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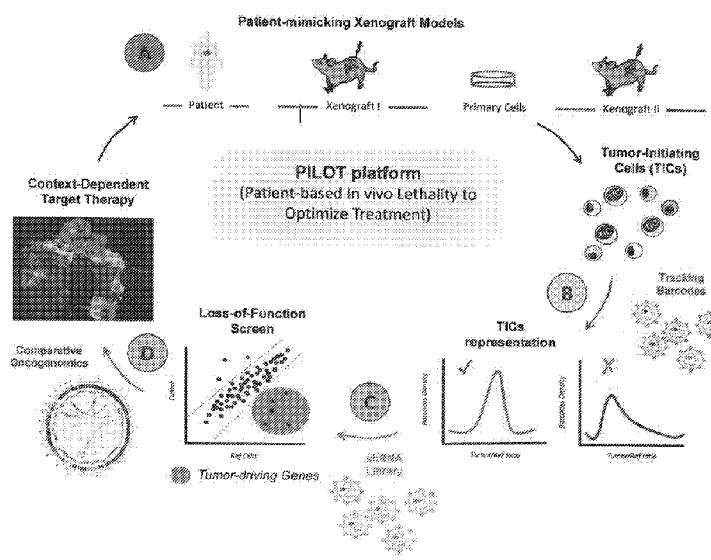


FIG. 1

(57) Abstract: Provided are methods for identification of therapeutic dependencies necessary for tumor initiation and maintenance.

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METHODS FOR THE IN VIVO DETECTION AND TREATMENT OF PATIENT-CENTRIC TUMOR DEPENDENCIES

BACKGROUND

[0001] Functional genomic approaches, such as short hairpin RNA (shRNA) or synthetic guide RNA (sgRNA) screens, hold great promise for the identification of genetic dependencies and clinically relevant co-extinction strategies. However, the reproducibility of large-scale screening efforts has been challenged by the prevalence of "off-target" effects and the lack of robust reagents.

[0002] Recent in vivo screens with human cancer cell lines have enforced the importance of capturing the role of key genetic elements in the hallmarks of cancer, including complex heterotypic cancer-host interactions, but were limited in their broad application by the inability to efficiently evaluate tumor-initiating potential in xenotransplantation settings (Possemato et al., 2012; Possik et al., 2014). Thus, there is a need to develop a system, or platform for identifying novel actionable molecular vulnerabilities in patient-derived tissue from each cancer indication and to dissect the context-specific nature of tumor dependencies. In addition, there is a need for customizing shRNA/sgRNA libraries that could be applied during co-clinical trials to predict the efficacy of current available therapeutic options providing clinicians the opportunity to define the most appropriate treatment paradigm for individual patients.

SUMMARY

[0003] The present disclosure provides methods for an in vivo platform for the identification of oncogenic drivers necessary for tumor maintenance in patient-derived xenografts. The methods described herein can be used to identify novel actionable molecular vulnerabilities in patient-derived xenograft (PDX) tissue from each cancer indication and to dissect context-specific nature of tumor dependencies. To this end, the methods herein provide a mechanism to prioritize oncology drug discovery targets as well as increase the probability of success new targeted therapies entering the clinic. The methods described herein can also be applied during co-clinical trials to predict the efficacy of current available therapeutic options providing clinicians the opportunity to identify co-extinction targets to inform the most appropriate treatment paradigm for individual patients with tumors of a specific genomic background.

[0004] In one aspect, the present disclosure provides a method of identifying a gene that modulates a function or a phenotype associated with tumorigenesis of a patient-derived cell with a defined genetic make-up. In some embodiments, the method includes introducing into a patient-derived cancerous cell population representative of a given genotype or histological type a shRNA or sgRNA library targeting specific protein targets. In some embodiments, the library includes a collection of genetic elements of interest. In some embodiments, the library also include one or more unique genetic barcode sequences. In some embodiments, the method produces a genetically engineered, patient-derived target cell population having a cancer cell genotype.

[0005] In some embodiments, the method includes transplanting the target cell population into a non-human mammal to produce a tumor in the mammal. In some embodiments, the method includes identifying the loss-of-expression of one or more of the genetic elements of interest in the outgrowing or surviving tumor. In some embodiments, identifying the loss-of-expression is determined by deep sequencing. In some embodiments, the genetic barcode sequences include from 4 to 8 nucleotides, from 6 to 10 nucleotides, 8 to 12 nucleotides, from 10 to 14 nucleotides, from 12 to 16 nucleotides, from 14 to 18 nucleotides, from 16 to 20 nucleotides, from 18 to 22 nucleotides, from 20 to 24 nucleotides, from 20 to 30 nucleotides, from 30 to 40 nucleotides, from 40 to 50 nucleotides, or from 60 to 100 nucleotides.

[0006] In some embodiments, the transplanting is orthotopic. In some embodiments, the transplanting is heterotopic. In some embodiments, the cell representative of a given genotype, phenotype, or histological type is a mammalian cell. In some embodiments, the cell representative of a given genotype, phenotype, or histological type is a progenitor cell or stem cell.

[0007] In some embodiments, the method includes patient-derived cell lines representative of a specific genotype or histological type in which one or more tumor suppressors protein pathways have been inactivated or suppressed. In some embodiments, the tumor suppressor protein pathway can include, but is not limited to RB, TP53, CDKN2A, SMAD4, PTEN, STK11 or a combination thereof. One of skill in the art will readily recognize that the methods described herein are not limited to those tumor suppressor protein pathways explicitly recited herein. In some embodiments, the method includes inactivating or suppressing one or more genetic elements of interest in the cell representative of a given genotype or histological type. In some embodiments, the method includes inactivating or

suppressing one or more epigenetic regulators in the cell representative of a given genotype or histological type.

[0008] In some embodiments, the patient-derived cell line is representative of cancers including, but not limited to, pancreatic cancer (e.g., pancreatic adenocarcinoma), melanoma, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like.

[0009] In some embodiments, the nucleic acid library comprises siRNA, shRNA, sgRNA microRNA or an antisense nucleic acids to the candidate genes or genetic elements of interest. In some embodiments, the genetic elements of interest are selected from the groups consisting of a PSMA1, RLP30, PHF5A, SMC2, BRD4, and WDR5. In some embodiments, the genetic element of interest is WDR5.

[0010] In some embodiments, the candidate genes or genetic elements of interest include enzymes. In some embodiments, the enzyme is a wildtype or activated mutant enzyme. In some embodiments, the enzyme is a metabolic enzyme. In some embodiments, the candidate genes or genetic elements of interest include kinase genes and/or genetic elements. In some embodiments, the kinase is a wildtype kinase or an activated mutant kinase. In some embodiments, the candidate genes or genetic elements of interest include a phosphatase gene and/or genetic elements. In some embodiments, the phosphatase is a wildtype or activated mutant phosphatase. In some embodiments, the candidate genes or genetic elements of interest include a methyltransferase gene and/or genetic elements. In some embodiments, the methyltransferase is a wildtype or activated mutant methyltransferase. In some embodiments, the candidate genes or genetic elements of interest include an epigenetic regulator gene and/or genetic elements. In some embodiments, the epigenetic regulator is a wildtype or activated mutant epigenetic regulator.

[0011] In some embodiments, the candidate genes or genetic elements of interest include genes and/or genetic elements involved in the PI3K signaling pathway. In some embodiments, the candidate genes or genetic elements of interest include genes and/or

genetic elements for membrane-bound proteins. In some embodiments, the candidate genes or genetic elements of interest include genes and/or genetic elements involved in a G-protein coupled receptor signaling pathway. In some embodiments, the candidate genes or genetic elements of interest include genes and/or genetic elements involved in the receptor tyrosine kinase signaling pathway. In some embodiments, the candidate genes or genetic elements of interest include genes and/or genetic elements for checkpoint-inhibitor proteins.

[0012] In some embodiments, the function or a phenotype associated with tumorigenesis is metastasis, cell migration, angiogenesis, extracellular matrix degradation, anchorage independent growth, or anoikis.

[0013] In one aspect, the present disclosure provides a method for identifying a genetic element of interest that synergizes (i.e., enhances) a biologically active agent that interacts with a tumorigenesis pathway. In some embodiments, the method includes producing a genetically engineered, patient-derived target cell having a cancer cell genotype. In some embodiments, the producing step includes introducing into a patient-derived cancerous cell population representative of a given phenotype or histological type a nucleic acid library. In some embodiments, the library includes a collection of genetic elements of interest and one or more unique genetic barcode sequences. In some embodiments, the method includes contacting the genetically engineered target cell with a candidate biologically active agent. In some embodiments, the method includes identifying the loss-of-expression of one or more of the genetic elements of interest in the tumor by determining whether the biologically active agent has increased anti-tumor activity as compared to agent-treated tumors that do not have the loss-of-function of the same genetic elements of interest.

[0014] In some embodiments, identifying the loss-of-expression is determined by deep sequencing. In some embodiments, the genetic barcode sequences include from 4 to 8 nucleotides, from 6 to 10 nucleotides, 8 to 12 nucleotides, from 10 to 14 nucleotides, from 12 to 16 nucleotides, from 14 to 18 nucleotides, from 16 to 20 nucleotides, from 18 to 22 nucleotides, from 20 to 24 nucleotides, from 20 to 30 nucleotides, from 30 to 40 nucleotides, from 40 to 50 nucleotides, or from 60 to 100 nucleotides.

[0015] In some embodiments, the patient-derived cancerous cell population is representative of cancers including, but not limited to, pancreatic cancer (e.g., pancreatic adenocarcinoma), melanoma, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central

nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like.

[0016] In some embodiments, the nucleic acid library comprises siRNA, shRNA, sgRNA microRNA or an antisense nucleic acids to the candidate genes or genetic elements of interest. In some embodiments, the genetic elements of interest are selected from the groups consisting of a PSMA1, RLP30, PHF5A, SMC2, BRD4, and WDR5. In some embodiments, the genetic element of interest is WDR5.

[0017] In some embodiments, the method includes a patient-derived cancerous cell population representative of a specific genotype or histological type in which one or more tumor suppressors protein pathways have been inactivated or suppressed. In some embodiments, the tumor suppressor protein pathway can include, but is not limited to RB, TP53, CDKN2A, SMAD4, PTEN, STK11 or a combination thereof. One of skill in the art will readily recognize that the methods described herein are not limited to those tumor suppressor protein pathways explicitly recited herein. In some embodiments, the method includes a patient-derived cancerous cell population having one or more epigenetic regulators activated or suppressed in the cell population representative of a given genotype or histological type.

[0018] In some embodiments, the genetic elements of interest include enzymes. In some embodiments, the enzyme is a wildtype or activated mutant enzyme. In some embodiments, the enzyme is a metabolic enzyme. In some embodiments, the genetic elements of interest include kinase genes and/or genetic elements. In some embodiments, the kinase is a wildtype kinase or an activated mutant kinase. In some embodiments, the genetic elements of interest include a phosphatase gene and/or genetic elements. In some embodiments, the phosphatase is a wildtype or activated mutant phosphatase. In some embodiments, the genetic elements of interest include a methyltransferase gene and/or genetic elements. In some embodiments, the methyltransferase is a wildtype or activated mutant methyltransferase. In some embodiments, the genetic elements of interest include an epigenetic regulator gene and/or genetic elements. In some embodiments, the epigenetic regulator is a wildtype or activated mutant epigenetic regulator.

[0019] In some embodiments, the genetic elements of interest include genes and/or genetic elements for membrane-bound proteins. In some embodiments, the genetic elements of interest include genes and/or genetic elements involved in a G-protein coupled receptor signaling pathway. In some embodiments, the genetic elements of interest include genes and/or genetic elements involved in the receptor tyrosine kinase signaling pathway.

[0020] In some embodiments, the tumorigenic phenotype is metastasis, cell migration, angiogenesis, extracellular matrix degradation, anchorage-independent growth, or anoikis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **FIG. 1** depicts a flow chart for discovery of patient-based, in vivo druggable gene targets.

[0022] **FIG. 2A** depicts histological sections of pancreatic ductal adenocarcinoma (PDAC) diagnosed patients stained with hematoxylin and eosin (H&E) as compared to sections from matched patient-derived xenograft (PDx) models. Primary (xenograft I) and secondary (xenograft II) xenografts were stained for cytokeratin 19 and vimentin (10x). Bright-field images of the stabilized cells were taken during in vitro amplification steps (20x).

[0023] **FIG. 2B** depicts the expression profiles of human (HLA) and mouse (Kd) major histocompatibility complexes (MHC) in xenograft-derived PDAC cells at passage 3 by flow cytometry.

[0024] **FIG. 2C** depicts bright-field and fluorescence micrographs xenograft-derived PDAC cells infected with different dilutions of a green fluorescent protein (GFP) pLKO.3g virus.

[0025] **FIG. 3A** depicts an experimental design outline for in vivo tumor-initiating cell (TIC) coverage study in patient-derived models of PDAC.

[0026] **FIG. 3B** (upper panel) depicts a schematic of a lentiviral vector cloning site carrying 12.5k molecular barcodes and a downstream polycistronic site carrying a puromycin resistance (Puro^R) and red fluorescent protein (RFP) report gene (TagRFP) separated by a 2A peptide; (lower panel) depicts a table summarizing the RFP-positive cells (percentage) from PDAC xenograft-derived models upon infection with different titers of the 12.5k barcoded library.

[0027] **FIG. 3C** depicts a density plot of the tumor/ref ratio (median between replicates) for xenograft-derived cells infected with the 12.5k barcoded library with a coverage of 400 cells/barcode.

[0028] **FIG. 3D** depicts a density plot of the tumor/ref ratio (median between replicates) for xenograft-derived cells infected with the 12.5k barcoded library with a coverage of 400 cells/barcode.

[0029] **FIG. 3E** depicts tables demonstrating the extreme limiting dilution analysis (ELDA) of PDAC human PDx cells.

[0030] **FIG. 4A** depicts an experimental design outline for deep-coverage shRNA screens in patient-derived and mouse models of PDAC.

[0031] **FIG. 4B** depicts a schematic of a lentiviral vector cloning site carrying 2.4k shRNA-coupled molecular barcodes with a downstream polycistronic site carrying a Puro^R and GFP reporter gene separated by a 2A peptide.

[0032] **FIG. 4C** depicts scatter-plots of PDx-derived cells screened in triplicate with 2000 cells/shRNA to determine the ability of each patient-derived model to maintain complexity of the genetic library. In this case, PATX53 maintains complexity whereas PATX66 fails to achieve complexity.

[0033] **FIG. 4D** depicts correlation plots between replicates of xenograft-derived tumors screened with the Epi shRNA library.

[0034] **FIG. 5A** depicts a cumulative distribution function (CDF) of shRNA's fold-change for the human MDA-PATX53 model.

[0035] **FIG. 5B** depicts an overlapped density plot of the TIC coverage studies and the corresponding shRNA screens in the xenograft-derived MDA-PATX53 model. Dashed grey lines indicate ± 2 standard deviations (SD).

[0036] **FIG. 5C** depicts a density plot of the p-value associated to the mean z-score (fold-change, average of the top 3 shRNAs) for each gene in the MDA-PATX53 screen.

[0037] **FIG. 5D** depicts heat maps of the top-scoring hits generated applying a p-value-based cut-off ($p < 0.05$) associated to the z-score (fold-change, average of the top 3 shRNAs) for each gene in the MDA-PATX53 and MDA-PATX43 screens. Ranking determined applying the mean z-score among all the screened samples. Negative controls (Luc1-4). Positive controls (Psm1, Rpl30, Pcn1).

[0038] **FIG. 5E** depicts a table demonstrating clinico-pathological (sex, age, pathology, stage, metastasis site) and mutational (Kras, TP53, CDKN2A, DPC4/Smad4) features for PDAC xenograft-derived samples.

DETAILED DESCRIPTION

[0039] Current treatment regimens for various cancers yield poor 5-year survival, emphasizing the critical need to identify druggable targets essential for tumor maintenance. Described herein are methods for an unbiased and in vivo target discovery approach to identify molecular vulnerabilities in low-passage and patient-derived tumor xenografts or genetically engineered mouse model-derived allografts.

[0040] In some embodiments the cancer is a solid-tumor cancer. In some embodiments the solid-tumor cancer is selected from the group consisting of breast cancer, prostate cancer, lung cancer, liver cancer, pancreatic cancer, and melanoma. In some embodiments, the solid-tumor cancer is pancreatic ductal adenocarcinoma (PDAC).

