

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
06 May 2021 (06.05.2021)



(10) International Publication Number
WO 2021/081663 A1

(51) International Patent Classification:

C12N 5/071 (2010.01) C12N 5/07 (2010.01)
C12N 5/02 (2006.01)

(21) International Application Number:

PCT/CA2020/051472

(22) International Filing Date:

30 October 2020 (30.10.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/928,706 31 October 2019 (31.10.2019) US

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(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN,
KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO,
NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW,
SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with information concerning incorporation by reference of
missing element or part (Rule 20.6)
- in black and white; the international application as filed
contained color or greyscale and is available for download
from PATENTSCOPE

(54) Title: MEDIUM AND METHODS FOR CULTURING ORGANIDS

(57) Abstract: There is described herein a cell culture medium comprising: a basal medium; an antibiotic; B27; Noggin; Y-27632; Human FGF10 or FGF7; preferably wherein there is an absence of a Wnt agonist. Methods and uses of the medium is also described.

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MEDIUM AND METHODS FOR CULTURING ORGANOIDS

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/928,706 filed on
5 October 31, 2019.

FIELD OF THE INVENTION

The invention relates to culture media, more particularly to media for culturing organoids.

10

BACKGROUND OF THE INVENTION

Non-small cell lung cancer (NSCLC) is the leading cause of cancer related death worldwide with a 5-year overall survival rate of 15% (1). Over the last decades, there has been tremendous effort in developing preclinical models of NSCLC, including 2-
15 dimensional (2D) cell lines, air-liquid interface cultures, genetically engineered mouse models (GEMM) and patient-derived xenografts (PDX) (2,3,4). These models have been used to accelerate our understanding of NSCLC biology and pathogenesis. Although cell lines are still widely used in preclinical studies, they often do not reflect the biology of their parental tumors or drug sensitivity to targeted therapeutics of their
20 patient tumors (5). In addition, although GEMMs and clinically relevant PDXs may be closer to the ideal models to study drug response in patients, studies using these models are labor intensive, costly and time consuming (6). Thus, research efforts are underway to develop novel preclinical models derived from NSCLC patient and PDX tissue that are economical, rapid to use, and accurately reflect the biology of the
25 disease.

Over the past few years, organoid cultures derived from primary patient tumors and PDXs of various cancers including the colon, pancreas, prostate, liver and breast have been described (7,8,9,10,11,12,13,14,15,16). These cancer organoids have been utilized for numerous applications, such as drug screening and biomarker identification

(17,18,19,20). They have been proposed to be better *in vitro* models than 2D cell lines due to higher rates of preservation of key histological and molecular traits of their parental tumors (14,15). Additionally, drug screening in patient-derived organoids has shown high concordance with that of the matched patient tumor (14,18). Some reports
5 have demonstrated the ability to generate normal lung organoids composed of airway cell lineages (21,22). These models were primarily generated from normal mouse and human airways to understand normal lung development and function. In addition, methods to generate lung organoids from pluripotent stem cells have been reported to aid in the study of genetic pulmonary diseases such as cystic fibrosis (21,23). A major
10 advance was outlined in recent reports describing protocols for the development of NSCLC organoids (24,25,26). However, while many of the models reported in these studies were cultured short-term and were useful for acute studies, lack of systematic documentation of organoid tumor cell purity was a significant issue and specific details regarding long-term growth of the models were not provided (25,26). Furthermore,
15 there still remains a great need to develop a NSCLC organoid platform suitable for drug screening and biomarker identification in lung cancer.

SUMMARY OF THE INVENTION

In an aspect, there is provided a cell culture medium comprising: a basal medium; an
20 antibiotic; B27; Noggin; Y-27632; Human FGF10 or FGF7; preferably wherein there is an absence of a Wnt agonist.

In an aspect, there is provided a medium comprising the medium components listed in Table 6 under Tsao M26.

In an aspect, there is provided a medium described herein for use in culturing
25 organoids.

BRIEF DESCRIPTION OF FIGURES AND TABLES

These and other features of the preferred embodiments of the invention will become more apparent in the following detailed description in which reference is made to the
30 appended drawings.

Figure 1. Establishment of NSCLC-derived organoids and characterization of short-term organoid cultures. **A**, Schematic of NSCLC organoid development from surgically resected tumors or PDX. Models propagated below 10 passages and under 3 months were considered to be short-term cultures, while models propagated beyond 10 passages and over 3 months were considered to be long-term cultures. **B**, Maximum number of days in culture of all models attempted visualized on a swimmer's plot. Models contaminated with mouse cells, normal cells, or were derived from metastasis were excluded. **C**, Selected short-term NSCLC organoid histology and immunohistochemistry staining. Note that LPTO126 patient tumor was both TTF-1 and p63 negative, while PDXO137 PDX was TTF-1 positive and p63 negative. The organoids reflected the TTF-1 and p63 staining of their parental tumors. Scale Bar = 100µm. **D**, Organoid cell growth of short-term organoid cultures. Each point on the graph represents a passage. Growth was calculated by plotting the time to passaging and the cumulative sum of the number of wells plated. **E**, Erlotinib testing in short-term organoid models.

Figure 2. Histological and growth characterization of long-term organoid cultures. **A**, Growth curves of seven long-term established organoid models. Each point on the graph represents a passage. Growth was calculated by plotting the time to passaging and the cumulative sum of the number of wells plated. **B**, Tumor cell purity in seven long-term established organoid models assessed by histological examination or flow cytometry analysis. H&E and IHC of representative **C**, LUAD and **D**, LUSC models demonstrating histological recapitulation of the patient tumor or PDX to the matched organoid. Scale Bar = 200µm.

Figure 3. Mutation, copy number and transcriptomic landscape of organoids and matched patient tumor/PDX. **A**, Mutational concordance and mutation burden between patient tumor and respective PDX and organoids. Heatmap represents the fraction of concordant mutations between corresponding samples. **B**, Copy number concordance heatmap on the global gene level. Pearson correlation of gene copy number was computed per sample. A panel of normal tissues was used for copy number calling for samples without matched normal tissue (Model 274, 54, 4056, 426, 126, 85, 344, 137). **C**, Gene expression Pearson correlation heatmap [95% confidence interval] showing gene expression clustering using 893 genes differentially expressed between LUAD and LUSC PDX models in 9 patient/PDX-organoid models (total of 23 samples). P=patient, X=patient-derived xenograft, O=organoid.

Figure 4. Drug testing in long-term NSCLC organoids. **A**, Trametinib, **B**, selumetinib, and **C**, afatinib drug testing in 3 LUAD and 1 LUSC organoid model performed in technical and biological triplicates. HCC827 cell line was used as an EGFR positive control. Error bars were determined as the standard error of the mean. Final drug curves were calculated as an average of three independent experiments. **D**, *In vivo* trametinib (1mg/kg) sensitivity curves at experimental endpoint in PDXO426 and PDXO274 PDX. Error bars were determined as the standard error of the mean. N=5 mice (PDXO426 PDX) and N=6 mice (PDXO274 PDX) were used for each arm. *p>0.05, **p<0.05.

Figure 5. Combination of FGFR1 and MEK inhibitors in LUSC organoid models. **A**, RT-qPCR of FGFR1 in PDXO274 PDX and organoid. FGFR1 expression was normalized to PDXO149 PDX. Error bars were determined as the standard error of the mean. **B**, FGFR1 protein expression in PDXO274 validated by western blot. **C**, *In vitro* screen of BGJ398 in PDXO274 and PDXO149 performed in technical and biological triplicates. Error bars were determined as the standard error of the mean. Final drug curves were calculated as an average of three independent experiments. **D**, Combination drug screen of BGJ398 with trametinib and BKM120 performed in technical and biological triplicates. ED50 is the drug synergy at 50% inhibition of cell viability and ED75 is the drug synergy at 75% inhibition of cell viability. Error bars were determined as the standard error of the mean. Final drug curves were calculated as an average of three independent experiments. Combination indices were determined in CompuSyn software. **E**, Targeted inhibition of FGFR1 downstream proteins with single agents and combination treatment at 1, 3 and 5 μ M for 24 hours. **F**, *In vivo* confirmation of the trametinib (1mg/kg) and BGJ398 (25mg/kg) combination in PDXO274 PDX. N=4-6 mice were used per arm. Error bars were determined as the standard error of the mean. **p<0.05 comparing between single agent and combination therapy using student's t-test at the 38-day timepoint.

Figure 6. CHIR99021 is not essential for the long-term growth of lung tumour organoids. XDO377 model was passaged 10 times in either M26 or a minimal media formulation that does not contain CHIR99021. MM=minimal media, contains adv. DMEM/F12+AA+Glutamax+B27+Y27+Noggin+FGF7

Figure 7. CHIR99021 is not essential for the short-term growth of lung tumour organoids. The proliferation was measured in four models grown either in M26 or M26 media without CHIR99021.

Figure 8. Histology and IHC images (TTF-1 and TP63) of formalin-fixed and paraffin embedded patient tumor and normal organoids (LPTO124 and LPTO125) derived from primary lung patient tumor. Scale bar=200µm.

Figure 9. Additional histology of long-term LUAD-derived organoids. (A) H&E, TTF-1 and p63 IHC of LPTO85 tumor and matched organoid. (B) TTF-1 IHC of LPTO54 tumor, PDXO426 PDX and PDXO4056 PDX. Scale bar=200µm.

Figure 10. Additional histology of long-term LUSC-derived organoids. (A) H&E, TTF-1 and p63 IHC of PDXO377 PDX and matched organoid. (B) CK5/6 and p63 IHC of PDXO149 PDX and PDXO274 PDX. (C) CK7 and TTF-1 IHC of PDXO149 and PDXO274 organoid. Scale bar=200µm.

Figure 11. Xenograft formation of NSCLC organoids. (A-D) Organoids derived from patient tumor and PDX models were re-injected back into NOD/SCID mice for xenograft growth potential assessment. Measurement began upon first appearance of a palpable tumor (at day 0) and was monitored for up to 20-30 days. Organoid-derived xenografts recapitulated the histology and IHC markers of their parental tumors. N=3-4 mice were used per injection for PDXO4056, PDXO274 and PDXO149 xenograft. N=1 mouse was used for LPTO85 xenograft. Scale bar=100µm.

Figure 12. Gene expression Pearson correlation between patient/PDX/organoids using 1492 differentially expressed genes with 2-fold gene expression change between LUAD and LUSC from the TCGA. 95% confidence intervals are expressed in []. Blue indicates a positive correlation and red indicates a negative correlation. The size and color of the circles indicate the strength of the correlation.

Table 1. Table summarizing patient disease stage, histology and organoid growth characteristics. Yellow=long-term models, gray=excluded models. LPTO = lung patient tumor organoid, which designates organoid models derived from primary lung patient tissue; PDXO = Patient-derived xenograft organoid, which designates organoid models derived from PDX.

Table 2. Table summarizing lung organoid establishment rate. Of the 13 patient-derived short-term organoid models, four were evaluated by histology. Three of the four evaluable models were contaminated with normal cells. Of the nine LUAD PDX-derived short-term models, 3 were evaluated by flow cytometry. Two of three
 5 evaluable models were contaminated with normal cells. Of the 19 LUSC PDX-derived short-term models, five were evaluated by flow cytometry. Two of the five models were contaminated with normal cells. All long-term models contained pure tumor populations.

Table 3. Table summarizing gene expression Pearson correlation coefficients in all 23
 10 samples between patient/PDX/organoids. Green are models from patients and orange are models from PDXs.

Table 4. Table summarizing the tumor features and mutations comparing between short-term and long-term patient-derived organoid models. Models lacking mutation information were excluded from the study.

15 **Table 5.** Table summarizing the tumor features and mutations comparing between short-term and long-term PDX-derived organoid models. Models lacking mutation information were excluded from the study.

Table 6. Media Comparison Chart

Table 7. Human Lung Tissue Collection Media

20

DETAILED DESCRIPTION

In the following description, numerous specific details are set forth to provide a thorough understanding of the invention. However, it is understood that the invention may be practiced without these specific details.

25 Non-small cell lung cancer (NSCLC) is the most common cause of cancer deaths worldwide. There is an unmet need to develop novel clinically relevant models of NSCLC to accelerate identification of drug targets and our understanding of the disease.

Thirty surgically resected NSCLC primary patient tissue and 35 previously established patient-derived xenograft (PDX) models were processed for organoid culture establishment. Organoids were histologically and molecularly characterized by cytology and histology, exome sequencing and RNA-sequencing analysis.

5 Tumorigenicity was assessed through subcutaneous injection of organoids in NOD/SCID mice. Organoids were subjected to drug testing using EGFR, FGFR and MEK-targeted therapies.

We have identified cell culture conditions favoring the establishment of short-term and long-term expansion of NSCLC organoids derived from primary lung patient and PDX

10 tumor tissue. The NSCLC organoids recapitulated the histology of the patient and PDX tumor. They also retained tumorigenicity as evidenced by cytologic features of malignancy, xenograft formation, preservation of mutations, copy number aberrations and gene expression profiles between the organoid and matched parental tumor tissue by whole exome and RNA-sequencing. NSCLC organoid models also preserved the

15 sensitivity of the matched parental tumor to targeted therapeutics, and could be used to validate or discover biomarker-drug combinations.

Our panel of NSCLC organoids closely recapitulates the genomics and biology of patient tumors, and is a potential platform for drug testing and biomarker validation.

Currently, there is an urgent need for clinically relevant preclinical models of non-small

20 cell lung cancer (NSCLC) for biomarker and drug discovery due to the lack of preclinical models that recapitulate the biology of the patient tumor. Three-dimensional (3D) organoids have become valuable preclinical models to study disease pathogenesis and identify novel drug targets. We have established a protocol for the development of NSCLC organoids from patient tumor and patient-derived xenograft

25 models. This protocol allowed for the efficient generation of organoids for multiple potential applications. Importantly, we showed that these organoids retained the histological and molecular features of their parental tumors and demonstrated their utility for drug testing. Our organoid platform provides additional preclinical models of NSCLC and may be useful for future drug screening biomarker identification.

30 In an aspect, there is provided a cell culture medium comprising: a basal medium; an antibiotic; B27; Noggin; Y-27632; Human FGF10 or FGF7; preferably wherein there is an absence of a Wnt agonist.

The Wnt signalling pathway is defined by a series of events that occur when the cell-surface Wnt receptor complex, comprising a Frizzled receptor, LRP and LGR is activated, usually by an extracellular signalling molecule, such as a member of the Wnt family. This results in the activation of Dishevelled family proteins which inhibit a
5 complex of proteins that includes axin, GSK-3, and the protein APC to degrade intracellular β -catenin. The resulting enriched nuclear β -catenin enhances transcription by TCF/LEF family transcription factors.

A Wnt agonist is defined as an agent that activates TCF/LEF-mediated transcription in
10 a cell. Wnt agonists are therefore selected from true Wnt agonists that bind and activate the Wnt receptor complex including any and all of the Wnt family proteins, an inhibitor of intracellular β -catenin degradation, a GSK inhibitor (such as CHIR9901) and activators of TCF/LEF.

15 In some embodiments, the Human FGF is FGF10 and is in a concentration of at least 500ng/ml.

In some embodiments, the medium further comprises a buffering agent. Preferably, the buffering agent is HEPES.

In some embodiments, the basal medium is Advanced DMEM/F12.

20 In some embodiments, the medium further comprises L-glutamine or GlutaMax™.

In some embodiments, the medium further comprises an antibiotic. Preferably, the antibiotic is penicillin, streptomycin or primocin, or combinations thereof.

In some embodiments, the medium further comprises N-acetyl-L-cystein.

In some embodiments, the medium further comprises A83 01.

25 In some embodiments, the medium further comprises N2.

In some embodiments, the medium further comprises human FGF4.

In some embodiments, the medium further comprises CHIR99021.

In some embodiments, the medium further comprises SAG.

In some embodiments, the medium further comprises human EGF.

In some embodiments, the concentration of the components correspond to those of the listed in Table 6 under Tsao M26 $\pm$ 30%. Preferably, the concentration of the components correspond to those of the listed in Table 6 under Tsao M26.

- 5 In an aspect, there is provided a medium comprising the medium components listed in Table 6 under Tsao M26.

In some embodiments, the concentration of the components correspond to those of the listed in Table 6 under Tsao M26 $\pm$ 30%. Preferably, the concentration of the components correspond to those of the listed in Table 6 under Tsao M26.

- 10 In an aspect, there is provided a medium described herein for use in culturing organoids.

In some embodiments, the medium is for use in culturing cancer organoids.

In some embodiments, the medium is for use in culturing lung cancer organoids.

- 15 The advantages of the present invention are further illustrated by the following examples. The examples and their particular details set forth herein are presented for illustration only and should not be construed as a limitation on the claims of the present invention.

EXAMPLES

20 Methods and Materials

Tumor tissue processing and organoid establishment.

