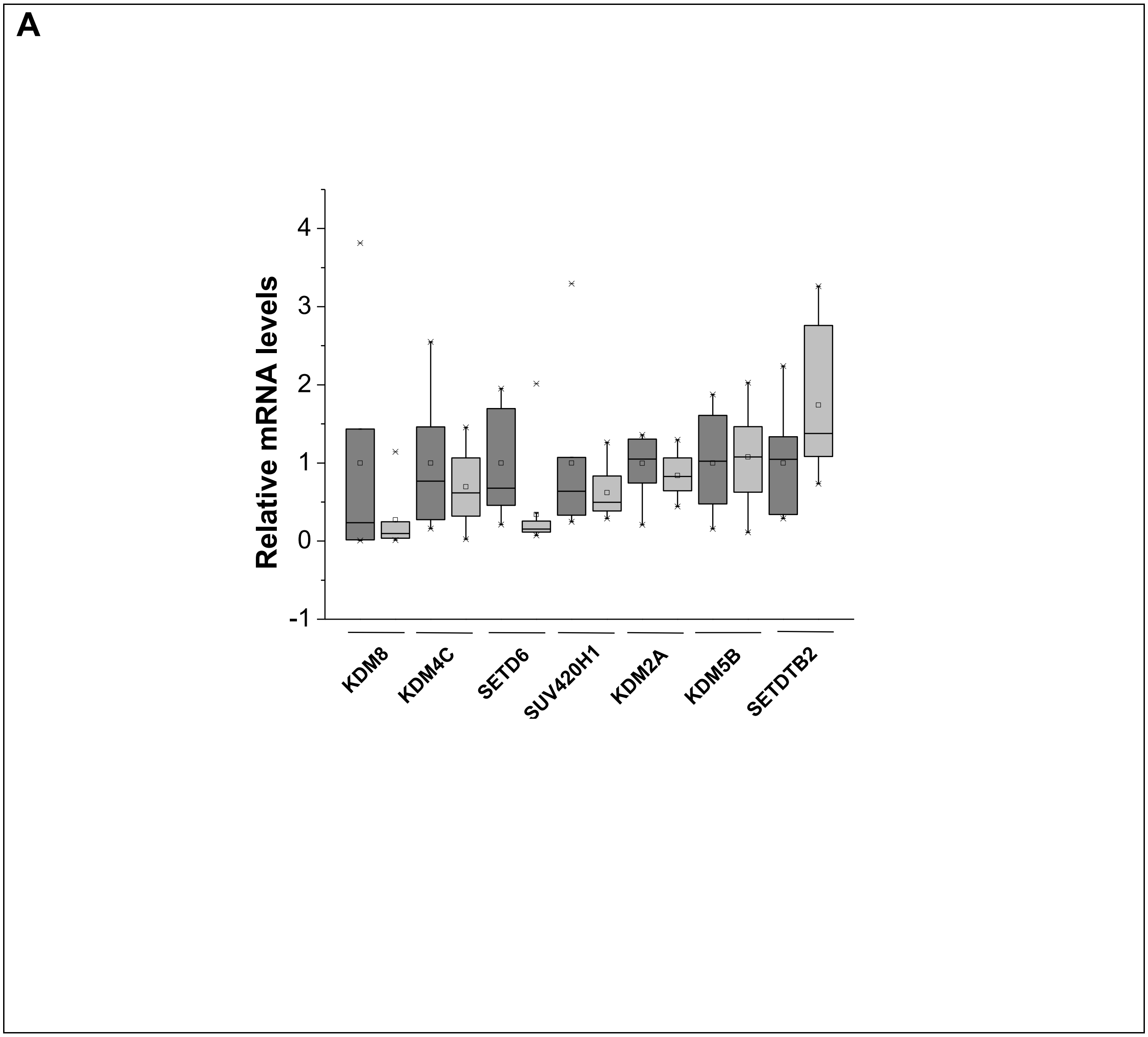
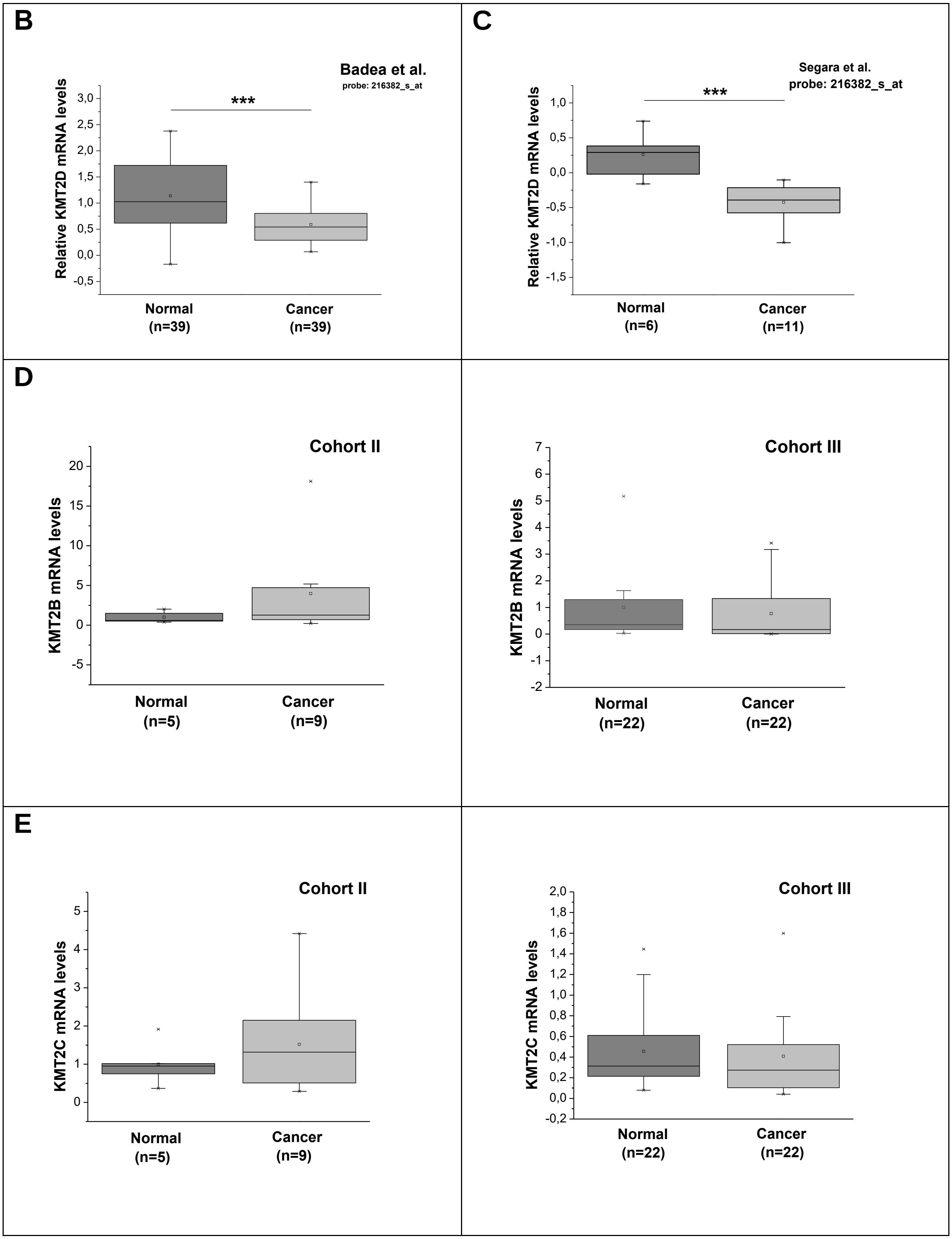
SUPPLEMENTARY INFORMATION

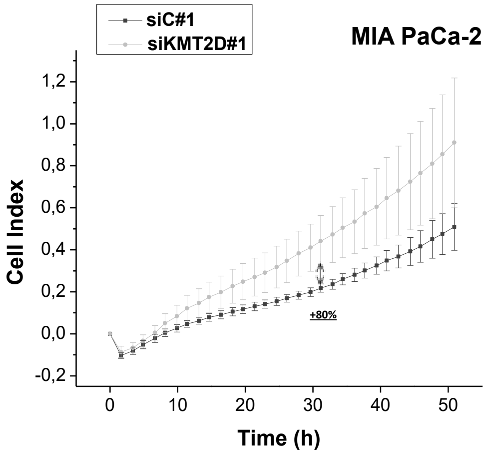
This appendix includes 16 Figures, 14 Tables, Supplementary Materials and Methods and Supplementary References.

1. SUPPLEMENTARY FIGURES

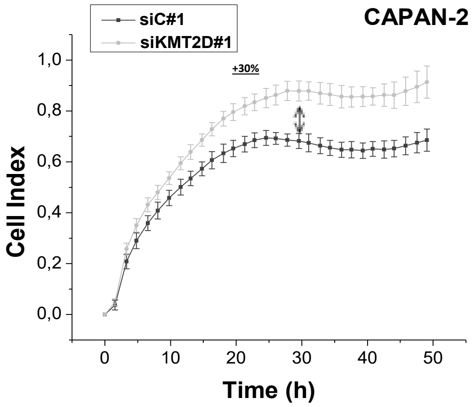



B
Badea et al.
probe: 216382_s_at
Relative KMT2D mRNA levels
3,0
2,5
2,0
1,5
1,0
0,5
0,0
-0,5
***
Normal (n=39)
Cancer (n=39)
C
Segara et al.
probe: 216382_s_at
Relative KMT2D mRNA levels
1,0
0,5
0,0
-0,5
-1,0
-1,5
***
Normal (n=6)
Cancer (n=11)
D
Cohort II
KMT2B mRNA levels
20
15
10
5
0
-5
Normal (n=5)
Cancer (n=9)
Cohort III
KMT2B mRNA levels
7
6
5
4
3
2
1
0
-1
-2
Normal (n=22)
Cancer (n=22)
E
Cohort II
KMT2C mRNA levels
5
4
3
2
1
0
Normal (n=5)
Cancer (n=9)
Cohort III
KMT2C mRNA levels
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
Normal (n=22)
Cancer (n=22)

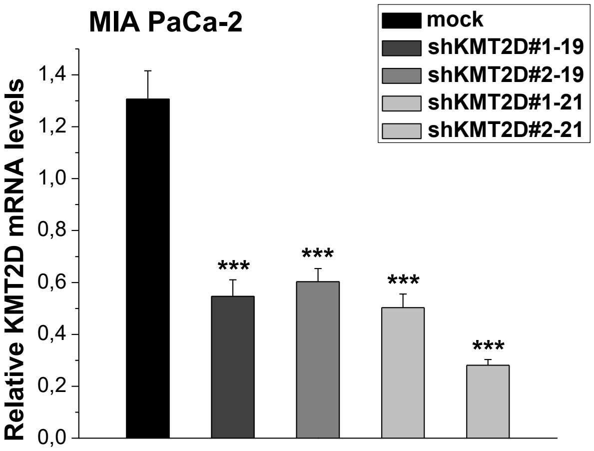
F


siC#1
siKMT2D#1
MIA PaCa-2
Cell Index
1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
+80%
0
10
20
30
40
50
Time (h)