[0041] In contrast to published approaches, the PILOT (patient-based in vivo lethality to optimize treatment) platform described herein leverages patient-derived primary cell cultures to identify tumor vulnerabilities that are more clinically relevant. Traditional approaches perform similar analysis with generic cell lines that fail to recapitulate the complexity and clonal heterogeneity of human tumors. Moreover, described herein is a protocol for the rapid determination of TIC frequency using clonal barcoding technology. In contrast to lengthy, resource intensive assay like the extreme limiting dilution assay (ELDA), the methods herein are more quantitative and robust for informing on TIC frequency.

[0042] To address these challenges, a synthetic lethality screen was developed to function in vivo in the context of an intact tumor microenvironment using patient-derived tumor models that more faithfully recapitulate the human disease. In this platform, target cells are transduced with custom designed, deep-coverage shRNA libraries (10-15 shRNAs targeting each gene), allowing for the simultaneous evaluation of hundreds of genes in a single experiment. Transduced cells were then implanted orthotopically into mice and the representation of shRNA pre/post tumor growth or drug treatment quantified by deep sequencing. shRNAs depleted in the context of drug treatment represent synthetic lethal interactions and provide rationale for co-extinction. The in vivo PILOT platform has been designed as a flexible system, allowing for target cells of different cellular and genetic context, and shRNA libraries targeting any aspect of the druggable genome.

[0043] Here, we describe, for the first time, a loss-of-function in vivo screening platform using patient-derived samples adjusted for the effective number of TICs. Among the multiple novel aspects of this platform is the opportunity to extend in vivo shRNA screening approaches to patient-derived primary cells that better reflect the state of a tumor at time of treatment.

[0044] Established cell lines and their transplanted tumors do not reflect the heterogeneity of human cancer biology and have been adapted to growth in non-physiological culture conditions. Primary cancer patient-derived xenografts are indeed able to capture a broader set of tumor cell features compared to conventional cancer cell lines, and they are being widely adopted to test efficacy of novel candidate therapeutic agents (Tentler et al., 2012). By focusing on targeting cell populations that can adequately form tumors in vivo, the methods described herein move the attention to those cells required to survive upon transplantation and to sustain tumor initiation and progression (Zhou et al., 2009; Viale et al., 2014). The stringency of the methods herein allow one to focus solely on the most promising “hits” on which can then be performed detailed clinical-pathological and functional validation as illustrated in the case of WDR5.

[0045] The results herein also display a critical role of WDR5 in PDAC, linked to sustaining a DNA replication checkpoint. Tumor cells possess an increased number of replication forks and endure elevated levels of replication stress compared to normal cells. Thus, WDR5 overexpression in tumors may be required to stabilize the replication machinery (Gaillard et al., 2015). Here, the confirmation of a direct and functional interaction between WDR5 and c-Myc clearly demonstrated that WDR5 is critical to recruit c-Myc on the chromatin and to enable Myc-dependent tumorigenic mechanisms in PDAC (Dominguez-Sola et al., 2007; Thomas et al., 2015). In PDAC, where mutations of Kras happen at a significantly high frequency, Myc is required downstream of the MAPK cascade to sustain tumorigenesis (Ischenko et al., 2014; Stellas et al., 2014), and its knockdown in Kras-driven pancreatic cells also reduced the expression of metabolic genes critical for tumor maintenance (Ying et al., 2012). The ability of Myc to promote cellular proliferation in S-phase appears to occur through cooperation of effects, some direct, e.g., those driving the regulation of nucleotide biosynthetic pathways and the control of active replication forks, others dependent on transcriptional activation, e.g., regulation of cell-cycle genes (Campaner and Amati, 2012). In the Examples herein, one of the Myc controlled mechanisms that emerge to restrain replication stress is the ATR/Chk1 pathway (Murga et al., 2011; Schoppy

et al., 2012), which support rather than suppress tumor development. The findings of MLL being phosphorylated and activated by ATR in order to allow for full activation of the S-phase checkpoint further highlight the functional interplay between oncogene-induced DDR and COMPASS complex (Liu et al., 2010). The Examples herein with two available low potency WDR5 inhibitors supported the centrality of the WDR5 network (OICR-9429), more than MLL-specific associations (MM-401), in driving PDAC cell proliferation, but also highlighted the requirement for a new generation of potent and selective Myc-oriented WDR5 inhibitors to deeply dissect the WDR5-Myc molecular dynamics and accelerate the bench-to-bedside translation process (Grebien et al., 2015; Cao et al., 2014). In further support of these mechanistic findings, it was previously demonstrated that ATR inhibition sensitizes PDAC cell lines to radiation or chemotherapy (Prevo et al., 2012).

[0046] Herein is also described a highly efficient technology for the rapid in vivo validation of any gene function in GEMM-derived models of PDAC. Compared to the traditional validation methods (constitutive or inducible RNAi tools), the methods herein have the advantage of knocking down a gene of interest in a time-restricted manner, in autochthonous pancreatic tumors originated from reprogrammed embryonic progenitors. Taken together, the Examples herein highlight the described methods as a powerful platform for the rapid identification of genetic vulnerabilities using patient-derived tumor samples. In some embodiments, the systematic evaluation of epigenetic regulators in specific genetic contexts and tumor molecular subtypes is performed.

Context-Specific Functional Genetic Screen Platform

[0047] In some embodiments, there is provided a genetic screen platform that can systematically assign upfront biological and clinical relevance in context of a functionality or phenotype to a library of GEOI (genetic elements of interest) for a specific clinically-definable genetic context. The genetic screen platform allows for the identification of new drug targets, and in parallel, the identification of new oncogenic drivers necessary for tumor initiation and maintenance which informs which additional pathways act cooperatively with those pathways altered in the predetermined genetic context and therefore informs the use of single or combination targeted therapies directed towards the new cancer pathway and/or the known cancer pathway. The PILOT platform was developed to identify high priority oncology targets that could be enrolled in drug discovery/development efforts. The platform aims to 1) prioritize drug discovery efforts towards higher probability targets, and 2) reposition clinical drugs to novel disease indications.

[0048] In some embodiments, the context-specific screen is composed of the following three elements: a population of patient-derived target cells; a tumorigenesis or metastasis phenotypic animal model, and a GEOI library.

[0049] The examples herein provide a description of one context-specific functional genetic screen according to the present embodiments that focuses on the identification of epigenetic regulators in the context of PDAC. The examples use human, patient-derived PDAC xenografts or genetically-engineered mouse model-derived allografts as the target cell with a highly relevant genetic context (i.e., epigenetic deregulation is a documented genetic hallmark of PDAC and developmental oncobiology overall (Omura and Goggins, 2009) and genetic lesions in chromatin regulators have been identified in a variety of cancers). In some embodiments, a focused epigenetic regulator library containing shRNAs targeting unique mouse or human epigenetic regulators can be cloned into a universal lentiviral vector. In some embodiments, the library can contain shRNAs targeting other oncogenic drivers. In some embodiments, lentiviruses expressing these shRNAs can be transduced into the target cells. In some embodiments, to enhance the robustness of the screen and facilitate hit prioritization, the library can be designed with ten unique shRNAs targeting each epigene. In some embodiments, the library can be designed with 1-5 unique shRNAs, 5-10 unique shRNAs, or 10-20 unique shRNAs targeting each oncogenic driver. To ensure adequate representation of the complexity of deep coverage epigenetics library in human and mouse

samples, mice can be implanted respectively with 2,000 cells/shRNA and 400 cells/shRNA, and barcode abundance quantified in established xenografts by deep sequencing.

Target Cells

[0050] In some embodiments, the target cells are mammalian cells (e.g., human cells or murine cells). In some embodiments, the target cells are patient-derived xenografts (PDX) that have been obtained by injecting patient-derived bioptic samples in NSG mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ). This genetic context defines the clinical path approach that can lead to an indication of the therapeutics, e.g. a disease type in a genetically defined subpopulation. Thus, the target cells are phenotypically indistinguishable from the patients' original tumors, as evaluated by histopathology and marker analyses.

Library of GEOIs

[0051] The term "genetic elements of interest" or "GEOI" refers to those genetic elements (e.g., genes) that have been linked or associated with cancer or associated with biological pathways of genes that drive cancer growth and metastasis. A library of genetic elements of interest refer to a plurality of specific genetic elements of interest or variations thereof (e.g., somatic or germline mutations) that have been linked to a human cancer or a tumorigenic phenotype or metastatic phenotype.

[0052] A collection of genetic elements (cDNAs, shRNAs), defined by different means, including genomically altered GEOIs such as ones resident in regions of genomic amplifications; somatic mutated genes such as "driver kinases" shown to harbor statistical significant mutations in diverse human cancers; components of a defined pathway or biological process or a class of molecules, such as metabolic pathway enzymes, or GPCRs.

[0053] The GEOIs may be categorized as genomics driven libraries, class based libraries, druggable genome libraries, or cellular process libraries, which are described in further detail below.

[0054] The libraries of the GEOIs are nucleic acid libraries. This includes nucleic acid libraries comprising nucleic acids that encode for the genes or genetic elements of interest. The nucleic acid libraries may also be made up of siRNA, shRNA, microRNA or an antisense nucleic acids to the genes or genetic elements of interest. In some embodiments, the nucleic acid library comprises nucleic acids encoding inactive or dominant negative versions of the genes or genetic elements of interest.

Druggable Genome Libraries

[0055] Druggable genome libraries are libraries including genes that are known druggable enzymes implicated in human cancer. For example, human kinases are frequently altered in human cancer, either by amplification, overexpression, or mutation and have been successfully inhibited with small molecule inhibitors (i.e., Gleevec). Examples of druggable genome libraries include, but are not limited to, libraries of genes encoding kinases, phosphatases, histone methyltransferases, histone demethylases, and histone acetyltransferases, and histone deacetylases.

Histone Methyltransferases

[0056] Histone methyltransferases (HMT) are enzymes, histone-lysine N-methyltransferase and histone-arginine N-methyltransferase, which catalyze the transfer of one to three methyl groups from the cofactor S-Adenosyl methionine to lysine and arginine residues of histone proteins. These proteins often contain a SET (Su(var)3-9, Enhancer of Zeste, Trithorax) domain. Histone methylation serves in epigenetic gene regulation. Methylated histones bind DNA more tightly, which inhibits transcription.

[0057] Catalyzed by histone methyltransferases, histone methylation plays a key role in regulation of chromatin status and global gene expression, especially during development and differentiation. Histone methylation can be dysregulated in cancer and other important diseases, including inflammatory, metabolic and neurologic disorders.

[0058] Genomic copy number aberrations, mutations, mRNA expression dysregulation of histone methyltransferases have been identified in various human cancers. Inhibition of histone methyltransferases re-program cells into more differentiated states, therefore this class of enzymes serves as attractive cancer therapeutic targets.

Epigenetic Regulators

[0059] Epigenetic regulators (e.g., histone acetyltransferases, methyltransferases, chromatin-remodelling enzymes, etc.) play a fundamental role in the control of gene expression by modifying the local state of chromatin. However, little is known about their own regulation.

[0060] Examples of epigenetic regulators include: Arid1a, Arid1b, Arid2, Asf1a, Asf1b, Ash1l, Ash2l, Atad2, Atad2b, Bahd1, Baz1a, Baz1b, Baz2a, Baz2b, Bmi1, Bptf, Brd1, Brd2, Brd3, Brd4, Brd7, Brd8, Brd9, Brdt, Brms1, Brpf3, Brwd3, Carm1, Cbx1, Cbx2, Cbx3,

Cbx4, Cbx5, Cbx6, Cbx7, Cbx8, Chaf1a, Chaf1b, Chd11, Chd2, Chd3, Chd4, Chd5, Chd5, Chd6, Chd7, Chd8, Chd9, Clock, Crebbp, Ctbp1, Ctbp2, Ctf, Ctsl, Dicer1, Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l, Dot11, Dub2a, Eed, Ehmt1, Ehmt2, Ep300, Ep400, Ezh1, Ezh2, Fbx119, Fbxw7, H2afv, H2afx, H2afz, Hdac1, Hdac10, Hdac11, Hdac2, Hdac3, Hdac4, Hdac5, Hdac6, Hdac8, Hdac9, Idh1, Idh2, Ing1, Ing2, Ing3, Ing4, Ing5, Ino80, Jarid2, Jhdm1d, Jmjd1c, Jmjd4, Jmjd5, Jmjd6, Kat2a, Kat2b, Kat5, Kdm1a, Kdm1b, Kdm2a, Kdm2b, Kdm3a, Kdm3b, Kdm4a, Kdm4b, Kdm4c, Kdm4d, Kdm5a, Kdm5b, Kdm5c, Kdm5d, Kdm6a, Mbd1, Mbd2, Mbd3, Mbd4, Mbd5, Mbd6, Mecom, Mecp2, Men1, Mll1, Mll2, Mll3, Mta1, Mta2, Mta3, Kat8, Myst2, Myst3, Myst4, Ncoa1, Ncoa3, Nsd1, Parp1, Parp2, Pbrm1, Phf1, Phf13, Phf2, Phf21a, Phf21b, Phf3, Phf5a, Phf6, Phf7, Phf8, Prdm1, Prdm10, Prdm11, Prdm12, Prdm13, Prdm14, Prdm15, Prdm16, Prdm2, Prdm4, Prdm5, Prdm6, Prdm8, Prdm9, Prmt1, Prmt2, Prmt3, Prmt5, Prmt6, Prmt7, Prmt8, Prmt8, Rnf20, Sertad1, Sertad2, Setd1a, Setd1b, Setd2, Setd3, Setd4, Setd7, Setd8, Setdb1, Setdb2, Sin3a, Sin3a, Sin3b, Sirt1, Sirt2, Sirt3, Sirt4, Sirt5, Sirt6, Sirt7, Smarca2, Smarca4, Smarcb1, Smc1a, Smc1b, Smc2, Smc3, Smc4, Smc5, Smc6, Smyd3, Smyd4, Smyd5, Sp100, Sp110, Sp140, Suv39h1, Suv39h2, Suv420h1, Suv420h2, Taf1, Tet1, Tet2, Tet3, Trim24, Trim28, Trim33, Trim66, Ube2a, Ube2b, Ube2e1, Ube2i, Usp22, Usp27x, Wdr11, Wdr11, Wdr11, Wdr5, Wdr82, Whsc1, Whsc111, Zmynd11.

Definitions

[0061] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only not intended to be limiting. Other features and advantages of the invention will be apparent from the following detailed description and claims.

[0062] For the purposes of promoting an understanding of the embodiments described herein, reference will be made to preferred embodiments and specific language will be used to describe the same. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention. As used throughout this disclosure, the singular forms "a," "an," and "the" include plural reference

unless the context clearly dictates otherwise. Thus, for example, a reference to "a composition" includes a plurality of such compositions, as well as a single composition, and a reference to "a therapeutic agent" is a reference to one or more therapeutic and/or pharmaceutical agents and equivalents thereof known to those skilled in the art, and so forth. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth. Further, the use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0063] Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0064] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[0065] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open ended and do not exclude additional, unrecited elements or method steps.

[0066] The terms "tumor" or "cancer" refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Cancer cells are often in the form of a tumor, but such cells may exist alone within an animal, or may be a non-tumorigenic cancer cell, such as a leukemia cell. As used herein, the term "cancer" includes premalignant as well as malignant cancers. Cancers include, but are not limited to, pancreatic cancer (e.g., pancreatic adenocarcinoma), melanoma, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer,

testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like.

[0067] The term “Patient-Derived xenograft (PDX)” refers to direct implant of surgically resected tumors in recipient hosts. These recipient hosts can be immune-compromised mice. These models maintain morphological similarities and recapitulate molecular profiling of the original tumors, thus representing a useful tool in evaluating anticancer drug response.

[0068] The term “GEMM” refers to genetically-engineered mouse models. GEMMs are transgenic animals in which gene knock-out and knock-in technologies have made it possible to more faithfully mimic the genetic and biological evolution of human cancers. GEMMs develop spontaneous, autochthonous tumors and have the microenvironment required for tumor progression, including degradation of the matrix and angiogenesis. GEMMs can be engineered to have a short latency yet with high penetrance. Also, in contrast with xenografts, GEMMs are immuno-competent animals.