- 25 The collection of surgically resected primary tumors from early stage NSCLC patients and the development of patient-derived xenografts were approved by the University Health Network Research Ethics Board (REB: 17-558) and Animal Care Committee (AUP: 5555). Informed written consent was received from all patients. All studies were performed in accordance with TRI-Council Policy Statement: Ethical Conduct for Research Involving Humans. Clinical diagnosis of NSCLC subtypes was validated by

pathological review. The protocol for establishing NSCLC PDXs was previously described (4,27,28). For organoid cultures, tumor tissues were processed into 4mm diameter pieces and washed with ice cold phosphate buffered saline (PBS). Tumor pieces were dissociated into single cells in Advanced DMEMF12 (GIBCO) with
5 Liberase TM (Sigma, St. Louis, MO, USA) for 1 hour followed by 10-minute incubation with TrypLE Express (Invitrogen, Carlsbad, CA, USA) in 37°C with gentle shaking. Mouse cell depletion in PDX samples was performed after tissue dissociation using H-2Kb/H-2Db antibody (#MA5-17998, Invitrogen) labeling and Streptavidin (BD Biosciences, Franklin Lakes, NJ, USA) bead magnetic separation. Cells were counted
10 and re-suspended in 100% growth factor reduced Matrigel (VWR, Radnor, PA, USA), plated in 24 well tissue culture plates as Matrigel domes and maintained in 37°C 5% CO₂ with media overlaying the Matrigel dome. Refer to Table 6 for a list of media components. Organoid growth was monitored weekly for the detection of initiated organoids, and organoids were kept in the same passage for no longer than four
15 weeks. The identity of PDX and organoids were authenticated by short tandem repeat (STR) analysis and matched to patient tissue. Organoid cultures were tested routinely for mycoplasma.

Immunohistochemistry

Fresh tumor tissue was fixed in 10% formalin for 24-48 hours, followed by fixation in
20 70% ethanol prior to paraffin embedding. Organoids were fixed with 10% formalin for 24-48 hours and 70% ethanol with eosin and embedded in HistoGel™ (Thermo Fisher Scientific, Waltham, MA) before processing for H&E and IHC. Formalin-fixed paraffin embedded tumor tissues and organoids were cut into 4µm thick slices and allowed to dry overnight at 60°C. Prepared tissue sections were stained with appropriate
25 antibodies using BenchMark XT autostainer (Ventana Medical System, Tucson, AZ). Primary antibody specific to CK5/6 (Ventana), TP63, TTF-1 and CK7 (Dako) were used for IHC analysis. The slides were scanned and imaged using Aperio Scanscope XT (Leica, Wetzlar, Germany).

DNA extraction and whole exome sequencing analysis

30 Snap frozen tumor tissues and fresh organoid pellets were lysed in tris buffered saline solution with 10% SDS and proteinase K (1mg/ml) overnight at 55°C. DNA was isolated and eluted on spin columns using proprietary solutions provided by a DNA extraction kit (Norgen Biotek, Thorold, ON, Canada). DNA quality was assessed using

Bioanalyzer, Tapestation and qPCR. 100-200ng of genomic DNA was used for library preparation (Agilent SureSelect Human All Exon v5 capture kit). DNA was sequenced using 125-cycle paired-end protocol and multiplexing to obtain 150X coverage on Illumina HiSeq2500 sequencer. Xenome (29) was used to eliminate reads pertaining to mouse stroma. Sequence reads were subsequently aligned to the human reference genome (GRCh37) using Burrows-Wheeler Aligner v0.7.12 (30). The mapped data were further processed for quality control using the standard GATK pipeline, including Picard v1.140. (31) Mutect v1.1.5 (32) and VarScan v2.3.8 (33) were used for mutation calling, while dbSNP (34), ExAC (35) and ESP (36) were used as filters for samples without matched normal tissue. Annovar (37), vcf2maf v1.6.14 and Variant Effect Predictor (VEP) v87 (38) were used to annotate final mutation calls, following which, the R package "ComplexHeatmap" (39) was used to generate oncoprints and visualize the data. CNVkit (40) was used to infer copy number from exome-sequenced samples by applying circular binary segmentation (CBS) (41) to make calls in both targeted regions and non-targeted regions. The targeted regions used for this algorithm were a combination of SureSelect Human All Exon V4 and V5 regions. A panel of normal lung tissue was used for samples lacking a matched normal. Subsequently, GISTIC2.0 (42) was run in order to identify genes affected by copy number alterations, while also taking into account the frequency and amplitude of the events. Whole exome sequencing data were deposited in the Sequence Read Archive (SRA), accession: SRP158596.

RNA extraction and RNA sequencing analysis

Organoids were extracted from Matrigel using Cell Recovery Solution (Corning) on ice for 1 hour. Total RNA from homogenized tumor tissue and pelleted organoids were extracted using TriZol (Invitrogen) method, followed by isolation and precipitation in chloroform and 70% ethanol. DNA cleanup was performed using DNA cleanup kit (Invitrogen). Total RNA was quality checked via BioAnalyzer (Agilent), Tapestation and qPCR. Library preparation was performed using Illumina TruSeq Stranded mRNA sample preparation kit (Illumina). RNA was sequenced using HiSeq 2000 sequencer with 75-cycle paired end protocol and multiplexing to obtain 40-80 million reads/sample. Xenome (29) (version 1.0.1 with standard parameters) was used to filter mouse reads from human reads. For transcript quantification, Salmon (43) (version 0.8.2 with default parameters) with quasi-mapping was applied to assign reads directly to transcripts to obtain transcripts per million (TPM) values. The $\log_2(\text{TPM}+1)$ were

used for all statistical analysis. ComBat (44) was applied to adjust for batch effects. For correlation analysis, genes that are differentially expressed between LUAD and LUSC at a 2-fold or greater cut-off were identified from profiling of PDX models (4) or primary patient tumors (TCGA). These gene sets consisted of 893 and 1492 differentially expressed genes, respectively, and were used to calculate correlation coefficients between patient, PDX and organoids. RNA-sequencing data were deposited in the Gene Expression Omnibus (GEO), accession: GSE119004.

In vitro drug studies

For *in vitro* drug testing, compounds were purchased from UHN Shanghai and dissolved in DMSO. Organoids were dissociated into single cells, counted and plated in Matrigel-coated 384 well plates (3000 cells per well) in triplicate for 24 hours prior to drug treatment. Organoids were treated with a range of drug concentrations (0.01-10 μ M) for 96 hours and cell viability was determined by Celltiter Glo 3D viability assay (protocol mentioned above). Drug response curves were graphed and IC₅₀ values were calculated using Graphpad Prism 6.0 (La Jolla, CA, USA). CompuSyn software (45) was used to calculate combination indices for combination drug studies.

RT-qPCR

Total RNA was extracted according to the methods mentioned above. RNA was reverse transcribed to cDNA using a reverse transcription kit (Thermo Fisher Scientific). Primers used for qPCR included *FGFR1* F 5'-GCATCAACCACACATACCAGC-3', *FGFR1* R 5'-CACGTTGCTACCCAGGGC-3', *ACTB* F 5'-TCCTAAAAGCCACCCCACTTCT-3', *ACTB* R 5'-GGGAGAGGACTGGGCCATT-3', *B2M* F 5'-GAGTGCTGTCTCCATGTTTGATGT-3', *B2M* R 5'-AAGTTGCCAGCCCTCCTAGAG-3'. The following conditions were used for qPCR: 94°C for 1 min, 60°C for 30-sec, and 72°C for 1 min for 35 cycles.

Western blotting

Matrigel/organoid suspension was dissociated with TrypLE Express and organoid pellets were lysed with RIPA buffer (Sigma) with PMSF, sodium vanadate, and protease inhibitor cocktail (Roche, Mississauga, ON, Canada). Protein was quantified via Bradford assay (Bio-rad, Mississauga, ON, Canada), denatured in sample buffer (Bio-rad) and loaded for SDS-PAGE. Proteins were transferred onto nitrocellulose

membranes (Bio-rad) and blocked in 5% skim milk for 1 hour and probed overnight with appropriate primary antibodies. The membrane was probed with secondary anti-rabbit/mouse IgG, HRP-linked antibodies (#7074, #7076, Cell Signaling) for one hour prior to imaging. ECL reagent (GE Healthcare, Chicago, IL, USA) was used to detect proteins of interest. The primary antibodies used in this study: pFGFR (Y653/654) (#3471), FGFR1 (#9740), pErk (T202/Y204) (#9101), Erk (#9102), pAkt (S473) (#9271), and Akt (#9272) were obtained from Cell Signaling (Danvers, MA, USA). β -actin antibody (#A1978) was obtained from Sigma.

In vivo organoid implantations

Dissociated organoids were isolated from growth factor reduced Matrigel using Cell Recovery Solution (Corning, NY, USA) for 1 hour on ice. Organoids were re-suspended with 500,000 cells in 200 μ l M26 media prior to injection in the subcutaneous flank of 4-6-week-old NOD/SCID mice. Tumor growth was monitored once or twice weekly by caliper measurement. Tumors were harvested, formalin-fixed paraffin embedded for histological analysis and snap frozen for DNA/RNA/protein isolation.

In vivo therapeutic studies

Cryopreserved PDX tissue (below passage 10) was thawed and implanted into the subcutaneous flank of NOD/SCID mice. The tumor was harvested and cut into 4mm diameter pieces at endpoint and expanded into experimental arms for drug testing when the average size reached 150-200mm³. Trametinib (1mg/kg) and BGJ398 (25mg/kg) were dissolved in 0.5% hydroxyethyl-cellulose with 0.2% tween80 in sterile H₂O and 10% tween80, respectively. Compounds were delivered once daily via oral gavage for 21-28 days. Tumor size was monitored twice weekly by caliper measurement.

Statistical analysis

All exome sequencing and RNA-sequencing analysis were performed in the open-source R Statistical Computing software (<http://www.r-project.org/>). All statistical analysis for obtaining IC50s for drug screening were performed in Graphpad prism 6.0. Combination indices for drug combination studies were performed in Compusyn

(<http://www.combosyn.com/>). P-values in the *in vivo* drug studies were obtained using student's t-test at specific time points.

Results and Discussion

5 *Organoid establishment from NSCLC patient tumor and patient-derived xenografts*

From December 2015-2017, 19 surgically resected lung adenocarcinomas (LUADs), 15 lung squamous cell carcinomas (LUSCs), 16 LUAD PDXs, and 26 LUSC PDXs were processed for organoid establishment (Fig. 1A, 1B, Table 1). Note that the nomenclature LPTO and PDXO were used to denote organoid models derived from
10 lung primary patient tumor and PDX, respectively. Of the 76 tissues processed, 11 models were excluded from the final count due to lack of starting tumor cell material, mouse/normal epithelial cell contamination, and sites of metastasis (Table 1). We attempted to grow organoids in Advanced DMEMF12 base media with additional supplements which we termed M26. Our M26 media was modified from the media
15 used to derive normal lung organoids from induced pluripotent stem cells (21). In terms of our success rates, 88% (57/65) of our dissociated NSCLC tissue successfully initiated organoid cultures, which is defined as organoid formation upon plating in passage zero (Table 2). Seventy-two percent (47/65) of the models exhibited a range of short-term growth (passage 1-9, 1~3 months), providing opportunities for most
20 tissues to be used in acute studies (Fig. 1B, Table 1, 2). In addition, 15% (10/65) of the models achieved long-term growth (Fig. 1B, Table 1, 2).

Tumor purity of short-term organoid cultures

A recurrent issue highlighted by previous work is the outgrowth of normal epithelial cells of organoid cultures derived from primary patient tumor (24,26). Consistent with
25 previous reports of NSCLC organoids, we observed the outgrowth of normal epithelial cells in 58% (7/12) of our short-term organoid cultures derived from patient tumor and PDX (Table 1, 2). Since surgically resected lung tumors or biopsies may contain entrapped normal lung airway/alveolar epithelial cells, we speculate that these normal organoids derived from patient tissue likely arose from this region. In contrast, normal
30 organoids arising from subcutaneously implanted PDX could have arisen from entrapped murine breast/sweat gland tissues at the implantation site. To determine

tumor purity in patient-derived organoids (PDO), we performed cytological evaluation by H&E and immunohistochemistry for the lung markers TTF-1 and TP63 of the cultured cells and the original patient tumor. We observed that the normal-like organoid models do not reflect the IHC results of their parental tumor (Table 1, Fig. 8).

5 For example, the LPTO124 patient tumor is an adenocarcinoma that stains negative for both TTF-1 and TP63, but the matched organoid stains positive for TP63 and negative for TTF-1. Since TP63 is a marker for lung basal cells, we speculate that the organoids derived from the LPTO124 patient tumor reflects a cell population growing from normal cells of basal cell origin. To assess the percentage of tumor cells vs.

10 mouse cells in the PDX-derived organoids (XDO), EpCAM+ (human epithelial cells) and H2K+ (mouse cells) cell populations were characterized by flow cytometry analysis. Overall, for short-term cultures, 75% (3/4) of the evaluable PDO models were contaminated with this normal cell population, while 50% (4/8) of the evaluable XDOs were contaminated with mouse cells (<2% human EpCAM, >60% H2K) (Table 1, 2).

15 PDO and XDO models that were deemed to not be largely contaminated with normal cell populations exhibited 75-97% and 50-90% tumor cell populations, respectively (Table 1). Finally, we were not able to detect the presence of fibroblasts and immune cells in the short-term organoid cultures by histological assessment.

20 *Recapitulation of histologic and cell lineage features of parental tumors by short-term NSCLC organoid cultures*

To assess the quality of our short-term NSCLC organoids for downstream applications, we assessed the organoid models by cytology/histology. Note that histological and tumor purity assessment were performed in the short-term cultured LPTO126 and PDXO137 organoids before they were later established as long-term models.

25 Histological evaluation of the short-term models revealed LUAD and LUSC representing various histological subtypes such as mucinous (LPTO126) and acinar (PDXO137) morphology in LUAD, and moderate differentiation (PDXO321) in LUSC (Fig 1C). The organoids also reflected the TTF-1 and TP63 staining pattern of their parental tumors, suggesting that they recapitulate the histology of their parental tumors

30 (Fig. 1C).

To demonstrate the utility of short-term organoid models for drug testing, we first determined whether there was sufficient number and growth of cells for these experiments. The four short-term models used in our drug test were propagable in the

first few passages and contained enough cells for plating (Fig. 1D). We evaluated the efficacy of clinically approved EGFR targeted therapy in NSCLC in four short-term organoid models. While some of the organoids later on became long-term models, the drug test was performed in early passages (P1) of those organoids for the purpose of assessing the ability of short-term models for drug testing. We evaluated the EGFR inhibitor erlotinib in three models with wildtype EGFR and one model with EGFR exon 19 deletion. The EGFR exon 19 deleted organoid model PDXO137 was the most sensitive to erlotinib, while the EGFR wild type models were less sensitive (Fig. 1E). The parental PDX of PDXO137 has also been previously shown to respond to erlotinib (27), demonstrating that organoid drug responses reflect those of its parental tumor. Therefore, we demonstrated as a proof-of-principle that short-term organoids contain sufficient cell numbers for drug testing and may be used as preclinical models for biomarker validation.

Characterization of long-term NSCLC organoid cultures (growth, purity, histologic/lineage marker)

Fifteen percent of NSCLC organoid models became long-term cultures, as defined by continuous cell growth that maintained the same split ratios in late passages (beyond 10 passages, over 3 months) and retained a high percentage of tumor cells (Fig. 1B and Fig. 1, 3). These cultures could be propagated beyond 10 passages for over one year in culture with a splitting ratio of at least 1:3, and without a decline in proliferation as the passage number increased (Fig. 2A). They were also recoverable from >1 year of cryopreservation and could be expanded in culture after thawing.

Using the same method to assess tumor purity as described for short-term, in long-term cultures, none were contaminated with normal or non-human cells. PDOs consisted of over 85% of tumor cells and the majority of the XDOs contained over 65% of EpCAM-positive cells (Fig. 2B, Table 2), with <8% of H2K positive cells in all of the long-term organoid models.

Long-term established NSCLC organoids also retained the histologic features of their parental tumors. LUAD tumors can be classified into multiple histological subtypes, which include acinar, lepidic, solid, papillary, and mixed histology. Four LUAD patient and PDX tumors (LPTO54 tumor, LPTO85 tumor, PDXO426 PDX and PDXO4056 PDX) collectively represented three histological subtypes of LUAD: acinar predominant, mucinous, and solid predominant (poorly differentiated) (Fig. 2C and Fig.

9A). These histological subtype patterns were reflected in the matched organoids of the tumor samples. In addition, expression of LUAD lineage markers such as TTF-1 was preserved. LPTO54 tumor and PDXO4056 PDX, as well as their matched organoids were positive for TTF-1, while LPTO85 tumor and PDXO426 PDX, along
 5 with their respective organoid models, were TTF-1 negative (Fig. 2C and Fig. 9A).