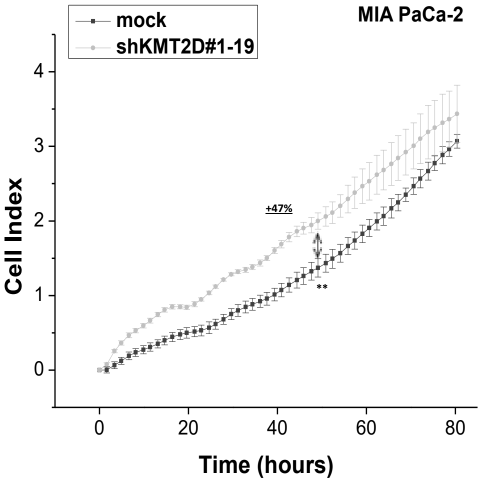
G


siC#1
siKMT2D#1
CAPAN-2
+30%
Cell Index
1,0
0,8
0,6
0,4
0,2
0,0
0
10
20
30
40
50
Time (h)

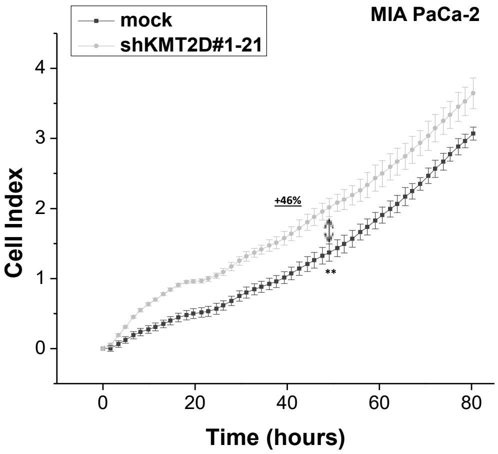
H

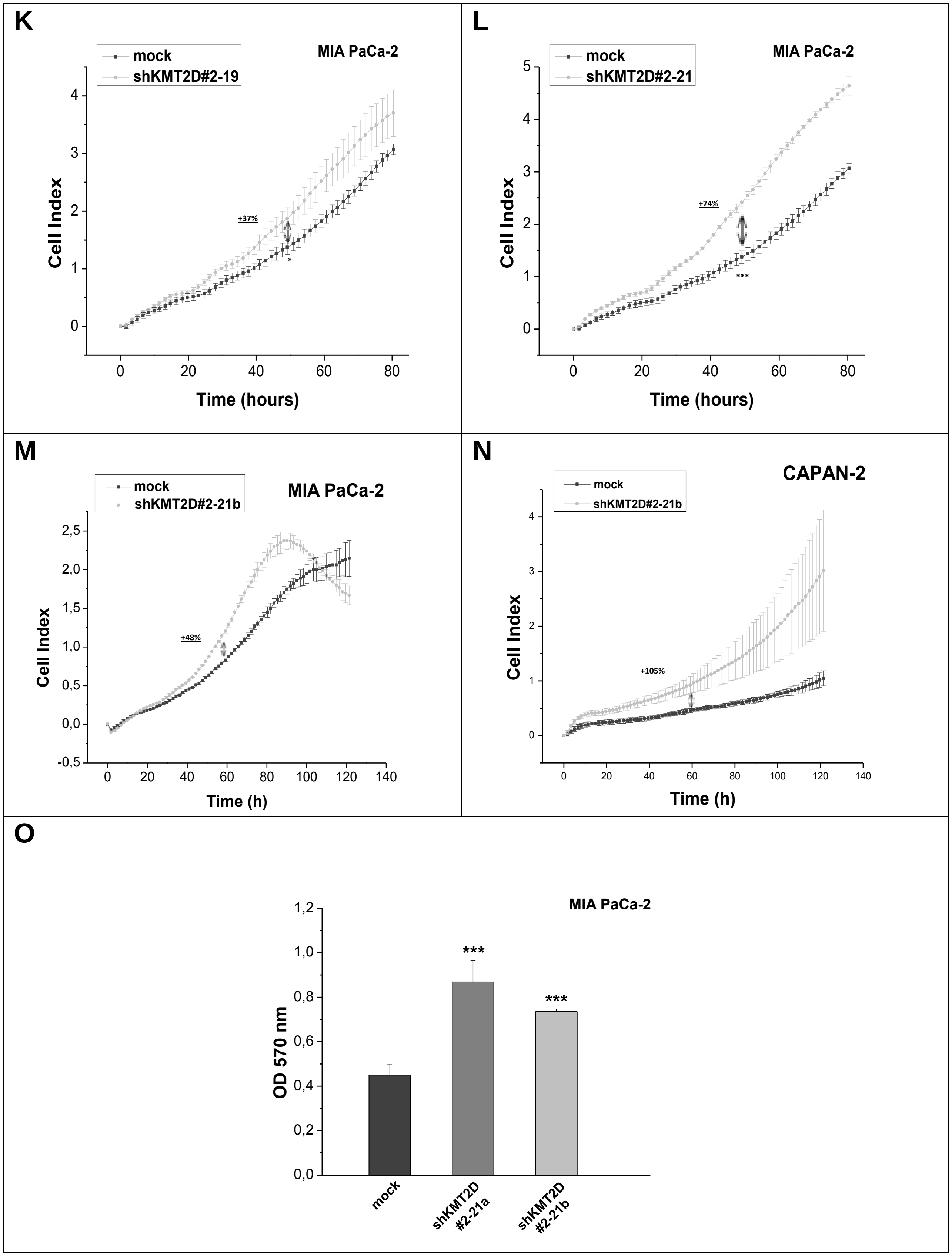

MIA PaCa-2
mock
shKMT2D#1-19
shKMT2D#2-19
shKMT2D#1-21
shKMT2D#2-21
Relative KMT2D mRNA levels
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
***
***
***
***

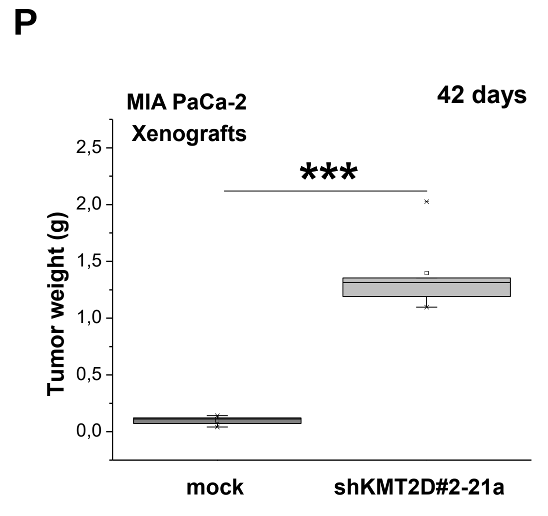
I

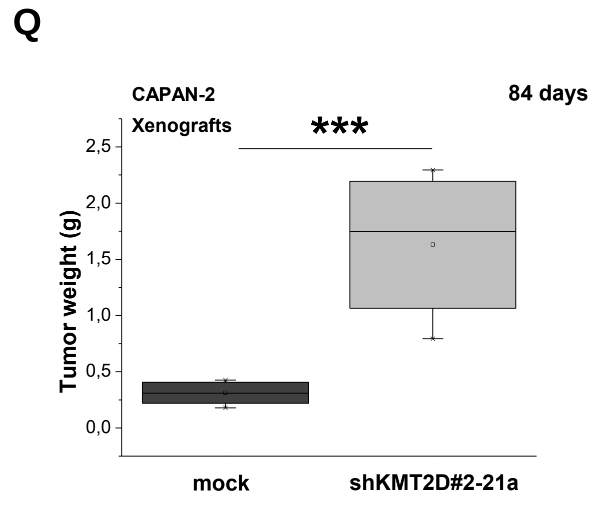

mock
shKMT2D#1-19
MIA PaCa-2
Cell Index
4
3
2
1
0
+47%
**
0
20
40
60
80
Time (hours)

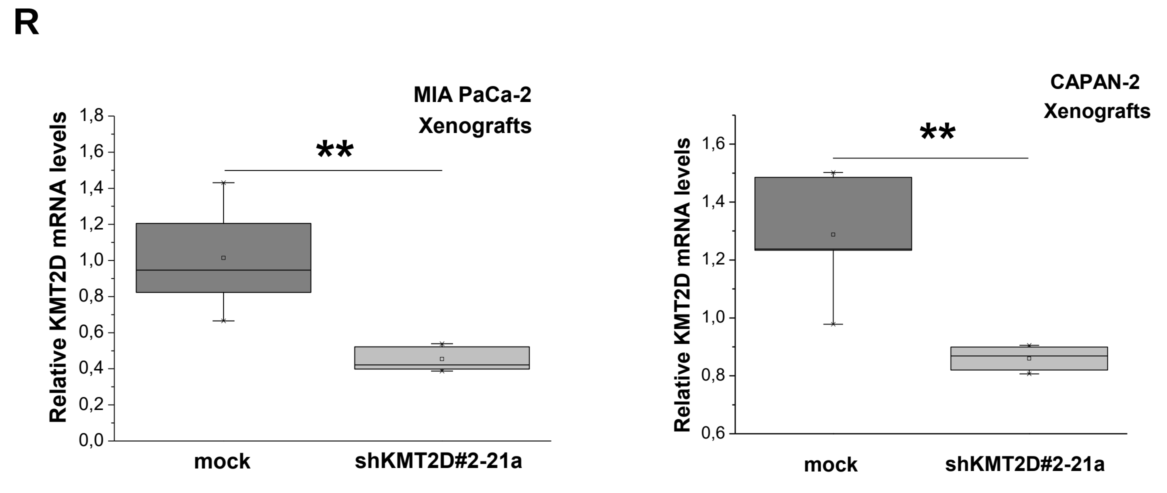
J

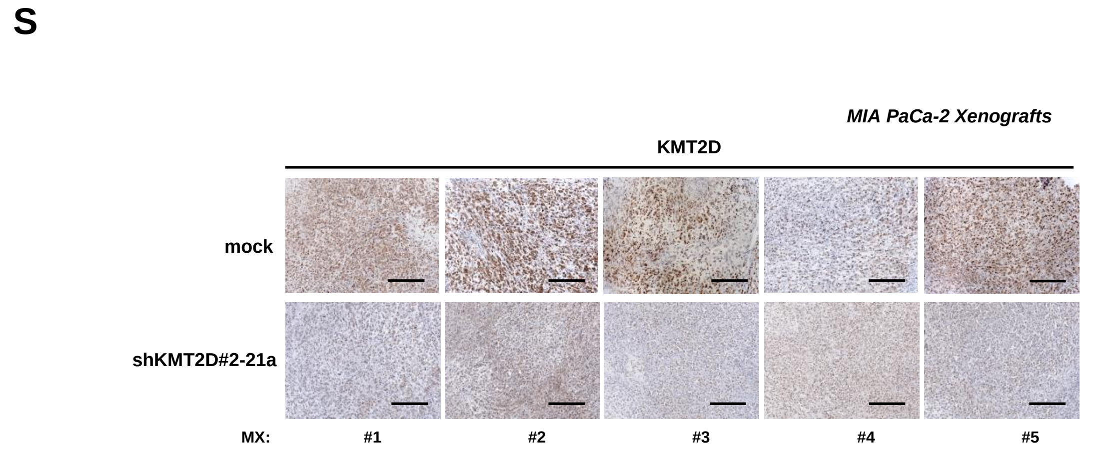

mock
shKMT2D#1-21
MIA PaCa-2
Cell Index
4
3
2
1
0
+46%
**
0
20
40
60
80
Time (hours)