[0069] The term “TIC” refers to the tumor-initiating cells (popularly known as cancer stem cells). The unique features of these cells are: long-term self-renewal ability, tumor-initiating capacity, and the ability to give rise to more differentiated progeny. Though the frequency of the TICs may vary among various kinds of tumors, they often represent a minor subset of tumor cells endowed exclusively with tumor-initiating ability. Tumor-initiating cells are practically defined by their ability to form tumors after xenotransplantation in immuno-deficient mice and appear to be relatively rare in most human cancers.

EXAMPLES

[0070] The following examples are included to demonstrate embodiments of the disclosure. The following examples are presented only by way of illustration and to assist one of ordinary skill in using the disclosure. The examples are not intended in any way to otherwise limit the scope of the disclosure. Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1: Tumor Cell Isolation and Culture from Human PDx.

[0071] Tumors from human primary xenografts (xenograft I) were harvested in HBSS (Gibco). Isolation of PDAC tumor xenograft (PATX) cells was performed by a combination of enzymatic (Tumor Dissociation Kit, Human, Miltenyi Biotec) and mechanical (mincing the tumor tissue in very small pieces with sterile scissors) dissociation protocols. Erythrocytes were removed through RBC Lysis Buffer 1X (eBioscience). The single-cell populations were seeded at high-confluency on collagen IV-coated plates (Corning) in DMEM/F12 (Gibco) supplemented with 10% FBS (Gibco), 1% BSA (Fisher Scientific), 0.5 μ M hydrocortisone (Sigma Aldrich), 10 mM HEPES (Invitrogen), 100 ng/ml cholera toxin (Sigma Aldrich), 5 mL/L insulin-transferrin-selenium (Becton Dickinson), 100 IU/mL penicillin (Gibco), 100 μ g/mL streptomycin (Gibco). In order to remove murine fibroblasts in the culture, brief trypsinization cycles were performed (0.25% Trypsin-EDTA (Gibco)) before each round of splitting. The enrichment for human components was confirmed by flow cytometry comparing the percentage of cells expressing human (HLA-ABC) or mouse (H-2Kd) histocompatibility complex antigens. The isolated human cells were maintained in culture for a maximum of three passages before being transplanted in a secondary host (xenograft II). Primary xenograft isolated cells were also kept in culture as spheres in semi-solid media. Single cell suspensions were plated in DMEM (Gibco) supplemented with 2 mM glutamine (Invitrogen), 10% FBS (Gibco), 40 ng/mL hEGF (PeproTech), 20 ng/mL hFGF (PeproTech), 5 μ g/mL h-insulin (Roche), 0.5 μ M hydrocortisone (Sigma Aldrich), 100 μ M β -mercaptoethanol (Sigma Aldrich), 4 μ g/mL Heparin (Sigma Aldrich), 100 IU/mL penicillin (Gibco), 100 μ g/mL streptomycin (Gibco). Methocult (StemCell Technologies) was added to SCM (0.8% final) to keep cells growing as clonal spheres versus aggregates. Fully-formed spheres were collected and digested with 0.25% trypsin-EDTA (Gibco) to single cells and re-plated.

Example 2: Libraries Design and Construction.

[0072] A custom library with 2410 shRNAs focused on chromatin remodeling enzymes was constructed by using chip-based oligonucleotide synthesis and cloned into the pRSI16 lentiviral vector (Cellecta) as a pool. The shRNA library targeted 236 genes with coverage of 10 shRNAs/gene. The shRNA included 2 G/U mismatches in the passenger strand, a 7-nucleotide loop and a 21-nucleotide targeting sequence. Targeting sequences were designed using a proprietary algorithm (Cellecta). The oligo corresponding to each shRNA was synthesized with a unique molecular barcode (18 nucleotides) for measuring representation by next-generation sequencing (NGS). The 12.5k barcoded library applied for the TIC covering studies was constructed using the same technology and cloned as a pool into the pRSI17 lentiviral plasmid (Cellecta).

Example 3: Genomic DNA Extraction and PCR for NGS Library Production.

[0073] Frozen tumors from in vivo experiments were mechanically minced to small pieces with sterile scalpels and suspended in buffer P1 (QIAGEN, 1 mL buffer/100 mg tumor) supplemented with 100 µg/mL RNase A (Promega). The dissociation step was performed in disposable gentleMACS M tubes (Miltenyi Biotec) with the gentleMACS dissociator (Miltenyi Biotec). The cell pellet obtained from the reference cells was suspended in 1 mL buffer P1/RNase A. Samples were transferred in a 15 ml polypropylene tube (Falcon) and lysed adding 1/20 volume of 10% SDS (Promega). After mixing, the reference cells lysates were incubated at room temperature (RT) for 5 minutes and the tumors for 20 minutes. Genomic DNA was sheared by passing the lysate 10-15 times through a 22-gauge syringe needle. Then, a first genomic DNA extraction step was executed adding 1 volume of phenol:chloroform pH 8.0 (Sigma Aldrich). After centrifugation (12000 rpm, 12 minutes), the upper phase was moved to a new tube and a second extraction step with chloroform (Sigma Aldrich) was performed. Again, the upper phase was transferred to a new tube and added with 0.1 volumes of 3M NaCl (Sigma Aldrich) and 0.8 volumes of isopropanol (Fisher Scientific) to precipitate the genomic DNA. Centrifugation of tumor samples was performed at 12000 rpm for 20 minutes, the samples from reference cells were stored overnight at -20°C before centrifugation. The DNA pellet was washed once in 70% ethanol (Fisher Scientific) and centrifuged again for 5 minutes at 12000 rpm. The DNA pellet was finally air-dried and dissolved overnight in UltraPure distilled water (Invitrogen). The final DNA concentration was assessed by NanoDrop 2000 (Thermo Scientific) quantification.

[0074] For NGS libraries generation, the barcodes were amplified starting from the total amount of genomic DNA in 2 rounds of PCR using the Titanium Taq DNA polymerase (Clontech-Takara) and pooling together the total material from the first PCR before proceeding with the second run. The first PCR reactions were performed for 16 cycles with the common primer 13K_R2 (5'-AGTAGCGTGAAGAGCAGAGAA-3') and the specific primers for in vivo TIC covering studies, FHTS3 (5'-TCGGATICAAGCAAAGACGGCATA-3') or in vivo screens, 13K_F2 (5'-TCGGATICGCACCAGCACGCTA-3'). The second PCR reactions were performed for 12 cycles with the common primer P5_NR2 (5'-AATGATACGGCGACCACCGAGACGAGCACCGACAACAACGCAGA-3') and the specific primers for in vivo TIC covering studies, Gx1_Bp (5'-TCAAGCAGAAGACGGCATACGAAGACA-3') or in vivo screens, P7 NF2 (5'-CAAGCAGAAGACGGCATACGATICGCACCAGCACGCCTACGCA-3'). The primers for the second PCR reactions were optimized in order to introduce the required adapters for Illumina NGS technology. The PCR amplifications were analyzed by agarose gel electrophoresis (2.5%, Lonza) to check for the expected 279 bp (in vivo TIC covering studies) or 272 bp (in vivo screens) products. Amplified PCR products from 2 replicates of the second PCR reactions were pooled together and extracted from agarose gel with the QIAquick gel purification kit (QIAGEN). The amount of purified PCR product was quantified using the High Sensitivity DNA Assay (Agilent Technologies) for the Agilent 2100 Bioanalyzer. Barcode representation was measured by NGS on an Illumina HiSeq2000 with a common sequencing primer for both the libraries, 13K_Seq (5'-AGAGGTICAGAGTICTACAGTCCGAA-3').

Example 4: In Vivo TIC Studies.

[0075] The volume of virus required to give a percentage of infection around 30% or below was determined sample by sample using a 3-points dose response in the presence of 8 µg/mL polybrene (Millipore): 0.15, 0.3, and 0.6 transducing units (TU)/cell for the human PDx cells; 0.3, 0.6, and 1.2 TU/cell for the genetically-engineered mouse model (GEMM) derived cells. Infectivity was determined as the % of RFP positive cells 2 days after infection as measured by fluorescence-activated cell sorting (FACS) analysis. In vivo TIC covering studies were performed at least in replicate. For large scale infection of human PDx cells, 6 X 10⁷ cells were plated in T-175 flasks (Corning) with fresh media containing 8 µg/mL polybrene and sufficient virus to guarantee a 25% infection rate based on precedent

calculations. For infection of GEMM-derived cells, 2×10^7 cells were plated in T-75 flasks (Corning) with fresh media containing 8 $\mu\text{g/mL}$ polybrene and sufficient virus to guarantee a 15% infection rate based on precedent calculations. 24 hours after infection, the culture media was replaced with fresh media containing puromycin (Gibco). For each cell line, the optimal puromycin dose to achieve more than 95% cell killing in 72 hours was determined by measuring cell viability with a Cell Titer Glo assay (Promega) for a 6 points dose response ranging from 0 to 8 μg of puromycin. 72 hours following puromycin addition, cells were trypsinized, pooled together, and counted. A representative portion of the total cells (normally 1/3 or 1/4) was collected as reference cells and immediately frozen as pellet at -80°C . The cells for the in vivo studies were separated into independent tubes (replicates or triplicates), suspended in 200 μL of a PBS:Matrigel (1:1) solution and injected subcutaneously into the flank of 4- to 6-week-old female immunodeficient mice (NOD scid gamma (NSG), The Jackson Laboratory). The experiments with the GEMM-derived cells were performed transplanting 10^6 cells per mouse ensuring an in vivo representation of 80 cells/barcode. For the human PDx experiments, each injection was performed with 5×10^6 cells to guarantee an in vivo coverage of 400 cells/barcode. Specifically, the TIC in vivo study with MDA-PATX53 to modulate the appropriate coverage in the human models was executed in triplicate with 1×10^6 , 3×10^6 and 5×10^6 cells from the same infection. Mice were monitored every 5 days and euthanized when the tumors reached a volume around 750 mm^3 as determined by caliper measurement. Tumor volume was calculated using the formula: $V = L^2 \cdot W/2$ (where L is length and W is width). The whole tumor was collected from each mouse under sterile conditions, weighed and snap-frozen in liquid nitrogen.

Example 5: Tumor Transplantation and Transplantation in Limiting Dilution.

[0076] Tumor cells were isolated from PDx tumors or GEMMs and stabilized in culture as described in Example 1. Generally, 10^6 tumor cells were used for routine transplantation from PDx. Tumor volume was calculated using the formula: $V = L^2 \cdot W/2$ (where L is length and W is width). For transplantation in limiting dilution 10^4 , 10^3 , 10^2 or 10 cells were used. Tumor cells were suspended in PBS (Gibco) and Matrigel (BD Biosciences) (1:1 dilution) and injected subcutaneously into the flank of 4- to 6-weekold female immunodeficient mice (NSG, The Jackson Laboratory). TIC frequencies and 95% confidence intervals were determined by the ELDA software (Hu et al., 2009). All manipulations were performed under Institutional Animal Care and Use Committee (IACUC) approved protocols.

Example 6: Bioinformatic Data Analysis.**Read Counting**

[0077] Illumina base calls were processed using CASAVA (version 1.8.2) and resulting reads were processed using an in-house pipeline. Raw FASTQ files were filtered for a 4 base pair spacer (CGAA) starting at the 18th base allowing for one mismatch to account for sequencing errors, such that only reads amplified using above mentioned PCR reactions were used for further processing. 23-40 bp of the above reads were then extracted for targeting libraries, and 1-18 bp for a non-targeting library. These reads were further aligned using Bowtie (2.0.2) to their respective libraries (2.4k mouse epigenome, 2.4k human epigenome and 12.5k non-targeting library) (Langmead et al., 2009). SAMtools were then used to count the number of reads aligned to each barcode (Li et al., 2009).

Complexity Analysis

[0078] Read counts were normalized for the amount of sequencing reads retrieved for each sample, using library size normalization (to 100 million reads).

Hit Analysis

[0079] A similar approach was employed as with complexity analysis, describe above. Using normalized counts, each sample was then compared with its respective reference and a Log₂ Fold Change (FC) was calculated. This was further normalizing using a robust Z-Score defined by: $(FC_i - \text{Median}) / \text{Median Absolute Deviation (MAD)}$ (Konig et al., 2007). To summarize the effect of knock-down at the gene level, RSA was employed to score each gene (Birmingham et al., 2009).

Example 7: Target Identification of Druggable Components that are Rate Limiting in a Defined Genetic Context.

[0080] To address the lack of effective treatment regimens in PDAC pancreatic, a systematic functional approach was created to target identification, with the goal of identifying druggable components that are rate limiting in defined genetic contexts.

[0081] Applying the PILOT platform (FIG. 1), short-term cultures isolated from PDx models were established. These cultures recapitulate the complex histology of human PDAC (i.e., glandular structures surrounded by dense desmoplasia) when implanted into immunocompromised mice (FIG. 2A). The purity of the PDx derived-cultures was determined using flow cytometry by estimating the percentage of human histocompatibility complex (HLA) positive cells (FIG. 2B). Before transplanting the PDx human cells in a secondary host, the ability of the culture to be infected with lentivirus and the possibility to modulate the infection rate (0, 3 and 10 μ L of pLK03.G-GFP virus, FIG. 2C), an important step for in vivo screening with shRNA libraries, was verified. To rapidly and accurately determine TIC frequency in PDAC primary models, a non-targeting "tracking" library was used expressing 12,500 unique molecular barcodes in early passage tumor samples to "tag" individual cells and assess their fate by comparing clone representation in infected cells with that emerging after tumor establishment in recipient mice (FIG. 3A). Next, cells isolated from early passage human PDAC xenografts (MDA-PATX43, MDA-PATX50, MDA-PATX53, MDA-PATX66) were infected with the tracking library at a low MOI (<1 integrant/cell) (FIG. 3B) and then the infected cells (puromycin selection) were implanted subcutaneously into NSG mice. To model optimal library distribution, tumors seeded with 80, 240, or 400 individual cells/barcode were analyzed. Tumors were isolated from mice and individual barcodes quantified by deep sequencing for comparison with the reference cell population. Variability was observed among PDx models, demonstrating coverage of the tracking library with implantation of 400 cells/barcode in MDA-PA TX53 and MDA-PA TX43 (FIG. 3C). Depletion of a majority of the tracking library in MDA-PA TX50 and MDA-PA TX66 was observed even with implantation of 400 cells/barcode (FIG. 3D), suggesting TIC frequency in these cell lines was too low to sustain the expression of a library of such complexity. Assessment of TIC frequency by extreme limiting dilution supported the results obtained from the tracking library (FIG. 3E). Thus, the approach facilitates rapid assessment of TIC frequency to optimize experimental design and ensure adequate library coverage, vastly elevating the utility of excised tumor samples for genetic screen approaches.

[0082] To explore the utility of the system in the discovery of therapeutic targets, an extensive collection of chromatin regulators was investigated in the context of PDAC. To probe epigenetic vulnerabilities in PDAC, and guided by the TIC results from the tracking library, MDA-PATX53 and MDA-PATX66 were enlisted in in vivo screens of an shRNA library targeting 236 unique mouse or human epigenetic regulators (FIG. 4A). To enhance the robustness of the screen and facilitate hit prioritization, the library was designed with 10 unique shRNAs targeting each epigene (2400 shRNA, FIG. 4B). To ensure adequate representation of the complexity of our deep-coverage epigenetics library in human samples, mice were implanted with 2,000 cells/shRNA, and barcode abundance quantified in established xenografts by deep sequencing (FIG. 4C). Comparison among replicates (Pearson's correlation factor) confirmed the usefulness of the tracking approach in predicting the TIC coverage and feasibility conditions sample-by-sample (FIG. 4D).