All three LUSC PDX models exhibited features of moderately differentiated LUSC. Likewise, our long-term LUSC PDXs and matched organoids were moderately differentiated and non-keratinizing squamous cell carcinomas (PDXO274 and PDXO377), except for PDXO149 which was a keratinizing LUSC. (Fig. 2D and Fig.
 10 10A, B). The LUSC organoids preserved the histology of their matched PDX models. LUSC organoids were positive for TP63 and CK5, and negative for TTF-1 and CK7, which is characteristic of LUSC (Fig. 10C).

To determine if the organoid culture conditions preserved the tumorigenic properties of the cancer cells, organoids derived from patient tissue and PDX were implanted into
 15 immunocompromised NOD/SCID mice. The NSCLC organoid models formed tumor xenografts that histologically recapitulated their parental tumors (Fig. 11A, B, C, D). Among LUAD models, the PDXO4056 xenograft formed a solid LUAD positive for TTF-1 while the LPTO85 xenograft exhibited features of a mucinous LUAD negative for TTF-1, which is typical of mucinous LUAD (46). Among LUSC models, both PDXO274
 20 and PDXO149 organoid xenografts formed LUSC expressing the LUSC markers CK5 and TP63 (Fig. 11A, B, C, D). Overall, our data indicate that even over long-term, our organoid culture conditions allow the cancer cells to retain key biological properties observed in the patient tumors, including histological differentiation and tumorigenicity.

NSCLC organoids preserve the mutation and copy number landscape of their parental
 25 *tumors*

To evaluate the genome profile concordance of organoid cultures to their source we compared the spectrum of somatic mutation and copy number aberrations between nine long-term organoid cultures and their parental tumors by WES. These samples included three patient-organoid pair (LPTO54, LPTO85, LPTO126), one PDX-organoid
 30 pair (PDXO4056) and five patient-PDX-organoid groupings (PDXO426, PDXO344, PDXO137, PDXO149, PDXO274). The spectrum of mutations was highly concordant between the organoid and their matched patient tumor and/or PDX tissue (Fig. 3A, and data not shown). Furthermore, the mutation burden in the five long-term established

organoids was also similar to that of their parental patient/PDX tumors (Fig. 3A), indicating that the culture conditions do not destabilize the cancer genomes. The WES data further revealed that our organoid models harbored common mutations that were previously identified in independent NSCLC patient profiling studies (47,48,49). The affected genes included *TP53*, *DDR2*, *KRAS*, *KEAP1*, *CUL3*, *NOTCH*, etc (data not shown).

Copy number variation (CNV) analysis also supported the tumor origin of the organoids and indicated that CNV profiles of the parental tumors were largely preserved during organoid culture (Fig. 3B). Notably, major chromosomal copy number changes associated with LUAD and LUSC including, chr.1q and 3q amplifications, respectively, were detected in our patient/PDX/organoid cohorts (data not shown). LPTO85 and LPTO344 patient tumors appeared to be highly correlated with one another by CNV. We confirmed the distinct identities of these samples by STR profiling. However, more detailed analysis of their genomes revealed that they were both close to copy number neutral, suggesting that this similarity largely accounted for their close correlation by CNV analysis. At the gene level, we also detected amplification in *KRAS* and deletion of *CDKN2A* in PDXO426, which are frequent occurrences in LUAD (47), as well as *FGFR1* amplification in PDXO274, which is commonly observed in LUSC (48). Overall, the cancer mutations and CNV detected in our study support the tumor origin of the organoids and indicate that they retain the genomic aberrations and potentially other key cancer properties of their parental tumors.

Gene expression profiles are similar between NSCLC organoids and parental tumors

To determine whether gene expression profiles are preserved in the organoids, we used RNA-seq to analyze gene expression of nine matched organoid-patient and/or PDX tumor pairs described in the genomic analysis. Due to the confounding situation of human stromal cells uniquely contributing to gene expression in the patient samples, we sought to identify a gene set that reduced the number of stromal-specific genes and enriched for genes expressed in tumor epithelial cells. To identify such a gene set, we used gene expression profiles for primary patient LUAD and LUSC growing as PDXs that were obtained using human-specific microarray chips (4). From these gene expression profiles, we obtained a list of 893 genes that are differentially expressed between LUAD and LUSC at a level of 2-fold or more. Using this list, we

determined that the overall gene expression correlation between patient tumor and organoids is 0.59, while PDX and organoids is 0.8 (Fig. 3C). Furthermore, gene expression between 5/6 PDX-derived organoids and 1/3 patient-derived organoids was more highly correlated with their matched tumor tissue from which the organoid was derived, than with any other organoid model (Table 3). Additionally, gene expression correlations were also calculated with 1492 differentially expressed genes (a cut-off of 2-fold change) between LUAD and LUSC patient samples from the TCGA. Using this gene set, the correlation coefficient between patient-organoids is 0.66, while PDX-organoids is 0.85 (Fig. 12) similar to what we observed using the tumor epithelial-enriched gene list. Overall, the molecular data indicate that our *in vitro* growth conditions allow organoid tumor cells to largely maintain key molecular properties of their parental tumors.

Utility of long-term NSCLC organoids for drug testing

To explore the utility of our long-term NSCLC organoids for drug testing, we first surveyed genomic data of the five well-characterized long-term models for potential sensitizing biomarker alterations. The *KRAS* G13C mutation and amplification was detected in the patient, PDX and organoid of PDXO426. Preclinical studies with *KRAS* mutant cell lines have suggested that such mutant tumor cells may be more sensitive to MEK inhibitors, as compared to *KRAS* wild type cell lines (50,51). To determine whether the *KRAS* mutation and amplification in the PDXO426 organoid confers sensitivity to the MEK inhibitor trametinib, we compared its response to the drug to that of three other organoid models without *KRAS* alterations. Consistent with previous studies, we found that the *KRAS* mutant PDXO426 was much more sensitive to the MEK inhibitor trametinib ($IC_{50} < 0.05 \mu M$) than three other organoids with wild-type *KRAS* ($IC_{50} > 0.5 \mu M$) (Fig. 4A). Similar results were also generated with the MEK inhibitor selumetinib (Fig. 4B). To confirm that the *KRAS* alterations in PDXO426 act specifically to confer sensitivity to targeted therapy, we also examined the responses of these four organoid models to the EGFR inhibitor afatinib (Fig. 4C). As expected, none of the 4 organoid models responded to afatinib relative to the HCC827 cell line, which has an EGFR mutation that sensitizes cells to EGFR inhibitors.

To determine whether the specific MEK inhibitor sensitivity displayed by the PDXO426 organoid model reflects a biological property of the PDX tumor from which it was derived, we evaluated trametinib sensitivity in the parental PDXO426 PDX model.

PDXO426 PDX exhibited trametinib sensitivity, while the KRAS wild type PDXO274 PDX was resistant, supporting ex vivo organoid drug responses being reflective of in vivo responses of non-culture adapted tumor cells (Fig. 4D). Overall, these results support organoids being clinically relevant surrogates for patient tumors for drug testing.

Combination therapy in NSCLC organoids

We next explored whether NSCLC organoids can also be used as discovery tools for novel biomarker and combination therapy approaches. CNV analysis revealed chromosome 8p amplification in the patient, PDX, and organoid model of PDXO274. *FGFR1* amplification in this region is a common occurrence in LUSC, which occurs in 20% of LUSC cases (48). However, *FGFR1* amplification by itself is not a good biomarker for FGFR inhibitor monotherapy in LUSC, as only 7-11% of pre-selected patients demonstrated durable response in clinical trials (52,53). Thus, we utilized PDXO274 to model potential combination therapies in *FGFR1* amplified LUSC.

FGFR1 mRNA and protein quantification by RT-qPCR and western blot revealed that PDXO274 exhibited more than a 10-fold increase in *FGFR1* mRNA expression and higher phospho-FGFR1 (pFGFR1) and total FGFR1 protein expression relative to PDXO149 (*FGFR1* wild type) (Fig. 5A, B). These results indicated that in the PDXO274 organoid model, *FGFR1* amplification correlated with increased *FGFR1* mRNA levels, protein expression and pathway activation. However, reflective of the low response rates to FGFR inhibitors in patients, *in vitro* drug testing of the FGFR inhibitor BGJ398 revealed that PDXO274 was largely insensitive to FGFR inhibition (Fig. 5C). Based on previous cell line studies showing efficacy of the combination of MEK and PI3K inhibitors with FGFR inhibitors in FGFR aberrant cancers (54,55), we tested trametinib and the PI3K inhibitor BKM120 with BGJ398 in our *FGFR1* amplified organoid model. Strong synergy (combination index<0.5) was observed in the BGJ398+trametinib combination, while weaker synergy (combination index>0.5) was observed in the BGJ398+BKM120 combination (Fig. 5D). Additionally, while single agent BGJ398 inhibited pFGFR and pAkt, and single agent trametinib inhibited only pErk, targeted inhibition of all three phosphoproteins was achieved with the combination of the two compounds (Fig. 5E). The efficacy of the trametinib and BGJ398 combination was further verified *in vivo*, in the parental PDXO274 PDX (Fig. 5F), which further supports our earlier contention that organoid models can retain the

targeted therapy sensitivity of its source tumor tissue. Our data thus support combining FGFR and MEK inhibitors in FGFR1 amplified LUSC. Collectively, our drug studies generally support the addition of organoid and PDX models to a validation/discovery pipeline from cell lines to the patient, that may vastly improve the clinical response rate.

Wnt Agonist such as CHIR99021 is not essential

As shown in Figure 6, CHIR99021 is not essential for the long-term growth of lung tumour organoids. XDO377 model was passaged 10 times in either M26 or a minimal media formulation that does not contain CHIR99021. MM=minimal media, contains adv. DMEM/F12+AA+Glutamax+B27+Y27+Noggin+FGF7 Further, as shown in Figure 7, CHIR99021 is not essential for the short-term growth of lung tumour organoids. The proliferation was measured in four models grown either in M26 or M26 media without CHIR99021.

Discussion

Organoid methodology has gained widespread popularity in the past few years for its utility in disease modeling and drug screening (56,57,58,59). We aimed to establish a protocol of culturing NSCLC organoids from patient tumors and PDXs, with the eventual goal of establishing an improved platform for drug testing and biomarker discovery in NSCLC. Recent reports described two distinct methods for generating NSCLC organoid cultures, which harbored a mixture of normal and tumor cell populations (24,26). In one method, the authors found it necessary to treat the organoid cultures with the MDM2 inhibitor nutlin-3a to enrich for tumor cells harboring *TP53* mutation, due to the large amount of contamination by non-tumor cells (26). However, long-term exposure to such chemicals may have unexpected consequences on tumor cell biology and although *TP53* mutations are common in NSCLC, they do not occur in all cases (47,48,60), precluding this method for such tumor samples. Additionally, there should be continued efforts to improve media to better enrich for tumor cells from a greater number of samples. Future development of organoid models and media formulations would benefit from a standardized set of parameters to calculate "success rates" of model establishment. Currently, different studies use different criteria for deeming their cultures to be initiated or established. The definition of established models, for example, is defined by continuous propagation for six months in one study (61), while others use one month (25) or ambiguous cut-offs (26)

to define the longevity of their organoid cultures. Using our methods, which includes histological and flow cytometric characterization of cultures, we were able to achieve a collective overall establishment rate of 88% for both short-term and long-term NSCLC organoids. One question that would be interesting to address in future studies is what are the differences between tumor samples that determine whether tumor cells can adapt at all versus short-term versus long-term to organoid culture. Reasons for these differences are currently unknown, although mitotic instability of cancer cells, lack of physical environmental support and essential media components, and the quiescent nature of lung stem cells have been speculated to account for some failures (22). We attempted to compare tumor features (histological subtype, size) and mutation status between long-term and short-term organoid models (Table 4 and 5). However, these association studies are currently limited by our small sample size, which may be overcome as more organoid models become available. Interestingly, we did observe that organoids were more easily formed from PDXs than primary patient tumors. This finding suggests that a prior selection pressure in the non-orthotopic *in vivo* PDX environment might enrich for tumor populations that are more likely survive *in vitro*. We also observed that it was more difficult to establish organoids from LUSC patient tumors than LUAD tumors. This suggests that the *ex vivo* conditions are still not optimal for LUSC and consequently, LUSC exhibits a marked preference for an *in vivo* environment, even non-orthotopic. Indeed, it has generally been more difficult to establish 2D cell lines from LUSC, as compared to LUAD, while LUSC patient tumors engraft much better as PDXs (60% vs 25%, respectively) (4, 28).

In addition to recapitulating the biology that drives histological appearance of their parental tumors growing *in vivo*, another attractive feature of our organoid models is that they have not yet drifted on the molecular level, as many cell lines have. Previous studies have shown that organoids from other cancers can retain the molecular profiles of their parental tumors, even with subsequent passaging (15,61,62,63). We examined the mutation and CNV profiles of our NSCLC organoids and identified major somatic alterations in lung cancer that were preserved in the parental tumors. We also observed that organoids of late passage (even after more than 10 passages) still retained the molecular features of their parental tumors. This suggests that mutation and CNV profiles are largely stable in organoid cultures. In addition, there have been reports of both concordance and discordance of gene expression profiles between organoids and their parental tumors (15). We showed that majority of our organoid

models (>10 passages) exhibited a strong correlation in gene expression with their parental tumors. However, it did appear that the gene expression correlations were better for organoids derived from PDXs than patient tumors. This may reflect the patient tumors having the most heterogeneity among cell populations, with the PDX
5 and organoid models potentially selecting for fewer clonal populations.

One of the attractive applications of our short-term cultures is that there is usually enough cell material for drug testing, which we have demonstrated for some targeted therapeutics. This may be particularly useful not only for biomarker validation studies, but also for quick assessment of which therapies would be suitable for patient use
10 when they fail on first or second-line therapies. Recent reports have demonstrated the utility of PDXs for drug screening and personalized medicine (64). However, PDXs are limited by slow growth rate and are economically challenging to maintain. Thus, our protocol for establishing NSCLC organoids from patient tumor and PDX could potentially provide additional models of NSCLC that are biologically relevant for future
15 drug screening studies. Indeed, we find that we are usually able to obtain enough cells even in short-term culture for drug testing, which would be particularly useful for quick assessment of alternative therapies when patients fail on first or second-line treatments. With short-term cultures, we verified a previous suggestion that MEK could be a potential clinically relevant target in some *KRAS* mutant LUADs (50,51).
20 Using our long-term organoid cultures, we also found evidence to support combining FGFR and MEK inhibitors in *FGFR1*-amplified LUSC, the basis for which was also initially suggested from cell line work (54). Importantly, we found that the organoid drug response is similar to that of the matched PDX, which was not adapted to *ex vivo* culture. Thus, organoids developed with our protocol appear to be good surrogates for
25 clinical tissue for drug screening and biological studies.

In conclusion, our study provides a methodology of developing short-term and long-term organoid cultures from NSCLC patient and PDX tumor tissue, and demonstrates the utility of the established organoids for drug testing and biomarker validation. Our collection of NSCLC organoids is also a novel addition to existing preclinical models of
30 NSCLC that may be useful for identifying viable therapeutic options for this disease.

Although preferred embodiments of the invention have been described herein, it will be understood by those skilled in the art that variations may be made thereto without departing from the spirit of the invention or the scope of the appended claims. All

documents disclosed herein, including those in the following reference list, are incorporated by reference.

Table 1. Table summarizing Tissue histology, features and organoid characterization.

