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate or less than about 500 mM NaCl and 50 mM trisodium citrate or less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, *e.g.*, formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide or at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C or at least about 37° C or at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, *e.g.*, sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a specific embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In some embodiments, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In certain aspects, hybridization may occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

[0056] For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps may be less than about 30 mM NaCl and 3 mM trisodium citrate, or more less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, for example of at least about 42° C, or at least about 68° C. In some embodiments, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In certain embodiments, wash steps will occur at 42° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a specific implementation, wash steps may occur at 68° C in

15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

[0057] Typically, substantially identical polypeptides or nucleic acids exhibit at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). In certain implementations, such a sequence is at least 60%, or at least 80% or 85% or at least 90% or at least 95% or at least 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

[0058] Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions may include substitutions within the following groups:

glycine, alanine;

valine, isoleucine, leucine;

aspartic acid, glutamic acid, asparagine, glutamine;

aspartic acid, glutamic acid;

asparagine, glutamine;

serine, threonine; lysine, arginine; and

phenylalanine, tyrosine.

In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

[0059] The term “pharmaceutical composition,” as used herein, represents a composition containing a compound (*e.g.*, a PDE3 modulator) formulated with a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of a disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (*e.g.*, a tablet, capsule, caplet, gelcap); for topical administration (*e.g.*, as a cream, gel, lotion, ointment); for intravenous administration (*e.g.*, as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); or in any other formulation described herein.

[0060] Useful pharmaceutical carriers for the preparation of the compositions hereof, can be solids, liquids, or gases. Thus, the compositions can take the form of tablets, pills, capsules, suppositories, powders, enterically coated or other protected formulations (*e.g.*, binding on ion-exchange resins), sustained release formulations, solutions, suspensions, elixirs, and aerosols. The carrier can be selected from the various oils including those of petroleum, animal, vegetable or synthetic origin, *e.g.*, peanut oil, soybean oil, mineral oil, and sesame oil. Water, saline, aqueous dextrose, and glycols are liquid carriers, particularly (when isotonic with the blood) for injectable solutions. For example, formulations for intravenous administration comprise sterile aqueous solutions of the active compound(s) which are prepared by dissolving solid active compound(s) in water to produce an aqueous solution, and rendering the solution sterile. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, talc, gelatin, malt, rice, flour, chalk, silica, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, and ethanol. The compositions may be subjected to conventional pharmaceutical additives, such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, and buffers. Suitable pharmaceutical carriers and their formulation are described in Remington's Pharmaceutical Sciences by E. W. Martin. Such compositions will, in any event, contain an effective amount of the active compound

together with a suitable carrier so as to prepare the proper dosage form for administration to the recipient.

[0061] As used herein, the term “pharmaceutically acceptable salt” refers to salts of any of the compounds mentioned herein that within the scope of sound medical judgment, are suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. Salts may be prepared from pharmaceutically acceptable non-toxic acids and bases including inorganic and organic acids and bases. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, dichloroacetate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glutamate, glycerophosphate, hemisulfate, heptonate, hexanoate, hippurate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, isethionate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, methanesulfonate, mucate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, and valerate salts. Representative basic salts include alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, and magnesium, aluminum salts, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, caffeine, and ethylamine.

[0062] As used herein, the term “subject” refers to any organism to which a composition in accordance with the disclosure may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include any animal (e.g., mammals, such as mice, rats, rabbits, non-human primates, and humans). The subject may be domesticated animals (e.g., cows, calves, sheep, lambs, horses, foals, pigs, piglets), or animals in the family

Muridae (e.g., rats, mice). A subject may seek or be in need of treatment, require treatment, be receiving treatment, may be receiving treatment in the future, or a human or animal that is under care by a trained professional for a particular disease or condition.

[0063] A “patient in need thereof” as used herein, refers to a human individual who may be identified as having a disease, disorder, or condition responsive to complex formation. As described herein, in some embodiments, an individual in need thereof is suffering from a proliferative disorder, such as cancer. In some embodiments, an individual in need thereof has been diagnosed by a medical doctor with a proliferative disorder requiring treatment. A patient in need or an individual in need are used interchangeably herein.

[0064] As used herein, the term “reference” or “reference level” refers to an amount or concentration of the indicated biomarker (e.g., SLFN12, PDE3A, PDE3B, AIP, TRRAP), which may be of interest for comparative purposes. For example, a reference level may be the level of the indicated biomarker expressed as an average of the level of the biomarker from samples taken from a control population of healthy subjects. In some embodiments, a reference level may be the level of the indicated biomarker expressed as an average of the level of the biomarker measured from a plurality cancer cell lines (e.g., the cancer cell lines measured in FIG. 5). In one embodiment, the “reference” is a standard or control condition. Suitable samples or references for determining reference levels include healthy cells, cells nonresponsive to chemically induced complex formation (e.g., cells nonresponsive to PDE3A modulation, cells nonresponsive to PDE3B modulation), non-cancerous cells, normal tissue, and the like. In various implementations, the reference may be the average expression level of cancerous cells. In some embodiments, the reference to determine the reference level of an indicated biomarker may be derived from the subject, a subject known not to suffer from cancer, such as a healthy subject, or a population of subjects. Suitable references include a concurrently run control, or a standard which may be created by assaying one or more non-cancer cells and collecting biomarker data. Thus, the control sample may optionally be a standard that is created and used continuously. The standard includes, for example, the average level of a biomarker in a sample from a non-cancer control group. In certain embodiments, the reference is derived from a sample of a subject known not to suffer from cancer.

[0065] As used herein, and as well understood in the art, “to treat” a condition or “treatment” of the condition (*e.g.*, the conditions described herein, such as cancer) is an approach for obtaining beneficial or desired results, such as clinical results. Treatment of a subject may include a decrease in the proliferation of cancer cells in the subject. Beneficial or desired results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions; diminishment of extent of disease, disorder, or condition; stabilized (*i.e.*, not worsening) state of disease, disorder, or condition; preventing spread of disease, disorder, or condition; delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition; and remission (whether partial or total), whether detectable or undetectable. “Palliating” a disease, disorder, or condition means that the extent and/or undesirable clinical manifestations of the disease, disorder, or condition are lessened and/or time course of the progression is slowed or lengthened, as compared to the extent or time course in the absence of treatment.

[0066] Chemical Inducers of PDE3A/B-SLFN12 Complexes

[0067] Without wishing to be bound by theory, it is believed that certain chemical agents are able to induce complexation in certain responsive cells between specific phosphodiesterase and schlafen proteins in the presence of AIP and TRRAP. As shown in WO 2017/027854, hereby incorporated by reference in its entirety, increased expression of PDE3A and SLFN12 has been shown to correlate with cytotoxicity of certain chemical agents (*i.e.*, “complex inducers” or “complex inducing active compounds”), such as PDE3A modulators. As shown herein, selective apoptosis of only malignant biomarker positive cells may occur following chemical induction of PDE3A/B-SLFN12 formation. The present disclosure is partially premised on the discovery that AIP and TRRAP peptides are implicated in the apoptotic response of cancer cells. Expression of combinations of these biomarkers have been shown to correlate with complex inducing active compound (*e.g.*, PDE3 modulator, PDE3A modulator, PDE3B modulator) sensitivity. Using AIP and TRRAP as biomarkers allows for further stratification of cells responsive to PDE3A-SLFN12 or PDE3B-SLFN12 complex formation, and particularly, chemically induced complexation. This increased stratification allows for more efficient identification of specific chemical agents for the treatment or prophylaxis of diseases, disorders, or conditions in that patient population with

responsive cells. Furthermore, it facilitates identification of patients who will benefit from a treatment with PDE3 modulators, such as a PDE3A modulator and/or a PDE3B modulator operative via PDE3A-SLFN12 and/or PDE3B-SLFN12 complex formation.

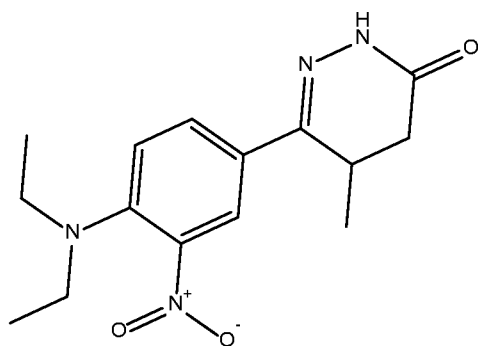
[0068] Once a cell is identified as responsive to complex formation by the methods described herein (*e.g.*, by expression of AIP and/or TRAPP; and increased expression of PDE3A or PDE3B relative to a reference, such as a healthy control cell or the average expression level of cancerous cells such as those cells measured in FIG. 5; and increased expression of SLFN12 relative to reference, such as a healthy control cell or the average expression level of cancerous cells), apoptosis of that cell may occur by chemical induction of complex formation by one or more PDE3 modulators. Complex inducing active compounds may be delivered to responsive cell lines to induce complexation between PDE3A-SLFN12 and/or PDE3B-SLFN12 which may result in apoptosis in only those cells susceptible to complex formation (*e.g.*, cancer cells). In some embodiments, the PDE3A-SLFN12 complex inducing active compound is a PDE3 modulator, such as a PDE3A modulator or a PDE3B modulator. In various implementations, the PDE3B-SLFN12 complex inducing active compound is a PDE3 modulator, such as a PDE3A modulator or a PDE3B modulator. For example, the complex inducing active compound may be DNMDP or a compound of WO2019/025562.

[0069] The identification of complex inducing active compounds including PDE3 (*e.g.*, PDE3A, PDE3B) modulators suitable for use as therapeutic agents to induce PDE3A/B-SLFN12 complex formation may be made with a phenotypic screen designed to identify cytotoxic small molecules that preferentially kill cancer cells over a healthy cell through complex formation of PDE3A-SLFN12 or PDE3B-SLFN12 upon administration. A predictive chemogenomics approach may complement target-driven drug development programs, which typically consist of extensive *in vitro* and *in vivo* target validation. Many U.S. Food and Drug Administration (FDA)-approved targeted therapies have been developed this way, among them small-molecule kinase inhibitors that target oncogenic somatic driver mutations. However, the discovery and development of targeted therapies is often hampered by limitations in knowledge of the biological function of the target, its mechanism of action, and the available chemical matter to selectively modulate the target. The present disclosure is related to increasing that knowledge base.

[0070] Phenotypic screening can discover novel targets for cancer therapy whose specific molecular mechanism is often elucidated by future studies. For example, a phenotypic screen developed to identify small molecules causing synthetic lethality in *tp53* mutant cancer cells enabled the serendipitous discovery of a class of cancer-selective cytotoxic agents which act as modulators of phosphodiesterase 3A (PDE3A). Many PDE3A modulators also directly bind PDE3B proteins and a PDE3A modulator may be used to induce complexation between SLFN12 and PDE3B. Cyclic nucleotide phosphodiesterases catalyze the hydrolysis of second messenger molecules cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), and are important in many physiological processes.

[0071] The present disclosure provides methods for identifying subjects that have a malignancy that is likely to respond to PDE3 modulator treatment based on the expression of AIP and/or TRRAP in a subject biological sample comprising a cancer cell which also expresses increased levels PDE3A and SLFN12 or PDE3B and SLFN12 relative to a reference.

[0072] Examples of PDE3A modulators include DNMDP (6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one)



and pharmaceutically acceptable salts thereof or a compound of WO2019/025562 such as (6S)-5-[4'-fluoro-2-(trifluoromethyl)biphenyl-4-yl]-6-methyl-3,6-dihydro-2H-1,3,4-oxadiazin-2-one (Compound X), 5-{4-[1-(difluoromethyl)-1H-pyrazol-4-yl]-3-(trifluoromethyl)phenyl}-3,6-dihydro-2H-1,3,4-oxadiazin-2-one, (6S)-5-[4-(2-aminopyridin-4-yl)-3-(trifluoromethyl)phenyl]-6-methyl-3,6-dihydro-2H-1,3,4-oxadiazin-2-one and (6S)-6-methyl-5-{3-(trifluoromethyl)-4-[3-(trifluoromethyl)-1H-pyrazol-1-yl]phenyl}-3,6-dihydro-2H-1,3,4-oxadiazin-2-one or a pharmaceutically acceptable salt thereof.

[0073] It will be understood that the modulators described above are known in the art. The structures are provided for illustrative purposes. Any discrepancy between the structure and the known drug will be resolved in favor of the known drug. The PDE3 modulator may be in the form of a pharmaceutically acceptable salt.

[0074] It is possible for the PDE3 modulators to have systemic and/or local activity. For this purpose, they can be administered in a suitable manner, such as, for example, via the oral, parenteral, pulmonary, nasal, sublingual, lingual, buccal, rectal, vaginal, dermal, transdermal, conjunctival, otic route or as an implant or stent. For these administration routes, it is possible for the compounds according to the disclosure to be administered in suitable administration forms.