[0083] To confirm an accurate representation of each shRNA, a cumulative distribution was function (CDF) was applied as a firstline filter to detect the top 15-30% most-depleted shRNAs (FIG. 5A). Results from CDF analysis of the epigenetics library identifying the proportion of shRNAs not depleted below the cutoff (-2Log_2) matched, with good approximation, what was observed in the corresponding TIC coverage study (FIG. 5B). "Hits," or top-scoring genes, emerging from the screen were prioritized by determining the fold-change of the top 3 shRNAs for each gene to generate a RSA (Redundant shRNA Activity) score and subsequently rank hits based on the corresponding p-value (FIG. 5C). Notably, two well-known essential genes encoding for a proteasome (PSMA 1) and a ribosomal (RLP30) protein added to the library as positive controls scored among the most-depleted hits (FIG. 5D), as did PHF5A, SMC2, and BRD4, all known to have a role in cancer (Hubert et al., 2013; Krattenmacher et al., 2014; Sahai et al., 2014). The majority of pancreatic adenocarcinomas harbor mutant KRAS, which plays a key role in reprogramming pancreatic cancer cells into duct-like lineages capable of progressing from pre-neoplastic lesions to advanced PDAC, as demonstrated in genetically engineered mouse models (GEMMs) (Almoguera et al., 1988). PDAC progression is accompanied by genomic deletion and/or loss-of-function mutations in tumor suppressor genes (TSGs), including TP53, CDKN2A, SMAD4, and PTEN (Hingorani et al., 2003; Hingorani et al., 2005). Recent large-scale genome profiling of human PDAC has identified low-frequency mutational events contributing to epigenetic deregulation and defects in DNA repair, and chromosome structure analysis has now classified human PDAC into 4 molecular subtypes with the potential to

define biomarkers of subtype-specific drug response (Omura and Goggins, 2009; Waddell et al., 2015). The genetic and transcriptional profiling of the PDx models (FIG. 5E) enrolled in the in vivo screening approach, together with the clinico-pathological data for the matched patients, gave the unique opportunity to prioritize the most relevant "hits" for targeting this dramatic disease and potentially predict the most responsive genetic context. Taken together, the data highlight PILOT as a powerful new tool to identify genetic vulnerabilities using PDAC patient-derived tumor samples.

[0084] Focusing on epigenetic regulators, WDR5 was identified using the methods described herein, a core member of the COMPASS histone H3 Lys4 (H3K4) MLL methyltransferase complex, as a top tumor maintenance hit required across multiple human and mouse tumors. Mechanistically, WDR5 functions to sustain proper execution of DNA replication in PDAC cells, as previously suggested by replication stress studies involving MLL1, and c-Myc, also found to interact with WDR5. Here, that interaction with c-Myc was demonstrated to be critical for this function. By showing that ATR inhibition mimicked the effects of WDR5 suppression, these data provide rationale to test ATR and WDR5 inhibitors for activity in this disease.

Example 8: Development of an In Vivo Functional Genomic Screen in Patient-Derived Xenografts

[0085] A rapid and reproducible protocol was established to isolate primary tumor cells from patient-derived xenograft (PDx) tissue or genetically engineered mouse models (GEMMs). These primary models more closely reflect the histologic and phenotypic complexity of human tumors. More importantly, short-term cultures isolated from primary models recapitulate the complex histology of human PDAC (i.e., glandular structures surrounded by dense desmoplasia) when implanted into immunocompromised mice.

[0086] In vivo shRNA screens rely on the specific elimination of individual shRNAs in a cell population and require therefore that the infected cell population be adequately endowed with tumor engraftment capacity when implanted into recipient mice. The ability of a tumor cell population to engraft and propagate itself when implanted in vivo varies dramatically among tumor types and must be accurately determined to ensure faithful representation of complex, pooled shRNA libraries (Quintana et al., 2008; Ishizawa et al., 2010). Most commonly, the engraftment efficiency is a measure of the TIC frequency, and it is assessed by in vivo transplantation upon extreme limiting dilution assays (Hu and Smyth, 2009;

Bonnefoix and Callanan, 2010), but this approach is time consuming and results are widely variable across biological replicates. This limits the use of biologically relevant PDx models, as low-passage human cells have a very low and variable TIC frequency relative to established human and murine cell lines.

[0087] To rapidly and accurately determine the engraftment efficiency required by each PDAC model, non-targeting “tracking” library expressing 12,500 unique molecular barcodes were used in early passage tumor samples to “tag” individual cells and assess their fate by comparing clone representation in infected cells with that emerging after tumor establishment in recipient mice.

[0088] To demonstrate the power of the approach, cells isolated from early passage human PDAC xenografts (MDAPATX43, MDA-PATX50, MDA-PATX53, MDA-PATX66) and PDAC GEMM-derived allografts (Ptf1a-Cre, Kras^{G12D_LSL/+}, Tp53^{+/L}; Ptf1a-Cre, Kras^{G12D_LSL/+}, Cdkn2a^{+/L}; Ptf1a-Cre, Kras^{G12D_LSL/+} escaper line), which display either epithelial or mesenchymal histological features, can be infected with the tracking library at a low MOI (less than one integrant/cell) and infected cells (puromycin selection) implanted subcutaneously into NSG mice. To model optimal library distribution, tumors seeded with 80, 240, or 400 individual cells/barcode were analyzed. Tumors were isolated from mice, and individual barcodes were quantified by deep sequencing for comparison with the reference cell population. Implantation of fewer cells/barcode was required to adequately represent the tracking library (reference/tumor Log₂ ratio resulted in a normal distribution) in murine cells (80 cells/barcode) compared to all four PDx cell populations. Variability was observed among PDx derived samples, demonstrating coverage of the tracking library with implantation of 400 cells/barcode in MDAPATX53 and MDA-PATX43, but not in MDA-PATX50 and MDA-PATX66 even with implantation of 400 cells/barcode, suggesting the engraftment efficiency in these cell lines was too low to sustain the expression of a library of such complexity. Assessment of TIC frequency by extreme limiting dilution was supportive of the conclusions from the tracking library. Thus, this approach facilitates rapid assessment of xenotransplantation potential in order to optimize experimental design and ensure adequate library complexity, vastly elevating the utility of excised tumor samples for genetic screen approaches.

Example 9: In Vivo Loss-of-Function Screens to Identify Epigenetic Vulnerabilities in Patient-Derived Xenografts and Mouse Models

[0089] To explore the utility of the system in the discovery of therapeutic targets, focus was placed on an extensive collection of chromatin regulators in the context of PDAC. Genetic lesions in chromatin regulators have been identified in a variety of cancers, and new epigenetic cancer dependencies are emerging as actionable vulnerabilities (Dawson and Kouzarides, 2012; Hoffman et al., 2014). More specifically, epigenetic deregulation is a documented genetic hallmark of PDAC and developmental oncobiology overall, implying a causal role in disease pathogenesis (Omura and Goggins, 2009). To probe epigenetic vulnerabilities in PDAC, and guided by the engraftment efficiency results from the tracking library, three murine cell lines and MDA-PATX53 and MDA-PATX43 were enlisted in in vivo screens of an shRNA library targeting 236 unique mouse or human epigenetic regulators. To enhance the robustness of the screen and facilitate hit prioritization, the library was designed with ten unique shRNAs targeting each epigene. To ensure adequate representation of the complexity of our deep coverage epigenetics library in human and mouse samples, mice were implanted respectively with 2,000 cells/shRNA and 400 cells/shRNA, and barcode abundance was quantified in established xenografts by deep sequencing. Comparison among replicates (Pearson's correlation factor) confirmed the usefulness of the tracking approach in predicting the engraftment efficiency.

[0090] To detect the top 15%–30% most-depleted shRNAs a cumulative distribution function (CDF) was applied as a first-line filter, using thresholds of $-2/-4 \text{ Log}_2$. Imposing the same thresholds on the previously introduced engraftment efficiency study with the tracking library, less than 2.5% barcode scoring was observed, substantiating that only significantly depleted hairpins were detected. Multiple methods were leveraged to evaluate “hits” (or top-scoring genes) emerging from our screens, including mean of the top-scoring three hairpins and p values from RSA (or redundant shRNA activity) scores. Notably, irrespective of the approach used, two well-known essential genes encoding for a proteasome (PSMA1) and a ribosomal (RLP30) protein added to the library as positive controls scored among the most-depleted hits, as did PHF5A, SMC2, and BRD4, all known to have a role in cancer (Hubert et al., 2013; Krattenmacher et al., 2014; Sahai et al., 2014). Unsupervised clustering analysis of all genes in the library validated replicates across tumors but also identified vulnerabilities unique to each model. Downregulation of PHF5A, SMC2, and WDR5 using two independent

singleton shRNAs significantly impaired new colony formation and tumor growth in both human and mouse PDAC models.

Example 10: WDR5 is Essential for PDAC Initiation and Proliferation.

[0091] One of the most robust hits to emerge across multiple screens was the WD repeat-containing protein 5 (WDR5), a core member of the COMPASS histone H3 Lys4 (H3K4) methyltransferase complex (Steward et al., 2006; Trievel and Shilatifard, 2009; Smith et al., 2011). Recently, WDR5 upregulation was detected in prostate and bladder cancers, where it was also found to be critical for cancer cell proliferation (Kim et al., 2014; Chen et al., 2015). Deregulated expression of WDR5 was first confirmed in human PDAC compared to normal control pancreas. WDR5 knockdown dramatically affected tumor growth of orthotopically implanted patient-derived PDAC cells and extended survival compared to non-targeting (NT) shRNA controls. The observed effects were confirmed to be on target, as expression of ectopic WDR5 cDNA lacking the 30 UTR targeted by the shRNA rescued the impairment in colony formation ability upon WDR5 knockdown (Figures 3C and S4A). Next, we confirmed an essential role across multiple primary patient-derived PDAC in vivo models. Moreover, pancreatic cancer spheres were also generated from both human and mouse PDAC samples using serum-free 3D growth conditions (Viale et al., 2014). Consistent with the observed in vivo response, WDR5 knockdown significantly impaired the spherogenic potential of these tumor-initiating cells, as demonstrated by calcein staining and spheroid counts.

[0092] To evaluate whether WDR5 was essential for proliferation of an established PDAC tumor, patient-derived cells (MDA-PATX53 and MDA-PATX66) were infected with Tet-inducible WDR5 shRNA (Sh1 hWDR5i, Sh2 hWDR5i) or control shRNA constructs (Sh NTi) and transplanted them in host mice (n = 5). A dramatic growth arrest of established tumors was observed upon WDR5 downregulation under doxycycline treatment that was maintained through the end of the study (Student's t test, $p < 0.05$). Immunohistochemistry staining confirmed that WDR5 knockdown was positively correlated with a robust reduction of the proliferation marker Ki67. Taken together, these data confirm that WDR5 is a critical regulator of tumor growth in human PDAC.

Example 11: WDR5 Inhibition Arrests Tumor Progression of Autochthonous PDAC Models.

[0093] To further validate the biological role of WDR5 in tumor maintenance, an autochthonous lentiviral-based somatic-mosaic (pLSM5) in vivo system was developed. A

modular system was designed by combining the Cre-LoxP and Flpo-Frt technologies in a single vector, thereby generating PDAC cells carrying a latent shRNA to allow time-restricted, acute inactivation of any gene of interest in established tumors generated from cells transplanted in the pancreas of host recipients. Specifically, early epithelial progenitor cultures were established from R26^{Cag-FlpoERT2/+}; KRas^{G12D_LSL/+}; Tp53^{L/L} or Rosa26^{mTmG/+} embryonic livers and expanded ex vivo (Zender et al., 2008). Cells were transduced with the pLSM5 system where a Frt-stop-Frt cassette containing the Cre recombinase under the Krt19 promoter was cloned between the U6 promoter and the shRNA and transplanted in immunocompromised Rag2^{-/-} mice pretreated with cerulein. Specifically, transplanting epithelial progenitors from Rosa^{26mTmG/+} mice, upon infection with the pLSM5-K19 lentiviral vector, no tumor formation and differentiation in pancreatic acini was observed, as demonstrated by double positivity for GFP and amylase of pancreatic sections, suggesting that early embryonic liver progenitors can be reprogrammed by the host microenvironment toward a pancreatic exocrine differentiation. Instead, R26^{Cag-FlpoERT2/+}; KRas^{G12D_LSL/+}; Tp53^{L/L} transplants generated tumors and expressed the epithelial markers Cytokeratin 19 and Sox9 and the pancreas-specific marker PDX1, suggesting that embryonic endodermal progenitors are remarkably adaptable and able to generate pancreatic tumors, which pathologically recapitulate the human counterpart. Upon tumor establishment, FlpoERT2 was activated by repeated tamoxifen (Tx) treatments to remove the stopper cassette and activate the shRNA. Consistent with the above findings, acute inactivation of Wdr5 in vivo resulted in a dramatic increase of overall survival and inhibition of tumor growth, characterized by a decrease in the numbers of Ki67 positive cells and accumulation of DNA damage (γ H2AX staining).

Example 12: The COMPASS Complex is a Critical Regulator of PDAC Development.

[0094] COMPASS and COMPASS-like complexes are characterized by their unique subunit composition, and individual subunits appear to dictate the biological functions of each complex (Trievel and Shilatifard, 2009; Smith et al., 2011). For example, even though both MLL1 and MLL2 are recruited to the Hox loci through MEN1-specific interactions, they also have non-redundant functions, as exemplified by the phenotypes of MLL1 and MLL2 knockout mouse models (Yu et al., 1995; Wang et al., 2009). The WDR5-RBBP5-ASH2L (WAR) core showed high protein expression level in human PDAC xenografts, associated with a hypermethylation phenotype. The functional non-redundant role of the COMPASS complex in human PDAC was proved by the significant impairment of colony formation ability we observed when ASH2L, RBBP5, and MLL1 were downregulated in PDX-derived samples using two independent shRNAs. In addition, the downregulation of each member of the so-called WAR module (WDR5-ASH2L-RBBP5) in human PDAC models affected the expression of a set of common genes highlighting a critical role of the complex in sustaining tumor proliferation. WDR5 association with methyltransferases in the COMPASS complex leads to H3K4 methylation, a validated marker of open-chromatin conformation and active transcription (Steward et al., 2006; Trievel and Shilatifard, 2009; Smith et al., 2011; Schuettengruber et al., 2011). Using H3K4me3 chromatin immunoprecipitation sequencing (ChIP-seq), a moderate overall reduction in global methylation levels in both human and mouse PDAC cells was observed. Mapping of tri-methylation profiles with functional elements of the genome confirmed that a relatively small fraction (4%–20%) of the methylation regions found altered upon WDR5 suppression was in proximity (<1 kb) of transcriptional start sites (TSSs). These results suggest that the COMPASS complexes may orchestrate the transcriptional control of cancer-relevant genes that support PDAC maintenance. cross-species RNA sequencing (RNAseq) analysis was performed to inform on transcriptional changes consequent to WDR5 knockdown. Gene set enrichment analysis (GSEA) identified genes involved in the control of DNA replication and cell-cycle progression.

Example 13: WDR5 Complex Protects PDAC Cells from Replicative Stress and DNA Damage through Myc Interaction.

[0095] Bromodeoxyuridine (BrdU)-labeling studies were performed in patient-derived PDAC cells and found that WDR5 knockdown resulted in a reduction in BrdU incorporation, with a paradoxical increase in overall DNA content, suggesting failure to sustain a DNA

replication checkpoint. It is well established that tumor cells are inherently more sensitive to S-phase perturbations, possibly due to an increased number of active replication forks and concomitant alterations in activating G1 checkpoints (Nghiem et al., 2001). Indeed, treatment with the ATR inhibitor, VE-821, caused accumulation of replicative damage in the same human PDAC cell lines. In both cases, the observed dramatic cell-cycle phenotype was associated with induction of DNA damage (gH2AX staining), which was also observed upon knocking down WDR5 in vivo. Subcellular protein fractionation indicated a reduction of chromatin-bound CDC45 and an increase in chromatin-bound CDT1 upon WDR5 knockdown, suggesting that prolonged stalling of replication forks may underlie the observed arrest of the replication machinery in the absence of WDR5. The analysis of the chromatin-bound fraction also highlighted a reduction in c-Myc as a consequence of WDR5 silencing. By performing immunoprecipitation experiments for exogenously expressed Flag-WDR5 or endogenous c-Myc, the physical interaction of these two proteins in PDAC cells was confirmed. Notably, WDR5 mutants carrying specific point mutations in the Myc binding site (L240K and V268E; Thomas et al., 2015) drew accumulation of DNA damage (gH2AX staining), similar to the effects seen upon knocking down WDR5, and most likely due to dominant-negative effects on PDAC cells. The induction of DNA damage observed upon infection of PDAC cells with these WDR5 mutants also resulted in a significant impairment of new colony formation, which was not detected when overexpressing wild-type WDR5. Finally, PDAC cells showed a greater sensitivity to pharmacological inhibition of the WDR5 interaction network (OICR-9429, 5-10 μ M) than the specific inhibition of the WDR5-MLL1 association (MM-401, 20-40 μ M) in colony formation assays.