Organoid model	Histology	****Predominant subtype/differentiation	Tissue source	Tissue IHC	Organoid IHC	Organoid split ratio	Organoid passage #
PDX0083	Adenocarcinoma	acinar	PDX	NA	NA		4
PDX0110	Adenocarcinoma	solid	PDX	NA	NA		8
PDX0137	Adenocarcinoma	acinar & papillary	PDX	TTF-1-/TP63	TTF-1+/TP63	1 to 4	>10
PDX0148	Adenocarcinoma	solid	PDX	NA	NA		1
PDX0256	Adenocarcinoma	Mucinous	PDX	NA	NA		4
PDX0309	Adenocarcinoma	acinar	PDX	NA	NA		2
PDX0314	Adenocarcinoma	solid & acinar	PDX	NA	NA		2
PDX0320	Adenocarcinoma	not specified	PDX	NA	NA		1
PDX0344	Adenocarcinoma	acinar & solid	PDX	TTF-1focal/TP63focal	TTF-1focal/TP63focal	1 to 4	>10
PDX0379	Adenocarcinoma	Mucinous	PDX	NA	NA		3
PDX0402	Adenocarcinoma	acinar & solid	PDX	NA	NA		2
PDX0425	Adenocarcinoma	solid & acinar	PDX	TTF-1	TTF-1	1 to 4	>10
PDX0484	Adenocarcinoma	Mucinous	PDX	NA	NA		0
PDX04208	Adenocarcinoma	acinar & solid	PDX	NA	NA		3
PDX04256	Adenocarcinoma	solid	PDX	TTF-1-	TTF-1+	1 to 4	>10
PDX04268	Adenocarcinoma	not specified	PDX	NA	NA		4
LPT054	Adenocarcinoma	acinar	Patient	TTF-1-	TTF-1+	1 to 4	>10
LPT057	Adenocarcinoma	solid	Patient	NA	NA		4
LPT080	Adenocarcinoma	acinar	Patient	NA	NA		2
LPT083	Adenocarcinoma	solid	Patient	NA	NA		4
LPT085	Adenocarcinoma	mucinous	Patient	TTF-1-/TP63	TTF-1-/TP63	1 to 4	>10
LPT086	Adenocarcinoma	lipidic	Patient	NA	NA		3
LPT090	Adenocarcinoma	acinar	Patient	NA	NA		4
LPT091	Adenocarcinoma	papillary	Patient	NA	NA		2
LPT092	Adenocarcinoma	acinar	Patient	NA	NA		2
LPT094	Adenocarcinoma	acinar	Patient	NA	NA		4
LPT095	Adenocarcinoma	Mucinous	Patient	TTF-1-/p63-	TTF-1-/p63+		8
LPT0124	Adenocarcinoma	solid	Patient	TTF-1-/p63-	TTF-1-/TP63+		8
LPT0125	Adenocarcinoma	not specified	Patient	TTF-1+/TP63+	TTF-1-/TP63+		8
LPT0126	Adenocarcinoma	solid	Patient	TTF-1-/TP63	TTF-1-/TP63	1 to 4	>10
LPT0127	Adenocarcinoma	acinar	Patient	NA	NA		4
LPT0128	Adenocarcinoma	not specified	Patient	NA	NA		2
LPT0129	Adenocarcinoma	solid	Patient	NA	NA		1
LPT0130	Adenocarcinoma	microcapillary	Patient	NA	NA		4
LPT0131	Adenocarcinoma	solid	Patient	TTF-1-/TP63	TTF-1-/TP63		0
PDX0086	Squamous	Poorly diff.	PDX	NA	NA		3
PDX0140	Squamous	moderately differentiated	PDX	NA	NA		>10
PDX0142	Squamous	moderately differentiated	PDX	NA	NA		1
PDX0149	Squamous	moderately differentiated	PDX	CK5+/TP63+	CK5+/TP63+	1 to 4	>10
PDX0152	Squamous	Poorly differentiated	PDX	NA	NA		2
PDX0162	Squamous	Poorly differentiated	PDX	NA	NA		1
PDX0168	Squamous	moderately differentiated	PDX	NA	NA		1
PDX0200	Squamous	Poorly differentiated	PDX	NA	NA		3
PDX0220	Squamous	moderately differentiated	PDX	NA	NA		1
PDX0252	Squamous	moderately differentiated	PDX	NA	NA		1
PDX0267	Squamous	moderately differentiated	PDX	NA	NA		1
PDX0268	Squamous	moderately differentiated	PDX	NA	NA		4
PDX0271	Squamous	Well differentiated	PDX	NA	NA		1
PDX0274	Squamous	moderately differentiated	PDX	CK5+/TP63+	CK5+/TP63+	1 to 4	>10
PDX0290	Squamous	moderately differentiated	PDX	NA	NA		0
PDX0296	Squamous	Poorly differentiated	PDX	NA	NA		1
PDX0321	Squamous	moderately differentiated	PDX	NA	NA		2
PDX0322	Squamous	moderately differentiated	PDX	NA	NA		2
PDX0328	Squamous	moderately differentiated	PDX	NA	NA		4
PDX0333	Squamous	moderately differentiated	PDX	NA	NA		3
PDX0356	Squamous	moderately differentiated	PDX	NA	NA		1
PDX0360	Squamous	Poorly differentiated	PDX	NA	NA		2
PDX0369	Squamous	Well differentiated	PDX	NA	NA		3
PDX0377	Squamous	moderately differentiated	PDX	TTF-1-/TP63-	TTF-1-/TP63+	1 to 4	>10
PDX0410	Squamous	Poorly differentiated	PDX	NA	NA		8
PDX0422	Squamous	moderately differentiated	PDX	NA	NA		1
LPT055	Squamous	moderately differentiated	Patient	NA	NA		1
LPT077	Squamous	moderately differentiated	Patient	NA	NA		4
LPT080	Squamous	poorly differentiated	Patient	NA	NA		4
LPT023	Squamous	poorly differentiated	Patient	NA	NA		0
LPT039	Squamous	moderately differentiated	Patient	NA	NA		3
LPT090	Squamous	moderately differentiated	Patient	NA	NA		0
LPT099	Squamous	poorly differentiated	Patient	NA	NA		3
LPT065	Squamous	Not specified	Patient	NA	NA		0
LPT071	Squamous	Poorly differentiated	Patient	NA	NA		0
LPT072	Squamous	moderately differentiated	Patient	NA	NA		0
LPT073	Squamous	moderately differentiated	Patient	NA	NA		2
LPT074	Squamous	Not specified	Patient	NA	NA		0
LPT075	Squamous	moderately differentiated	Patient	NA	NA		0
LPT076	Squamous	moderately differentiated	Patient	NA	NA		0
LPT077	Squamous	moderately differentiated	Patient	NA	NA		0

*Percent tumor cells for patient derived organoids is determined by correlation of TTF-1 and TP63 staining patterns; Percent tumor cells for PDX derived organoids is determined by eye under

**Yellow = long term established models

***Gray = excluded models

****Predominant subtype/differentiation for PDX is based on primary patient tumor

Table 1 cont'd

Organoid model	Latency (P0-P1 days)	Total days in culture	Media	Outcome	Percent tumor cells in organoids (method) [†]	Comment	Disease stage
PDX0083	8	58	M2E	Short term	NA		4
PDX0110	14	50	M2E	Short term	NA		2B
PDX0117	8	>100	M2E	Long term	50.9% LpCAM, 2% H2K (flow)	Estab. shed	2B
PDX0148	12	12	M2E	Short term	NA		3A
PDX0255	2	47	M2E	Short term	NA		1B
PDX0309	7	20	M2E	Short term	63% human LpCAM, 4.3% H2K (flow)		3A
PDX0314	14	43	M2E	Short term	NA		3B
PDX0325	20	20	M2E	Short term	NA		4
PDX0344	7	>100	M2E	Long term	75.5% human LpCAM, 0% H2K (flow)	Estab. shed	1A
PDX0379	1	35	M2E	Excluded	1.2% human EpCAM, 68.9% H2K (flow)	mouse	1A
PDX0402	26	50	M2E	Short term	NA		3A
PDX0425	10	>100	M2E	Long term	58% human LpCAM, 0% H2K (flow)	Estab. shed	3A
PDX0484	5	36	M2E	Excluded	0.1% human EpCAM, 75.4% H2K (flow)	mouse	1A
PDX04205	5	45	M2E	Short term	NA		4
PDX04254	5	>100	M2E	Long term	92% human LpCAM, 0.2% H2K (flow)	Estab. shed	4
PDX04268	7	69	M2E	Excluded	NA	Not primary lung	Metastasis from colon
LPT054	12	>100	M2E	Long term	90% (histology and IHC)	Estab. shed	1A
LPT057	5	48	M2E	Short term	NA		3A
LPT080	23	49	M2E	Short term	NA		1A
LPT083	23	58	M2E	Short term	NA		N/A
LPT085	23	>100	M2E	Long term	95% (histology and IHC)	Estab. shed	1B
LPT086	23	70	M2E	Short term	NA		1B
LPT090	23	52	M2E	Short term	NA		3B
LPT091	23	42	M2E	Short term	NA		1A
LPT092	23	42	M2E	Short term	NA		N/A
LPT094	23	52	M2E	Short term	NA		2A
LPT095	23	140	M2E	Excluded	20% (histology and IHC)	Normal	2A
LPTD124	7	185	M2E	Excluded	20% (histology and IHC)	Normal	2B
LPTD125	8	85	M2E	Excluded	30% (histology and IHC)	Normal	N/A
LPTD126	15	>100	M2E	Long term	85% (histology and IHC)	Estab. shed	1A
LPTD127	15	75	M2E	Short term	NA		1B
LPTD128	13	54	M2E	Short term	NA		1B
LPTD129	15	15	M2E	Short term	NA		1B
LPTD130	12	54	M2E	Short term	NA		2A
LPTD131	8	53	M2E	Short term	55% (histology and IHC)		1A
PDX080	16	42	M2E	Short term	NA		2B
PDXD140	4	>100	M2E	Excluded	NA	Not primary lung	Metastasis from H&N
PDXD142	4	N/A	M2E	Excluded	NA	Not primary lung	Metastasis from cervix
PDX0149	4	>100	M2E	Long term	95% human LpCAM, 4.2% H2K (flow)	Estab. shed	1B
PDX0152	2	3	M2E	Short term	NA		1A
PDX0162	4	10	M2E	Short term	NA		1B
PDX0168	1	7	M2E	Short term	NA		1B
PDX0200	6	12	M2E	Short term	NA		3A
PDX0225	1	40	M2E	Short term	NA		2A
PDX0252	23	23	M2E	Short term	50.8% human LpCAM, 0.7% H2K (flow)		2B
PDX0267	3	35	M2E	Short term	NA		1A
PDX0268	1	14	M2E	Short term	NA		2B
PDX0271	6	33	M2E	Short term	NA		2A
PDX0274	5	>100	M2E	Long term	90% human LpCAM, 1.4% H2K (flow)	Estab. shed	2B
PDX0295	1	22	M2E	Short term	NA		3B
PDX0296	5	6	M2E	Short term	74.1% human LpCAM, 5.9% H2K (flow)		3B
PDX0321	1	53	M2E	Short term	83.8% human LpCAM, 12.9% H2K (flow)		2B
PDX0322	2	3	M2E	Short term	NA		1B
PDX0328	1	18	M2E	Excluded	1.7% human EpCAM, 84.4% H2K (flow)	mouse	3B
PDX0353	16	50	M2E	Short term	NA		3A
PDX0355	1	14	M2E	Short term	NA		2A
PDX0365	26	50	M2E	Short term	NA		3A
PDX0369	16	50	M2E	Short term	NA		2A
PDX0377	19	>100	M2E	Long term	87% human LpCAM, 0.1% H2K (flow)	Estab. shed	1A
PDX0410	1	>30	M2E	Excluded	0% human EpCAM, 91.8% H2K (flow)	mouse	2B
PDX0422	2	6	M2E	Short term	NA		3A
LPT055	13	13	M2E	Short term	NA		1B
LPT057	4	35	M2E	Short term	NA		2B
LPT060	10	52	M2E	Short term	NA		2B
LPT023	0	N/A	M2E	Excluded	NA	No tumor cells	3
LPT039	3	14	M2E	Short term	NA		1B
LPT040	0	15	M2E	Failed to initiate	NA		1B
LPT059	8	23	M2E	Short term	NA		3A
LPT065	0	16	M2E	Failed to initiate	NA		1B
LPT071	0	15	M2E	Failed to initiate	NA		3A
LPT072	0	22	M2E	Failed to initiate	NA		2A
LPT073	18	45	M2E	Short term	NA		1A
LPT074	0	4	M2E	Failed to initiate	NA		N/A
LPT075	0	3	M2E	Failed to initiate	NA		1B&3
LPT076	0	14	M2E	Failed to initiate	NA		2A
LPT077	0	14	M2E	Failed to initiate	NA		1A

[†]Percent tumor cells: human LpCAM—cells by flow cytometry

**Yellow = long term

***Gray = exclude

****Predominant

Table 2: Table summarizing lung organoid establishment rate

	Patient		PDX		Total
Tissue	LUAD	LUSC	LUAD	LUSC	
Initiation	16/16 (100%)	6/14 (43%)	13/13 (100%)	22/22 (100%)	57/65 (88%)
Short-term (<3 months, P1-9)	13/16 (81%)	6/14 (43%)	9/13 (69%)	19/22 (86%)	47/65 (72%)
Long-term (>3 months, >P10)	3/16 (19%)	0/14 (0%)	4/13 (31%)	3/22 (14%)	10/65 (15%)
Normal epithelial cell contamination (short- term)	3/4 (75%)	-	2/3 (67%)	2/5 (40%)	-
Normal epithelial cell contamination (long- term)	0/3 (0%)	-	0/4 (0%)	0/3 (0%)	-

Table 3: Gene expression correlation coefficients in patient/PDX/organoids

ORGANOID	Source								
Patient	LPTO54_Patient	LPTO85	LPTO126	PDXO4056	PDXO426	PDXO344	PDXO137	PDXO149	PDXO274
LPTO54	0.491	0.636	0.555	0.664	0.622	0.758	0.546	0.642	0.654
	LPTO85_Patient	LPTO54	LPTO126	PDXO4056	PDXO426	PDXO344	PDXO137	PDXO149	PDXO274
LPTO85	0.666	0.636	0.537	0.735	0.771	0.737	0.638	0.675	0.739
	LPTO126_Patient	LPTO54	LPTO85	PDXO4056	PDXO426	PDXO344	PDXO137	PDXO149	PDXO274
LPTO126	0.661	0.555	0.537	0.554	0.598	0.427	0.546	0.607	0.554
PDX									
	PDXO4056_PDX	LPTO54	LPTO85	LPTO126	PDXO426	PDXO344	PDXO137	PDXO149	PDXO274
PDXO4056	0.827	0.664	0.735	0.554	0.593	0.593	0.649	0.669	0.687
	PDXO426_PDX	LPTO54	LPTO85	LPTO126	PDXO4056	PDXO344	PDXO137	PDXO149	PDXO274
PDXO426	0.841	0.622	0.771	0.598	0.593	0.679	0.636	0.701	0.723
	PDXO344_PDX	LPTO54	LPTO85	LPTO126	PDXO4056	PDXO426	PDXO137	PDXO149	PDXO274
PDXO344	0.823	0.758	0.737	0.427	0.755	0.679	0.554	0.668	0.710
	PDXO137_PDX	LPTO54	LPTO85	LPTO126	PDXO4056	PDXO426	PDXO344	PDXO149	PDXO274
PDXO137	0.845	0.546	0.638	0.546	0.649	0.636	0.554	0.626	0.649
	PDXO149_PDX	LPTO54	LPTO85	LPTO126	PDXO4056	PDXO426	PDXO344	PDXO137	PDXO274
PDXO149	0.779	0.642	0.675	0.607	0.669	0.701	0.668	0.626	0.565
	PDXO274_PDX	LPTO54	LPTO85	LPTO126	PDXO4056	PDXO426	PDXO344	PDXO137	PDXO149
PDXO274	0.693	0.654	0.739	0.554	0.687	0.723	0.710	0.649	0.565

Table 4: Patient tumor features & mutation in patient-derived organoids

*Features		Number of samples	Long-term	Short-term
Histology (LUAD)	Acinar	6	17% (1/6)	83% (5/6)
	Solid	5	20% (1/5)	80% (4/5)
	Lepidic	1	0% (0/1)	100% (1/1)
	Papillary	1	0% (0/1)	100% (1/1)
	Micropapillary	1	0% (0/1)	100% (1/1)
	Mucinous	1	100% (1/1)	0% (0/1)
	N/A	1	0% (0/1)	100% (1/1)
Histology (LUSC)	Poorly diff	2	0% (0/2)	100% (2/2)
	Moderately diff	4	0% (0/4)	100% (4/4)
Stage	I	13	23% (3/13)	77% (10/13)
	II	4	0% (0/4)	100% (4/4)
	III	3	0% (0/3)	100% (3/3)
	N/A	2	0% (0/2)	100% (2/2)
Tumor size (cm)	Median (range)	22	2.5 (2.1-2.7)	2.9 (1.8-6.5)
Gene mutation (LUAD)	EGFR	4	0% (0/4)	100% (4/4)

*Models that failed to initiate or lacking mutation information were excluded from the study