[0075] For oral administration, it is possible to formulate the compounds according to the disclosure to dosage forms known in the art that deliver the compounds of the disclosure rapidly and/or in a modified manner, such as, for example, tablets (uncoated or coated tablets, for example with enteric or controlled release coatings that dissolve with a delay or are insoluble), orally-disintegrating tablets, films/wafers, films/lyophilisates, capsules (for example hard or soft gelatine capsules), sugar-coated tablets, granules, pellets, powders, emulsions, suspensions, aerosols or solutions. It is possible to incorporate the compounds according to the disclosure in crystalline and/or amorphised and/or dissolved form into the dosage forms.

[0076] Parenteral administration can be effected with avoidance of an absorption step (for example intravenous, intraarterial, intracardial, intraspinal or intralumbal) or with inclusion of absorption (for example intramuscular, subcutaneous, intracutaneous, percutaneous or intraperitoneal). Administration forms which are suitable for parenteral administration are, inter alia, preparations for injection and infusion in the form of solutions, suspensions, emulsions, lyophilisates or sterile powders.

[0077] Examples which are suitable for other administration routes are pharmaceutical forms for inhalation (*e.g.*, powder inhalers, nebulizers), nasal drops, nasal solutions, nasal sprays; tablets/films/wafers/capsules for lingual, sublingual or buccal administration; suppositories; eye drops, eye ointments, eye baths, ocular inserts, ear drops, ear sprays, ear powders, ear-rinses, ear tampons; vaginal capsules, aqueous suspensions (lotions, mixturae agitandae), lipophilic

suspensions, emulsions, ointments, creams, transdermal therapeutic systems (such as, for example, patches), milk, pastes, foams, dusting powders, implants or stents.

[0078] Diagnostics

[0079] The present disclosure features diagnostic assays for the characterization of cancer. Levels of AIP and/or TRRAP, particularly in connection with levels of PDE3A and/or PDE3B, and levels SLFN12 may be measured in a subject sample and used as an indicator of cancer that is responsive to treatment with a PDE3 modulator. Levels of AIP, TRRAP, PDE3A, PDE3B, or SLFN12 polynucleotides may be measured by standard methods, such as quantitative PCR, Northern Blot, microarray, mass spectrometry, and in situ hybridization. Standard methods may be used to measure levels of AIP, TRRAP, PDE3A, PDE3B, or SLFN12 polypeptides in a biological sample derived from a tumor. Such methods include immunoassay, ELISA, western blotting using an antibody that binds AIP, TRRAP, PDE3A, PDE3B, or SLFN12, and radioimmunoassay. Elevated levels of PDE3A, SLFN12, AIP and/or TRRAP; or PDE3B, SLFN12, AIP and/or TRRAP polynucleotides or polypeptides are considered a positive indicator of a disease, disorder, or condition (*e.g.*, cancer) that is responsive to treatment with a PDE3 modulator.

[0080] Types of biological samples

[0081] In characterizing the responsiveness of a malignancy in a subject to modulation to induce complex formation treatment, the level of AIP, TRRAP, PDE3A, PDE3B, and/or SLFN12 expression is measured in different types of biologic samples. In one embodiment, the biologic sample is a tumor sample.

[0082] In most embodiments, PDE3A and SLFN12 or PDE3B and SLFN12 expression is higher in a sample obtained from a subject that is responsive to PDE3 modulator treatment than the level of expression in a non-responsive subject. In certain implementations, PDE3A, PDE3B, and SLFN12 expression is independently at least about 2, 5, 10, 20, or 30-fold higher in a subject with a malignancy than in a reference condition (*e.g.*, a healthy control). In some embodiments, fold change is determined by calculating the difference in expression of the biomarker (*e.g.*, AIP, TRRAP, PDE3A, PDE3B, SLFN12) in a cancer cell vs the level present in a non-responsive cancer

cell or the level present in a corresponding healthy control cell. Additionally, the present disclosure is partially premised on the discovery that PDE3A-SLFN12 or PDE3B-SLFN12 complex formation (and thus apoptosis of cells) occurs when the cells also express AIP. It has also been discovered that TRRAP is required for sensitivity to DNMDP. Accordingly, in addition to increased PDE3A and SLFN12 biomarkers, the cells responsive to complex formation may express no alteration or loss, minimal alteration or loss, or increased expression of AIP and/or TRRAP expression as compared to a reference. For example, the responsive cells may have more than 50% or more than 60% or more than 70% or more than 80% or more than 90% or more than 100% expression of AIP and/or TRRAP as compared to a reference. In certain embodiments, the cell may be considered to not express AIP and/or TRRAP if the number of copies of the biomarker per cellular genome is less than 1 or less than 2^{-1} or less than or less than 2^{-2} or or less than 2^{-3} or less than 2^{-4} or less than 2^{-5} . Conversely, the cell may be considered to express AIP and/or TRRAP if the number of copies of the biomarker per cellular genome is greater than 1 or greater than 2^{-1} or greater than or greater than 2^{-2} or greater than 2^{-3} or greater than 2^{-4} or greater than 2^{-5} . In certain embodiments, the reference is the average expression level of an indicated biomarker (*e.g.*, PDE3A, PDE3B, SLFN12, AIP, TRRAP) in all cell lines for which data is shown in FIG. 5. In various implementations AIP and/or TRAPP is considered to be expressed in a cell if the expression is greater than the the average expression level of the biomarker in all cell lines for which data is shown in FIG. 5 (*i.e.*, the reference for AIP and TRRAP may be the average expression level of the biomarker in all cell lines for which data is shown in FIG. 5). In some embodiments, PDE3A, PDE3B, and/or SLFN12 is considered to have increased expression in a cell if the expression is greater than a healthy control cell (*i.e.* the reference for PDE3A, PDE3B, and/or SLFN12 may be a healthy control cell). In some embodiments, PDE3A, PDE3B, and/or SLFN12 is considered to have increased expression in a cell if the expression is greater than the average expression levels of the cancer cells in FIG. 5.

[0083] Selection of a treatment method

[0084] As reported herein, subjects suffering from a malignancy may be tested for AIP and/or TRRAP expression in the course of selecting a treatment method or during the treatment method. In some embodiments, patients characterized as having:

(i) AIP and/or TRRAP expression (*e.g.* as determined by the average expression level in cancer cells such as that shown in FIG. 5),

(ii) increased expression of PDE3A or PDE3B relative to a reference (*e.g.*, a healthy cell, a value determined from the average expression level from a healthy sample population, as determined by the average expression level in cancer cells such as that shown in FIG. 5), and

(iii) increased expression of SLFN12 relative to a reference (*e.g.*, a healthy cell, a value determined from the average expression level from a healthy sample population, a value determined from the average expression level in cancer cells for example as determined from the cells measured in FIG. 5);

are identified as responsive to complex formation and PDE3 modulator treatment. For example, those patients characterized as having:

(i) AIP and TRRAP expression (*e.g.* as determined by the average expression level in cancer cells such as that shown in FIG. 5),

(ii) increased expression of PDE3A or PDE3B relative to a reference (*e.g.*, a healthy cell, a value determined from the average expression level from a healthy sample population, a value determined from the average expression level in cancer cells for example as determined from the cells measured in FIG. 5), and

(iii) increased expression of SLFN12 relative to a reference (*e.g.*, a healthy cell, a value determined from the average expression level from a healthy sample population, a value determined from the average expression level in cancer cells for example as determined from the cells measured in FIG. 5);

may be identified as responsive to complex formation and PDE3 modulator treatment. In certain implementations, patients characterized as having:

(i) AIP and/or TRRAP expression (*e.g.* as determined by the average expression level in cancer cells such as that shown in FIG. 5),

(ii) increased expression of PDE3A relative to a reference (*e.g.*, a healthy cell, a value determined from the average expression level from a healthy sample population, a value

determined from the average expression level in cancer cells for example as determined from the cells measured in FIG. 5), and

(iii) increased expression of SLFN12 relative to a reference (*e.g.*, a healthy cell, a value determined from the average expression level from a healthy sample population, a value determined from the average expression level in cancer cells for example as determined from the cells measured in FIG. 5);

are identified as responsive to complex formation and PDE3 modulator (*e.g.*, PDE3A modulator, PDE3B modulator) treatment. Those patients characterized as having:

(i) AIP and TRRAP expression (*e.g.* as determined by the average expression level in cancer cells such as that shown in FIG. 5),

(ii) increased expression of PDE3A relative to a reference (*e.g.*, a value determined from the average expression level from a healthy sample population, a value determined from the average expression level in cancer cells for example as determined from the cells measured in FIG. 5), and

(iii) increased expression of SLFN12 relative to a reference;

may be identified as responsive to complex formation and PDE3 modulator (*e.g.*, PDE3A modulator, PDE3B modulator) treatment. In various implementations, patients characterized as having:

(i) AIP and/or TRRAP expression,

(ii) increased expression of PDE3B relative to a reference, and

(iii) increased expression of SLFN12 relative to a reference;

are identified as responsive to complex formation and PDE3 modulator (*e.g.*, PDE3A modulator, PDE3B modulator) treatment. In some embodiments, patients characterized as having:

(i) AIP and TRRAP expression (*e.g.* as determined by the average expression level in cancer cells such as that shown in FIG. 5),

(ii) increased expression of PDE3B relative to a reference (*e.g.*, a value determined from the average expression level from a healthy sample population, as determined by the average expression level in cancer cells such as that shown in FIG. 5), and

(iii) increased expression of SLFN12 relative to a reference (*e.g.*, a value determined from the average expression level from a healthy sample population, as determined by the average expression level in cancer cells such as that shown in FIG. 5);

are identified as responsive to complex formation and PDE3 modulator (*e.g.*, PDE3A modulator, PDE3B modulator) treatment.

[0085] In certain embodiments for the selection of treatment methods described above the reference is the average expression level of all cell lines for which data is shown in FIG. 5.

[0086] In certain embodiments, the disclosure provides a method for identifying a subject having cancer responsive to treatment with a PDE3 modulator, particularly a compound of WO2019/025562, hereby incorporated by reference in its entirety and specifically in relation to compounds of formula (I) such as those on page 49, line 35-page 75, line 11. In various implementations, the PDE3 modulator may be selected from (6S)-5-[4'-fluoro-2-(trifluoromethyl)biphenyl-4-yl]-6-methyl-3,6-dihydro-2H-1,3,4-oxadiazin-2-one, 5-{4-[1-(difluoromethyl)-1H-pyrazol-4-yl]-3-(trifluoromethyl)phenyl}-3,6-dihydro-2H-1,3,4-oxadiazin-2-one, (6S)-5-[4-(2-aminopyridin-4-yl)-3-(trifluoromethyl)phenyl]-6-methyl-3,6-dihydro-2H-1,3,4-oxadiazin-2-one and (6S)-6-methyl-5-{3-(trifluoromethyl)-4-[3-(trifluoromethyl)-1H-pyrazol-1-yl]phenyl}-3,6-dihydro-2H-1,3,4-oxadiazin-2-one or a salt thereof,

the method comprising

determining

- a) AIP and TRRAP expression,
- b) increased expression of PDE3A or PDE3B relative to a reference (*e.g.*, the level present in a corresponding healthy/control cell or the average expression level of cancer cell lines such as those illustrated in FIG. 5), and

c) increased expression of SLFN12 relative to a reference (e.g., the level present in a corresponding healthy/control cell or the average expression level of cancer cell lines such as those illustrated in FIG. 5);

in a sample; thereby identifying the subject as having a cancer responsive to treatment with a PDE3 modulator.

[0087] In certain embodiments, the method above can be used to identify a subject as having a cancer that is less likely to respond to treatment comprising a PDE3 modulator mentioned herein the method comprising:

- a) determining AIP and/or TRRAP expression, PDE3a or PDE3B expression and SLFN12 expression in a sample from said subject and
- b) identifying the subject as being less likely to respond to treatment comprising a PDE3 modulator when AIP and/or TRRAP are absent.

[0088] The present disclosure also relates to the use of AIP for stratifying in vitro a cancer patient or a sample from a cancer patient disposed to respond treatment with a PDE3 modulator mentioned herein.

[0089] The use of a capture reagent, such as an antibody, that binds to or interacts with AIP for stratifying in vitro a cancer patient or sample from a cancer patient disposed to respond to a PDE3 modulator treatment mentioned herein is contemplated within the present disclosure.

[0090] The use of a capture reagent that binds to or interacts with TRRAP for stratifying in vitro a cancer patient or sample from a cancer patient disposed to respond to a PDE3 modulator treatment mentioned herein is contemplated within the present disclosure as well.

[0091] The use of a capture reagent that binds to or interacts with SLFN12 for stratifying in vitro a cancer patient or sample from a cancer patient disposed to respond to a PDE3 modulator treatment mentioned herein is contemplated within the present disclosure as well.

[0092] The use of a PDE3 modulator mentioned herein for the treatment of cancer in a subject characterized by the expression of AIP, TRRAP and increased expression of PDE3A or PDE3B and SLFN12 is contemplated within the present disclosure as well.