Other Embodiments

[0096] The detailed description set forth above is provided to aid those skilled in the art in practicing the present disclosure. However, the disclosure described and claimed herein is not to be limited in scope by the specific embodiments disclosed herein because these embodiments are intended as illustration of several aspects of the disclosure. Any equivalent embodiments are intended to be within the scope of this disclosure. For example, while the preceding examples were targeting PDAC, one of skill in the art would readily recognize that various other cancer types could be targeted by the methods disclosed herein. Examples include, but are not limited to, melanoma, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer,

cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like. Indeed, various modifications of the disclosure in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description, which do not depart from the spirit or scope of the present inventive discovery. Such modifications are also intended to fall within the scope of the appended claims.

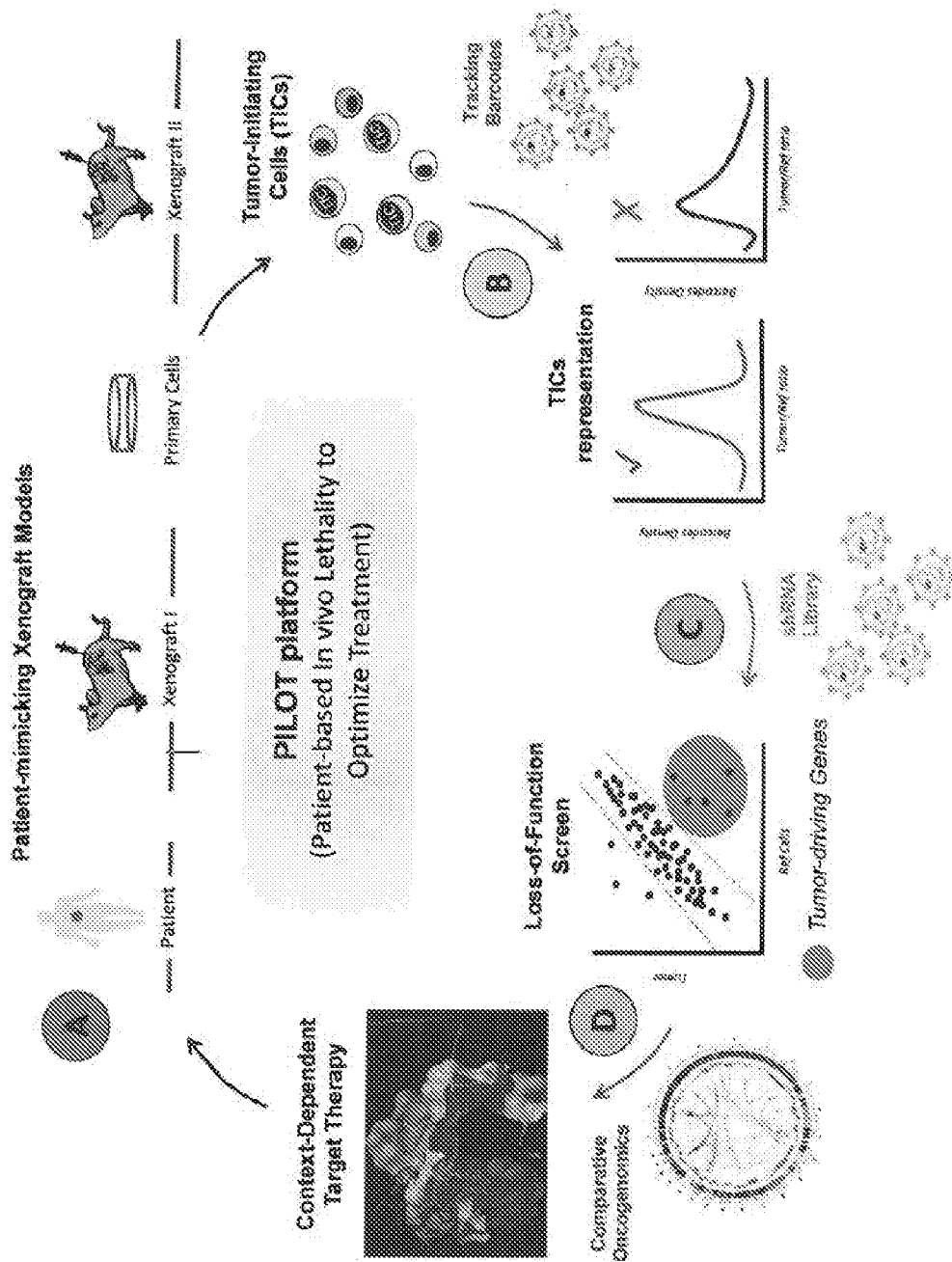
CLAIMS

What is claimed is:

1. A method for identifying a gene that modulates a function or a phenotype associated with tumorigenesis of a patient-derived cell of defined genotype comprising the following steps:
 - a) introducing into a patient-derived cancerous cell population representative of a given phenotype or histological type:
 - i) a nucleic acid library wherein said library comprises a collection of genetic elements of interest; and
 - ii) one or more unique genetic barcode sequences, thereby producing a genetically engineered target cell population having a cancer cell genotype;
 - b) transplanting the target cell population into a non-human mammal to produce a tumor in the mammal; and
 - c) identifying the loss-of-expression of one or more of the genetic elements of interest in the outgrowing/surviving tumor.
2. The method of claim 1, wherein step (c) is determined by deep sequencing.
3. The method of claim 1, wherein said transplanting is orthotopic or heterotopic.
4. The method of claim 1, wherein the genetic barcode sequences comprises from 4 to 100 nucleotides.
5. The method of claim 1, wherein the cell representative of a given genotype or histological type is a mammalian cell.
6. The method of claim 1, wherein the cell representative of a given genotype or histological type is a progenitor cell or stem cell.
7. The method of claim 1, further comprising inactivating or suppressing one or more tumor suppressor protein pathways in the cell representative of a given genotype or histological type.
8. The method of claim 7, wherein the tumor suppressor protein pathway is RB, TP53, CDKN2A, SMAD4, PTEN, STK11 or a combination thereof.

9. The method of claim 1, further comprising inactivating or suppressing one or more genetic elements of interest in the cell representative of a given genotype or histological type.
10. The method of claim 1, wherein said nucleic acid library comprises siRNA, shRNA, sgRNA, microRNA or an antisense nucleic acids to candidate genes or genetic elements of interest coding for specific proteins.
11. The method of claim 10, wherein the candidate genes or genetic elements of interest comprise enzymes.
12. The method of claim 11, wherein the enzymes comprise metabolic enzymes.
13. The method of claim 12, wherein said metabolic enzymes are wildtype or activated mutant metabolic enzymes.
14. The method according to claim 10, wherein the candidate genes or genetic elements of interest comprise kinase genes and/or genetic elements.
15. The method of claim 14, wherein said kinase is a wildtype kinase or an activated mutant kinase.
16. The method according to claim 10, wherein the candidate genes or genetic elements of interest comprise a phosphatase gene and/or genetic elements.
17. The method of claim 16, wherein said phosphatase is a wildtype or activated mutant phosphatase.
18. The method according to claim 10, wherein the candidate genes or genetic elements of interest comprise an epigenetic regulator gene and/or genetic elements.
19. The method of claim 18, wherein said epigenetic regulator is a wildtype or activated mutant epigenetic regulator.
20. The method according to claim 10, wherein the candidate genes or genetic elements of interest comprise genes and/or genetic elements for membrane-bound proteins.
21. The method according to claim 10, wherein the candidate genes or genetic elements of interest comprise genes and/or genetic elements involved in a G-protein coupled receptor signaling pathway.

22. The method according to claim 10, wherein the candidate genes or genetic elements of interest comprise genes and/or genetic elements involved in the receptor tyrosine kinase signaling pathway.
23. The method of claim 10, wherein said function or phenotype associated with tumorigenesis is metastasis, cell migration, angiogenesis, extracellular matrix degradation, anchorage independent growth, or anoikis.
24. A method for screening or identifying a genetic element of interest that synergizes with a biologically active agent that interacts with a tumorigenesis pathway comprising the following steps:
 - a) producing a genetically engineered target cell having a cancer cell genotype, said producing step comprising introducing into a patient-derived cancerous cell population representative of a given phenotype or histological type a nucleic acid library, wherein said library comprises a collection of genetic elements of interest and one or more unique genetic barcode sequences;
 - b) contacting the genetically engineered target cell with a candidate biologically active agent; and
 - c) identifying the loss-of-expression of one or more of the genetic elements of interest in the tumor by determining whether the biologically active agent has increased anti-tumor activity as compared to tumors without the loss-of-expression of the one or more genetic elements of interest.
25. The method of claim 24, wherein the tumorigenic phenotype is metastasis, cell migration, angiogenesis, extracellular matrix degradation, anchorage-independent growth, or anoikis.



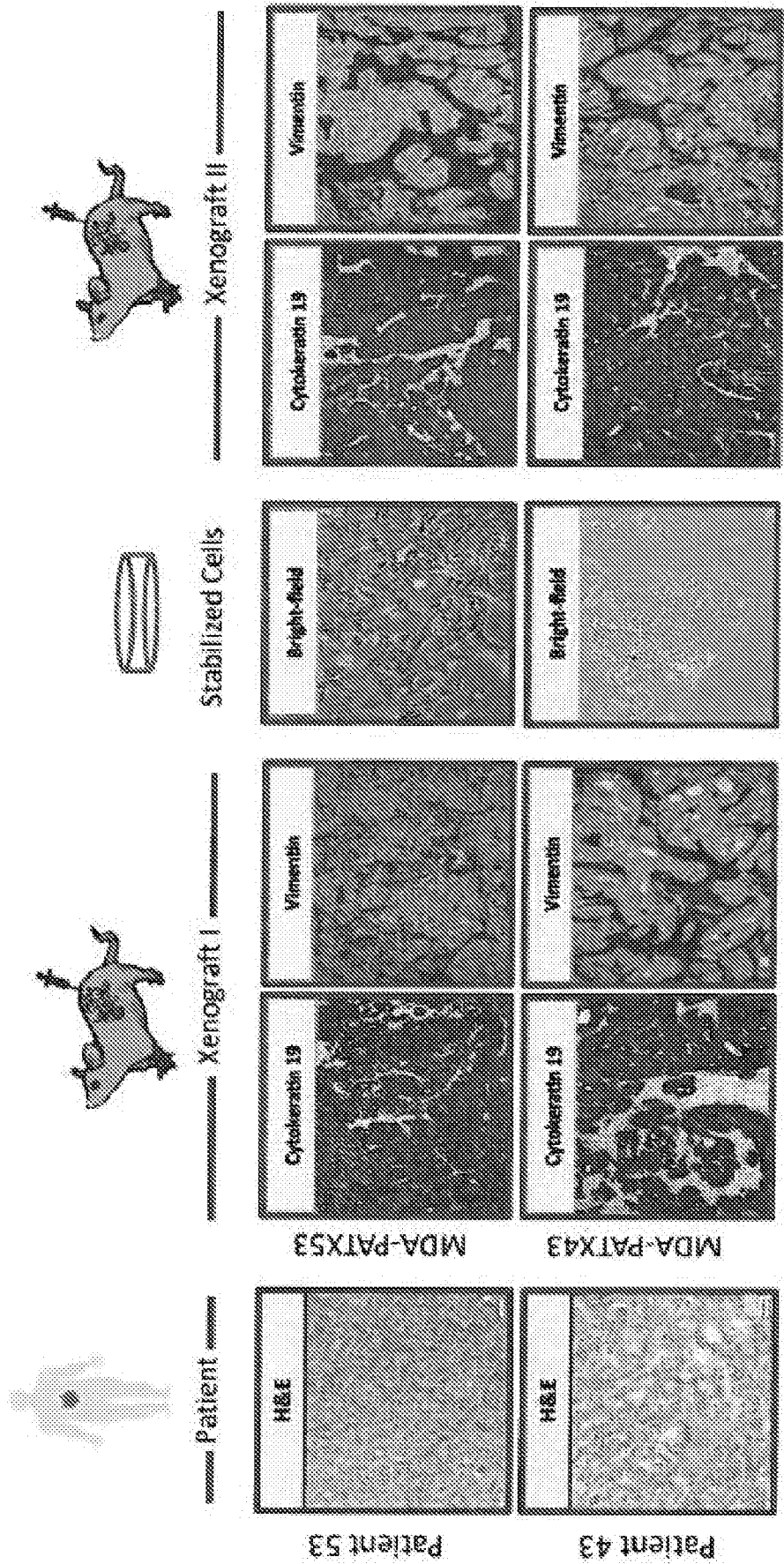
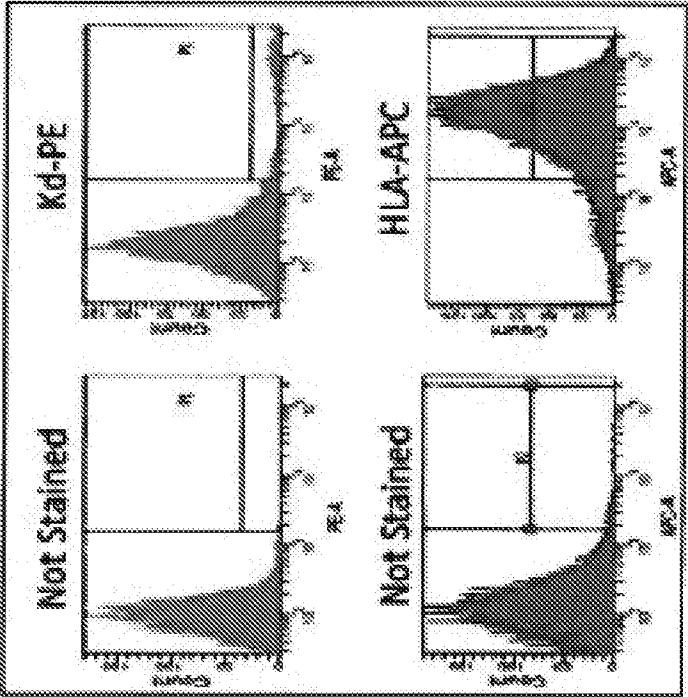