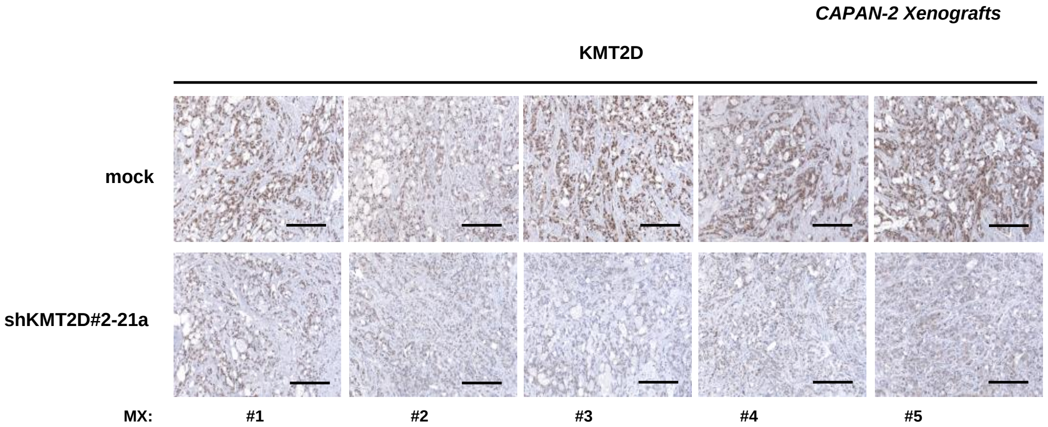

K
mock
shKMT2D#2-19
MIA PaCa-2
Cell Index
+37%
*
Time (hours)
0
1
2
3
4
0
20
40
60
80
L
mock
shKMT2D#2-21
MIA PaCa-2
Cell Index
+74%
***
Time (hours)
0
1
2
3
4
5
0
20
40
60
80
M
mock
shKMT2D#2-21b
MIA PaCa-2
Cell Index
+48%
Time (h)
-0,5
0,0
0,5
1,0
1,5
2,0
2,5
0
20
40
60
80
100
120
140
N
mock
shKMT2D#2-21b
CAPAN-2
Cell Index
+105%
Time (h)
0
1
2
3
4
0
20
40
60
80
100
120
140
O
MIA PaCa-2
OD 570 nm
0,0
0,2
0,4
0,6
0,8
1,0
1,2
***
***
mock
shKMT2D #2-21a
shKMT2D #2-21b


P
MIA PaCa-2
Xenografts
42 days
***
Tumor weight (g)
2,5
2,0
1,5
1,0
0,5
0,0
mock
shKMT2D#2-21a

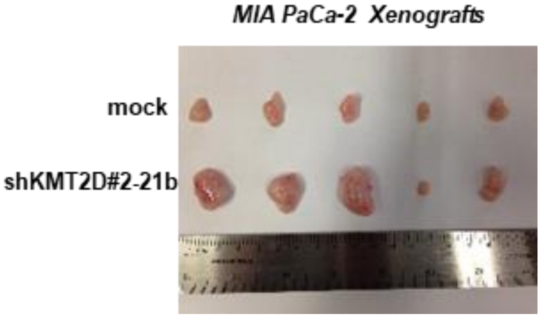

Q
CAPAN-2
Xenografts
84 days
***
Tumor weight (g)
2,5
2,0
1,5
1,0
0,5
0,0
mock
shKMT2D#2-21a

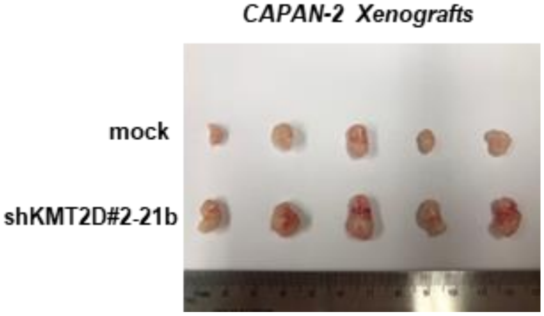

R
MIA PaCa-2
Xenografts
**
Relative KMT2D mRNA levels
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
mock
shKMT2D#2-21a
CAPAN-2
Xenografts
**
Relative KMT2D mRNA levels
1,6
1,4
1,2
1,0
0,8
0,6
mock
shKMT2D#2-21a