[0093] The cells identified as being responsive to complex formation (*e.g.*, as chemically induced by PDE3 modulation) may be hyperproliferative cells related to a hyperproliferative disease, disorder, or condition. Such identification may be used in the treatment and/or prevention of various hyperproliferative diseases, disorders, or conditions, such as a myeloproliferative disorder or cancer. In specific embodiments, the cell may be a cancer cell. In some implementations, the may be a cancer cell selected from bladder-, brain-, breast-, cervical-, colorectal-, endometrial-, esophageal-, gallbladder-, gastric-, glioblastoma-, kidney-, leukemia- (*e.g.*, acute myelogenous leukemia-, chronic myelogenous leukemia-, chronic lymphocytic leukemia-), liver- (*e.g.*, hepatocellular carcinoma-, intrahepatic cholangiocarcinoma-, angiosarcoma-, hemangiosarcoma-, hepatoblastoma-), lung- (*e.g.*, non-small cell lung cancer-, small cell lung cancer-, mesothelioma-), melanoma-, ovarian-, pancreatic-, prostate-, multiple myeloma-, sarcoma- (*e.g.*, osteosarcoma-, soft-tissue sarcoma-), thyroid-, urinary tract-, uterine cancer cells. In certain implementations the cell may be a hematopoietic cancer cell, such as acute lymphoblastic leukemia-, acute myelogenous leukemia-, chronic lymphocytic leukemia-, chronic myelogenous leukemia-, acute monocytic leukemia-, Hodgkin's lymphoma-, or non-Hodgkin's lymphoma cells. Other hyperproliferative disease, disorder, or conditions considered within the scope of the disclosure include myeloproliferative diseases, such as essential thrombocytosis.

[0094] Kits

[0095] The disclosure provides kits for characterizing the responsiveness of a subject to complex formation and PDE3 modulator treatment.

[0096] In certain embodiments, the kit may include a therapeutic composition containing an effective amount of a PDE3 modulator (*e.g.*, PDE3A modulator, PDE3B modulator) in unit dosage form.

[0097] In certain implementations, a diagnostic kit of the disclosure provides one or more reagents for measuring expression of AIP, TRRAP, PDE3A, PDE3B, SLFN12, and combinations thereof. Such reagents include one or more capture molecules (*e.g.*, antibodies that recognize a polypeptide selected from AIP, TRRAP, PDE3A, PDE3B or SLFN12). In some embodiments, the kit comprises a reagent for measuring the expression of AIP, a reagent for measuring the

expression of PDE3A, and a reagent for measuring the expression of SLFN12. In some embodiments, the kit comprises a reagent for measuring the expression AIP, a reagent for measuring the expression of PDE3B, and a reagent for measuring the expression of SLFN12. In some embodiments, the kit comprises a reagent for measuring the expression of TRRAP, a reagent for measuring the expression of PDE3A, and a reagent for measuring the expression of SLFN12. In some embodiments, the kit comprises a reagent for measuring the expression TRRAP, a reagent for measuring the expression of PDE3B, and a reagent for measuring the expression of SLFN12. In some embodiments, the kit comprises a reagent for measuring the expression of AIP, a reagent for measuring the expression of TRRAP, a reagent for measuring the expression of PDE3A, and a reagent for measuring the expression of SLFN12. In some embodiments, the kit comprises a reagent for measuring the expression AIP, a reagent for measuring the expression of TRRAP, a reagent for measuring the expression of PDE3B, and a reagent for measuring the expression of SLFN12. In some embodiments, the kit comprises a reagent for measuring the expression AIP, a reagent for measuring the expression of TRRAP, a reagent for measuring the expression of PDE3A, a reagent for measuring the expression of PDE3B, and a reagent for measuring the expression of SLFN12.

[0098] In some embodiments the kit comprises a PDE3 modulator mentioned herein together with reagents for measurement of expression of AIP, TRRAP, PDE3A or PDE3B, and SLFN12.

[0099] The kit may comprise a sterile container which contains a therapeutic or diagnostic composition—such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. In certain implementations, the container may be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments. If desired, the kit further comprises instructions for measuring biomarker (*e.g.*, PDE3A, PDE3B, SLFN12, TRRAP, AIP) expression and/or instructions for administering the PDE3 modulator to a subject having a malignancy, *e.g.*, a malignancy selected as responsive to PDE3A modulator treatment. In particular embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of malignancy or symptoms thereof; precautions; warnings; indications; counter-indications; over dosage information; adverse reactions; animal pharmacology; clinical

studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

[0100] The practice of the present disclosure employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. These techniques are applicable to the production of the polynucleotides and polypeptides of the disclosure, and, as such, may be considered in making and practicing the disclosure. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

[0101] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the disclosure, and are not intended to limit the scope of what the inventors regard as their disclosure.

EXAMPLES

[0102] The following examples illustrate specific aspects of the instant description. The examples should not be construed as limiting, as the example merely provides specific understanding and practice of the embodiments and its various aspects.

[0103] Example 1: Profiling for Sensitivity to PDE3A modulation

[0104] To measure cancer cell death in response to 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) treatment, cells were plated in 384w assay plates at the following cell density per well: 500 cells of HeLa (DMEM), A2058 (DMEM), HMCB (EMEM), IGR37 (DMEM), NCIH1734 (RPMI), 750 cells of CAL51 (DMEM), COLO741 (RPMI), DKMG (RPMI), GB1 (EMEM), HEL (RPMI), HEL9217 (RPMI), JHUEM1 (DMEM+F12), L3.3 (RPMI) and TE4 (RPMI), HCC15 (RPMI), UACC257 (RPMI), 1000 cells for HUT78 (IMEM), NCIH1563 (RPMI), NCIH2122 (RPMI), NCIH2172 (RPMI), RVH421 (RPMI) and SKMEL3 (McCoy's 5A), 1500 cells for C32 (EMEM), HS578T (DMEM) and JHOM1 (DMEM+F12). Cells were incubated at 37°C overnight and then treated with a DNMDP dose dilution series using an HP D300 digital dispenser. After 72 hours, the viability of cells in

each well were measured by Cell Titer Glo (Promega G755B and G756B). Percent viability values were determined using the values from untreated wells and AUC values were calculated using a 4-parameter fit. DNMDP was purchased from Life Chemicals (F1638-0042) and trequinsin was purchased from Sigma-Aldrich (T2057).

[0105] 1400 cells per well were seeded in a 96 well plate in media that had been centrifuged at $500 \times g$ for 5 min to remove particulates. The next day, the red fluorescent DNA-staining dye, Incucyte Nuclite Rapid Red, and green fluorescent apoptosis dye, Incucyte Caspase-3/7 Green Apoptosis Reagent (Essen Biosciences), were added in 2 μ l FBS to a final concentration of 1:1000 and 1:1500, respectively. Two hours later, [2 μ M DNMDP + 0.2% DMSO] or 0.2% DMSO was added. Because even sensitive cells sometimes divided before 24 hours, cells were tracked starting at 24 hours, although cells that apoptosed before 24 hours were also counted. For the washout study, the media was removed from DNMDP treated cells at 72 h, the cells were rinsed with media, and incubation was continued in the absence of DNMDP. Cells were tracked starting at 72 h. Images were taken every 1 h up to 96 h, and every 2 h thereafter, with an Incucyte S3 machine (Essen Biosciences). Three channels were recorded: phase contrast, red fluorescence (DNA), and green fluorescence (apoptosis). For cell tracking, a movie superimposing all three channels was analyzed. To avoid effects due to depletion of media components over time, cells were followed up to the last hour before DMSO control cells started to show slowed division or increased apoptosis (136 h for HeLa, 194 h for SKMEL3, 160 h for GB1, 130 h for TE4, 130 h for A2058, 144 h for DKMG, 106 h for HS578T, 186 h for H2172, 220 h for C32).

[0106] Melanoma cell lines were tested for sensitivity to DNMDP and the biomarkers of these cells were identified. Biomarker expression thresholds were optimized for positive predictive value and sensitivity. Of the 49 melanoma cell lines tested, seven expressed elevated levels of PDE3A and SLFN12 and one expressed elevated levels of the related protein PDE3B. FIG. 1 compares the reads per kilobase of transcript per million mapped reads (RKPM) for PDE3A and SLFN12 across hundreds of cancer cell lines and identifies the biomarker positive cell lines. Table 1 illustrates the biomarker expression and DNMDP response for 7 melanoma cell lines among the biomarker-positive cell lines in FIG. 1. The eighth sensitive melanoma cell line, RVH421, expressed elevated levels of the related protein, PDE3B. In Table 1, the biomarker mRNA levels

expressed as $\log_2(\text{RPKM}+1)$ and the area under the curve (AUC) is calculated on a scale from 0-4. Previous analysis of sensitivity data defined the positive predictive value (PPV) of the combined SLFN12/PDE3A biomarker to be about 50%, but the optimized biomarker thresholds result in a PPV of 62%, with sensitive defined by an AUC of less than 2.8, equivalent to an AUC of 1.6 on a scale of 0-4.

Table 1

Cell Line	Lineage	PDE3A expression	PDE3B expression	SLFN12 expression	AUC
HeLa	cervical	5.65	1.68	2.85	0.36
IGR37	melanoma	5.29	1.18	2.50	1.17
COLO741	melanoma	4.30	1.20	2.41	1.19
SKMEL3	melanoma	2.69	1.28	2.96	1.74
HMCB	melanoma	3.82	1.72	2.09	1.85
A2058	melanoma	4.64	1.32	2.02	2.11
C32	melanoma	2.97	1.32	3.37	3.13
UACC257	melanoma	4.90	1.95	2.39	4.00
RVH421	melanoma	0.16	2.66	2.16	3.02

[0107] All but one of these eight melanoma cell lines were sensitive to DNMDP. FIG. 2A illustrates the dose response of Cell Titer-Glo assay measurements on these eight cell lines with administration of DNMDP, whereas FIGS. 2B and 2C illustrate the response of all tested PDE3A/SLFN12 biomarker-positive cell lines from multiple disease indications (RVH421 is PDE3B/SLFN12 biomarker-positive). As can be seen in FIG. 2A, C32 and RVH421 are only partially sensitive, and UACC257 cells are completely insensitive. Further experimentation has linked the UACC257 insensitivity to its lack of AIP expression (*see, e.g., Example 3*). Additionally, it can be seen that sensitivity is not binary; rather dose response curves showed a continuous gradient of inhibition. Based on maximum viability values, the tested cell lines could be split into strongly sensitive cell lines (13 cell lines with <25% maximum viability, FIG. 2B), partially sensitive lines (7 cell lines with 25-75% maximum viability; FIG. 2C), and insensitive cell lines (2 cell; lines with 100% maximal viability; FIG. 2C).

[0108] Example 2: CRISPR Knockout

[0109] PDE3A CRISPR KO cells (sgRNA#2) were generated according to de Waal et al, 2016 (2), hereby incorporated by reference in its entirety. CRISPR target sites for PDE3B and AIP were identified using the CHOPCHOP CRISPR Design Tool (chopchop.cbu.uib.no). For cloning of sgRNAs, forward and reverse oligos were annealed, phosphorylated and ligated into a BsmBI-digested lentiCRISPRv2 vector. Lentivirus carrying each guide construct was packaged as described above and used to infect target cells. Transduced target cells were selected using 1 µg/ml puromycin and passaged for 7 days before use.

[0110] FIG. 3A illustrates the survival of cells in the presence of DNMDP that have had CRISPR knockout of SLFN12. FIG. 3B illustrates the survival of cells in the presence of DNMDP that have had CRISPR knockout of PDE3A (with and without ectopic expression of PDE3B). As can be seen, SLFN12 knockout abrogates all measured sensitivity to PDE3 modulators. Moreover, PDE3B can support DNMDP sensitivity in the absence of PDE3A expression, and ectopic expression of PDE3B can furthermore support DNMDP sensitivity in PDE3A knockout HeLa cells. The survival in FIGS. 3A and 3B was measured with a 72 h Cell Titer-Glo assay and CRISPR was performed with sg4, SLNF12 CRISPR guide RNA #4.