FIG. 2A

MDA-PATX43 Cells



MDA-PATX53 Cells

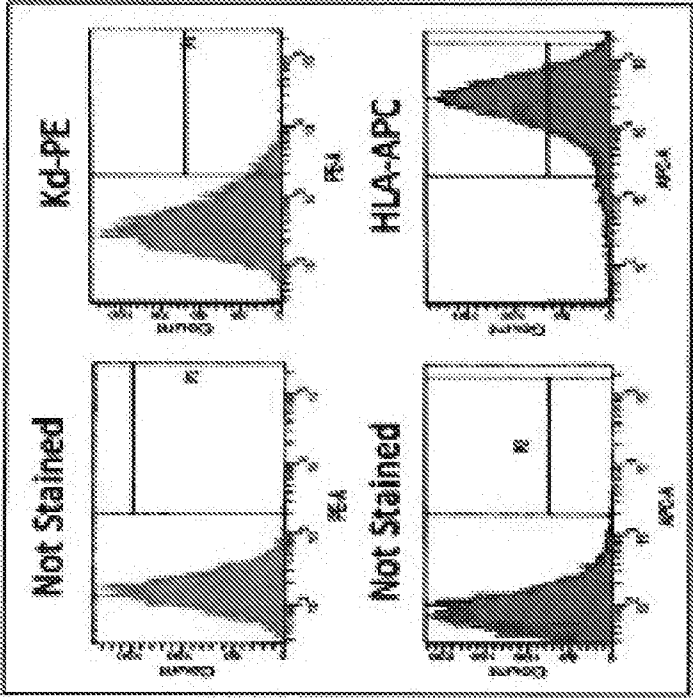


FIG. 2B

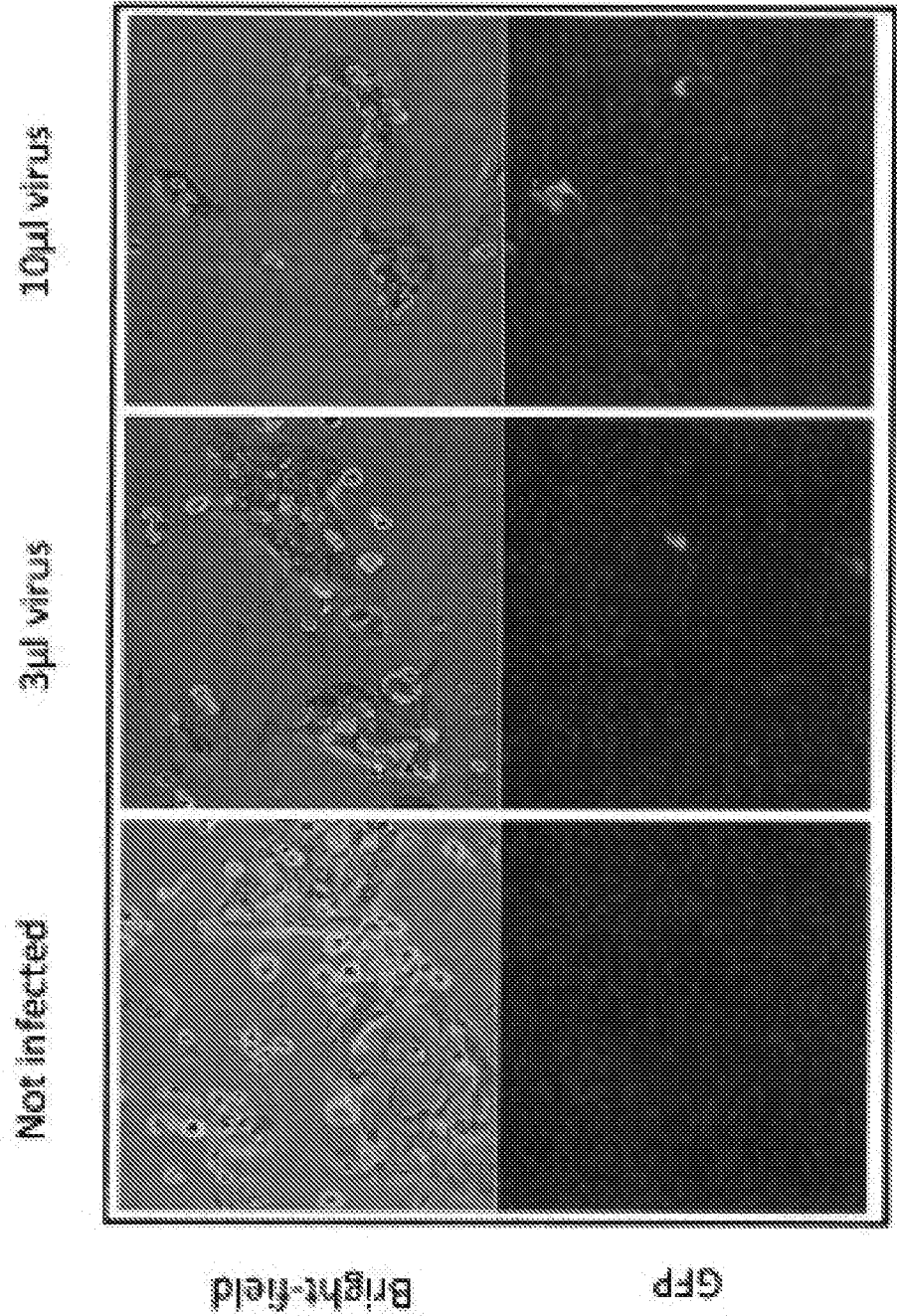


FIG. 2C

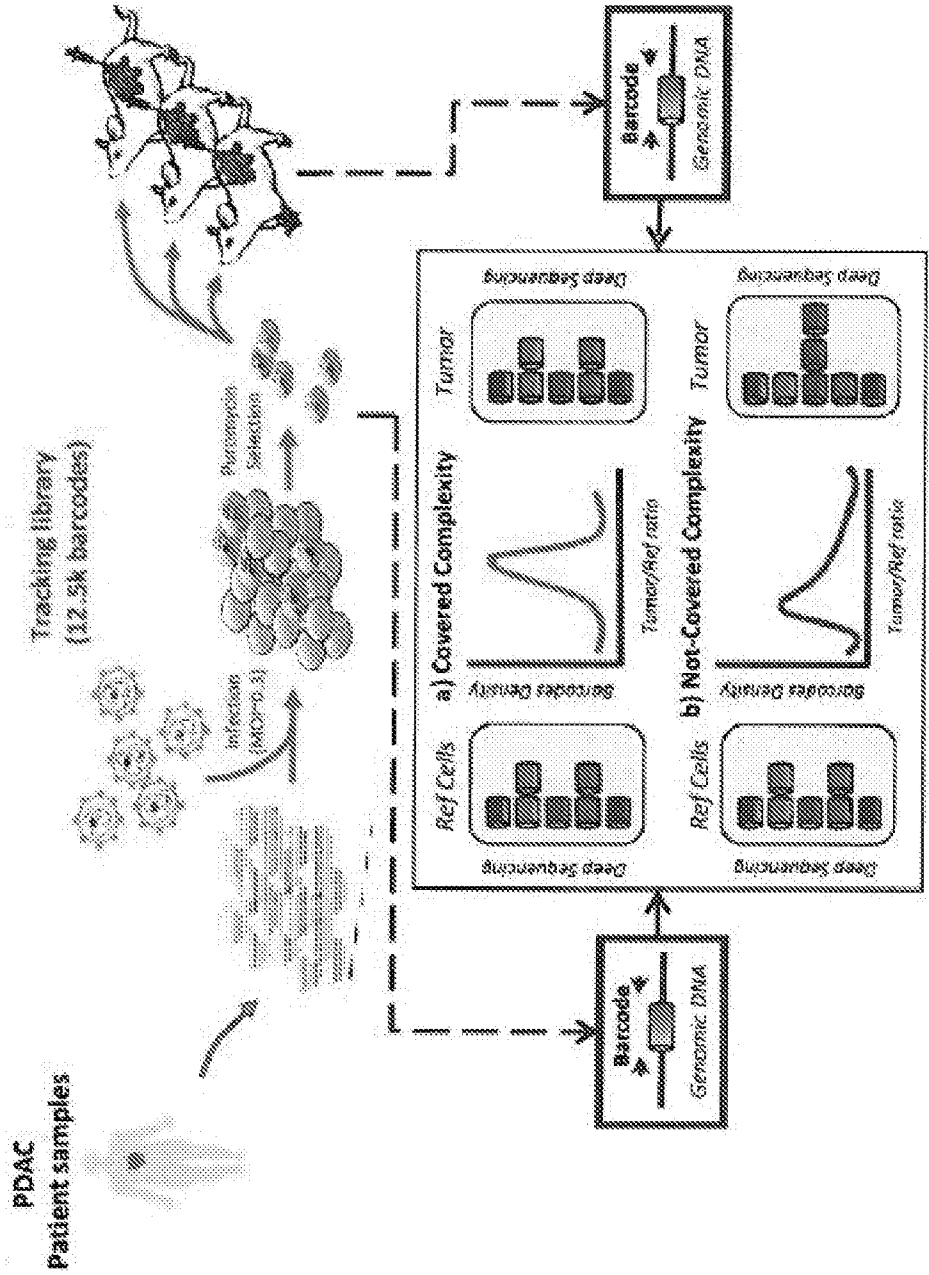


FIG. 3A

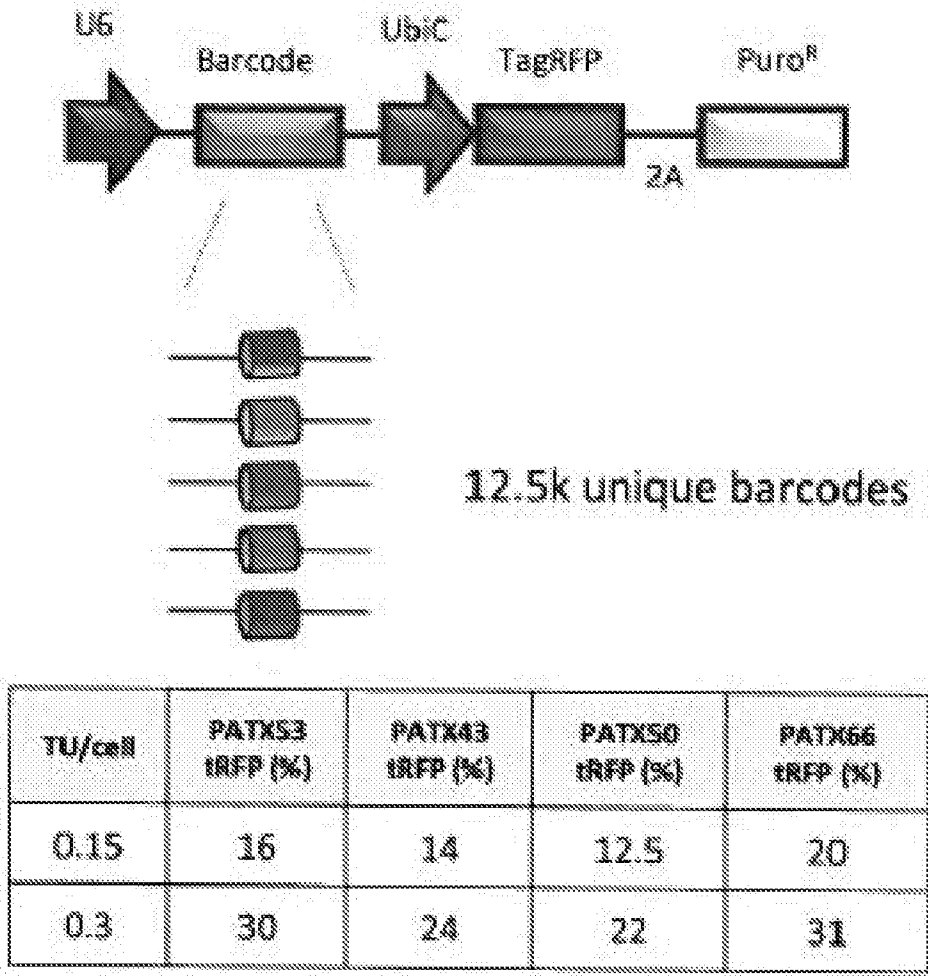


FIG. 3B

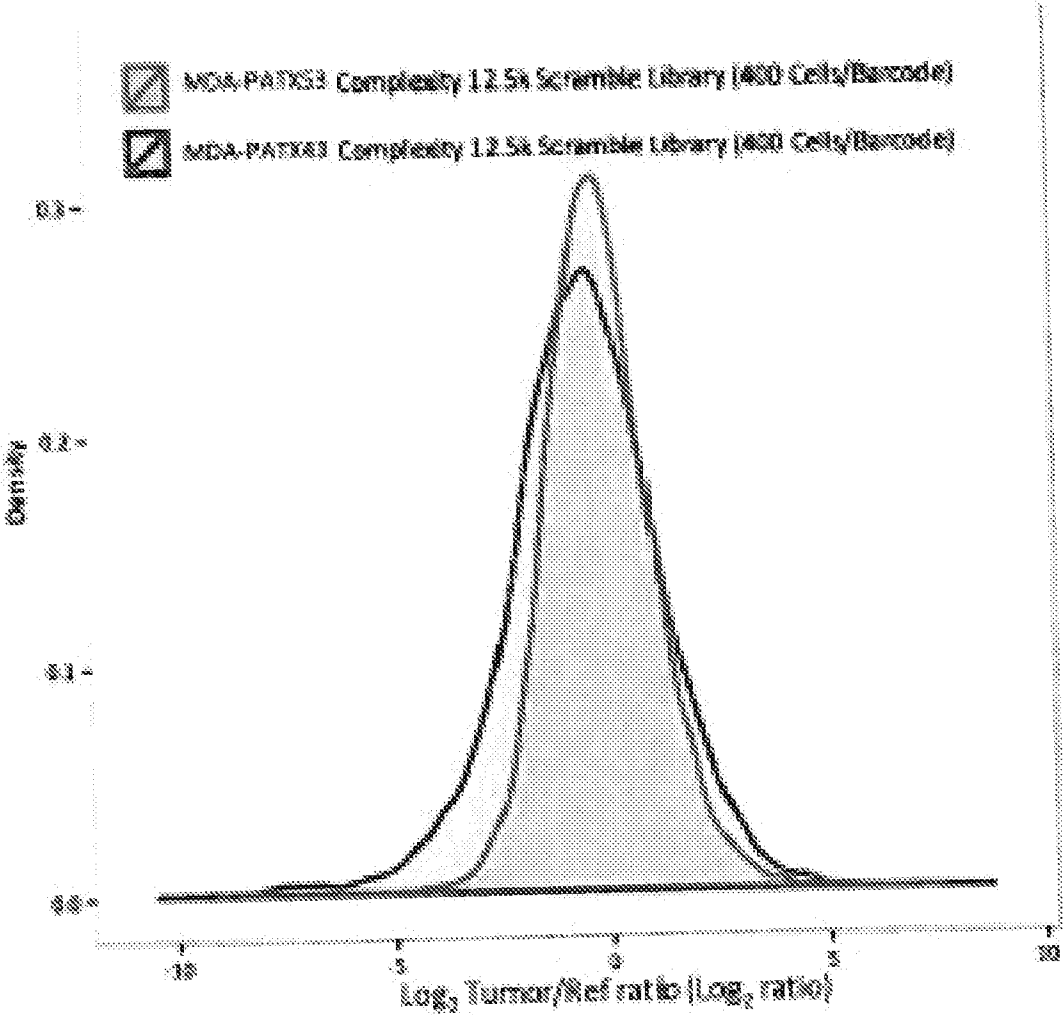


FIG. 3C

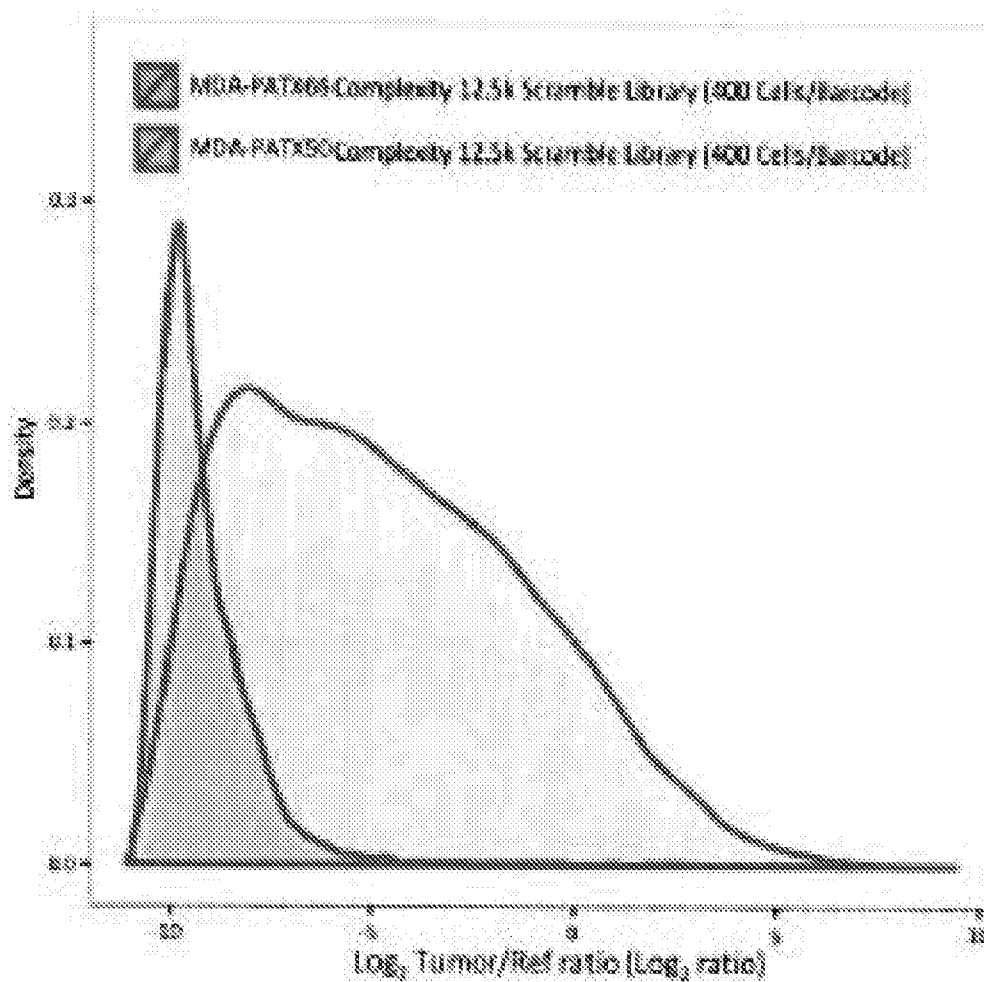


FIG. 3D

MDA-PATX53		MDA-PATX43	
TEST SET	FAILURES	TEST SET	FAILURES
100,000	2/2	100,000	2/2
10,000	4/4	10,000	4/4
1,000	4/4	1,000	3/4
100	2/10	100	3/10
10	1/10	10	0/10
TIC Frequency: 1/288 (95% C.I.: 1/118-1/704)		TIC Frequency: 1/495 (95% C.I.: 1/197-1/1245)	