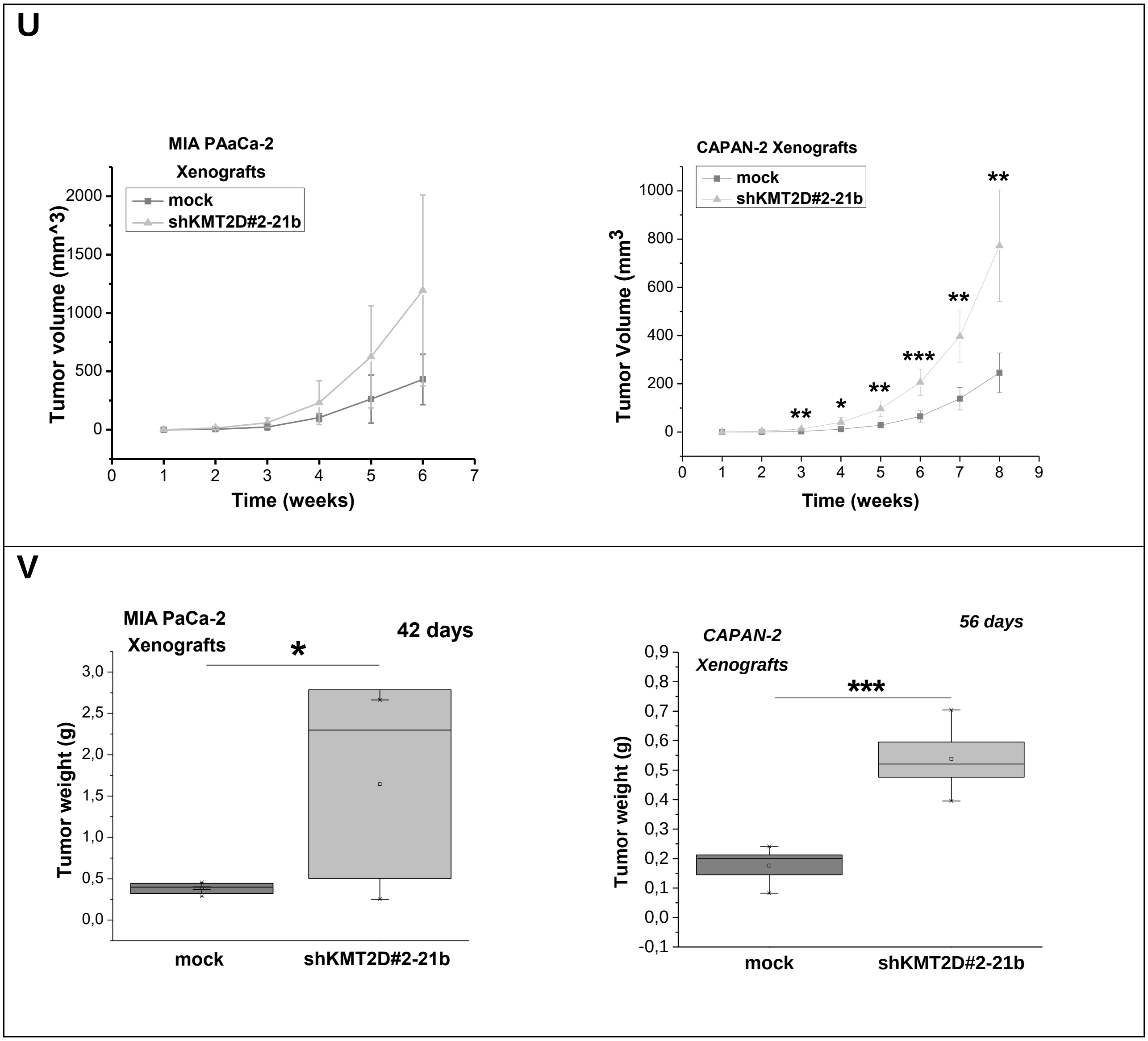

S
MIA PaCa-2 Xenografts
KMT2D
mock
shKMT2D#2-21a
MX:
#1
#2
#3
#4
#5


CAPAN-2 Xenografts
KMT2D
mock
shKMT2D#2-21a
MX:
#1
#2
#3
#4
#5

T


MIA PaCa-2 Xenografts
mock
shKMT2D#2-21b


CAPAN-2 Xenografts
mock
shKMT2D#2-21b

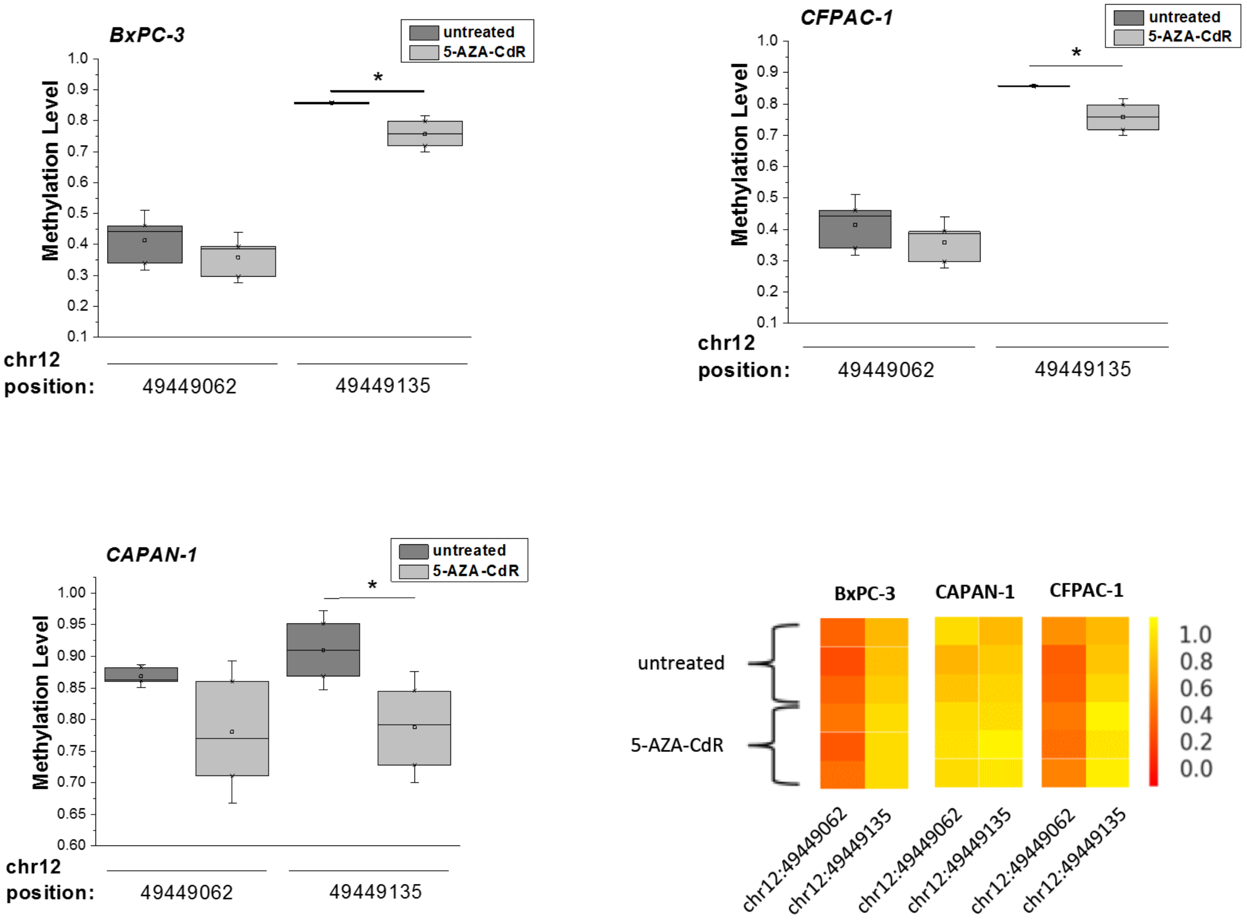


Supplementary Figure S1. Histone methyltransferase KMT2D acts as a tumor suppressor in pancreatic cancer.

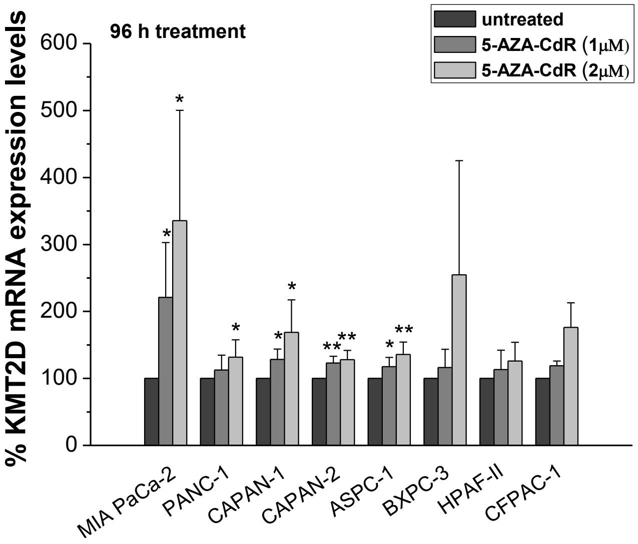
(A) Differential expression of chromatin regulators including *KDM8, KDM4C, SETD6, SUV420H1, KDM2A, KDM5B and SETDB2* in pancreatic carcinoma versus normal tissues originating from Cohort II, as assessed by RT-qPCR. **(B, C)** Differential expression of *Lysine (K)-Specific Methyltransferase 2D (KMT2D)* in pancreatic carcinoma versus normal tissues, based on 2 studies listed in Oncomine database [1 2]. **(D, E)** Relative mRNA levels of KMT2B and KMT2C in Cohorts II and III, as assessed by RT-qPCR. **(F, G)** Effect of transient *KMT2D* suppression by using siKMT2D#1 and the respective scramble control on pancreatic cancer cell proliferation, as assessed by the xCELLigence system. **(H)** Efficiency of *KMT2D* stable depletion by using 4 different shRNAs in MIA PaCa-2 cells, as assessed by RT-qPCR. **(I-L)** Effect of stable *KMT2D* suppression by using 4 different shRNAs on pancreatic cancer cell proliferation, as assessed by the xCELLigence system. **(M, N)** Assessment of the proliferative capacity of shKMT2D#2-21b clonal cell lines versus mock transfected cells. **(O)** Quantification of the colonies formed by shKMT2D#2-21 a and b clonal cell lines versus mock transfected cells. **(P, Q)** Tumor weight graphs of xenografts bearing KMT2D stably suppressed MIA PaCa-2 and CAPAN-2 cells (5 mice/group). *KMT2D* expression in MIA PaCa-2 and CAPAN-2 xenografts from mice injected with mock or shKMT2D#2-21a cells, as assessed by RT-qPCR **(R)** and IHC analysis **(S)**. **(T)** Representative images of the excised tumors and **(U)** tumor volume (mm^3) **(V)** and tumor weight graphs of xenografts from mice injected with mock or shKMT2D#2-21b cells. For establishing shKMT2D#2-21b xenografts, $3.5*10^6$ MIA PaCa-2 and $4.5*10^6$ CAPAN-2 cells were injected subcutaneously in the right flank of NOD-SCID mice (5 mice/group). siC#1, cells transfected with a negative control scramble siRNA; siKMT2D#1, cells transfected with siRNA#1 for KMT2D; mock, cells transfected with shRNA empty vector; shKMT2D#1-19, cells transfected with #1-19 shRNA for KMT2D; shKMT2D#1-21, cells transfected with #1-21 shRNA for KMT2D; shKMT2D#2-19, cells transfected with #2-19 shRNA for KMT2D; shKMT2D#2-21, cells transfected with #2-21 shRNA for KMT2D;

shKMT2D#2-21a or b, cells transfected with #2-21 shRNA for KMT2D that underwent clonal selection resulting in clones a and b; MX, Mouse Xenograft. Statistical analyses were performed using one-way ANOVA. Asterisks denote statistically significant differences, * *P*<.05, ** *P*<.01, *** *P*<.001