[0111] In a genome wide CRISPR screen, genes were identified as important for cancer cell killing in HeLa cells. The Brunello CRISPR library was used for the DNMDP resistance screen. Lentiviral infection was carried out in duplicate and for each replicate with enough HeLa cells to achieve >1000 infected cells per library member (80000 sgRNAs, $>8 \times 10^7$ cells total) and at low multiplicity of infection (MOI) to achieve transduction of a single sgRNA per cell. Infection efficiencies for the two replicates were 24% and 31% respectively, corresponding to a MOI of about 0.3, meaning about 85% of infected cells would be predicted to have single sgRNA integration. At the time of infection, HeLa cells were resuspended in media and mixed with Brunello library virus in the presence of 8 µg/ml polybrene (library lentivirus provided by the Genetic Perturbation Platform at the Broad Institute), plated in 12 well dishes at 3×10^6 cells per well, and spun at $931 \times g$ for 2 h at 30°C. 2 h after the spin infection, virus-containing media was removed and fresh media was added for incubation overnight. The day after the infection, cells were trypsinized and pooled into T225 flasks at 50% confluence (1.6×10^7 cells per flask) and

puromycin was added to 1 $\mu\text{g/ml}$ to select for infected cells. At the same time, in-line infection efficiency assays were performed by comparing cell counts after puromycin selection to those without selection. After 4 days of puromycin selection, infected cells were collected and passaged in T225 flasks at 25% confluence (8×10^6 cells per flask) for three additional days to allow CRISPR KO to complete. Cells were collected at 8 days after infection, and 8×10^7 cells each were split into DMSO control arm (plating at 8×10^6 cells per T225 flask) or 25 nM DNMDP treatment arm (plating at 2×10^7 cells per T225 flask). Cells were passaged every 3 to 4 days at 25% confluence for the next 14 days. For the DMSO arm, 8×10^7 cells were maintained at every passage, whereas all surviving cells were passaged for the DNMDP arm. After 14 days of compound treatment, cells were harvested, washed with cold PBS and flash frozen at 2×10^7 cells (DMSO arm) or less portions for genomic DNA isolation. Genomic DNA was isolated using the Nucleospin Blood XL kit (DMSO samples, 4 preps to cover 8×10^7 cells, Machere-Nagel 740950.50) or the QIAamp DNA Blood Mini kit (DNMDP-treated samples, Qiagen 51104). PCR amplification of sgRNA tags and pooled library sequencing were carried out as described in Sanson, et al. "Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities" *Nat Commun* 9(1):5416 ("Sanson"), hereby incorporated by reference in its entirety.

[0112] CRISPR screen data analysis was done largely as described in Sanson. Briefly, deconvolution of sequencing reads yielded read counts for each sgRNA under each replicate treatment condition. Log2-Normalized-Reads for each guide per condition was calculated using the formula $\log_2(\text{guide}/\text{total} \times 1000000 + 1)$ and averaged across the two replicates. Subtracting DMSO values from those for 25 nM DNMDP generated Log-Fold-Change values for each sgRNA, which were then averaged across all sgRNAs targeting the same gene to generate gene-level Average-Log-Fold-Change score. To statistically evaluate gene-level enrichment in DNMDP treatment relative to DMSO, sgRNAs were rank ordered based on Average-Log-Fold-Change, and p-values for each sgRNA relative to the rank order were determined by running a hypergeometric distribution without replacement, equivalent to a one-sided Fisher's exact test. The average of the negative $\log_{10}$ p values for each sgRNA targeting the same gene was calculated to generate the average negative $\log_{10}$ p-value for each gene. A volcano plot was generated using the average-

log₂-fold-change and the average negative log₁₀ p-value for all genes with 3 to 8 sgRNAs per gene to visualize gene enrichments after the positive selection of 25 nM DNMDP treatment.

[0113] AIP, SLFN12, and PDE3A knockout cause the greatest increase in cell survival in the presence of 25 nM DNMDP. FIG. 4 illustrates the results of the CRISPR screen comparing the log fold change (LFC) of gene CRISPR guide representation as compared to -log(p-value) of the screen (each as compared to HeLa cells). The gRNA best supporting survival in the presence of DNMDP was specific for the AIP. As expected, SLFN12 and PDE3A knockout also strongly supported cell survival in the presence of DNMDP, ranking second and third behind AIP, respectively. Knockout of the histone acetyltransferase complex protein, transformation/transcription domain associated protein (TRRAP), exhibited as significant but much weaker phenotype as well.

[0114] The CRISPR screen allowed for identification of the aryl hydrocarbon receptor interacting protein as having potent effect on cell survival rates. AIP is a co-chaperone protein that regulates stability and subcellular localization of the aryl hydrocarbon receptor and other proteins, as described in Trivellin, G. and M. Korbonits, *J Endocrinol* 210 (2011): 137-55, hereby incorporated by reference in its entirety. Of the cancer cell lines tested, only a single cancer cell line, UACC257, the biomarker-positive but DNMDP-resistant melanoma cell line lacked AIP expression. This can be seen in FIG. 5 where the x axis is plotted by log₂ copy number, and the y axis by gene expression. As can be seen, UACC257 does not express AIP while the remaining cell lines do.

[0115] Furthermore, that AIP knockout eliminates HeLa cell response to DNMDP was validated with independent gRNAs as shown in FIG. 6A. Similar results were observed upon knockout of AIP from the melanoma cell line A2058 (FIG. 6B). Additionally, AIP knockout in these cell lines resulted in decreased PDE3A protein expression (FIG. 6C).

[0116] Example 3: AIP Knockout and Complex Formation

[0117] Because decreased PDE3A protein expression could impact DNMDP-induced PDE3A-SLFN12 complex formation, the effects of AIP knockout on this complex formation were also measured. As there is no good antibody for SLFN12 protein, we ectopically expressed V5-tagged

SLFN12 in parental or AIP-knockout HeLa cells, immunoprecipitated endogenous PDE3A, and assessed whether V5-SLFN12 could be detected in the immunoprecipitates. Cells were plated in 10 cm petri dishes and collected at 50-90% confluence. For PDE3A immunoblotting in biomarker positive cells and in AIP KO cells, cells were seeded in 15cm plates at similar density as in viability assays with a vessel scaling factor of 5000, e.g., 500 cells per well was scaled to 10^6 cells per 10cm plate or 2.5×10^6 cells per 15cm plate, and then cultured for 72 hours before collection. Cell pellets were lysed at 4°C for 20 minutes in modified RIPA buffer (150 mM NaCl, 10% glycerol, 50 mM Tris-Cl pH 8.0, 50 mM MgCl₂, 1% NP-40) supplemented with EDTA-free protease inhibitors (Sigma-Aldrich 4693159001) and PhosSTOP phosphatase inhibitors (Sigma-Aldrich 4906837001). Lysates were clarified by centrifugation at 13,000 rpm $\times$ 10 min at 4°C and quantified using BCA protein assays (Thermo Fisher Scientific 23225). Clarified lysates were resolved on 4–12% Bis-Tris PAGE gels, transferred to nitrocellulose membranes (Thermo Fisher Scientific IB23001) and immunoblotted with primary antibodies against PDE3A (Bethyl 302-740A, 1:2000), V5 (Life Technologies R96205 at 1:5,000), AIP (Thermo Fisher Scientific MA3-16515 at 1:2000), Vinculin (Sigma-Aldrich V9264 at 1:5,000), GAPDH (Cell Signaling Technology 2118 at 1:2000) and secondary antibodies from LiCOR Biosciences (92632210 and 926068021, each at 1:10,000). Blots were washed and imaged using a LiCOR Odyssey infrared imager, and fluorescent signals quantified using the Image Studio software provided by the LiCOR manufacturer.

[0118] Genomic DNA was isolated from cells using QIAamp DNA mini kit (Qiagen 51304) and SLFN12 genomic region was amplified by PCR using Q5 High-Fidelity 2X Master Mix (New England Biolabs M0492) and primers SLFN12_2_F or SLFN12_428_F and SLFN12_858_R. PCR products were purified using QIAquick PCR Purification Kit (Qiagen 28104) and send for sequencing using Forward or Reverse primers used for PCR. Sequencing reads were aligned to reference sequence using Benchling alignment tools.

[0119] AIP knockout completely abolished PDE3A-SLFN12 complex formation in response to DNMDP (FIG. 7), confirming that AIP functions upstream of DNMDP-induced complex formation. The partial decrease in PDE3A protein levels shown in FIG. 6C cannot fully account for the effects of AIP knockout on PDE3A-SLFN12 complex formation. For example, A549 cells,

which express sufficient endogenous PDE3A to support partial sensitivity to complex inducing active compounds upon ectopic expression of SLFN12, express similar levels or even less PDE3A than these AIP knockout cells.

[0120] AIP was required for cancer cell killing in response to Compound X (also termed (6S)-5-[4'-fluoro-2-(trifluoromethyl)biphenyl-4-yl]-6-methyl-3,6-dihydro-2H-1,3,4-oxadiazin-2-one) (FIG. 8A). Cervical cancer cell viability was reduced at increasing concentrations of Compound X (FIG. 8A). Cancer cell killing required AIP, as no cancer cell killing was observed in HeLa cells lacking AIP, i.e., HeLa cells having an AIP knock out. AIP knockout also abolished PDE3A-SLFN12 complex formation in response to treatment with Compound X as shown at FIG. 8B. In FIG. 8B an anti-PDE3A antibody was used to pull down a PDE3A-SLFN12 complex. Complex formation between PDE3A and FLAG-tagged SLFN12 was induced in the presence of Compound X and in the presence of DNMDP. Complex formation was not observed when AIP was knocked out using Crispr (KO sg2, KO sg3). HeLa cells were treated with 10 μ M DNMDP or 10 μ M Compound X. These results indicate that AIP can act as a marker for responsiveness to Compound X.

[0121] As various changes can be made in the above-described subject matter without departing from the scope and spirit of the present disclosure, it is intended that all subject matter contained in the above description, or defined in the appended claims, be interpreted as descriptive and illustrative of the present disclosure. Many modifications and variations of the present disclosure are possible in light of the above teachings. Accordingly, the present description is intended to embrace all such alternatives, modifications and variances which fall within the scope of the appended claims.

CLAIMS

1. A method of identifying a subject having a hyperproliferative disease, disorder, or condition responsive to PDE3A-SLFN12 complex formation or PDE3B-SLFN12 complex formation are provided comprising obtaining one or more cells of the hyperproliferative disease, disorder, or condition from the subject and detecting:

(i) the expression of aryl hydrocarbon receptor interacting protein (AIP) polypeptides or polynucleotides and/or transformation/transcription domain associated protein (TRRAP) polypeptides or polynucleotides in the cell;

(ii) the expression of phosphodiesterase 3A (PDE3A) polypeptides or polynucleotides or the expression of phosphodiesterase 3B (PDE3B) polypeptides or polynucleotides in the cell relative to a reference, and

(iii) the expression of Schlafen family member 12 (SLFN12) polypeptides or polynucleotides in the cell relative to the reference;

wherein said hyperproliferative disease, disorder, or condition is characterized as responsive to said complex formation if:

(i) AIP and/or TRRAP are expressed in the cell,

(ii) the expression of PDE3A and/or PDE3B is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference.

2. The method according to claim 1, wherein said method comprises detecting:

(ii) the expression of PDE3A polypeptides or polynucleotides in the cells relative to a reference, and

the disease, disorder, or condition characterized as responsive to is selected as responsive to PDE3A-SLFN12 complex formation if:

(i) AIP and/or TRRAP are expressed in the cell,

(ii) the expression of PDE3A is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference.

3. The method according to claim 1, wherein said method comprises detecting:

(ii) the expression of PDE3B polypeptides or polynucleotides, and

the disease, disorder, or condition characterized as responsive to is selected as responsive to PDE3B-SLFN12 complex formation if:

(i) AIP and/or TRRAP are expressed in the cell,

(ii) the expression of PDE3B is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference.

4. The method according to any one of claims 1-3 comprising the detection of AIP and TRRAP expression in the cell, wherein the hyperproliferative disease, disorder, or condition is characterized as responsive to said complex formation if:

(i) AIP and TRRAP are expressed,

(ii) the expression of PDE3A and/or PDE3B is increased relative to the reference, and

(iii) the expression of SLFN12 is increased relative to the reference.

5. A method of killing or reducing the survival of a cell selected as responsive to PDE3A-SLFN12 complex formation or PDE3B-SLFN12 complex formation comprising contacting said cell with a PDE3 modulator, wherein said cell is selected as responsive to said PDE3 modulator when said cell:

(i) expresses AIP and/or TRRAP polypeptides or polynucleotides,

(ii) has increased expression of PDE3A and/or PDE3B polypeptides or polynucleotides relative to a reference, and

(iii) has increased expression of SLFN12 polypeptides or polynucleotides relative to the reference.

6. The method according to claim 5, wherein said cell is selected as responsive to said PDE3 modulator when said cell:

(i) expresses AIP and TRRAP polypeptides or polynucleotides,

(ii) has increased expression of PDE3A and/or PDE3B polypeptides or polynucleotides relative to a reference, and

(iii) has increased expression of SLFN12 polypeptides or polynucleotides relative to the reference.

7. The method according to claim 5, wherein said cell is selected as responsive to said PDE3 modulator when said cell:

(i) expresses AIP and TRRAP polypeptides or polynucleotides,

(ii) has increased expression of PDE3A polypeptides or polynucleotides relative to a reference, and

(iii) has increased expression of SLFN12 polypeptides or polynucleotides relative to the reference.

8. The method according to claim 5, wherein said cell is selected as responsive to said PDE3 modulator when said cell:

(i) expresses AIP and TRRAP polypeptides or polynucleotides,

(ii) has increased expression of PDE3B polypeptides or polynucleotides relative to a reference, and

(iii) has increased expression of SLFN12 polypeptides or polynucleotides relative to the reference.