MDA-PATX50		MDA-PATX66	
TEST SET	FAILURES	TEST SET	FAILURES
100,000	2/2	100,000	2/2
10,000	3/4	10,000	3/4
1,000	2/4	1,000	0/4
100	0/10	100	0/10
10	0/10	10	0/10
TIC Frequency: 1/4848 (95% C.I.: 1/687-1/13936)		TIC Frequency: 1/9138 (95% C.I.: 1/2921-1/28583)	

FIG. 3E

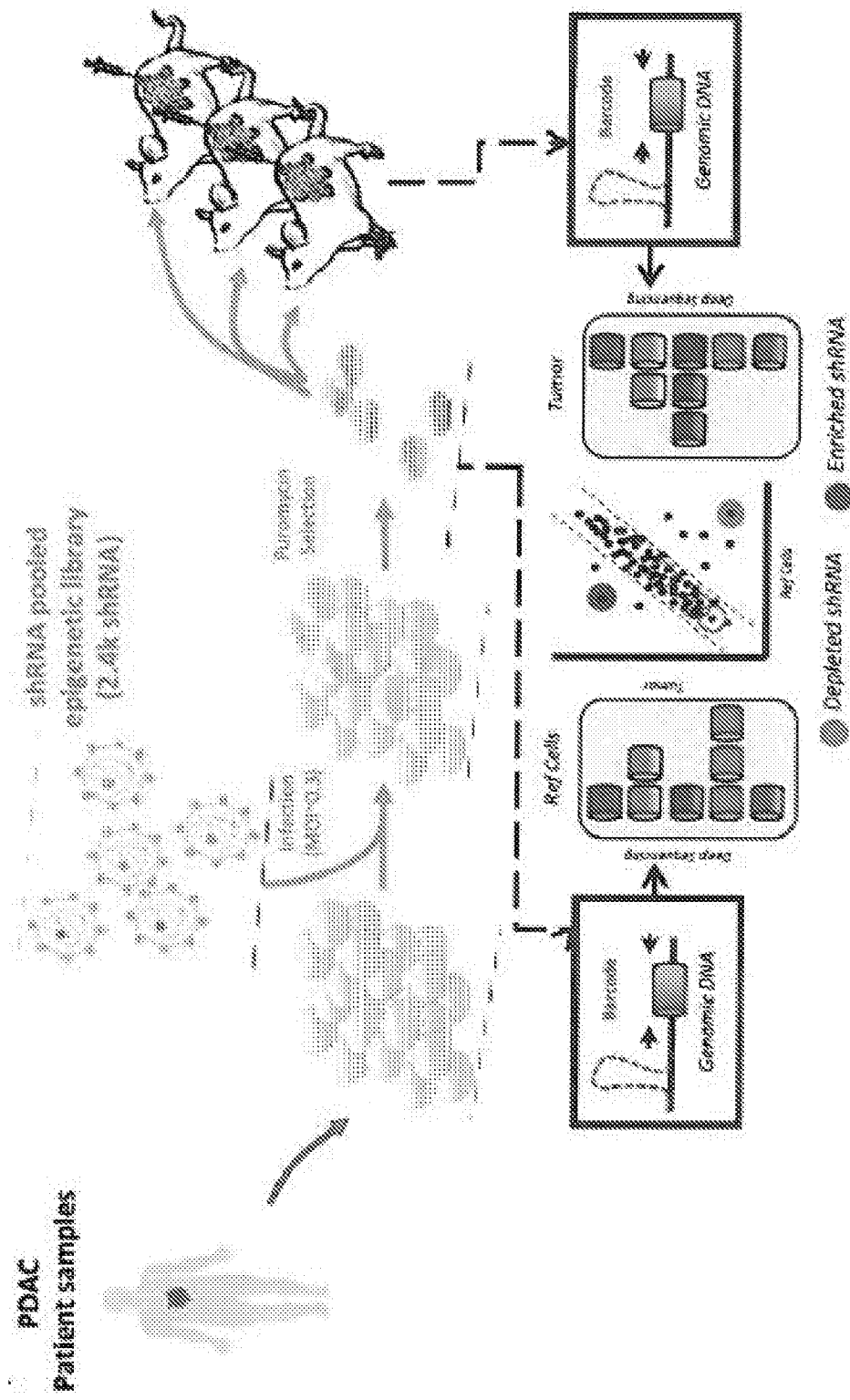


FIG. 4A

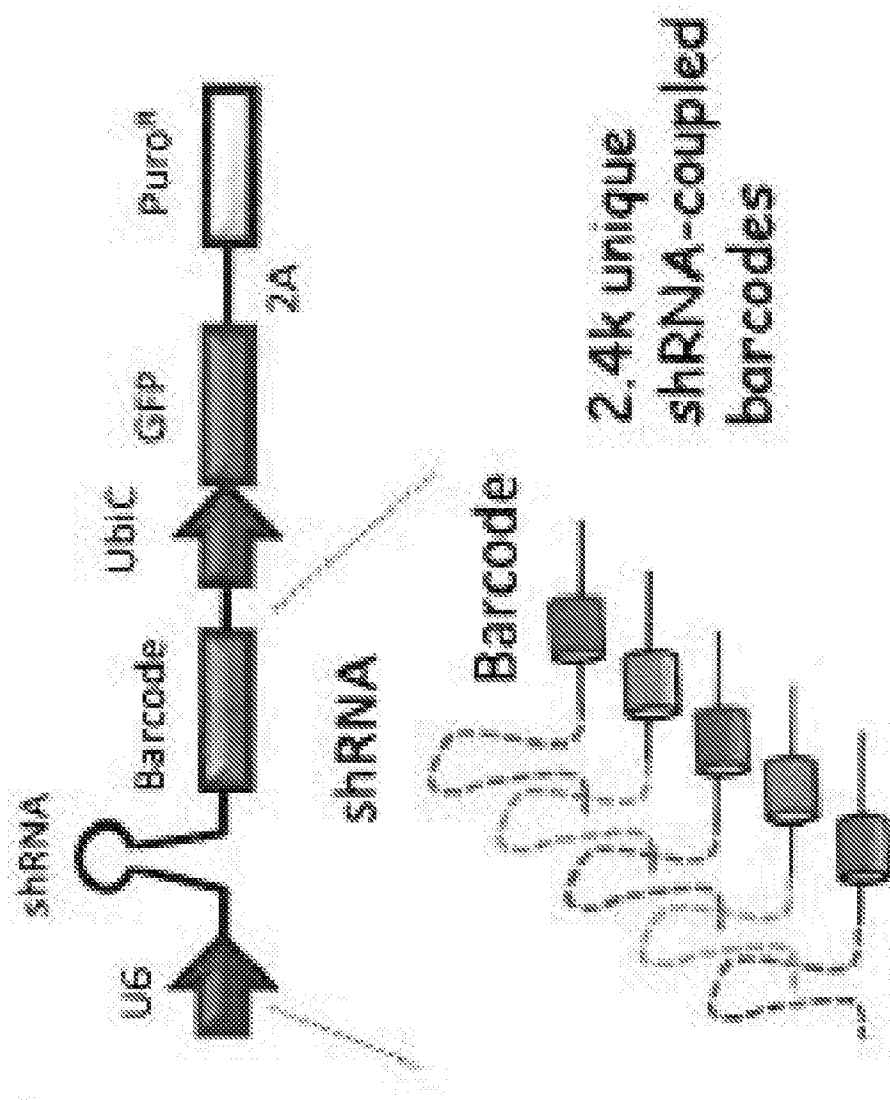


FIG. 4B

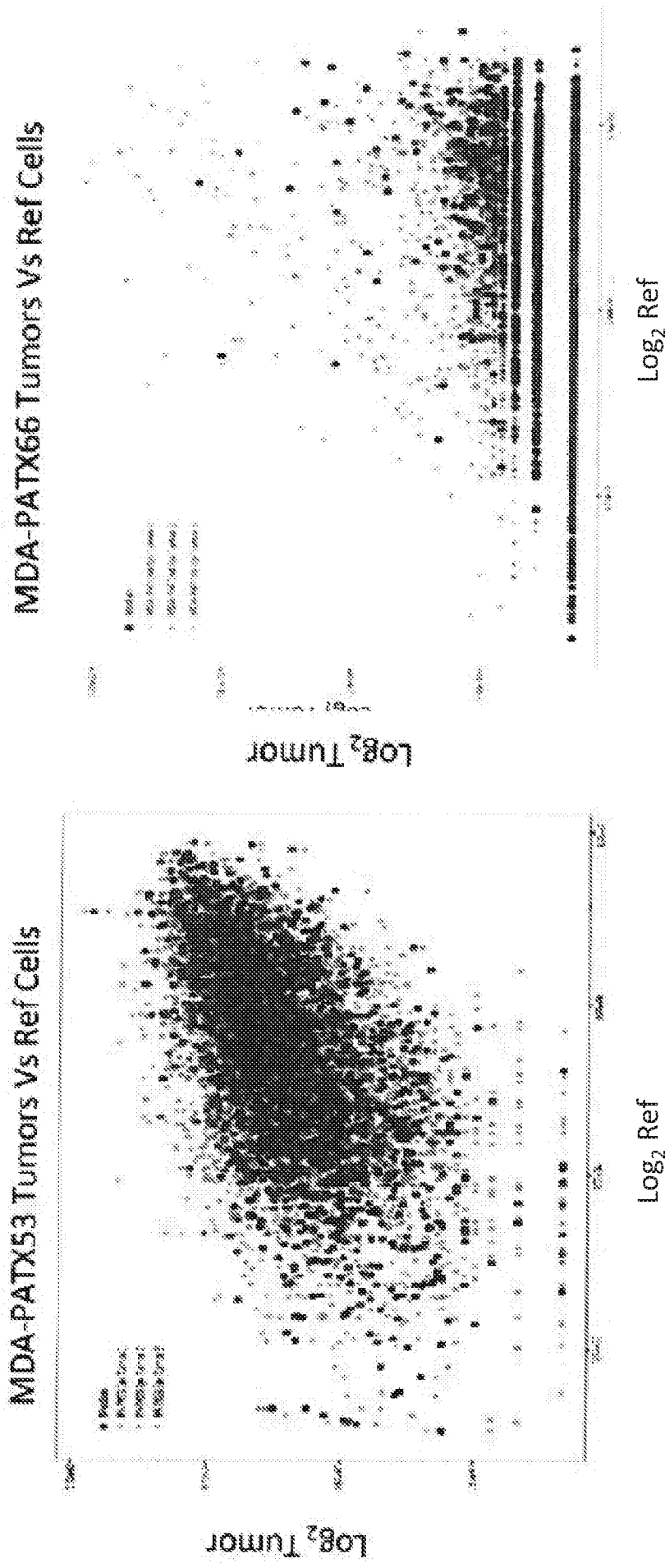


FIG. 4C

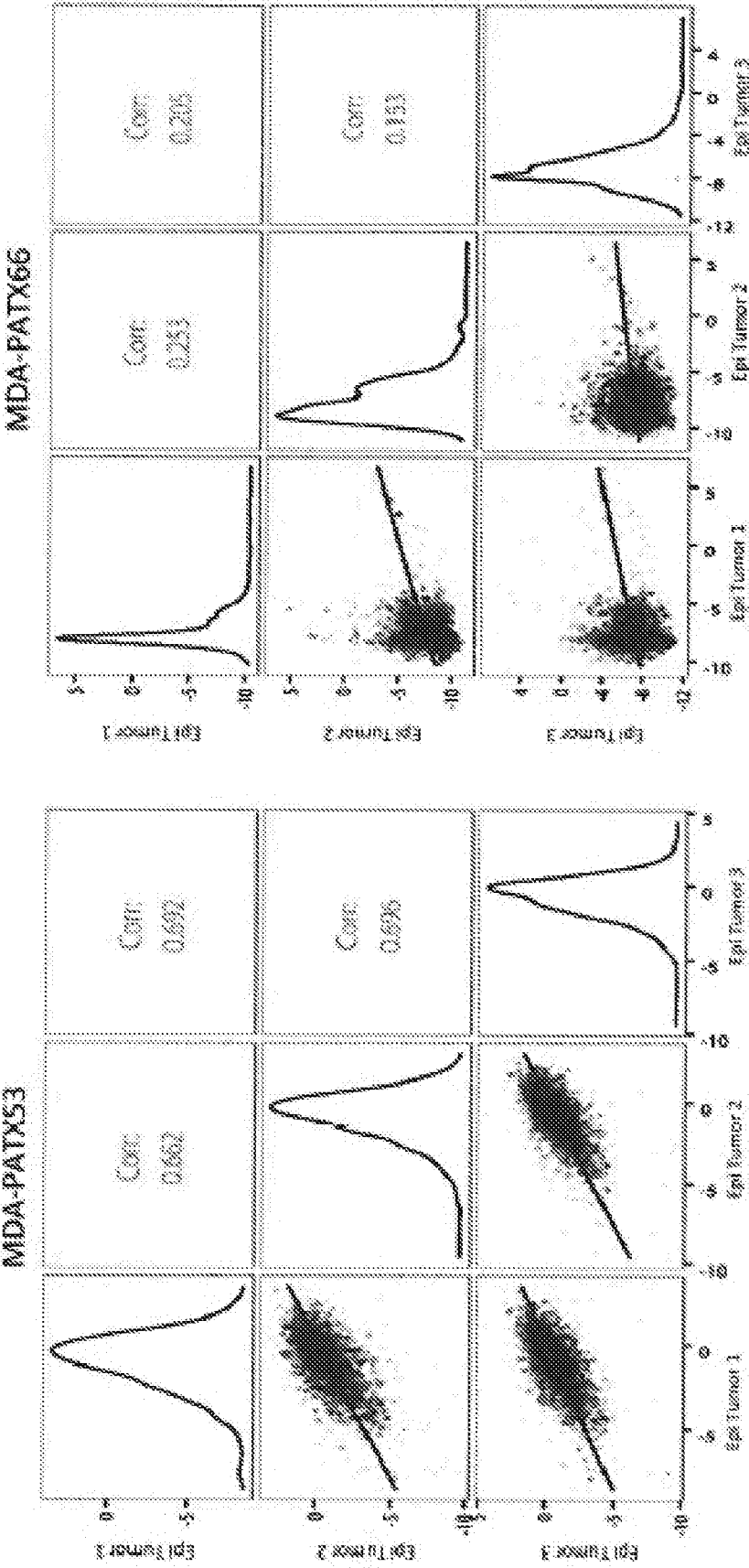


FIG. 4D

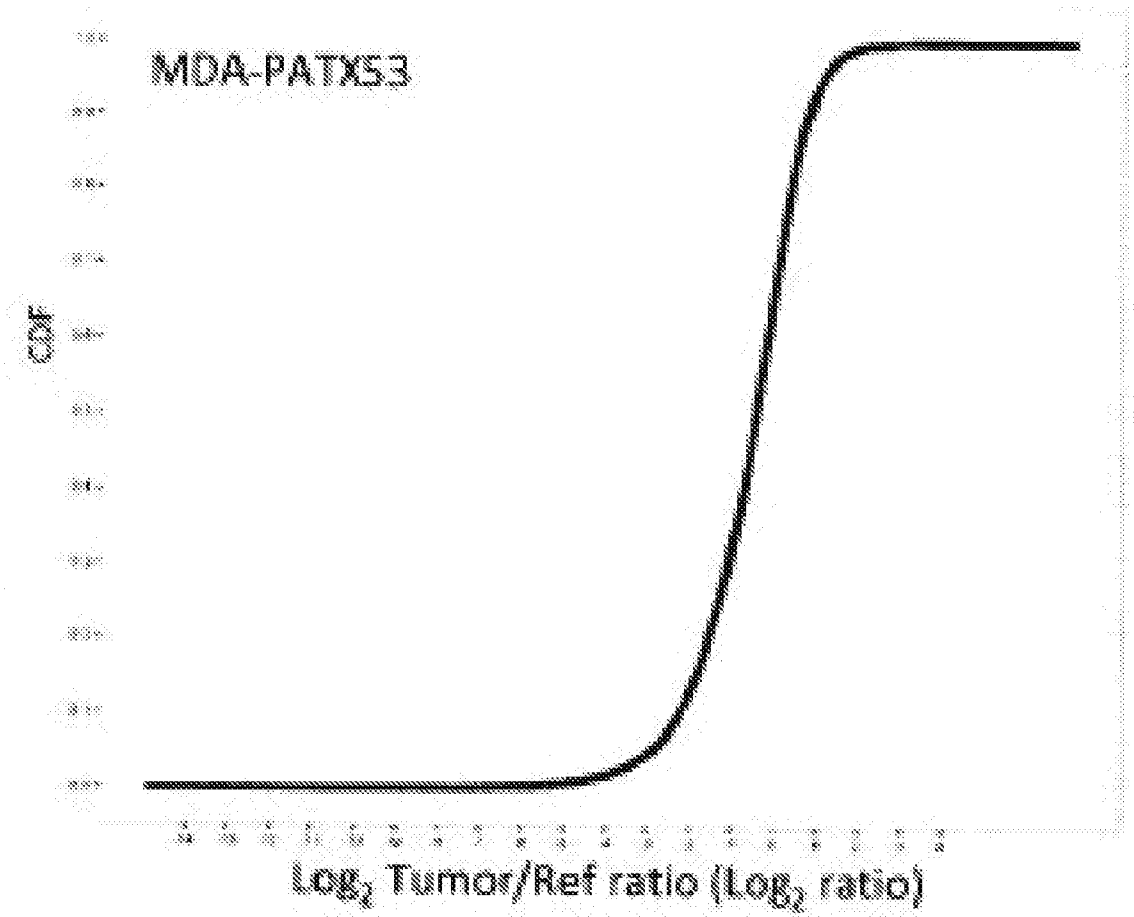


FIG. 5A

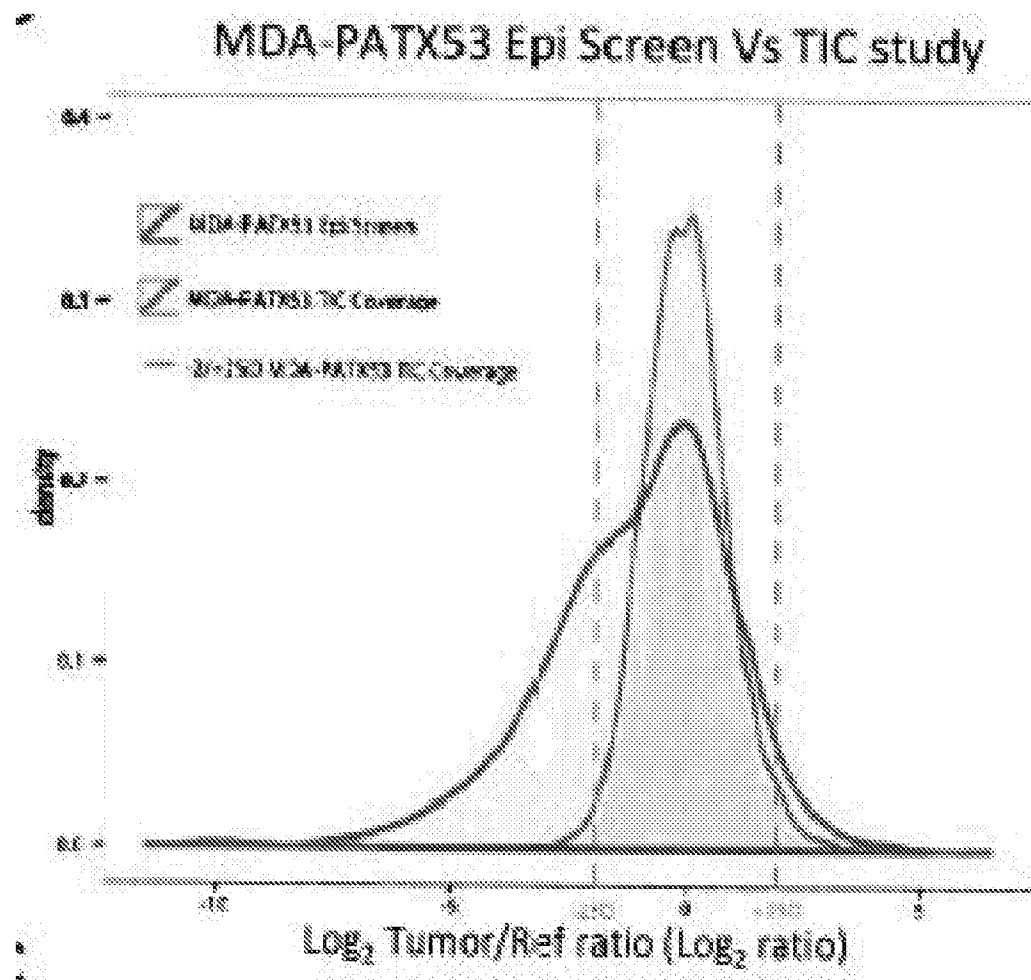


FIG. 5B

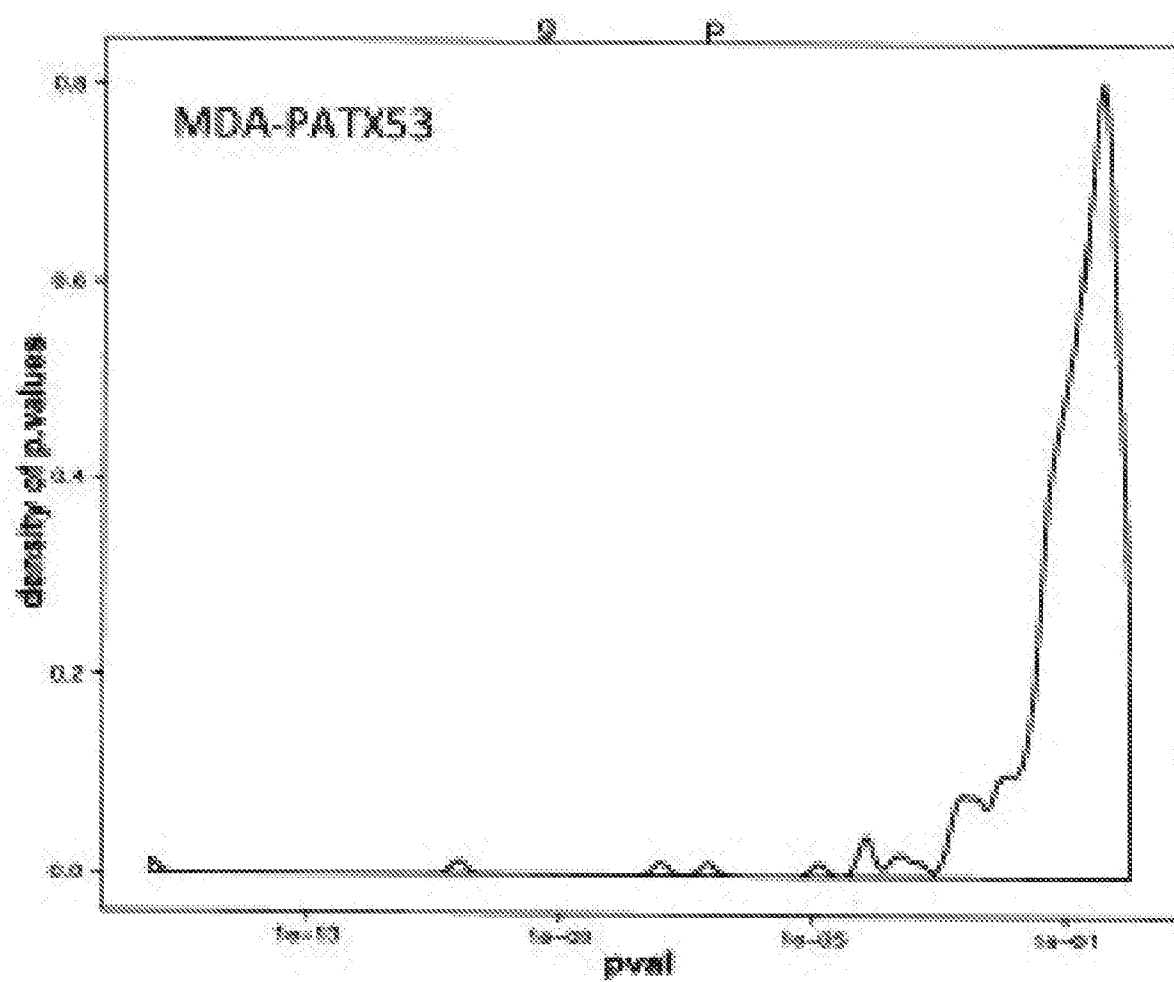


FIG. 5C

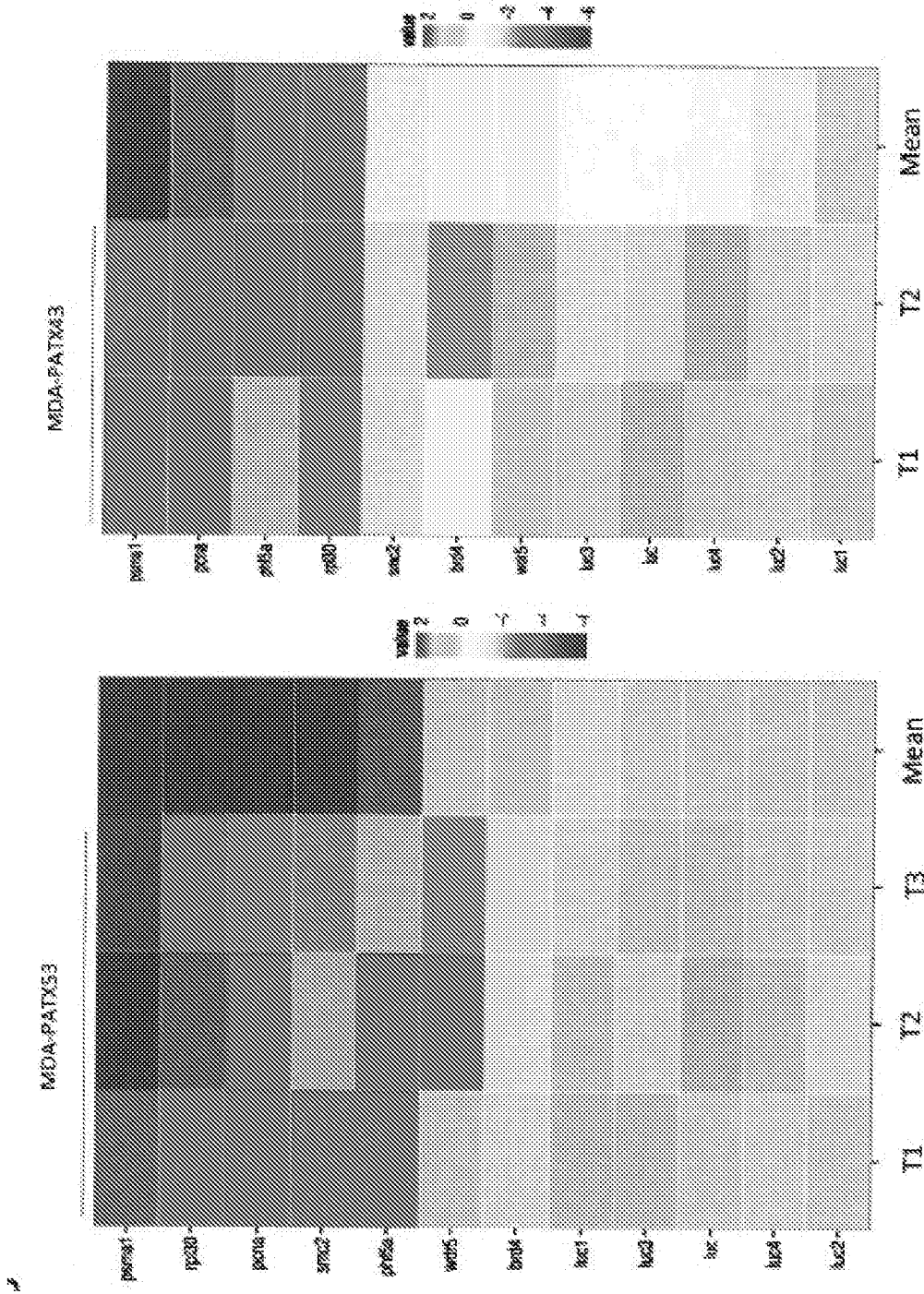


FIG. 5D

Patient Sample	Sex	Age	Stage	Metastasis Site	Kras	TP53	CDKN2A	DPC4/Smad4
MDA-PATX43	F	71	3.0.0	No	Cod 12: GGT;CGT	WT	Del	Del
MDA-PATX50	F	60	3.1.0	Liver & Lymph nodes	Cod 12: GGT;CGT	C(945,1061)>T	Del	Del
MDA-PATX53 ²	M	65		Liver	Cod 12: GGT;GAT	Del	WT	WT
MDA-PATX66	F	63	3.1.0	Lymph nodes	Cod 12: GGT; GAT	T(909,591)>A	Del	Del

FIG. 5E

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/32453

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 16/30, 16/28, 16/18; C12Q 1/68 (2017.01)

CPC - C07K 16/303, 16/3076, 16/28, 16/18; C12Q 1/6869, 1/6883, 1/6886

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	(CARUGO, A et al.) In Vivo Functional Platform Targeting Patient-Derived Xenografts Identifies WDR5-Myc Association as a Critical Determinant of Pancreatic Cancer. Cell Reports. 16 June, 2016; Vol. 16, No. 1; pages 133-147; abstract; page 134, column 2, paragraph 3- page 135, column 2, paragraph 1; page 137, column 1, paragraph 3 - column 2, paragraph 3; page 140, column 2, paragraph 1; page 142, column 2, paragraph 3; page 144, column 1, paragraphs 1-2; figures 1A-1F, 2A, S2A, S2B; DOI: 10.1016/j.celrep.2016.05.063	1-11, 18-20, 22-25 ----- 12-17, 21