Table 5: PDX tumor features and mutation in PDX-derived organoids

Features		Number of samples	Long-term	Short-term
Histology (LUAD)	Acinar	6	33% (2/6)	67% (4/6)
	Solid	5	40% (2/5)	60% (3/5)
	Mucinous	1	0% (0/1)	100% (1/1)
	N/A	1	0% (0/1)	100% (1/1)
Histology (LUSC)	Poorly diff	6	0% (0/6)	100% (6/6)
	Moderately diff	14	21% (3/14)	79% (11/14)
	Well diff	2	0% (0/2)	100% (2/2)
Stage	I	9	33% (3/9)	67% (6/9)
	II	11	18% (2/11)	82% (9/11)
	III	11	9% (1/11)	91% (10/11)
	IV	4	25% (1/4)	75% (3/4)
Tumor size (cm)	Median (range)	35	4 (1.7-5.9)	3.5 (1.5-11)
*Gene mutation	TP53	25	20% (5/25)	80% (20/25)
	CDKN2A	3	33% (1/3)	67% (2/3)
	KRAS	4	50% (2/4)	50% (2/4)
	KEAP1	6	16% (1/6)	84% (5/6)
	STK11	4	0% (0/4)	100% (4/4)
	EGFR	5	40% (2/5)	60% (3/5)
	NF1	28	21% (6/28)	79% (22/28)
	BRAF	2	0% (0/2)	100% (2/2)
	SETD2	1	0% (0/1)	100% (1/1)
	MGA	12	25% (3/12)	75% (9/12)
	MET	12	16% (2/12)	84% (10/12)
	ARID1A	3	0% (0/3)	100% (3/3)
	PIK3CA	7	14% (1/7)	86% (6/7)
	RB1	11	27% (3/11)	73% (8/11)
	U2AF1	2	50% (1/2)	50% (1/2)
	RIT1	4	50% (2/4)	50% (2/4)
	NFE2L2	7	14% (1/7)	86% (6/7)
	NOTCH1	5	40% (2/5)	60% (3/5)

*Models lacking mutation information were excluded from the study

Table 6

Media component	Stock Concentration	Tsao M26	Notes	Sachs (PMID:30643021)	Dijkstra (PMID:30100188)	Kim (PMID:31488816)
Medium (Advanced DMEM/F12)		1x	Basal medium that allows the culture of mammalian cells without Fetal Bovine Serum supplementation	1x	50% final volume	
DMEM/F12						
HEPES	1 M	10mM	Required supplement for Advanced DMEM/F12	10mM	1mM	
GlutaMAX	200 mM	1x	Required supplement for Advanced DMEM/F12	1x	1x	
Antibiotic	100 U/ml	Anti-Anti 1x	Antibiotic-Antimycotic - to prevent microbial contamination should contain at the minimum penicillin, streptomycin and amphotericin	Pen/Strep (100U/ml) and Primocin (50ug/ml)	Pen/Strep (100U/ml)	Pen/Strep (100U/ml)
Noggin	100 ug/ml	100ng/ml	Noggin antagonizes bone morphogenic protein (BMP) ligand of the transforming growth factor family. It is essential for maintenance of undifferentiated stem cells in vitro. Noggin conditioned media can be used as an alternative to recombinant Noggin.	100 ng/ml	L-WRN conditioned media*	
N-acetyl-L-cysteine	500 mM	1.25mM	Supplement form of cysteine, a semi-essential amino acid; antioxidant and mucolytic agent; prevents apoptosis	1.25mM	1mM	
A83 01	0.5 mM	0.5uM	A selective inhibitor of the Transforming Growth Factor beta type I receptor, the a-pla1stic lymphoma kinase 4 and 7 - permits expansion of adult epithelial cells from a wide array of tissues	0.5uM	0.5uM	
B27	50x	1x	Gibco B-27 supplement containing 27 supplements originally developed for culturing primary neurons; adapted and used in many organoid cultures	1x	1x w/o vitamin A	1x
N2	50x					1x
Human FGF4	100 ug/ml	100ng/ml	Fibroblast Growth Factor 4 involved in FGF signaling. Used to support the maintenance and self-renewal properties of stem cells.			
CHIR99021	5 mM	0.25uM	An aminopyrimidine derivative and a potent glycogen synthase kinase (GSK) 3 inhibitor. GSK3 is a serine/threonine kinase that is a key inhibitor of the WNT pathway. CHIR99021 is a WNT activator. WNT plays a key role during lung development.			
SAG	1 mM	0.1uM	Small molecule agonist of Smoothened receptor involved in Sonic hedgehog signaling. Inhibition of smoothened activates hedgehog signaling which plays a key role during lung development. Shh is activated in lung tumours.			
Y-27632	10 mM	10uM	Selective ROCK inhibitor. Increases survival rate of stem cells during propagation and cryopreservation.	5uM		10uM
Human FGF10	100 ug/ml	500ng/ml	Fibroblast growth Factor 10 involved in FGF signaling. During early lung development, FGF10 is expressed in distal mesenchyme and acts on the distal lung epithelial progenitors to prevent them from differentiating into proximal epithelial cells. This growth factor is required for initiation and maintenance of lung organoid cultures. The alternative for FGF10 is FGF7 which belongs to the same FGF7 subfamily. They both activate the b isoforms of EGFR1 and EGFR2 present in the epithelium.	100-ng/ml		
Human EGF	100 ug/ml	50ng/ml	Epidermal growth factor promotes proliferation of cells.		50ng/ml	50ng/ml
Human FGF2	100 ug/ml			25ng/ml		20ng/ml
Human FGF7	100 ug/ml			500ng/ml		
Human RSP0-1	500 ug/ml			0.5 uM	10uM	
SB202190	15 mM			5mM	10mM	
Nicotinamide	1 M				10-M	
Gastrin	100 uM					
Nutlin 3-a	10 mM			5 uM		

Red italic font (components not found in other lung media)

Table 7: Human Lung Tissue Collection Media

RPMI Medium 1640 (Gibco) comprises 90% of the final collection media.

Additive	Final Concentration	Stock concentration	100 ml CM
FBS	10%		10 ml

Table 2. Human Lung Tissue Freezing Media

RPMI Medium 1640 (Gibco) comprises 70% of the final collection media.

Additive	Final Concentration	Stock concentration	50 ml CM
FBS	20%		10 ml
DMSO	10%		5 ml

Table 3. Human Lung culture media (M26)

Additive	Final Concentration	Stock concentration		10 ml of CGM
GlutaMAX	2 mM	200 mM	100x	100 μ l
HEPES	10 mM	1 M	100x	100 μ l
Antibiotic-Antimycotic	100 U/ml	10 000 U/ml	100x	100 μ l
B-27 Supplement	1X	50x	50x	200 μ l
N-Acetyl-L-cysteine	1.25 mM	500 mM	400x	25 μ l
Recombinant Human γ EGF	50 ng/ml	100 μ g/ml	2000x	5 μ l
Recombinant Human γ FGF-10	100 ng/ml	100 μ g/ml	1000x	10 μ l
Recombinant Human γ FGF-4	100 ng/ml	100 μ g/ml	1000x	10 μ l
Recombinant Human γ Noggin	100 ng/ml	100 μ g/ml	1000x	10 μ l
A 83-01	0.5 μ M	0.5 mM	1000x	10 μ l
Y-27632	10 μ M	10 mM	1000x	10 μ l
CHIR 99021	250 nM	5 mM	20 000x	0.5 μ l
SAG	100 nM	1 mM	10 000x	1 μ l

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CLAIMS:

1. A cell culture medium comprising:
 - a. a basal medium;
 - b. an antibiotic;
 - 5 c. B27;
 - d. Noggin;
 - e. Y-27632;
 - f. Human FGF10 or FGF7;

preferably wherein there is an absence of a Wnt agonist.
- 10 2. The medium of claim 1, wherein the Human FGF is FGF10 and is in a concentration of at least 500ng/ml.
3. The medium of any one of claims 1 and 2, further comprising a buffering agent.
4. The medium of claim 3, wherein the buffering agent is HEPES.
5. The medium of any one of claims 1-4, wherein the basal medium is Advanced
15 DMEM/F12.
6. The medium of any one of claims 1-5, further comprising l-glutamine or GlutaMax™.
7. The medium of any one of claims 1-6, further comprising an antibiotic.
8. The medium of claim 7, wherein the antibiotic is penicillin, streptomycin or
20 primocin, or combinations thereof.
9. The medium of any one of claims 1-8, further comprising N-acetyl-L-cystein.
10. The medium of any one of claims 1-9, further comprising A83 01.
11. The medium of any one of claims 1-10, further comprising N2.

12. The medium of any one of claims 1-11, further comprising human FGF4.
13. The medium of any one of claims 1-12, further comprising CHIR99021.
14. The medium of any one of claims 1-13, further comprising SAG.
15. The medium of any one of claims 1-14, further comprising human EGF.
- 5 16. The medium of any one of claims 1-15, wherein the concentration of the components correspond to those of the listed in Table 8 under Tsao M26 $\pm$ 30%.
17. The medium of claim 16, wherein the concentration of the components correspond to those of the listed in Table 8 under Tsao M26.
- 10 18. A medium comprising the medium components listed in Table 8 under Tsao M26.
19. The medium of claim 18, wherein the concentration of the components correspond to those of the listed in Table 8 under Tsao M26 $\pm$ 30%.
20. The medium of claim 19, wherein the concentration of the components
15 correspond to those of the listed in Table 8 under Tsao M26.
21. The medium of any one of claims 1-20, for culturing organoids.
22. The medium of claim 21, for culturing cancer organoids.
23. The medium of claim 22, for culturing lung cancer organoids.