9. A method for the treatment or prevention of a hyperproliferative disease, disorder, or condition in a subject comprising administering to said subject a PDE3 modulator, wherein said subject is identified as having a hyperproliferative disease, disorder, or condition that is responsive to the PDE3 modulator with the method according to any one of claims 1-4.

10. The method of any one of claims 5-9, wherein the PDE3 modulator is 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (DNMDP) or (6S)-5-[4'-fluoro-2-(trifluoromethyl)biphenyl-4-yl]-6-methyl-3,6-dihydro-2H-1,3,4-oxadiazin-2-one (Compound X).

11. The method of any one of claims 1-10, wherein the expression of AIP polynucleotide or TRRAP polynucleotide is detected by a method selected from the group consisting of quantitative PCR, Northern Blot, microarray, mass spectrometry, immunoblotting, immunohistochemistry, ELISA, and in situ hybridization.
12. The method of any of claims 1-11, wherein the increase in the level of SLFN12 polynucleotide is detected by a method selected from the group consisting of quantitative PCR, Northern Blot, microarray, mass spectrometry, immunoblotting, immunohistochemistry, ELISA, and in situ hybridization.
13. The method of any of claims 1-12, wherein the increase in the level of PDE3A and/or PDE3B polynucleotide is detected by a method selected from the group consisting of quantitative PCR, Northern Blot, microarray, mass spectrometry, immunoblotting, immunohistochemistry, ELISA, and in situ hybridization.
14. The method of any one of claims 1-13, wherein said hyperproliferative disease, disorder, or condition is a myeloproliferative disease.
15. The method of any one of claims 1-13, wherein said cell is a cancer cell.
16. The method of claim 15, wherein said cancer cell is a melanoma-, endometrium-, lung-, hematopoietic-/lymphoid-, ovarian-, cervical-, soft-tissue- sarcoma-, urinary tract, pancreas-, thyroid-, kidney-, glioma-, glioblastoma-, or breast-cancer cell.
17. The method of claim 15, wherein said cancer cell is a melanoma-, glioma-, glioblastoma-, ovarian-, sarcoma-, acute myeloid leukemia-, or lung adenocarcinoma cell.
18. The method of any one of claims 5-17, wherein said PDE3 modulator is administered orally.
19. The method of any one of claims 5-17, wherein said PDE3 modulator is administered by intravenous injection.
20. The method of any of claims 1-19, wherein said cancer cell is collected from a tissue sample, a blood sample, or a plasma sample.

21. A kit for identifying a subject having cancer as responsive to PDE3A-SLFN12 complex formation or PDE3B-SLFN12 complex formation, the kit comprising a first capture reagent that binds AIP polypeptide and/or a second capture reagent that binds TRRAP polypeptide.
22. The kit according to claim 21, wherein said kit comprises a third capture reagent that binds PDE3A polypeptide and/or a fourth capture reagent that binds PDE3B polypeptide and a fifth capture reagent that binds SLFN12 polypeptide.
23. The kit according to claim 21, wherein said kit comprises a third capture reagent that binds PDE3A polypeptide or a fourth capture reagent that binds PDE3B polypeptide and a fifth capture reagent that binds SLFN12 polypeptide.
24. The kit according to claim 21, wherein said kit further comprises a third capture reagent that binds SLFN12 polypeptide.

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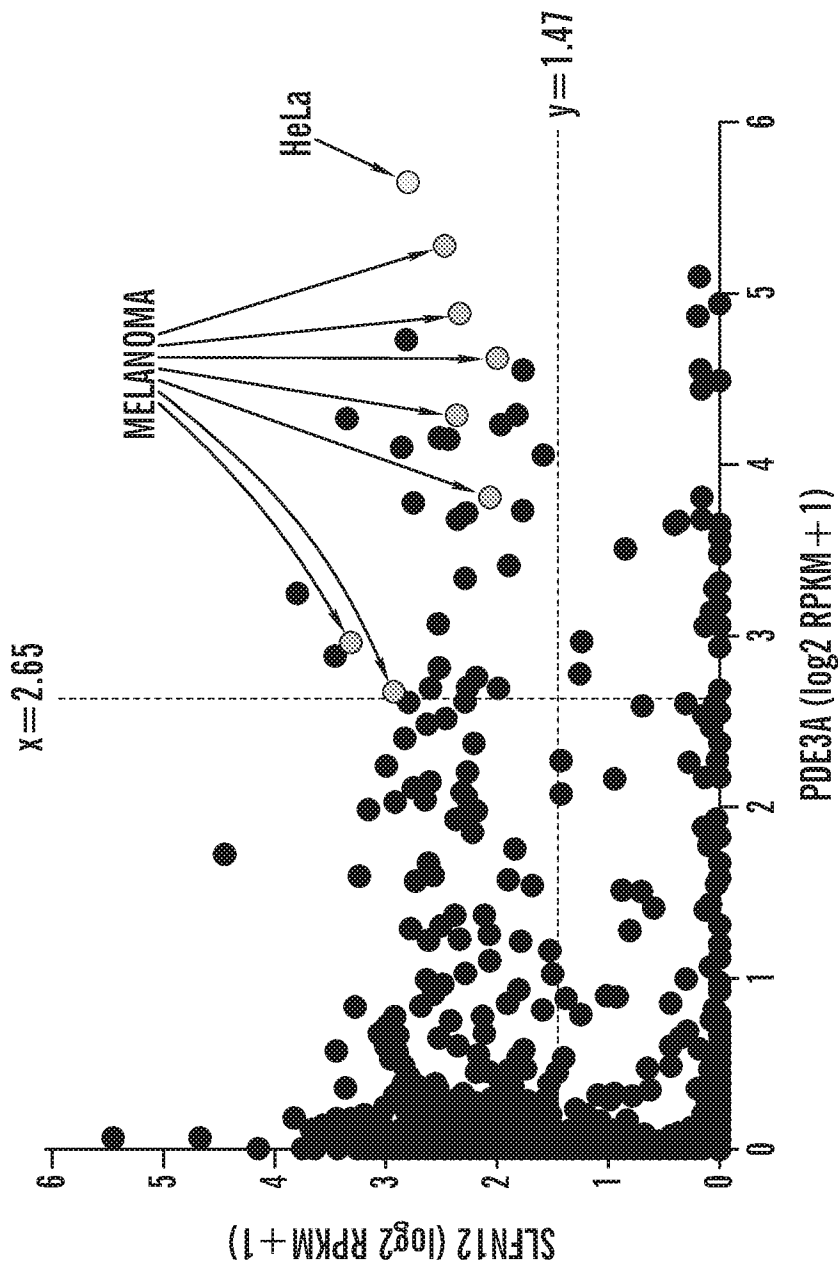