Y	(OLIVARES, O et al.) Metabolic rewiring of Pancreatic Ductal Adenocarcinoma: New Routes to Follow within the Maze. International Journal of Cancer. 18 March, 2015; Vol. 138, No. 4; pages 787-796; page 790, column 2, paragraph 2	12-15
Y	(MAITRA, A et al.) Pancreatic Cancer. Annual Review of Pathology. 2008; Vol. 3; pages 1-30; page 13, paragraph 2; DOI: 10.1146/annurev.pathmechdis.3.121806.154305	16-17
Y	(ZHOU, L et al.) G-Protein-Coupled Receptor Kinase 2 in Pancreatic Cancer: Clinicopathologic and Prognostic Significance. Human Pathology. 23 June, 2016; Vol. 156; pages 171-177; page 172, column 1, paragraphs 2-3; DOI: 10.1016/j.humpath.2016.06.012	21
Y	(LIU, X et al.) Membrane Proteomic Analysis of Pancreatic Cancer Cells. Journal of Biomedical Science. 13 September, 2010; Vol. 17, No. 74; pages 1-13; page 6, column 1, paragraph 1; DOI: 10.1186/1423-0127-17-74	20
Y	(KANG, CM et al.) The RON Receptor Tyrosine Kinase in Pancreatic Cancer Pathogenesis and Its Potential Implications for Future Targeted Therapies. Pancreas. March, 2014; Vol. 43, No. 2; pages 1-16; abstract; page 4, paragraph 2; DOI: 10.1097/MPA.0000000000000088	22



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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"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

11 August 2017 (11.08.2017)

Date of mailing of the international search report

01 SEP 2017

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Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/32453

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(TAN, X et al.) PI3K/AKT-Mediated Upregulation of WDR5 Promotes Colorectal Cancer Metastasis by Directly Targeting ZNF407. Cell Death and Disease. 16 March, 2017; Vol. 8, No. 3; pages 1-12; page 7, column 1, paragraph 4 – column 2, paragraph 1; DOI: 10.1038/cddis.2017.111	23, 25