A

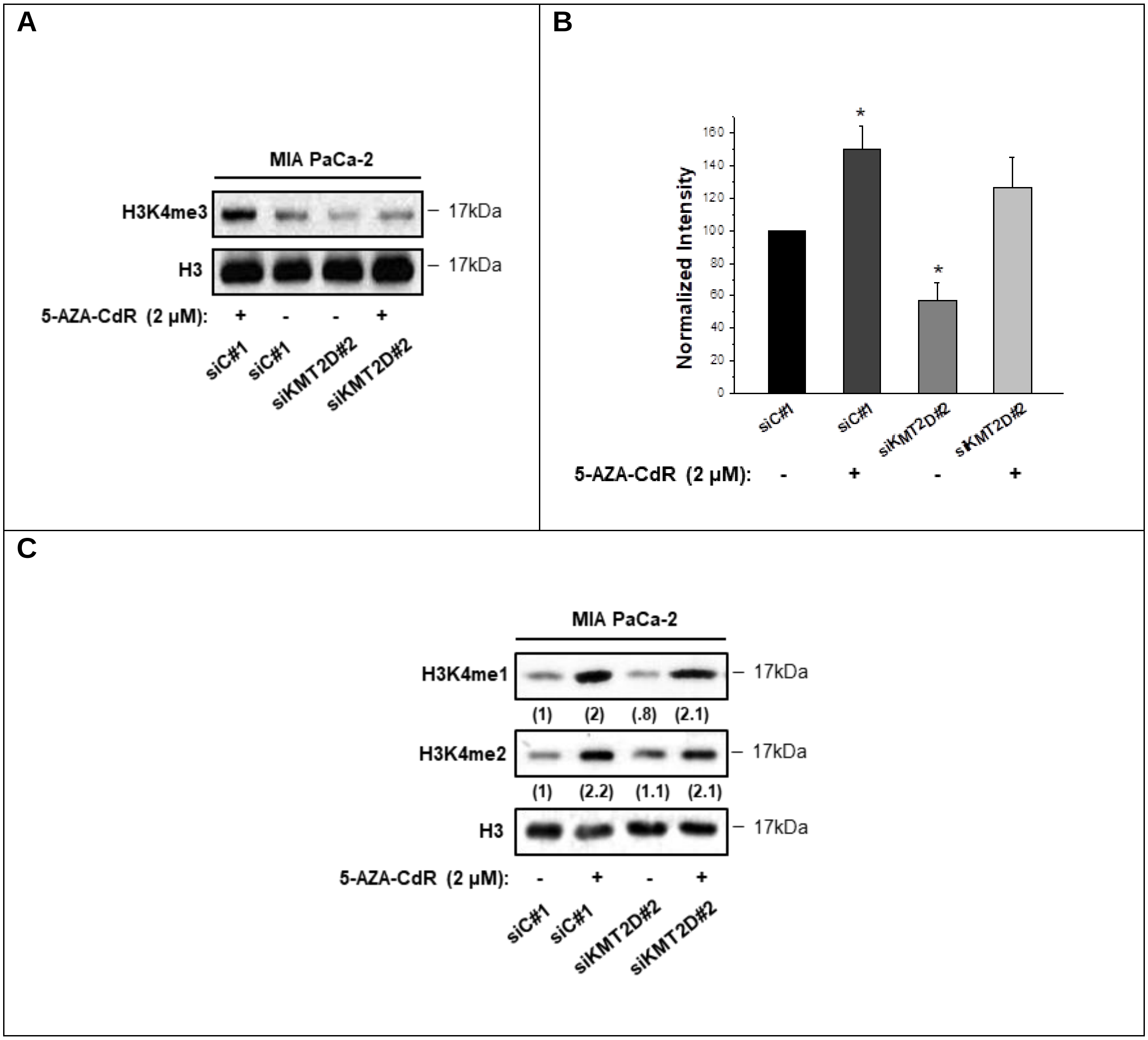


B



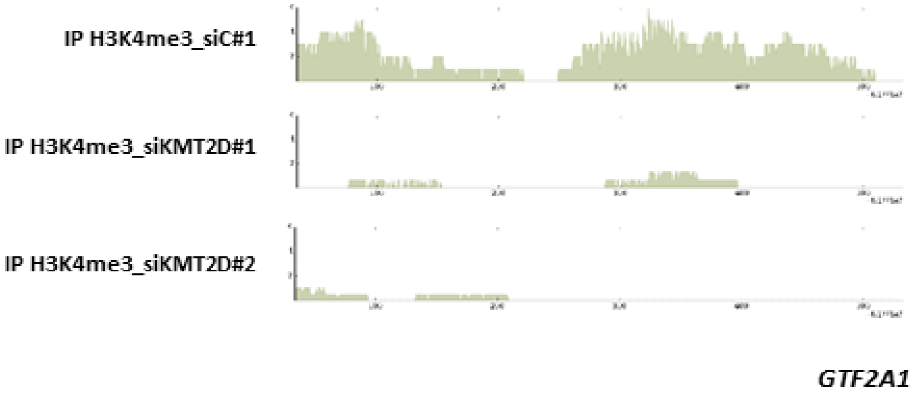
Supplementary Figure S2. Epigenetic regulation of KMT2D levels through DNA methylation of 2 CpG sites.

(A) CpG methylation validation via Targeted Bisulfite Sequencing for the selected region of interest (ROI) (chr12: 49448986-49449286). Quantitative methylation measurements at the single-CpG-site level for untreated or 5-AZA-2'-deoxycytidine (5-AZA-CdR)-treated BxPC-3, CAPAN-1 and CFPAC-1 cells are box plotted or depicted as heatmaps of the methylation ratio. The color indicates the level of methylation from higher to lower in yellow > orange > red order. **(B)** Dose response evaluation of 5-AZA-CdR treatment for 96 h of KMT2D mRNA levels, as assessed by RT-qPCR. Statistical analyses were performed using one-way ANOVA. Asterisks denote statistically significant differences, * *P*<.05, ** *P*<.01

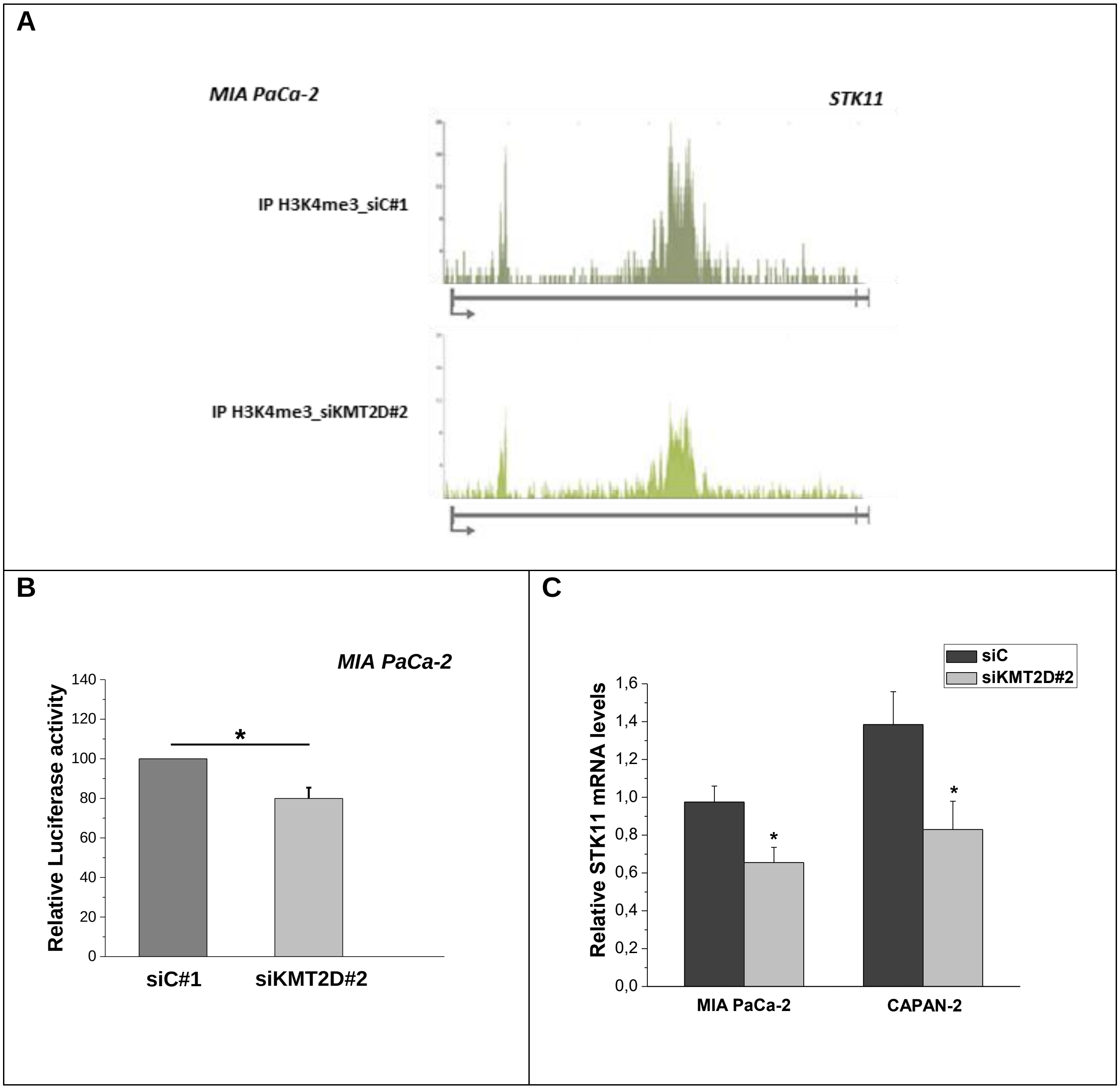


Supplementary Figure S3. Assessment of the global H3K4me3 levels of control or *KMT2D*-silenced cells upon 5-AZA-CdR treatment.

Effect of 5-AZA-CdR treatment for 48 h on the tri-methylated form of histone H3 at lysine 4 (H3K4me3) levels in control or *KMT2D*-silenced cells, as assessed by **(A)** Immunoblot (IB) analyses and **(B)** the quantification of the respective immunoreactive bands of 2 independent experiments. Statistical analyses were performed using one-way ANOVA. Asterisk denotes statistically significant differences, * $P<.05$. **(C)** Numbers in parentheses denote the average-fold change of the ratio H3K4me1:CREB total protein (upper lane) and H3K4me2:CREB (lower lane) of 5-AZA-CdR-treated cells versus non-treated cells (set as default 1). siKMT2D#2, cells transfected with siRNA#2 for KMT2D.



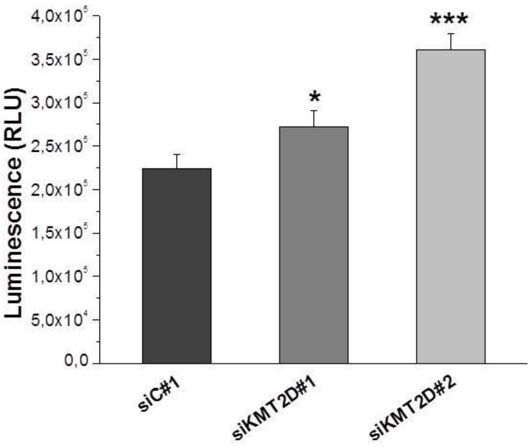
Supplementary Figure S4. ChIP-seq signals of H3K4me3 in *KMT2D*-silenced cells. Effects of KMT2D silencing measured by Chromatin Immunoprecipitation-Sequencing (ChIP-seq) on H3K4me3 occupancy of the *General Transcription Factor IIA Subunit 1 (GTF2A1)* genomic region. The enrichment values were tested with the Mann-Whitney U test ($P<.001$).