FIG. 1

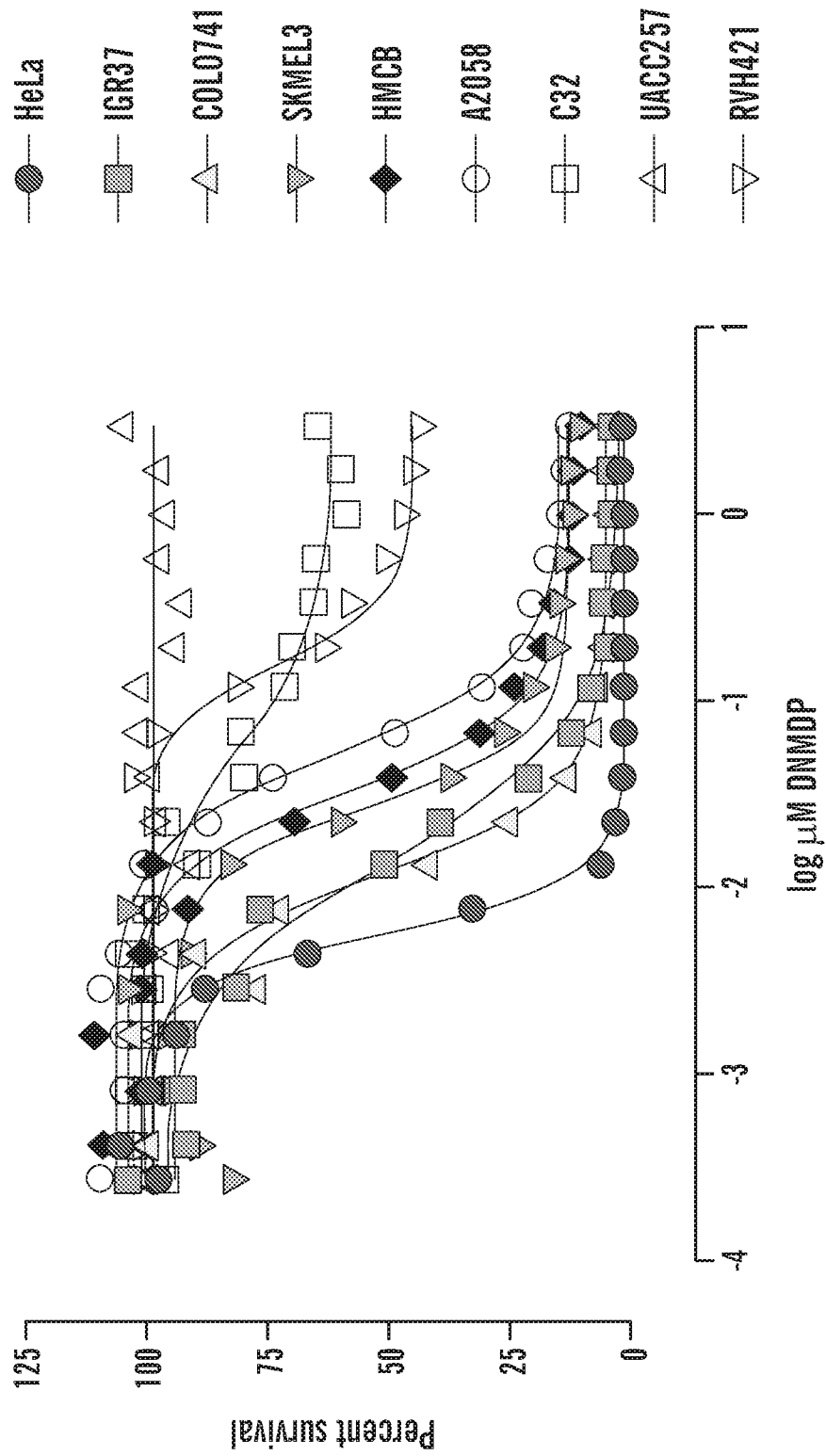


FIG. 2A

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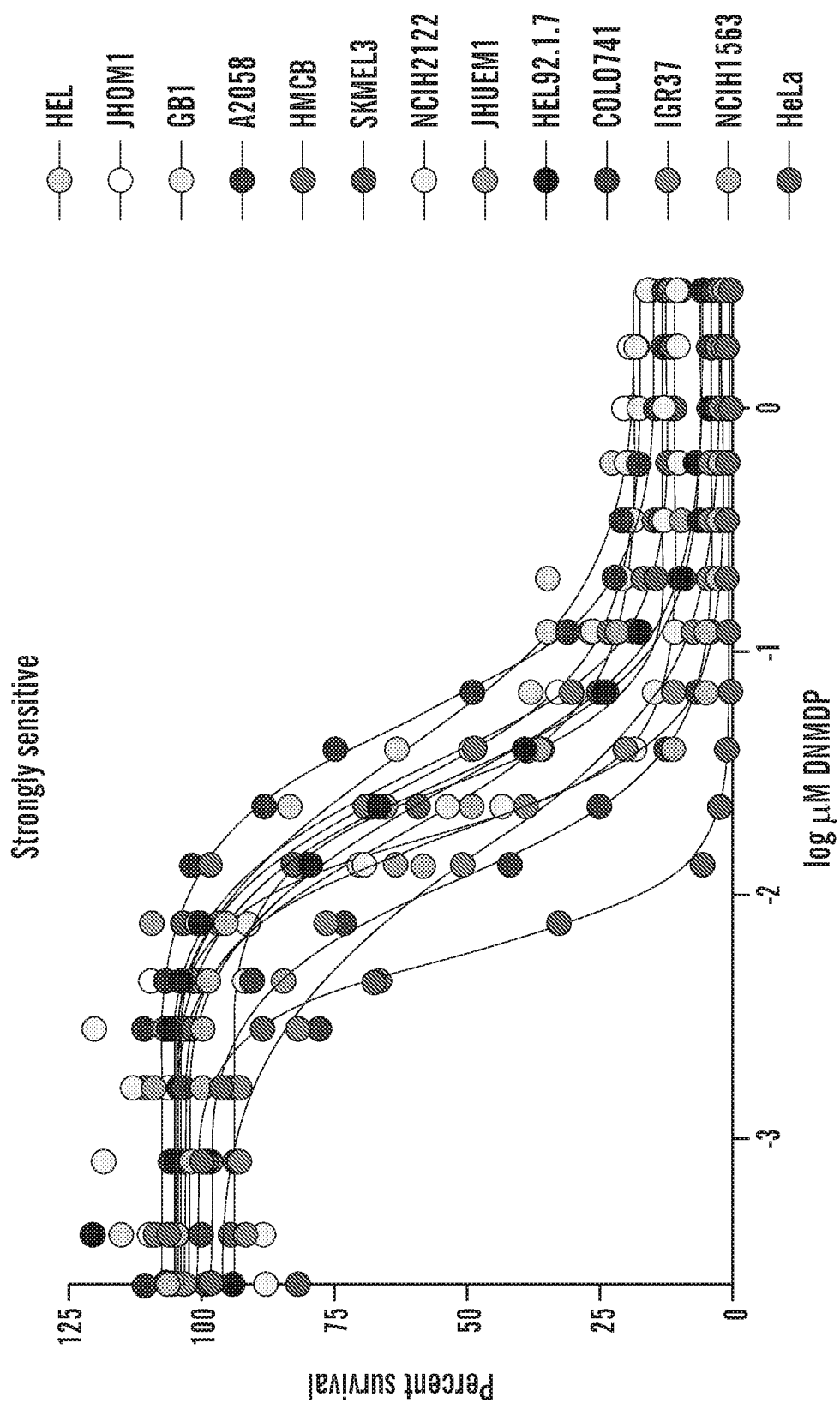
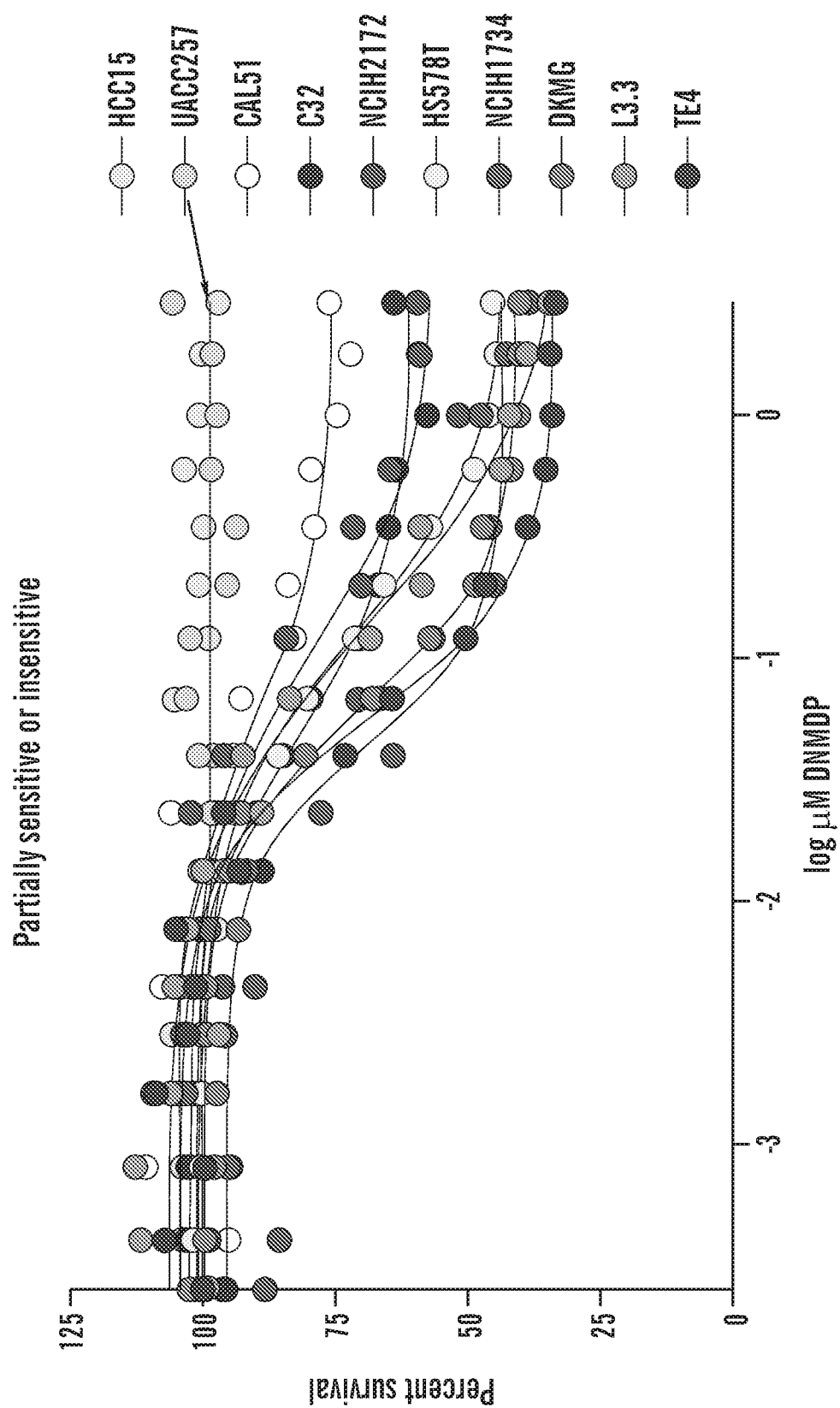