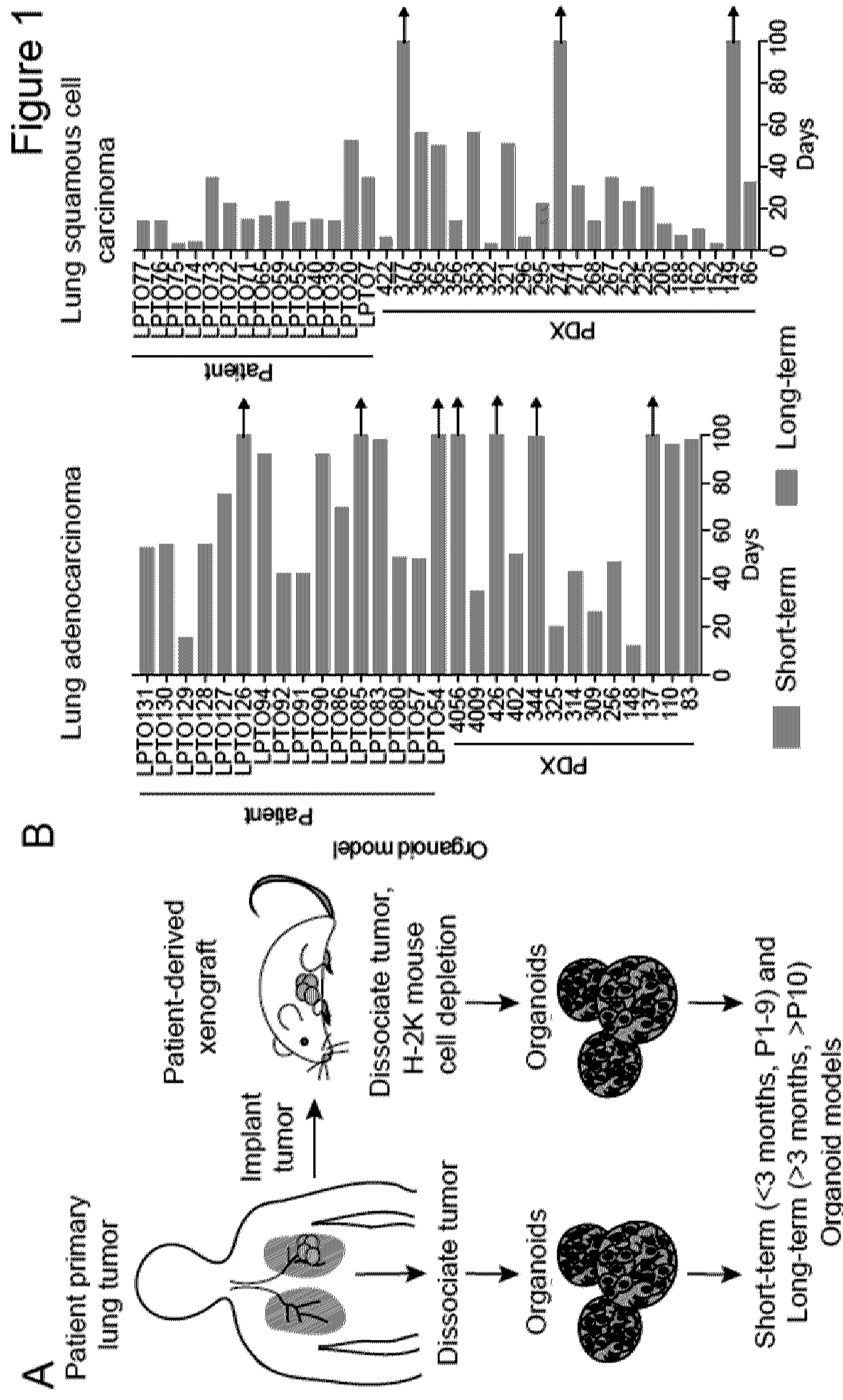


Figure 1

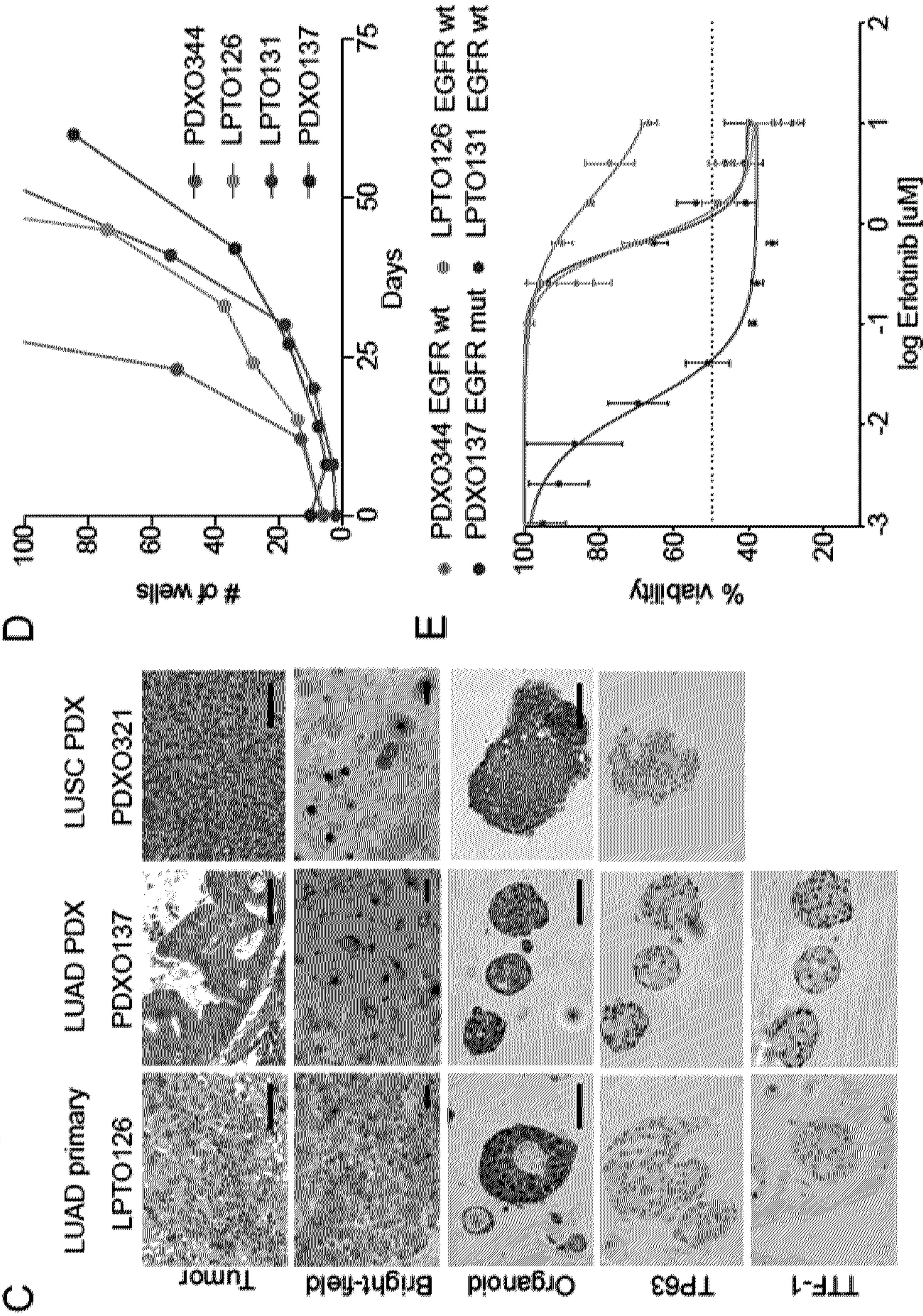


Figure 2

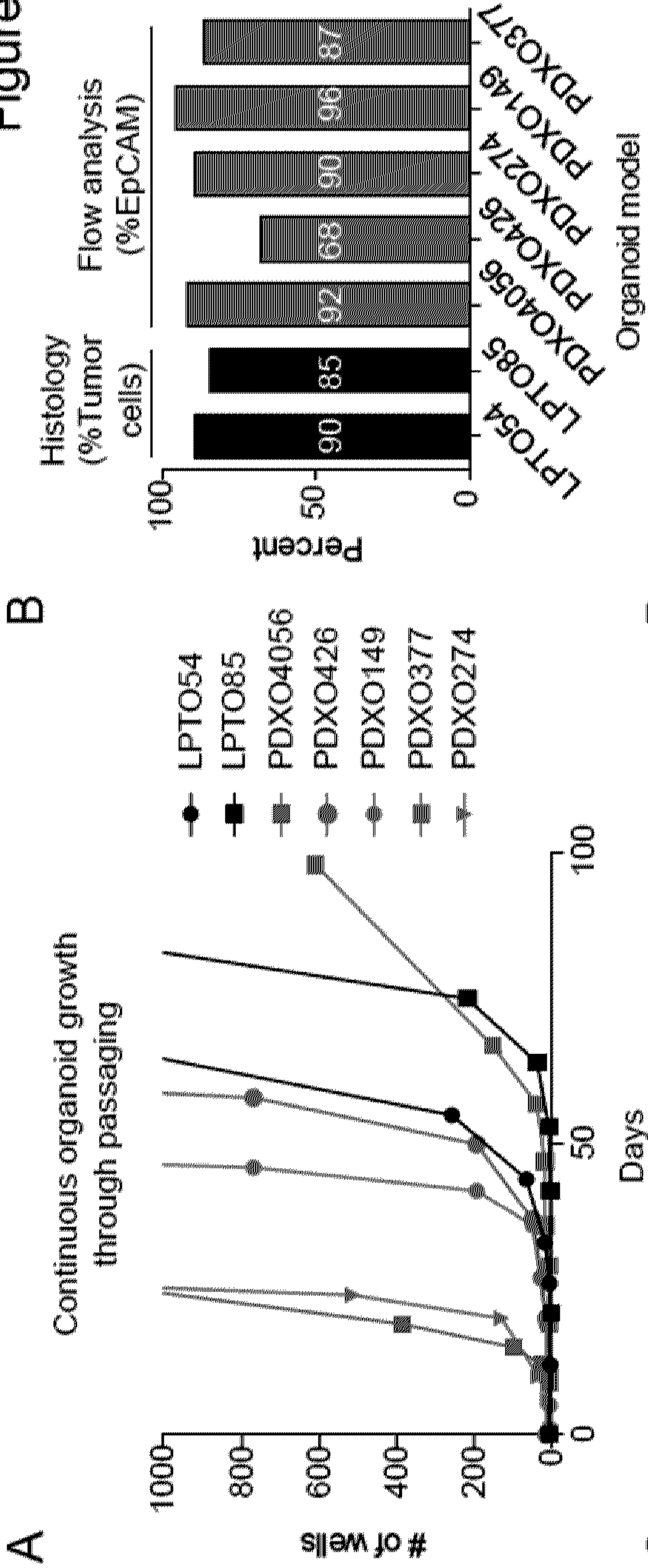
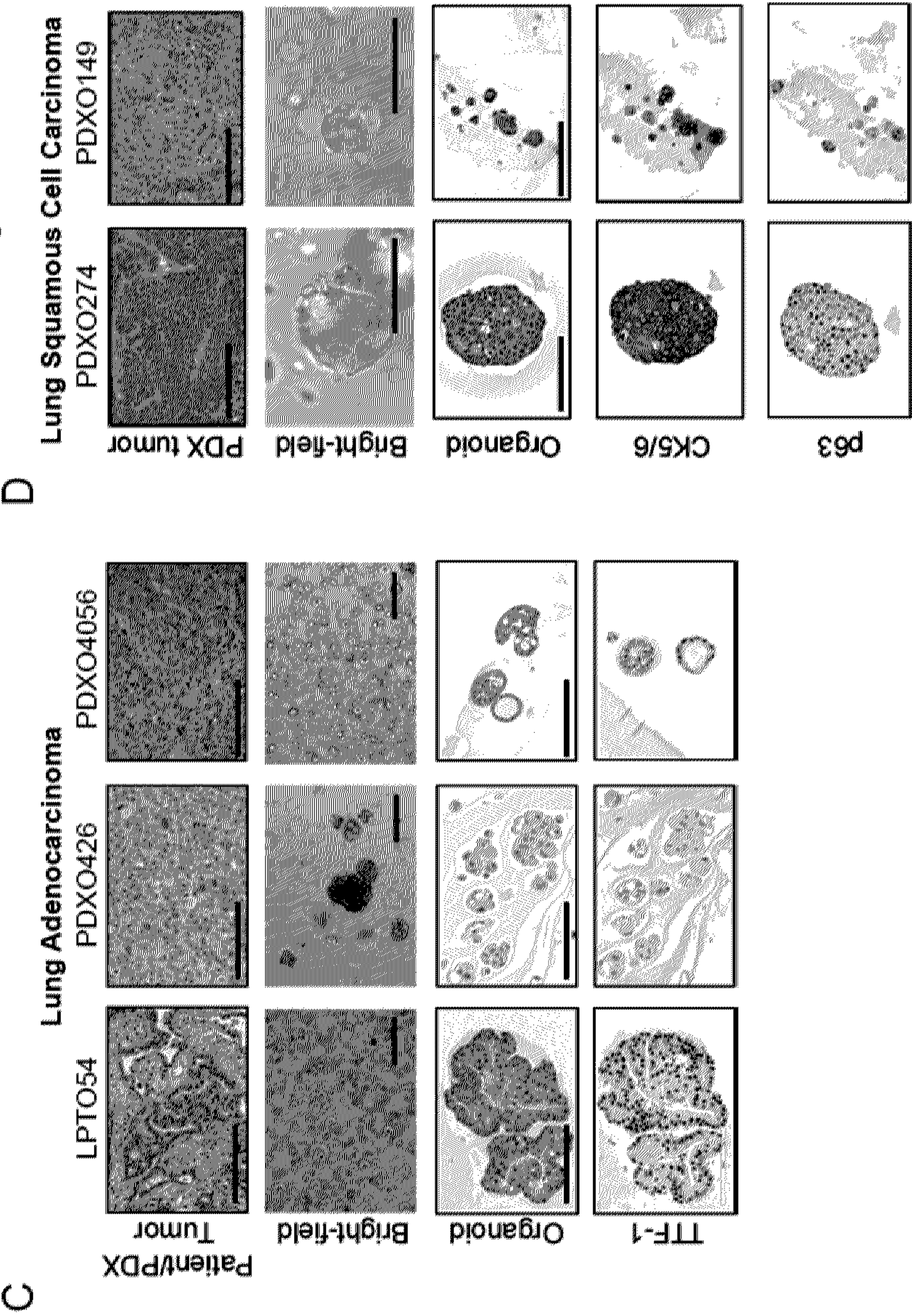


Figure 2



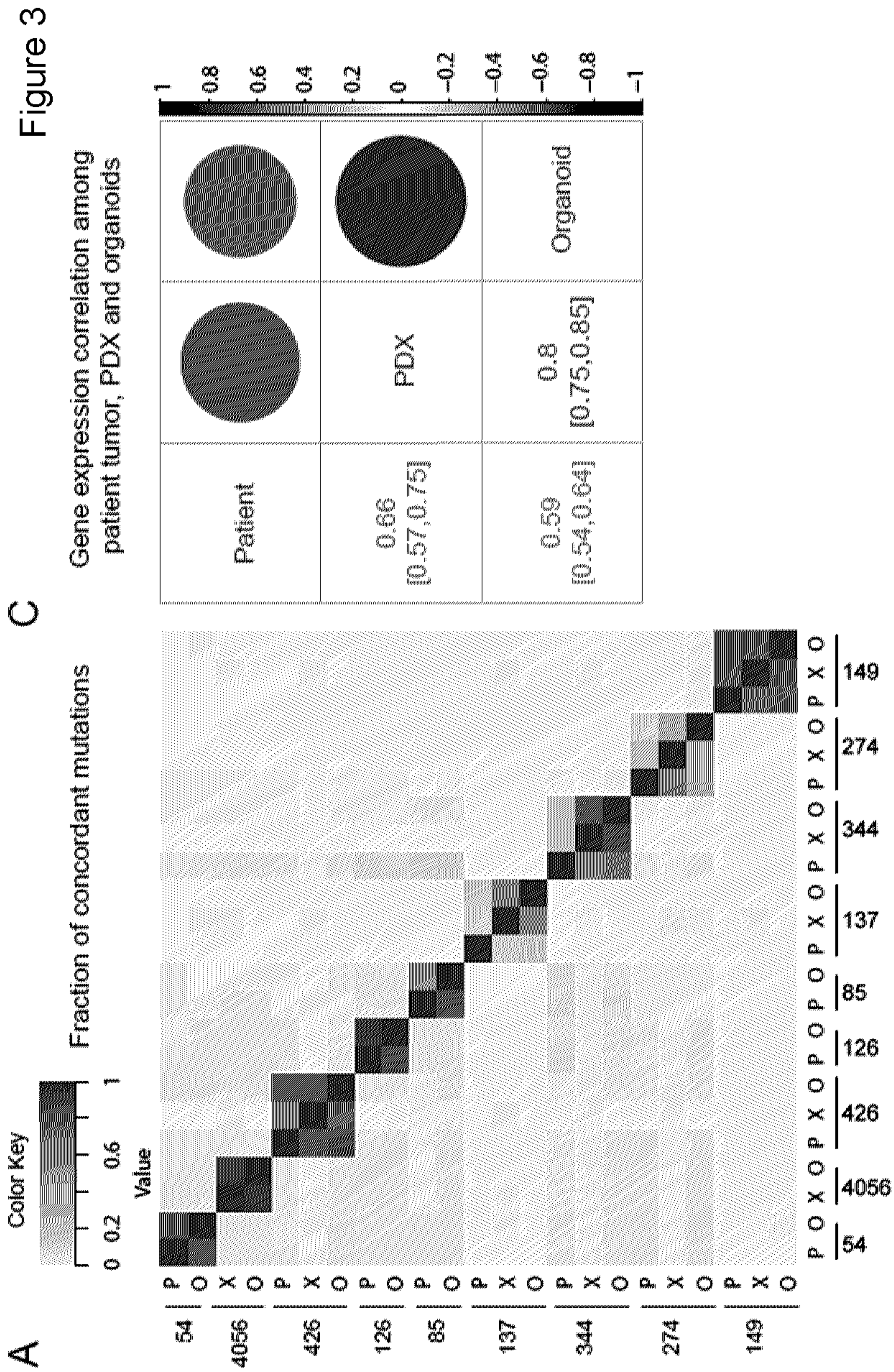


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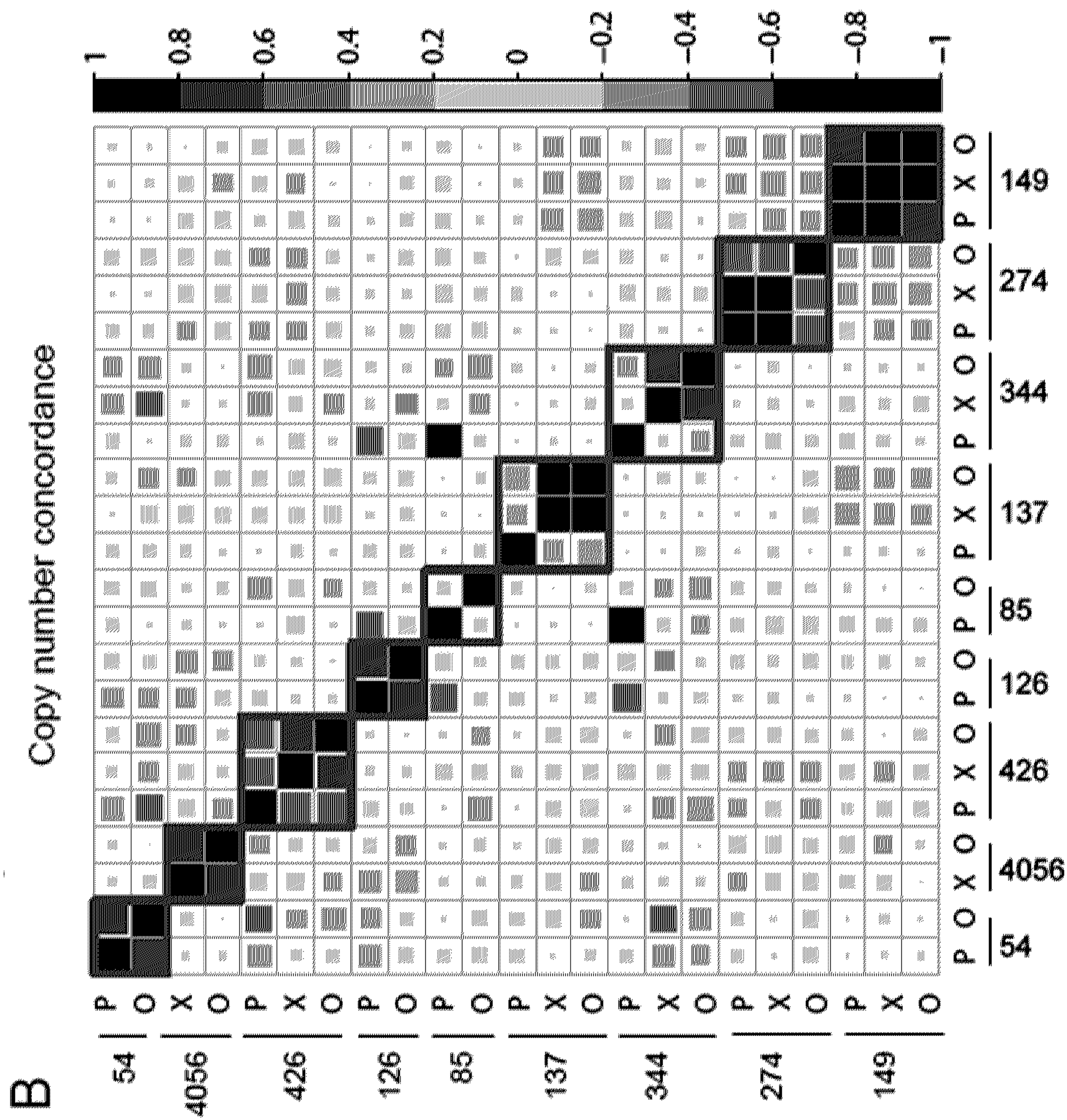


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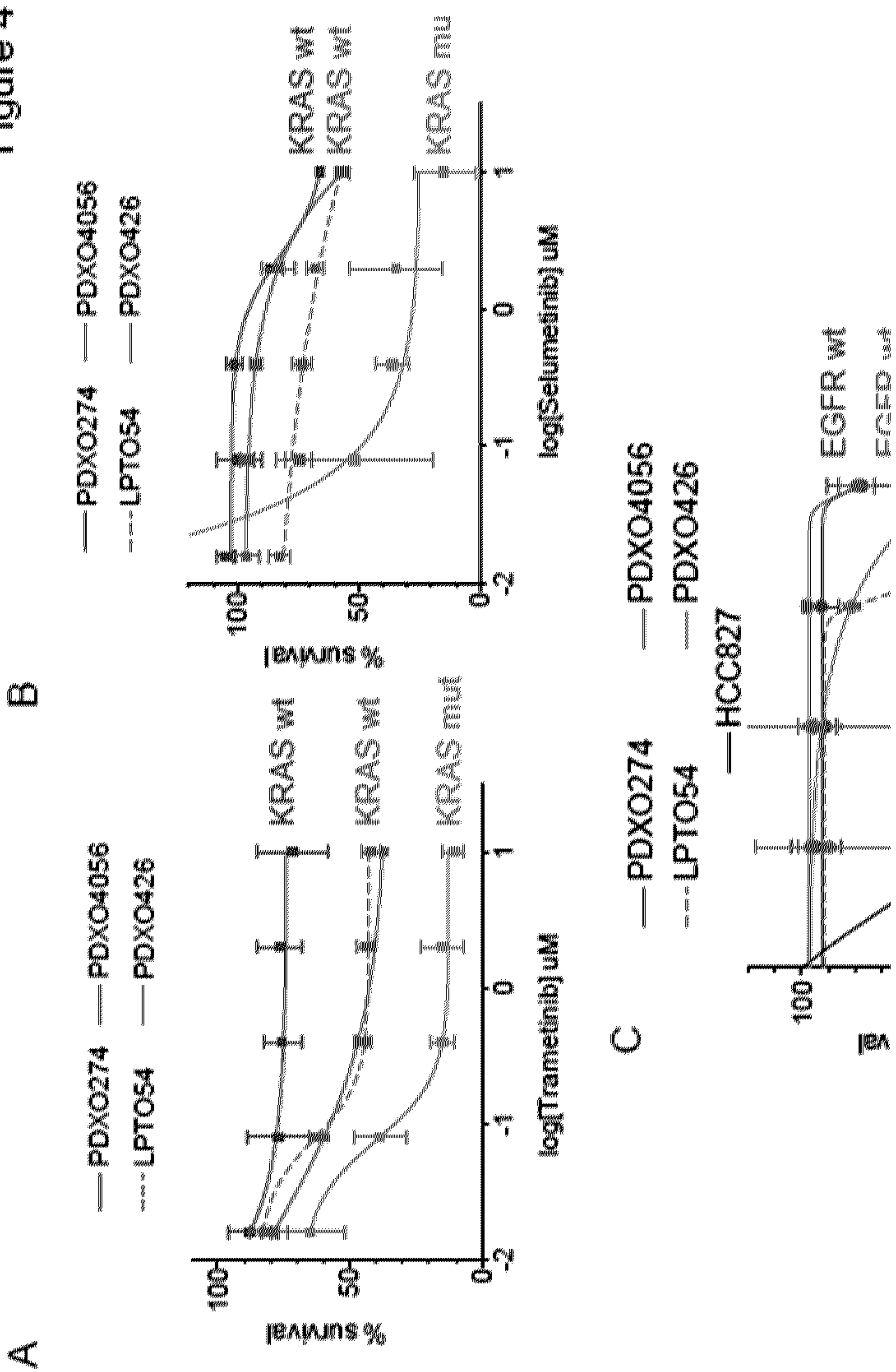