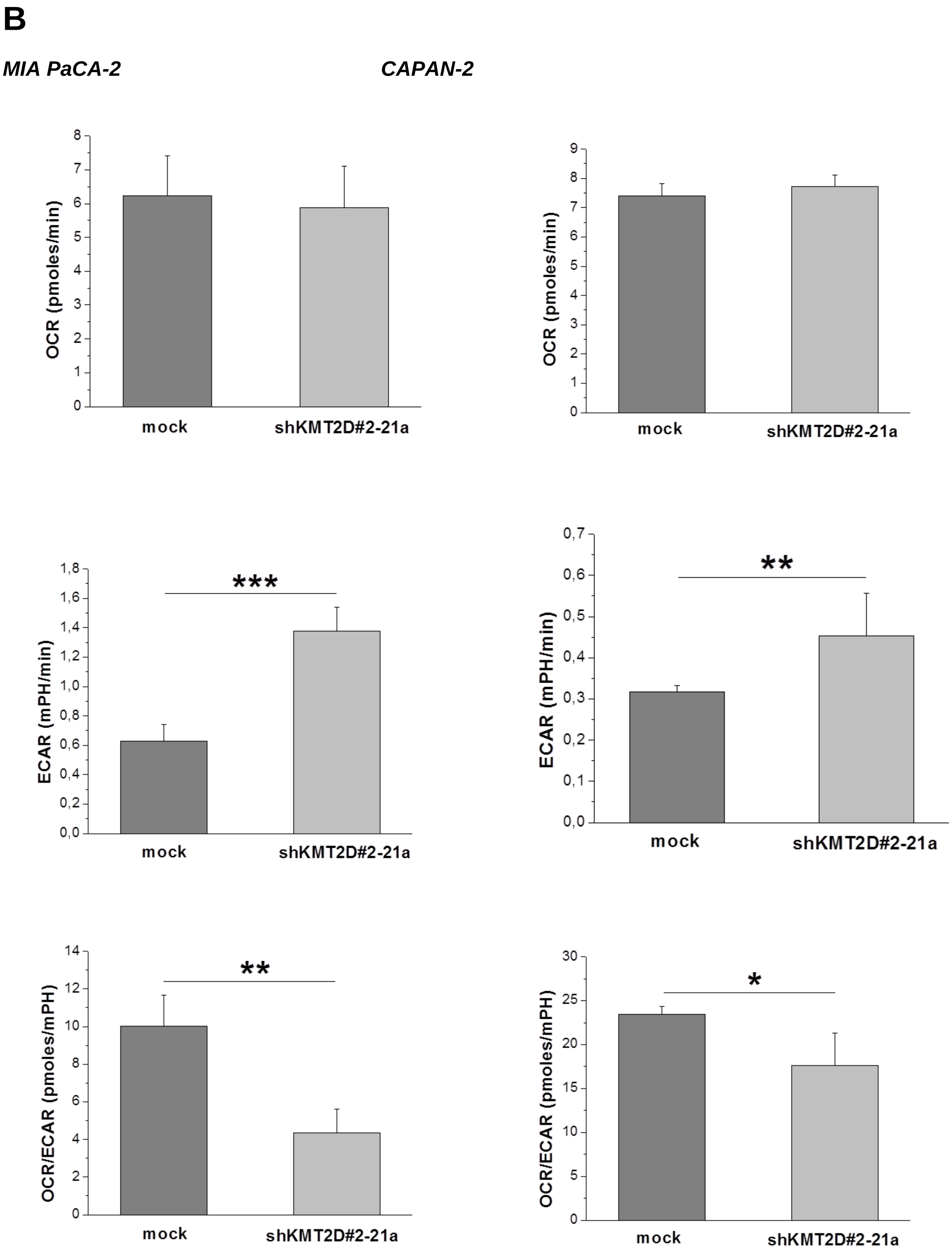


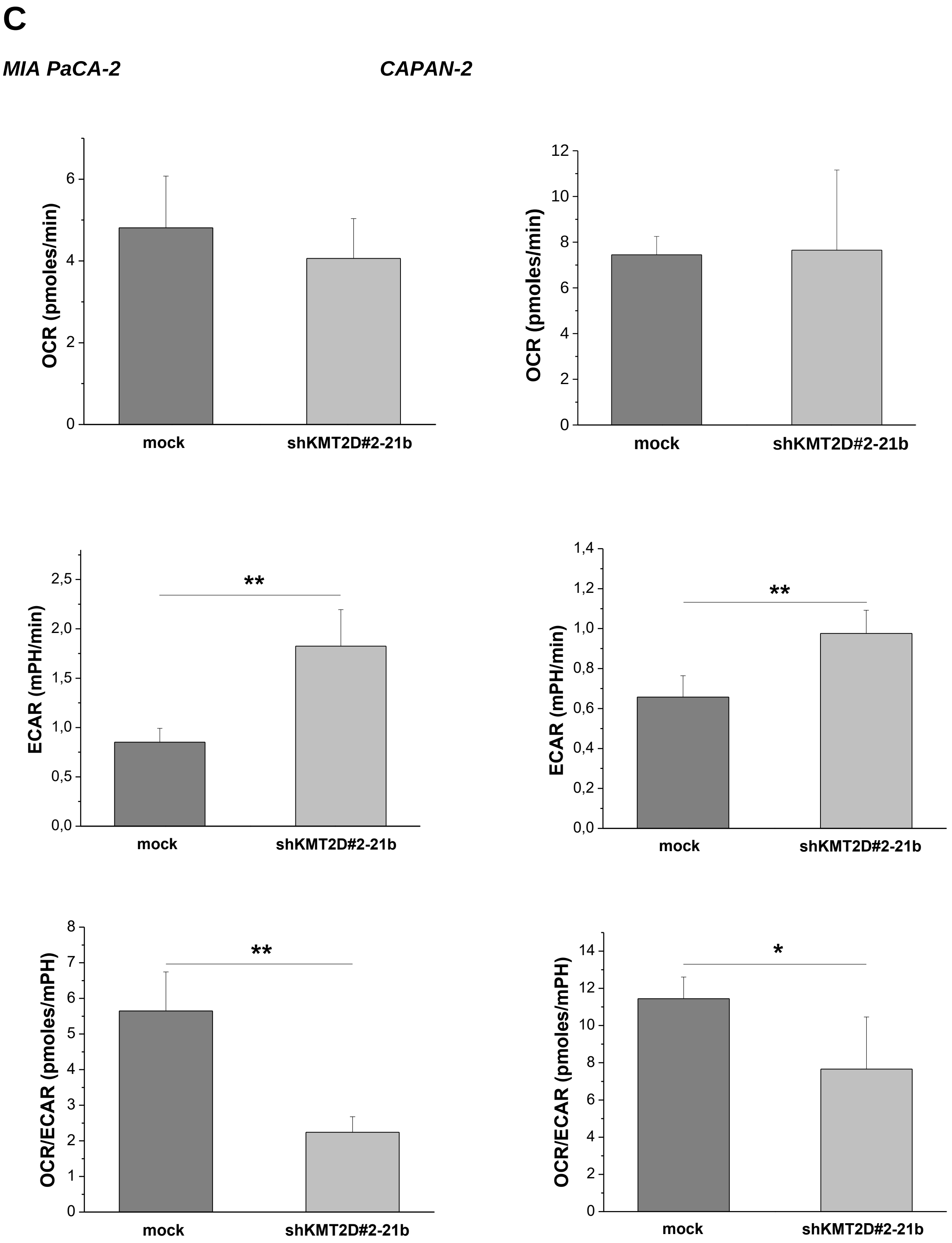
Supplementary Figure S5. Evaluation of *STK11* as a direct KMT2D transcriptional target.

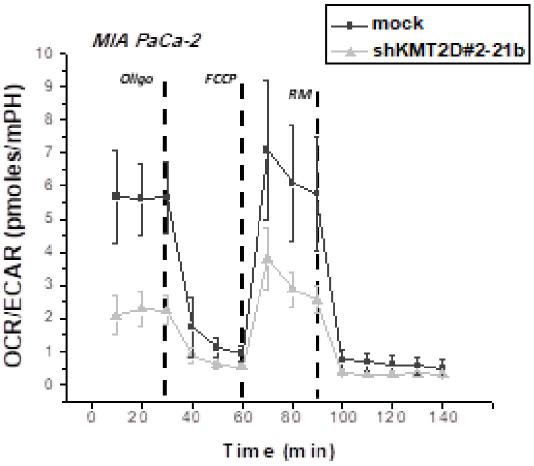
(A) Profiles of H3K4me3 ChIP-seq peaks at the *Serine/Threonine Kinase 11 (STK11)* locus upon *KMT2D* suppression. The x-axes indicate the genomic region. The y-axes represent the fold enrichment of H3K4me3 peaks compared with 2% input control. The enrichment values were tested with the Mann-Whitney U test (*P*<.001). **(B)** Luciferase activity mediated by *STK11* promoter upon KMT2D silencing was evaluated 28 h after transfection of the STK11_pLightSwitch_ Prom Reporter or pLenti CMV Puro LUC vectors in MIA PaCa-2 cells. **(C)** *STK11* mRNA levels in pancreatic cancer cell lines transiently-silenced of KMT2D, as assessed by RT-qPCR. Statistical analyses were performed using one-way ANOVA. Asterisk denotes statistically significant differences, * *P*<.05

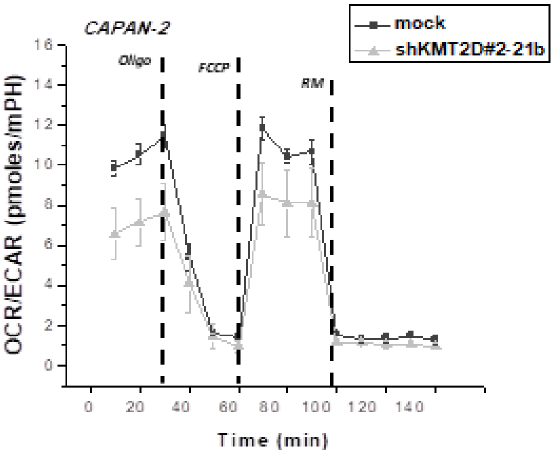
A



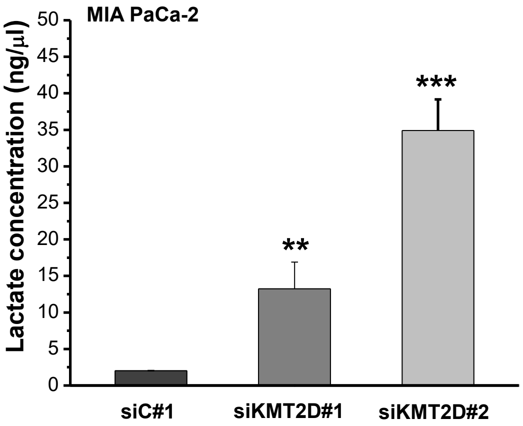

B
MIA PaCA-2
CAPAN-2
OCR (pmoles/min)
0
1
2
3
4
5
6
7
8
mock
shKMT2D#2-21a
OCR (pmoles/min)
0
1
2
3
4
5
6
7
8
9
mock
shKMT2D#2-21a
ECAR (mPH/min)
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
1,6
1,8
***
mock
shKMT2D#2-21a
ECAR (mPH/min)
0,0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
**
mock
shKMT2D#2-21a
OCR/ECAR (pmoles/mPH)
0
2
4
6
8
10
12
14
**
mock
shKMT2D#2-21a
OCR/ECAR (pmoles/mPH)
0
5
10
15
20
25
30
*
mock
shKMT2D#2-21a


C
MIA PaCA-2
CAPAN-2
OCR (pmoles/min)
0
2
4
6
mock
shKMT2D#2-21b
OCR (pmoles/min)
0
2
4
6
8
10
12
mock
shKMT2D#2-21b
ECAR (mPH/min)
0,0
0,5
1,0
1,5
2,0
2,5
**
mock
shKMT2D#2-21b
ECAR (mPH/min)
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
**
mock
shKMT2D#2-21b
OCR/ECAR (pmoles/mPH)
0
1
2
3
4
5
6
7
8
**
mock
shKMT2D#2-21b
OCR/ECAR (pmoles/mPH)
0
2
4
6
8
10
12
14
*
mock
shKMT2D#2-21b

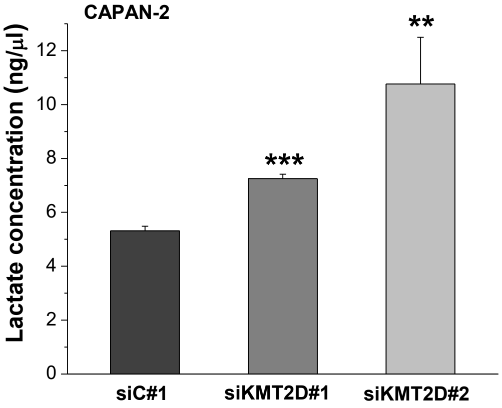

MIA PaCa-2
mock
shKMT2D#2-21b
Oligo
FCCP
RM
OCR/ECAR (pmoles/mPH)
Time (min)
0
1
2
3
4
5
6
7
8
9
10
0
20
40
60
80
100
120
140