FIG. 2B

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**FIG. 2C**

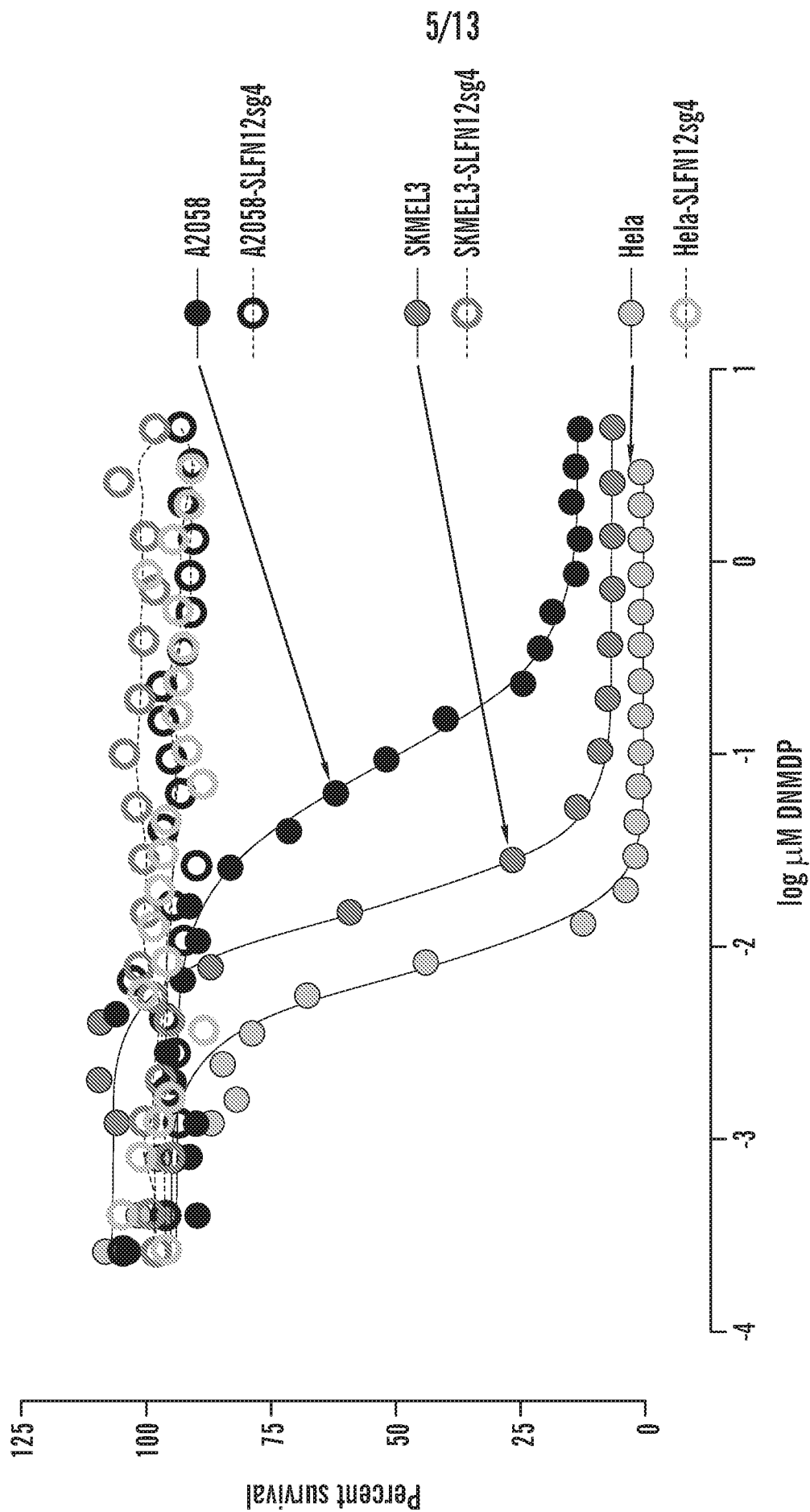


FIG. 3A

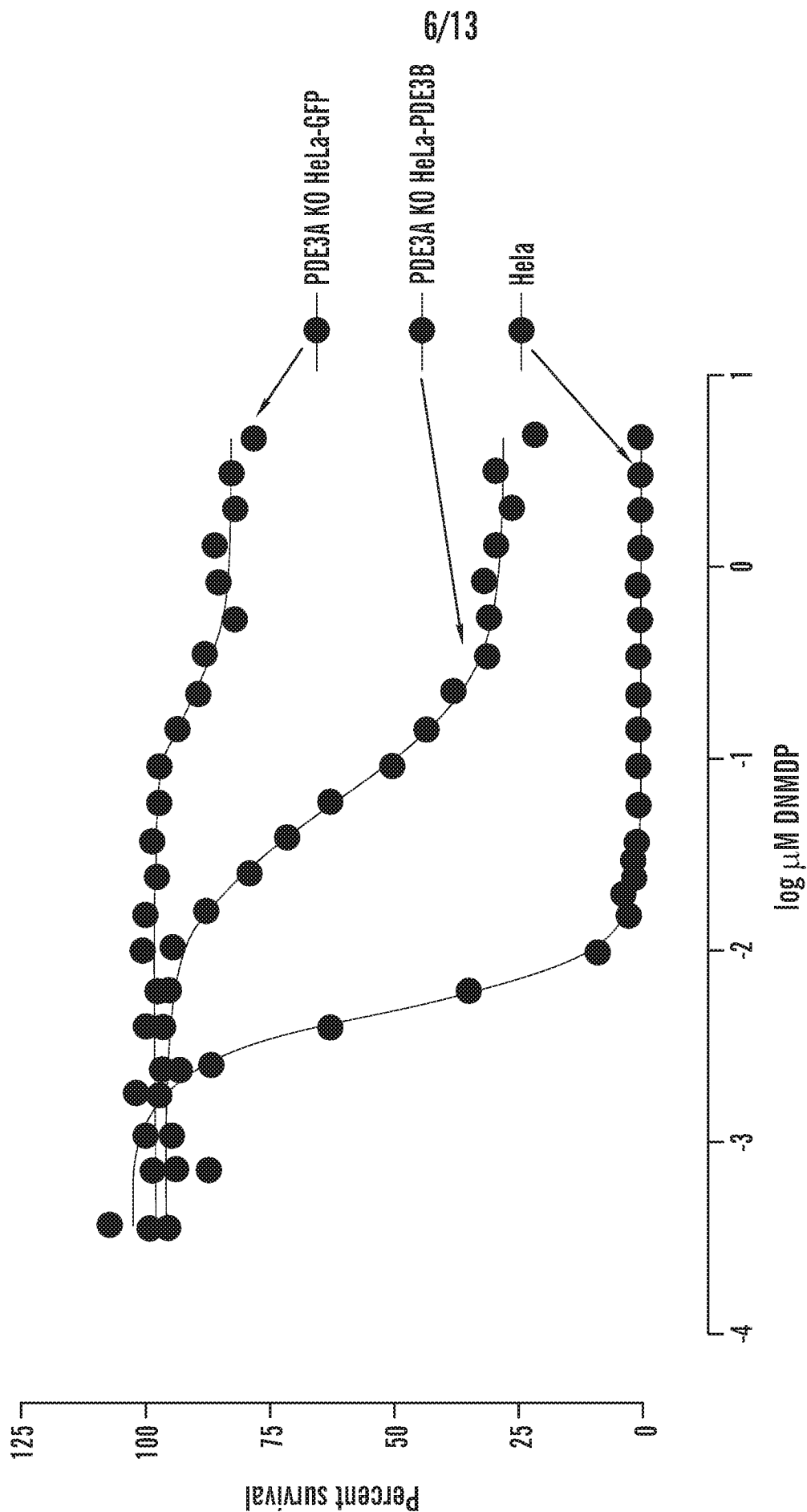


FIG. 3B

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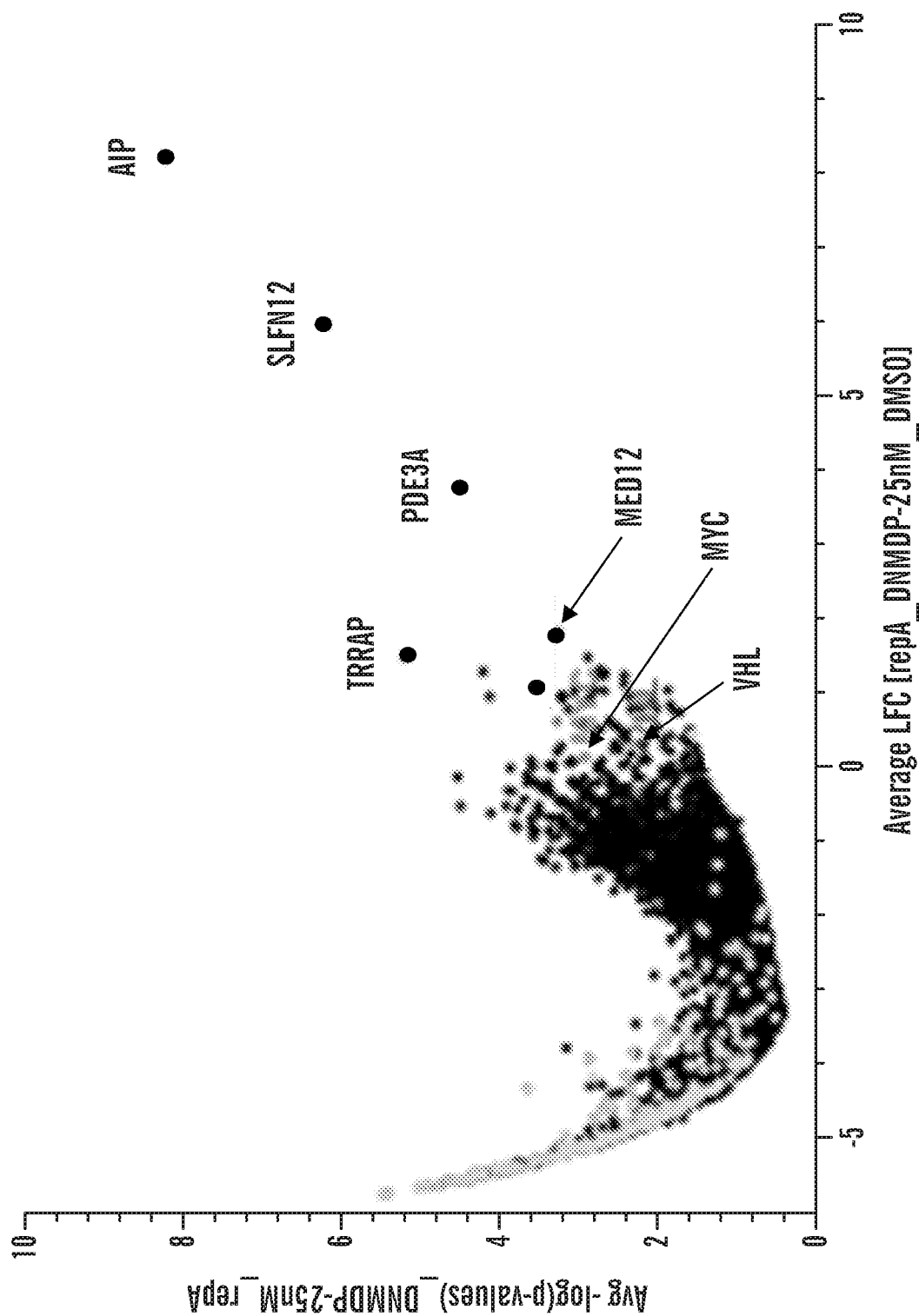


FIG. 4

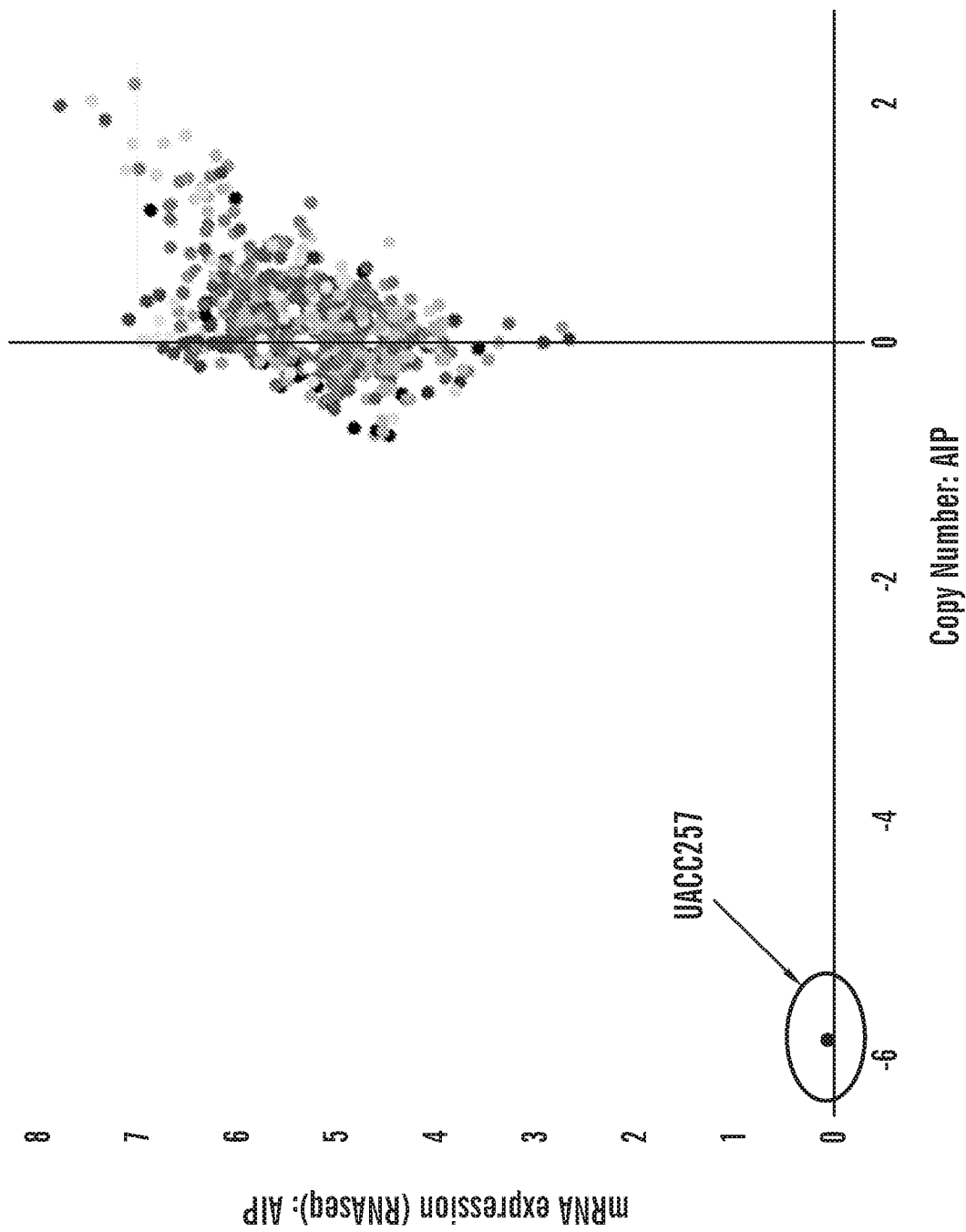


FIG. 5

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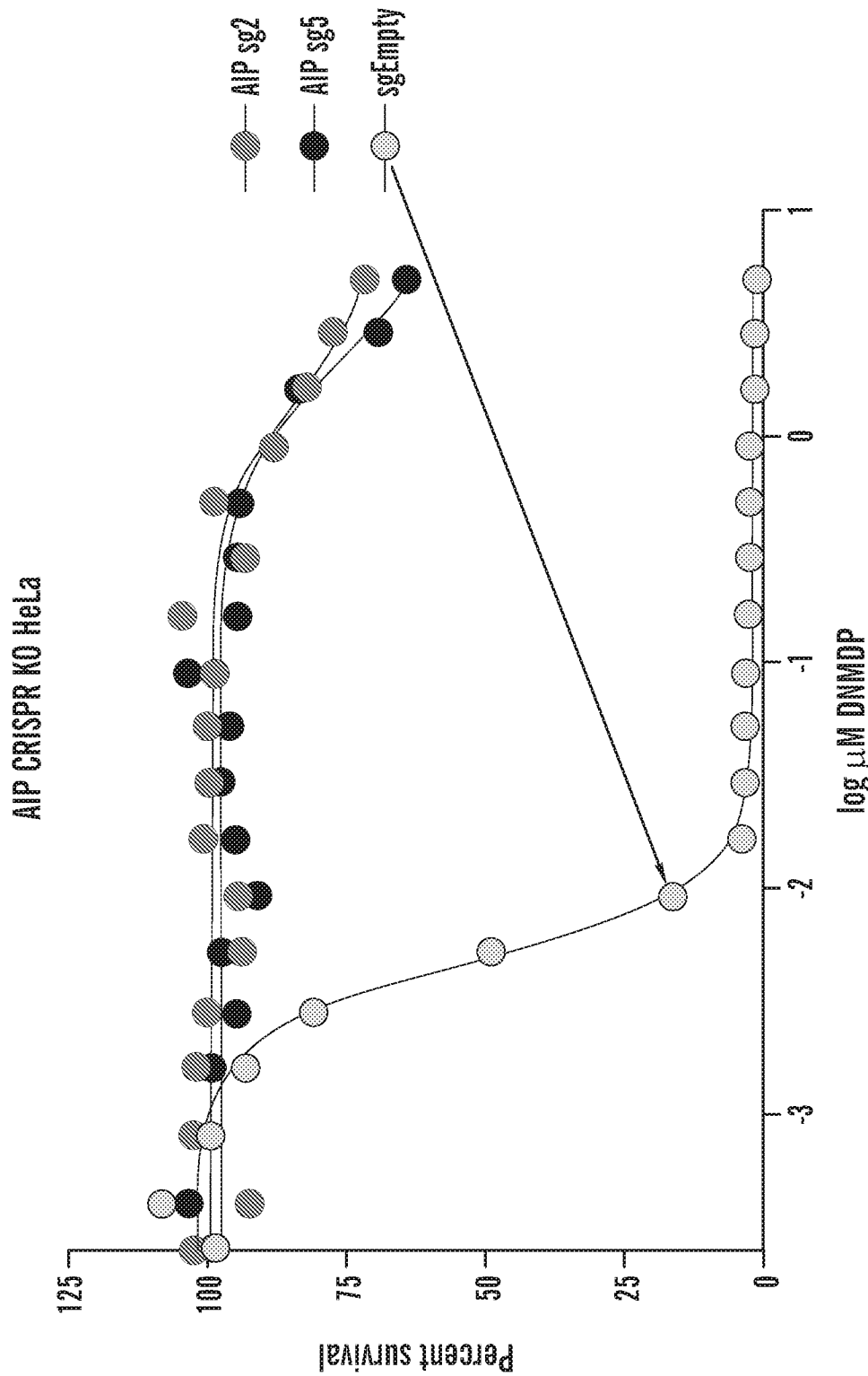