Figure 4

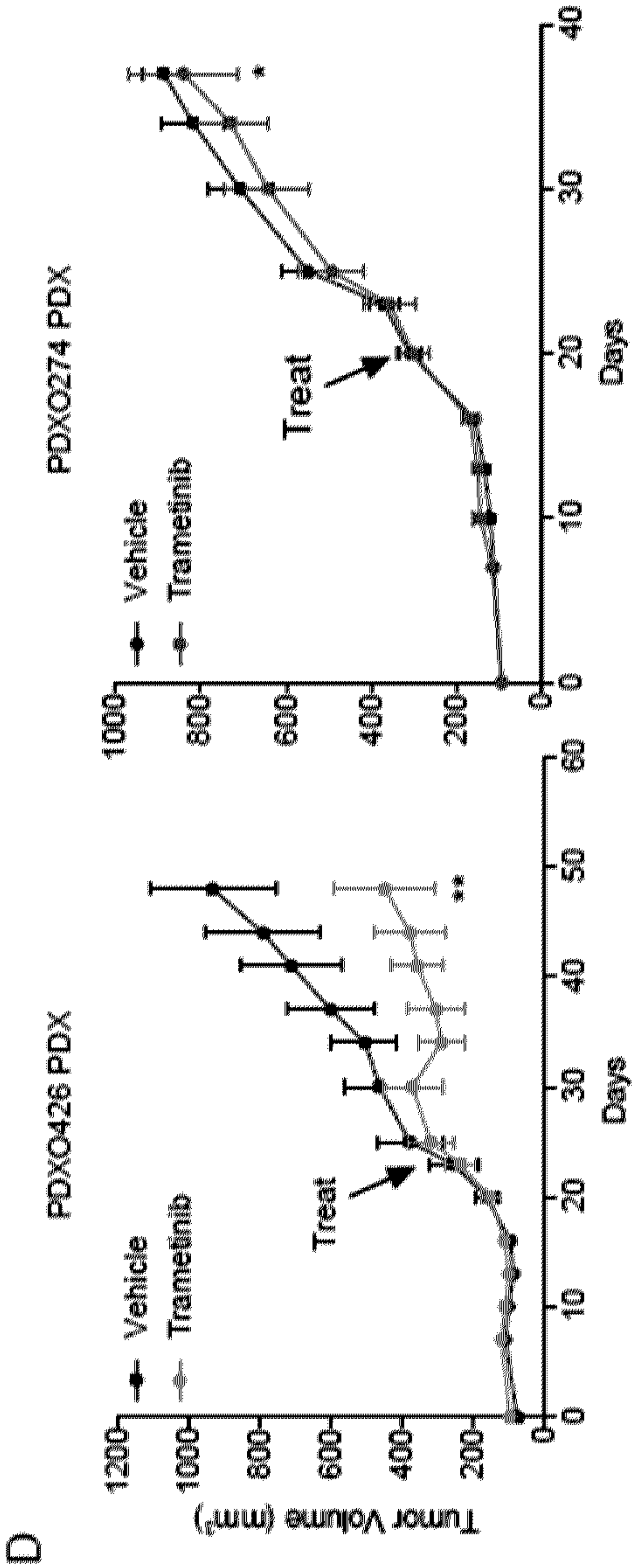


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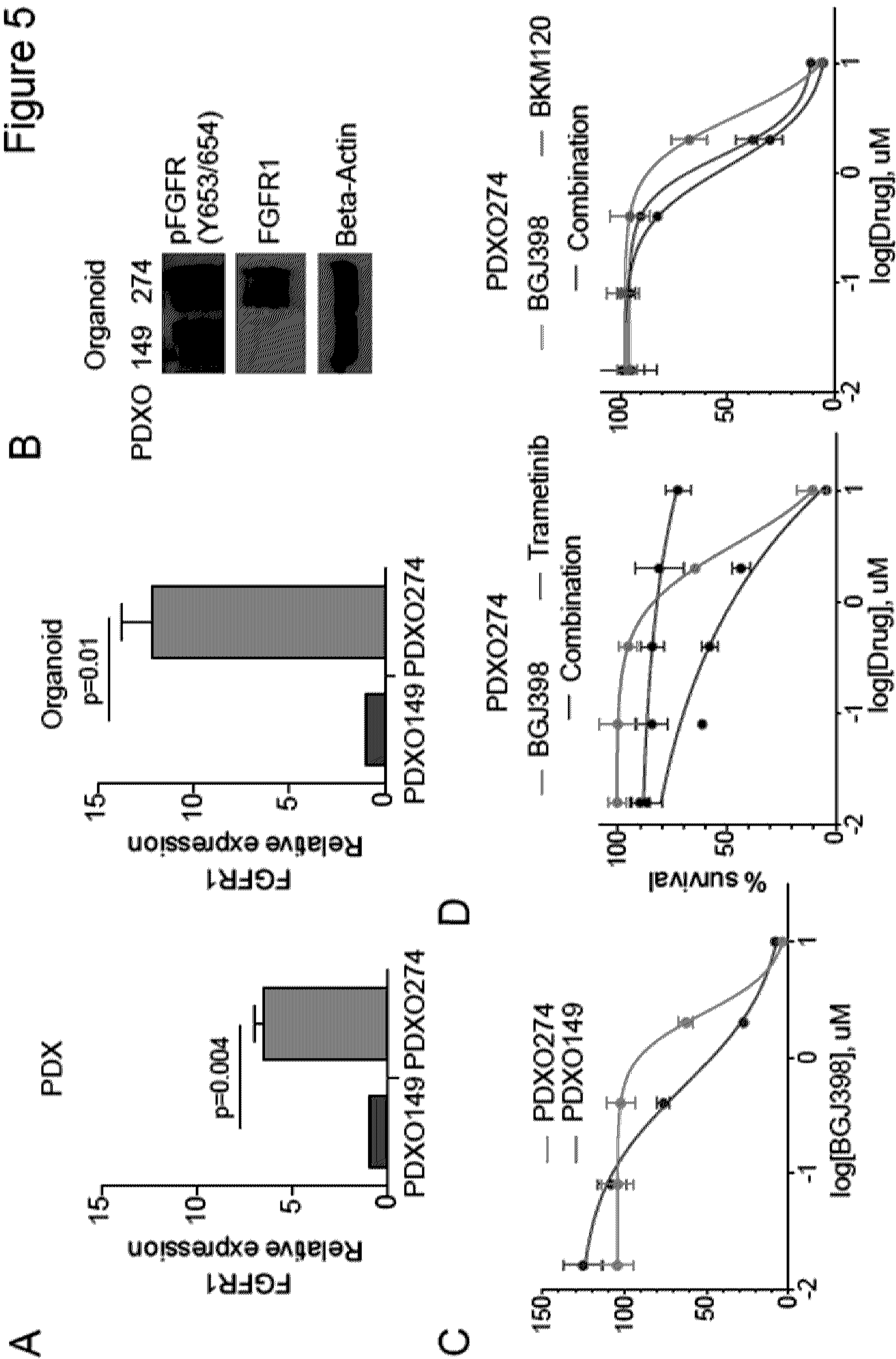