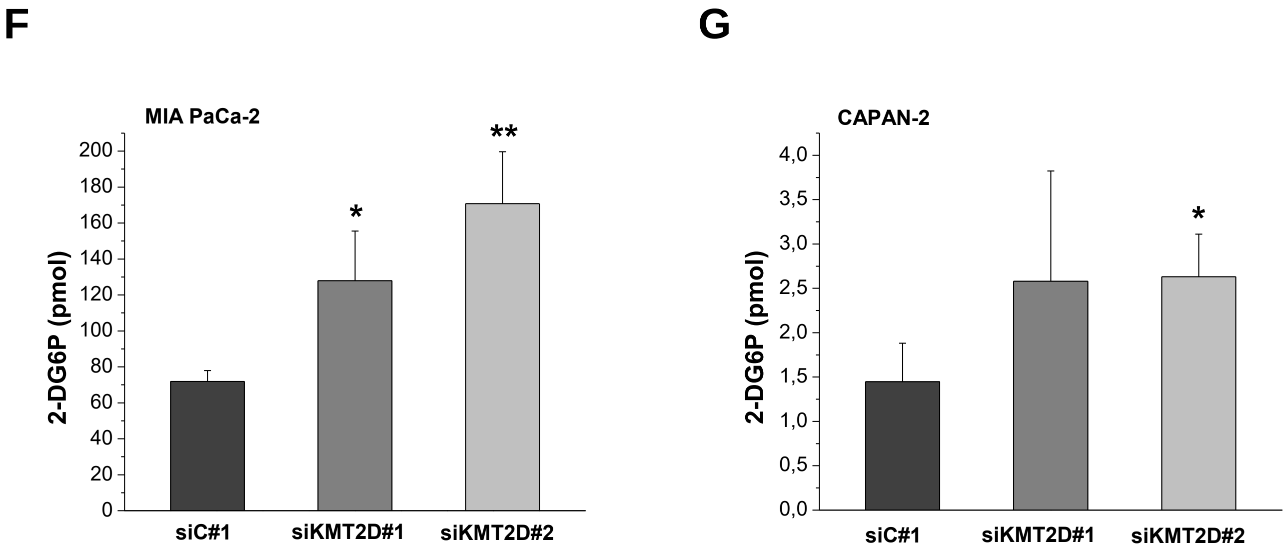

CAPAN-2
mock
shKMT2D#2-21b
Oligo
FCCP
RM
OCR/ECAR (pmoles/mPH)
Time (min)
0
2
4
6
8
10
12
14
16
0
20
40
60
80
100
120
140

D


MIA PaCa-2
Lactate concentration (ng/µl)
0
5
10
15
20
25
30
35
40
45
50
**
***
siC#1
siKMT2D#1
siKMT2D#2

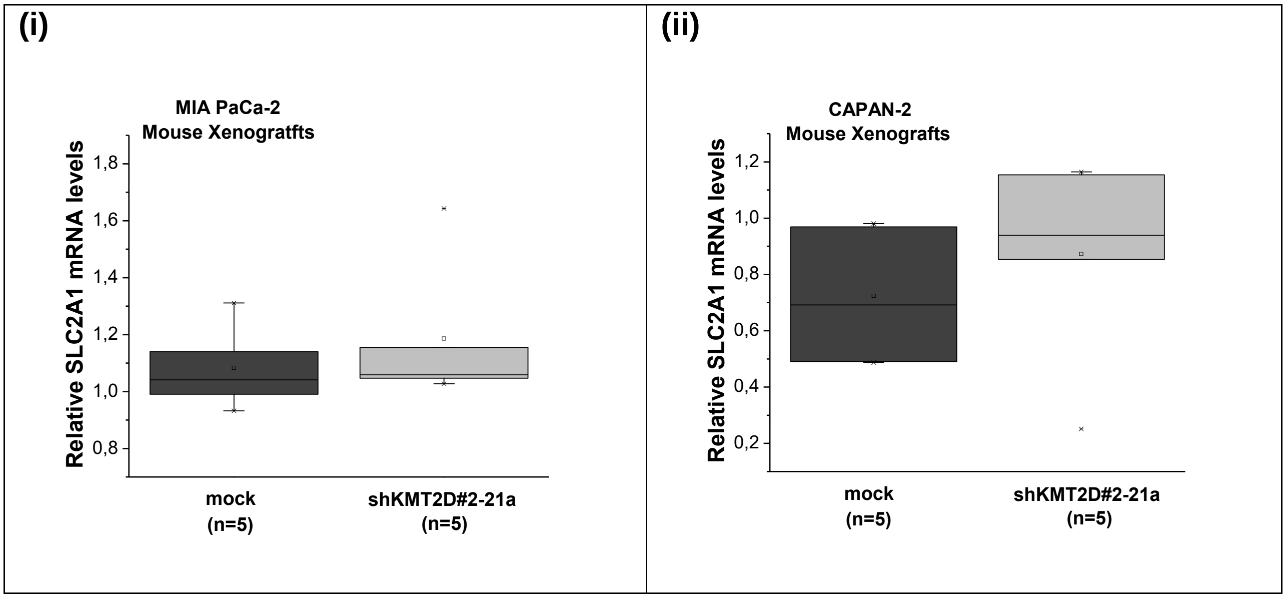
E


CAPAN-2
Lactate concentration (ng/µl)
0
2
4
6
8
10
12
***
**
siC#1
siKMT2D#1
siKMT2D#2



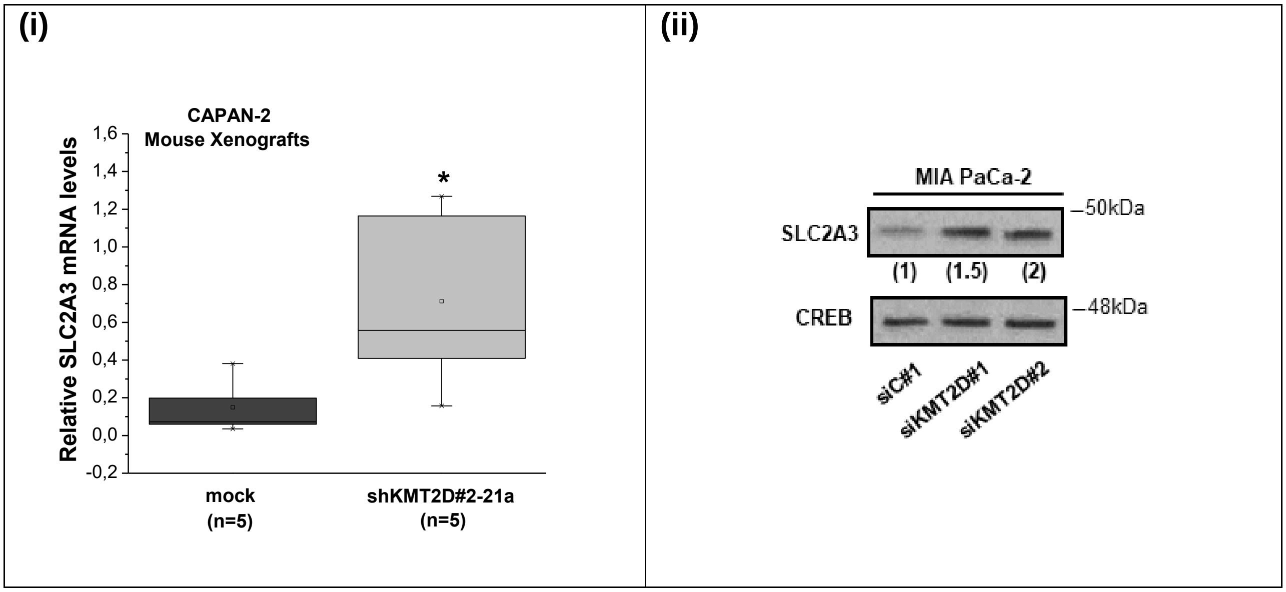
Supplementary Figure S6. KMT2D histone methyltransferase regulates pancreatic cancer cell metabolism.

(A) Endogenous nicotinamide adenine dinucleotide phosphate (NADPH) levels of MIA PaCa-2 cells pretreated with scramble siRNA or 2 different siRNAs against KMT2D, as assessed by the NADP/NADPH-Glo™ bioluminescent Assay. **(B-C)** Effects of *KMT2D* suppression, by using 2 different shKMT2D-stably transfected populations for each cell line on pancreatic cancer cells' bioenergetic profile, as assessed by the XF24-3 Analyzer. Effects of *KMT2D* silencing, by using 2 different siRNAs in **(D and E)** lactate production and **(F and G)** glucose uptake. Average basal OCR and ECAR were further normalized per protein for KMT2D stably-depleted cells. OCR, Oxygen Consumption Rate; ECAR, Extracellular Acidification Rate; 2-DG6P, the 2-deoxyglucose (2-DG) glucose analogue phosphorylated by hexokinase to 2-DG6P. Statistical analyses were performed using one-way ANOVA. Asterisks denote statistically significant differences, * *P*<.05, ** *P*<.01, *** *P*<.001



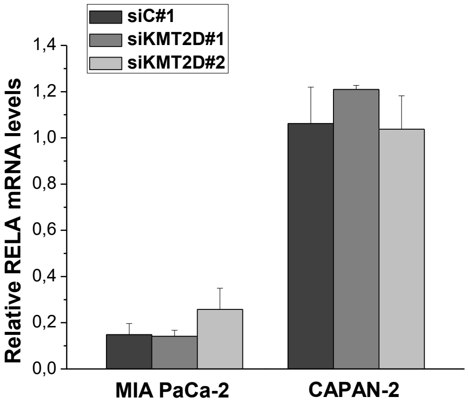
Supplementary Figure S7. Assessment of SLC2A1 mRNA levels in mouse xenografts.

Solute Carrier Family 2 Member 1 (SLC2A1) expression levels in xenografts from mice injected with mock or shKMT2D#2-21a **(i)** MIA PaCa-2 or **(ii)** CAPAN-2 cells, as assessed by RT-qPCR analysis. Statistical analyses were performed using one-way ANOVA.



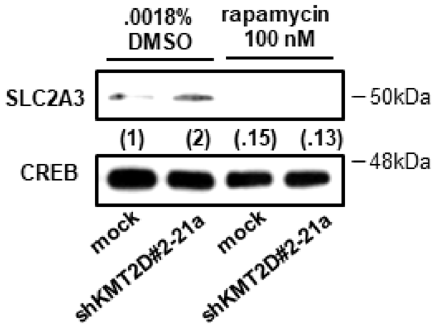
Supplementary Figure S8. Assessment of SLC2A3 mRNA levels and protein levels upon KMT2D genetic manipulation.