FIG. 6A

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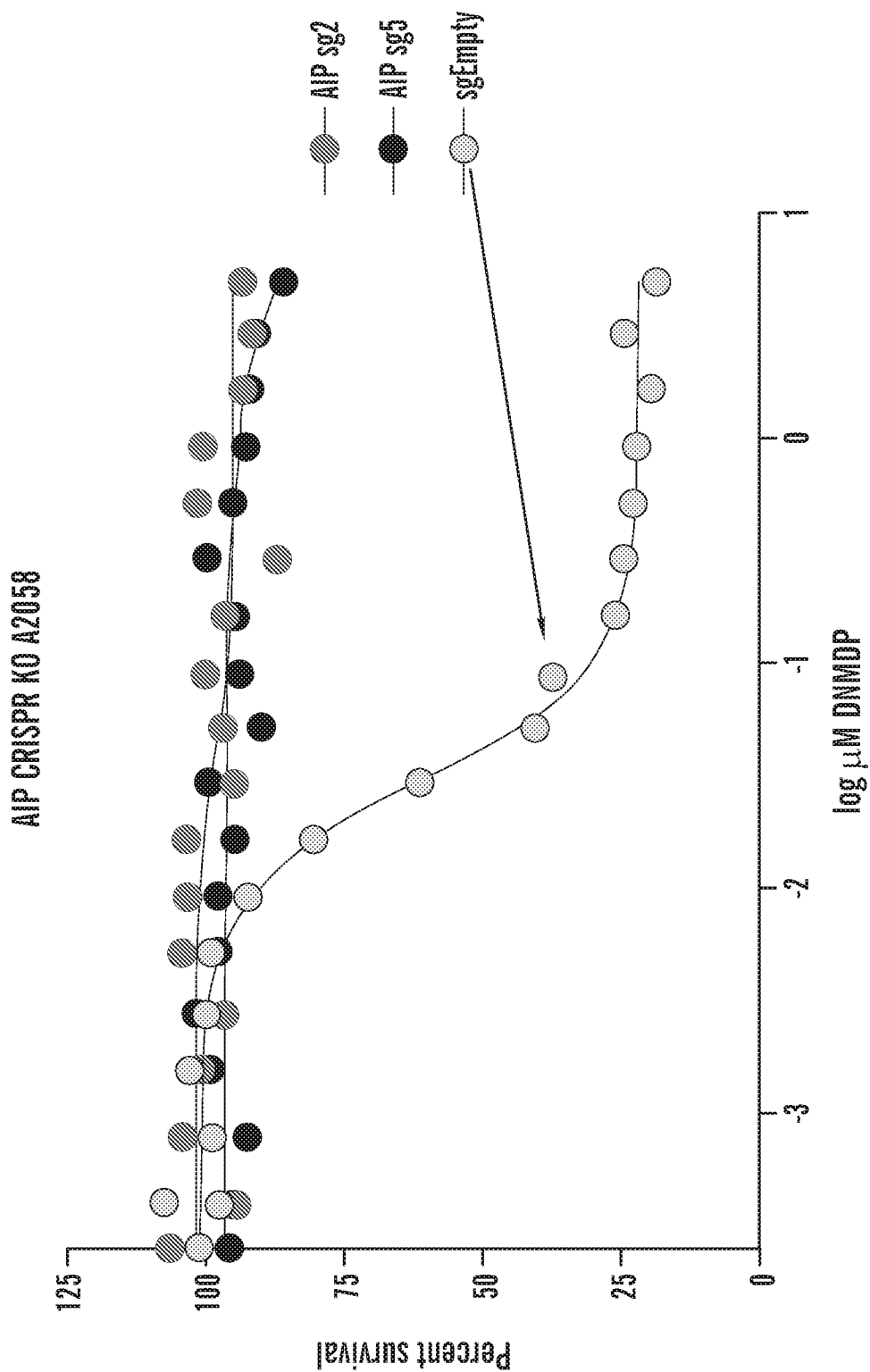
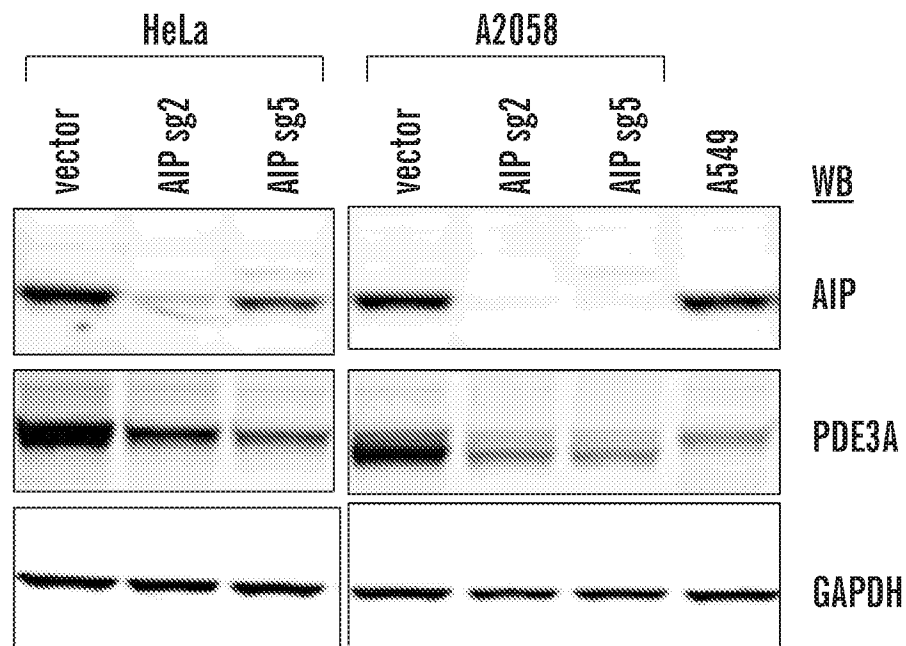
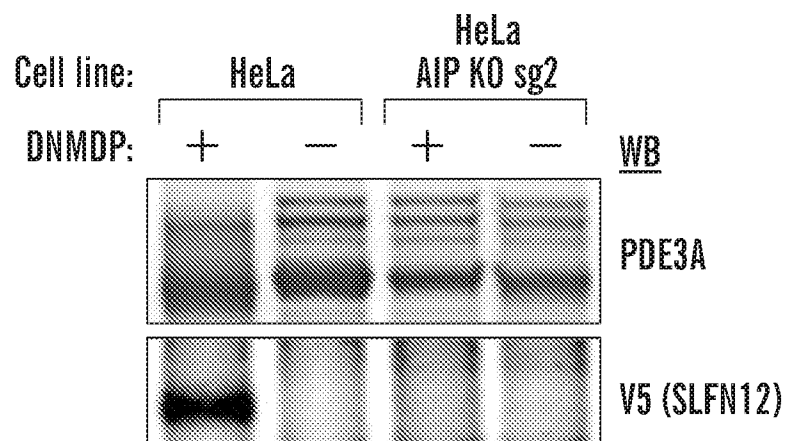


FIG. 6B

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**FIG. 6C****FIG. 7**

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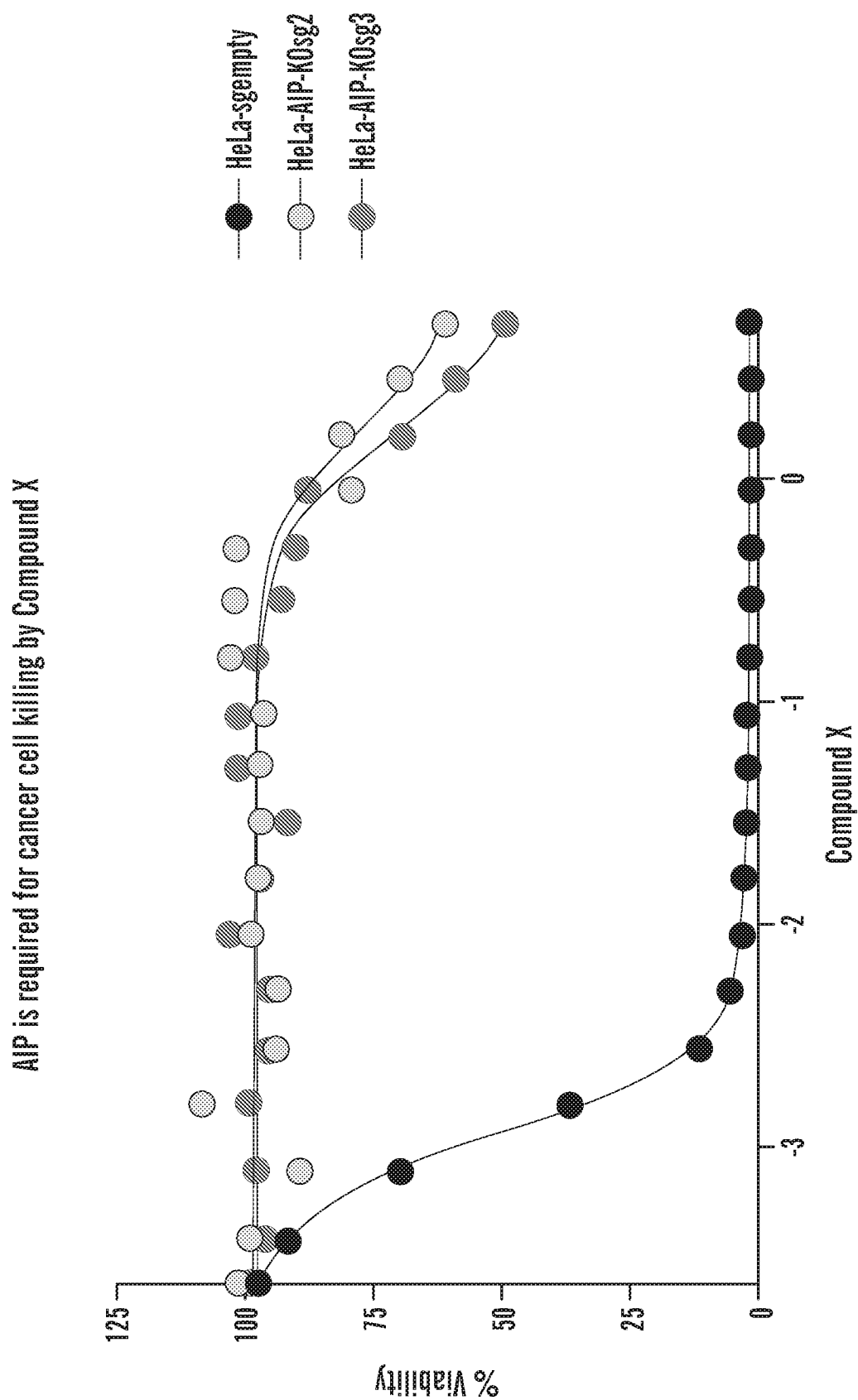


FIG. 8A

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AIP is required for induction of PDE3A-SLFN12 complex formation by Compound X

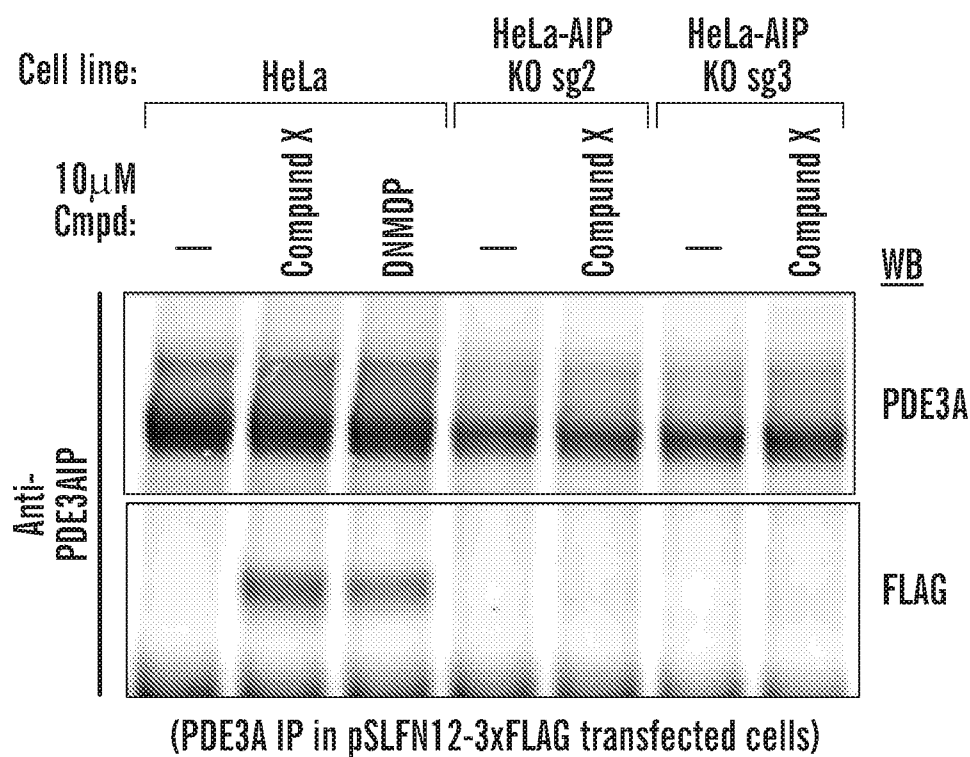


FIG. 8B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/059526

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/47; C12N 9/16; C12Q 1/6886 (2020.01)

CPC - C12Q 2600/158; C12Y 301/04017; G01N 2333/916; G01N 2800/52 (2020.01)

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

USPC - 435/6.18; 435/6.14 (keyword delimited)

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 2018/0235961 A1 (THE BROAD INSTITUTE, INC. et al) 23 August 2018 (23.08.2018) entire document	1-8, 21-24
Y	US 2018/0045727 A1 (CARIS MPI, INC.) 15 February 2018 (15.02.2018) entire document	1-8, 21-24
Y	US 2011/0257034 A1 (BARANY et al) 20 October 2011 (20.10.2011) entire document	4, 6-8
A	US 2018/0153975 A1 (THE BROAD INSTITUTE INC. et al) 07 June 2018 (07.06.2018) entire document	1-8, 21-24
A	— FRANCIS et al. "Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions," Physiological Reviews, 30 April 2011 (30.04.2011), Vol. 91, No. 2, Pgs. 651-690. entire document	1-8, 21-24
A	← HIRSCH et al. "Twice upon a time: PI3K's secret double life exposed," Trends in Biomedical Sciences, 17 April 2009 (17.04.2009), Vol. 34, No. 5, Pgs. 244-248. entire document	1-8, 21-24

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

08 January 2020

Date of mailing of the international search report

30 JAN 2020

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Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/059526

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.: 9-20
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.