Figure 8

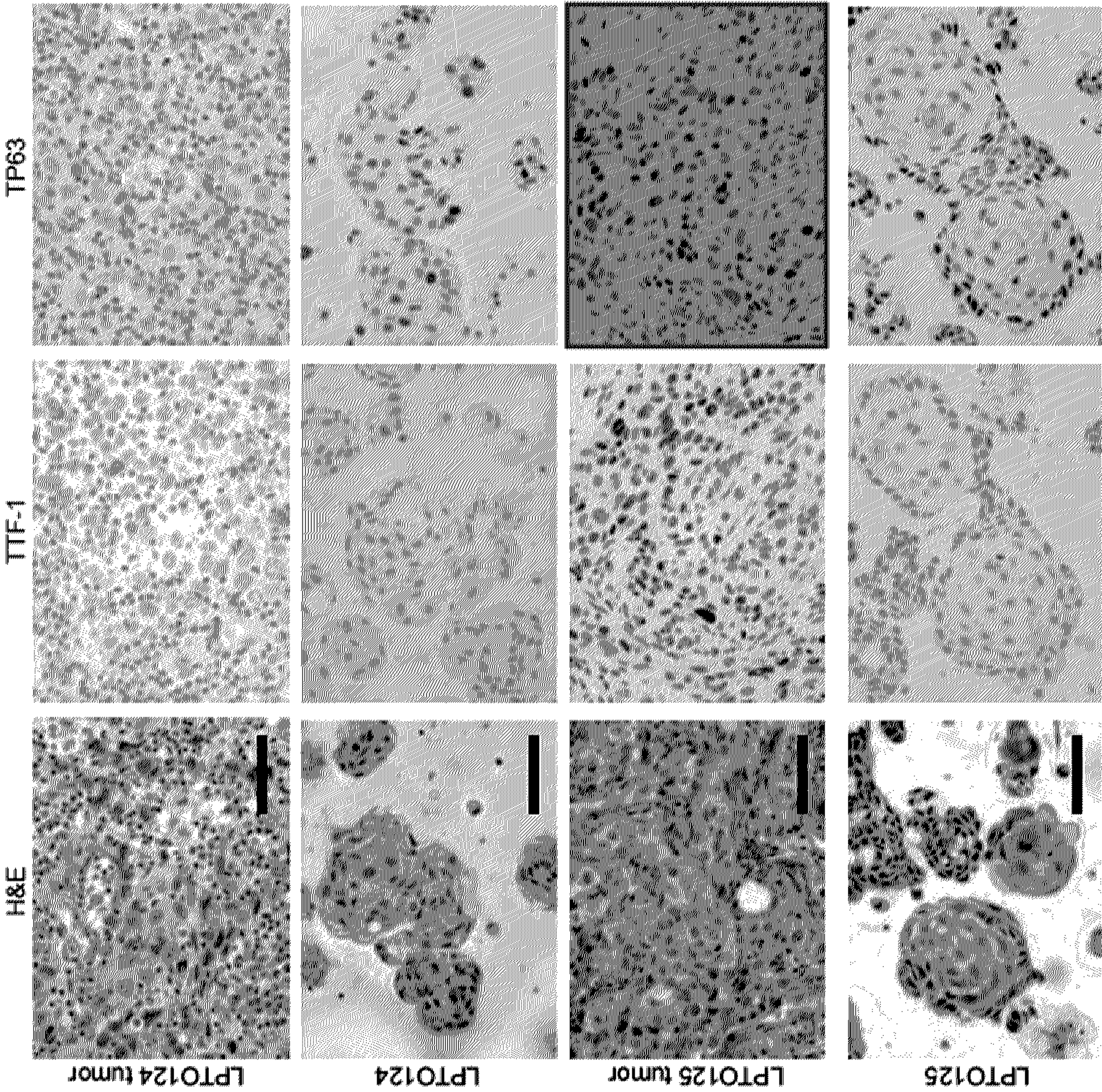


Figure 9

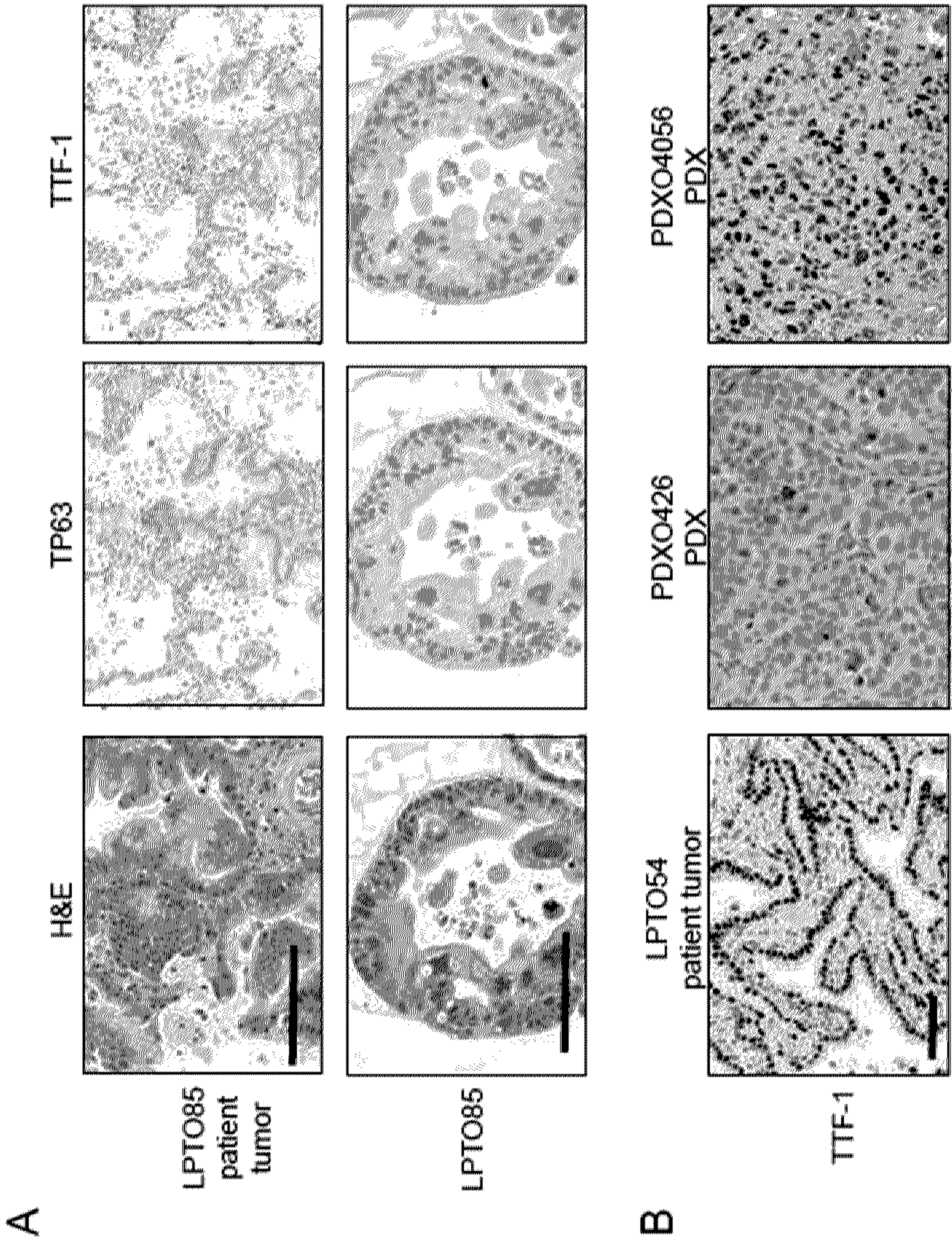


Figure 10

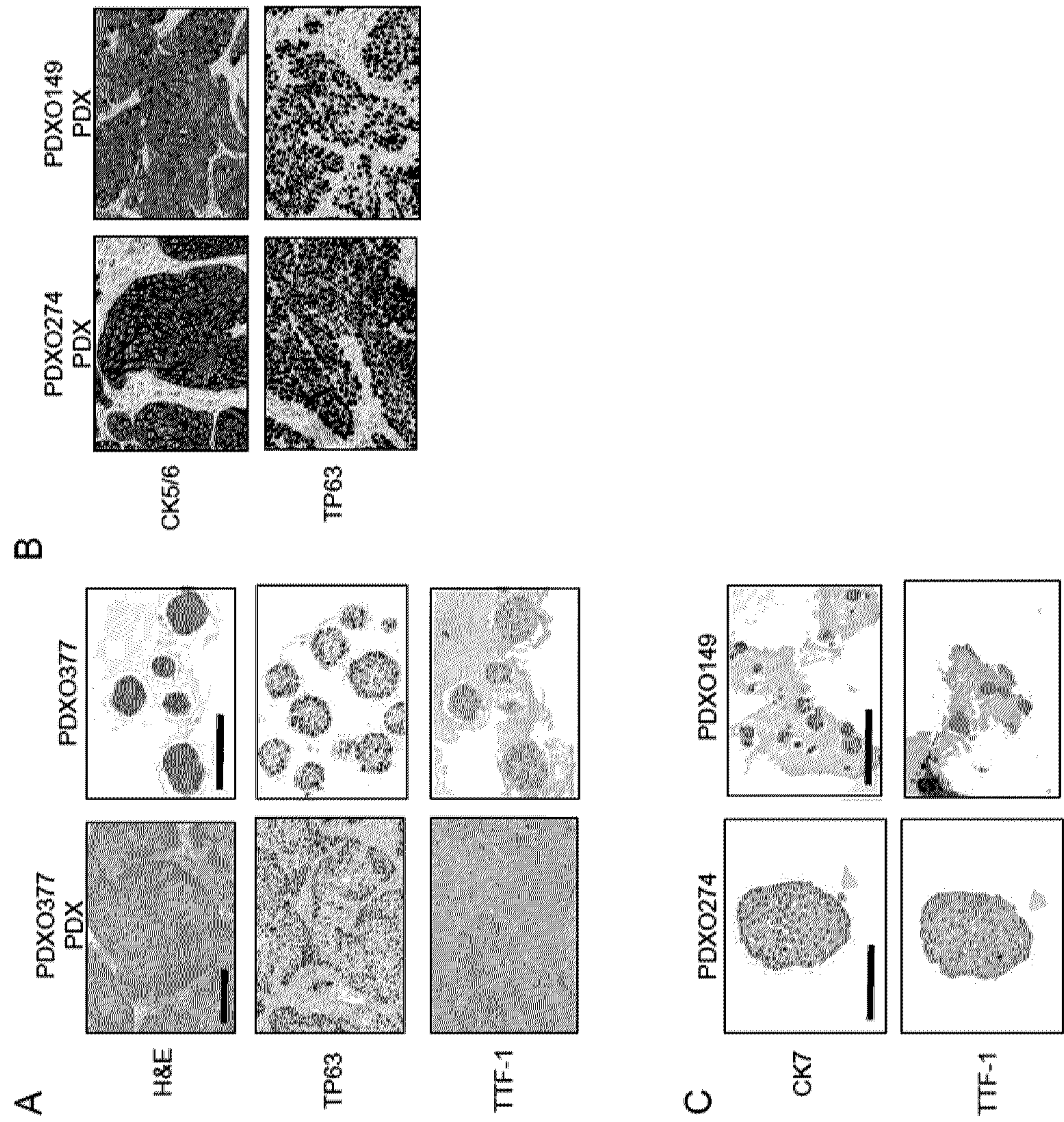


Figure 11

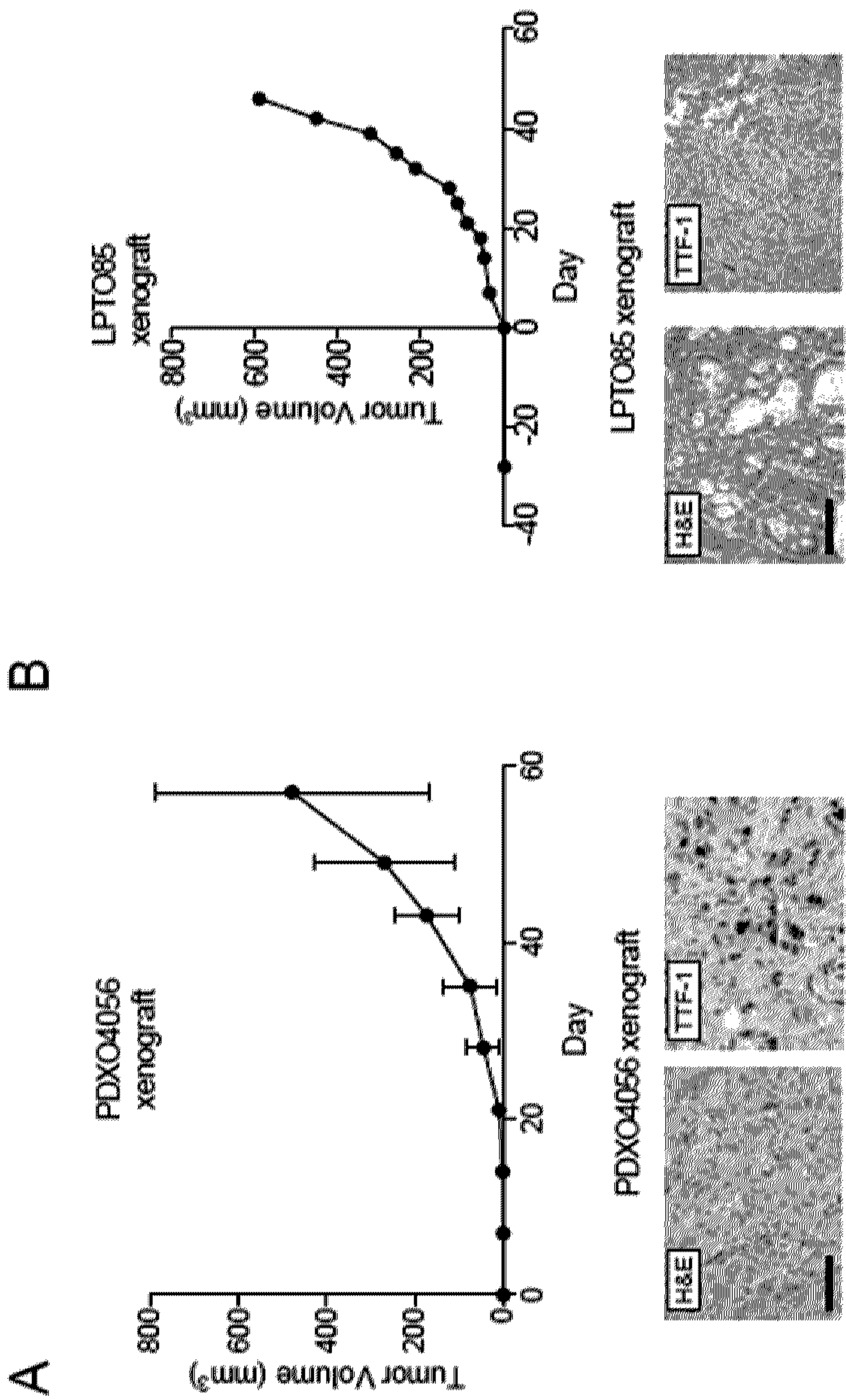


Figure 11

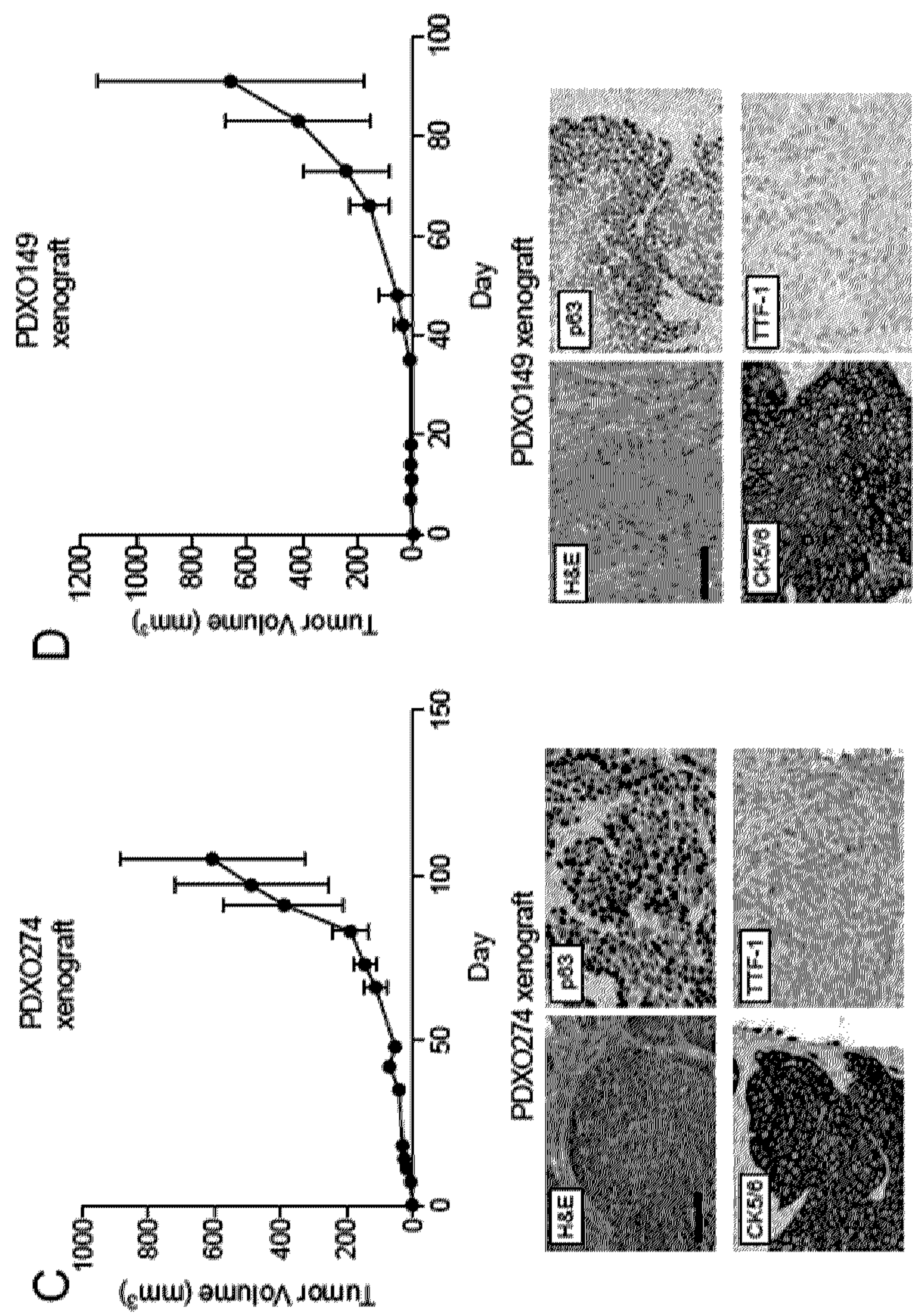
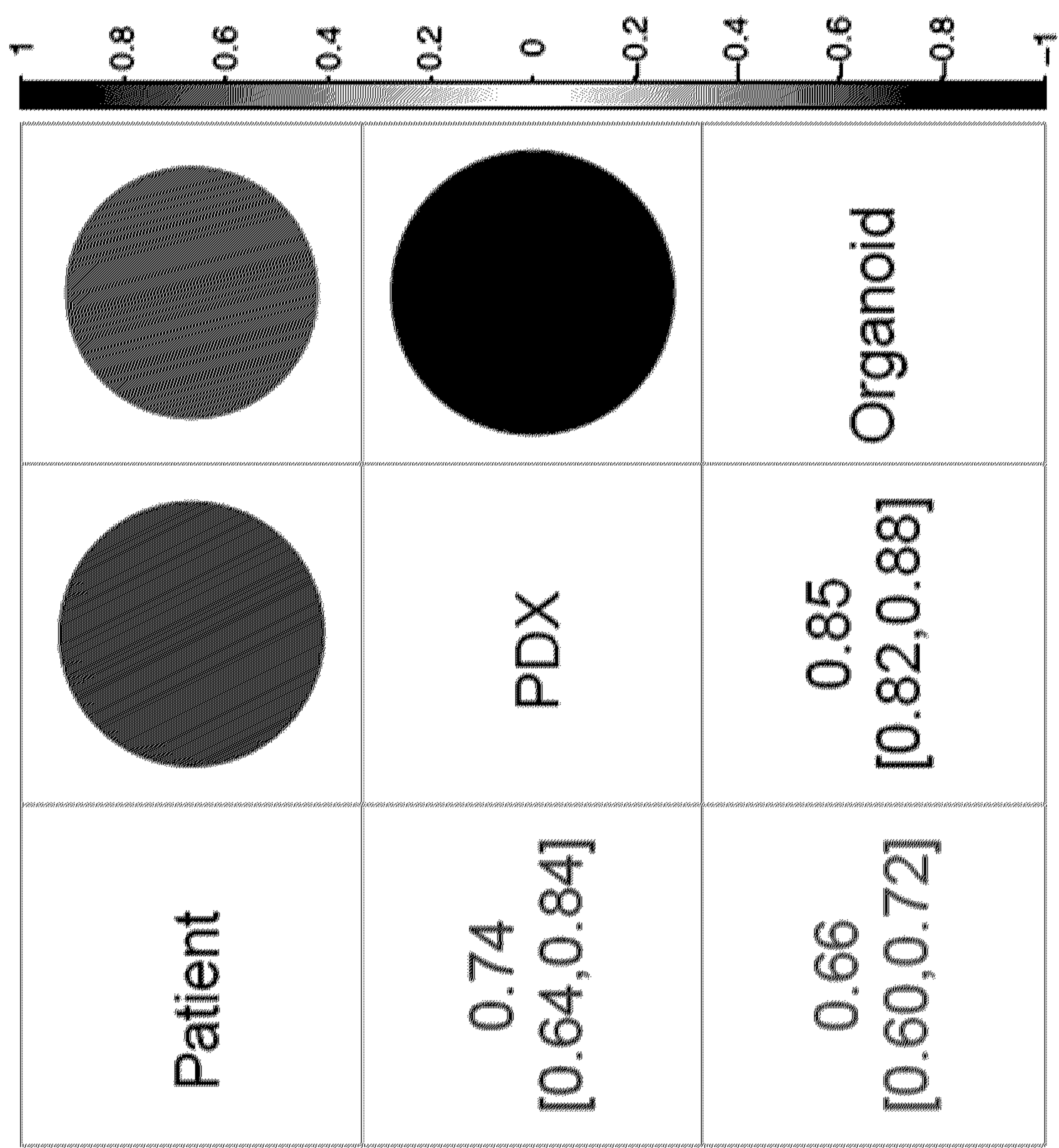


Figure 12



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2020/051472

A. CLASSIFICATION OF SUBJECT MATTER
IPC: *C12N 5/071* (2010.01) , *C12N 5/02* (2006.01) , *C12N 5/07* (2010.01)

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)
IPC: ALL

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

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

Databases: Questel Orbit, Scopus, STNext

Keywords: B27; noggin; Y-27632; FGF10 ORFGF7; antibiotic; basal medium; cell culture; culture media; organoid; Tsao M26; lung cancer; NSCLC OR non-small cell lung cancer ; FGF4; CHIR99021; SAG OR smoothened agonist

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2016/083613 A2 (SACHS, LN. et al.) 2 June 2016 (02-06-2016) *Entire document*	1-11, 13, 15-17 and 20-23
X,D	DIJKSTRA, KK. et al., " <i>Generation of tumor-reactive T cells by co-culture of peripheral blood lymphocytes and tumor organoids</i> ". Cell, 6 September 2018 (06-09-2018), Vol. 174(6), pp. 1586-1598, doi:10.106/j.cell.2018.07.009, ISSN: 1097-4172 *see pages 11-12*	1-11, 15-17 and 20-23
P,X	SHI, R. et al., " <i>Organoid cultures as preclinical models of non-small cell lung cancer</i> ". Clinical Cancer Research, 1 March 2020 (01-03-2020), Vol. 26(5), pp.1162-1174, doi:10.1158/1078-0432.CCR-19-1376, ISSN: 1557-3265 *see page 1163 and Supplementary Materials and Methods*	1-23

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

☒ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date 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) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	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 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 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
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Date of the actual completion of the international search
29 December 2020 (29-12-2020)

Date of mailing of the international search report
05 January 2021 (05-01-2021)

Name and mailing address of the ISA/CA
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2020/051472

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	P, X doc - CN110734894 A (CAO, Z et al.) 31 January 2020 (31-01-2020) *non-official translation*	1-11, 15-17 and 20-23
P, X	P, X doc – CN111575237 A (LUO, H. et al.) 25 August 2020 (25-08-2020) *non-official translation*	1-11, 15-17 and 20-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/CA2020/051472

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2016083613A2	02 June 2016 (02-06-2016)	WO2016083613A3	18 August 2016 (18-08-2016)
		AU2015352351A1	15 June 2017 (15-06-2017)
		CA2968736A1	02 June 2016 (02-06-2016)
		EP3224346A2	04 October 2017 (04-10-2017)
		GB201507834D0	17 June 2015 (17-06-2015)
		GB2532814A	01 June 2016 (01-06-2016)
		GB201421092D0	14 January 2015 (14-01-2015)
		HK1245323A1	24 August 2018 (24-08-2018)
		JP2018503360A	08 February 2018 (08-02-2018)
		US2017275592A1	28 September 2017 (28-09-2017)
CN110734894A	31 January 2020 (31-01-2020)	None	
CN111575237A	25 August 2020 (25-08-2020)	None	