(i) *Solute Carrier Family 2 Member 3* (*SLC2A3)* expression levels in xenografts from mice injected with mock or shKMT2D#2-21a CAPAN-2 cells, as assessed by RT-qPCR analysis. **(ii)** Effect of *KMT2D* silencing by using 2 different siRNAs on SLC2A3 protein levels, as assessed by IB analysis. Numbers in parentheses denote the average-fold change of the ratio SLC2A3:CREB total protein of siKMT2D-transiently transfected cells compared with siC#1-treated cells (set as default 1) of 2 independent experiments, as assessed by densitometric analysis of the immunoreactive bands. Statistical analyses were performed using one-way ANOVA. Asterisk denotes statistically significant differences, * *P*<.05



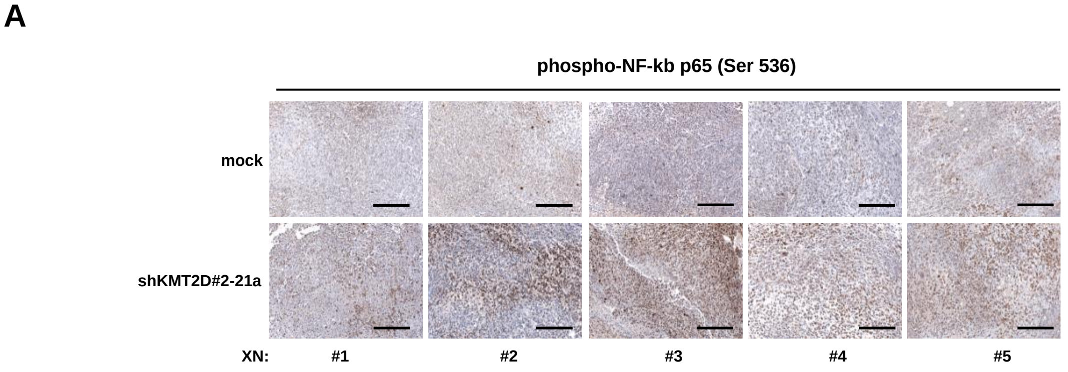
Supplementary Figure S9. Assessment of REL-associated protein mRNA levels upon KMT2D genetic manipulation.

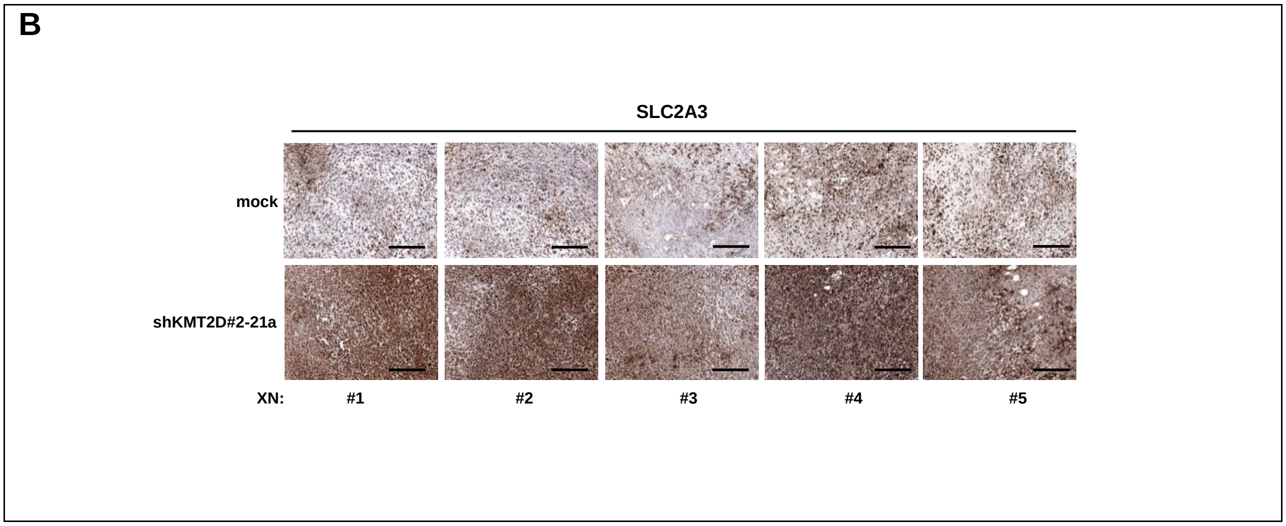
Effect of *KMT2D* silencing on *REL-associated protein (p65)* expression, as assessed by RT-qPCR analysis. Statistical analyses were performed using one-way ANOVA.



Supplementary Figure S10. Effect of pharmacological inhibition of mTORC1 on the SLC2A3 levels upon KMT2D suppression.

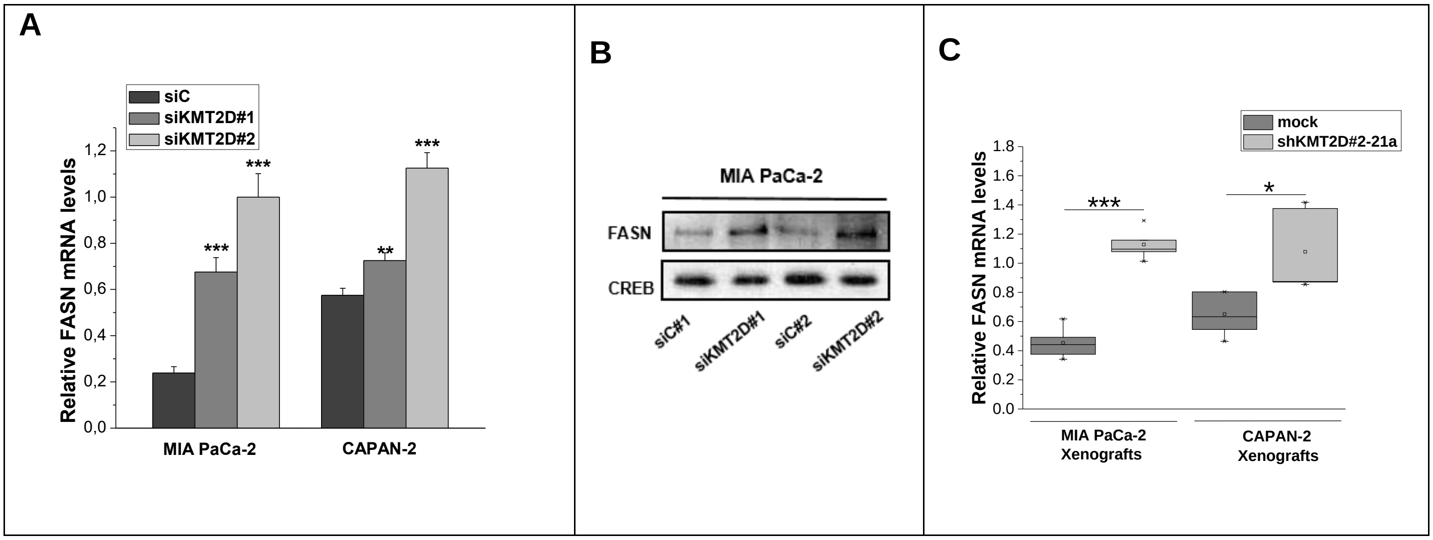
Representative IB images for the indicated antibodies upon treatment of MIA PacA-2 cells harboring differential KMT2D levels with 100 nM rapamycin for 24 h. Numbers in parentheses denote the average-fold change of the ratio SLC2A3:CREB total protein of drug-treated cells versus non-treated cells (set as default 1).





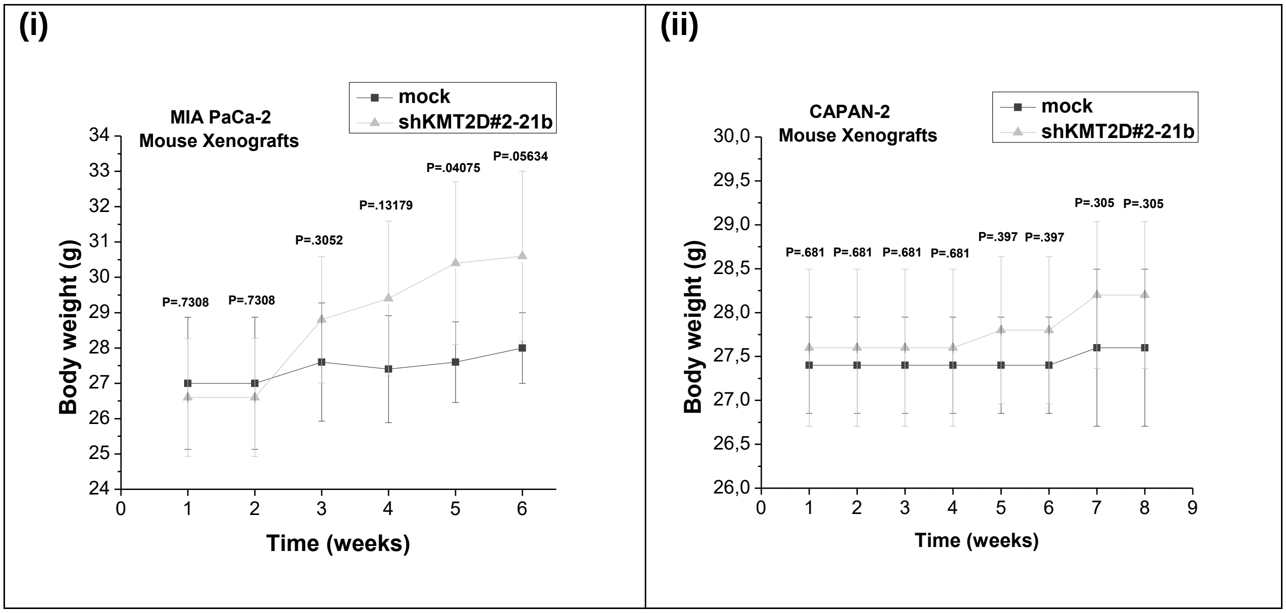
Supplementary Figure S11. Phospho-NF-kB (Ser 536) and SLC2A3 staining patterns in mouse xenografts bearing KMT2D-depleted or mock-transfected pancreatic cancer cells.

Representative images (10x magnification) of **(A)** phospho-NF-kb p65 (Ser 536) and **(B)** SLC2A3 expression, as assessed by IHC analysis, in tumors from xenografts bearing KMT2D stably-suppressed or mock-transfected MIA PaCa-2 cells. Scale bars represent 50 µm.



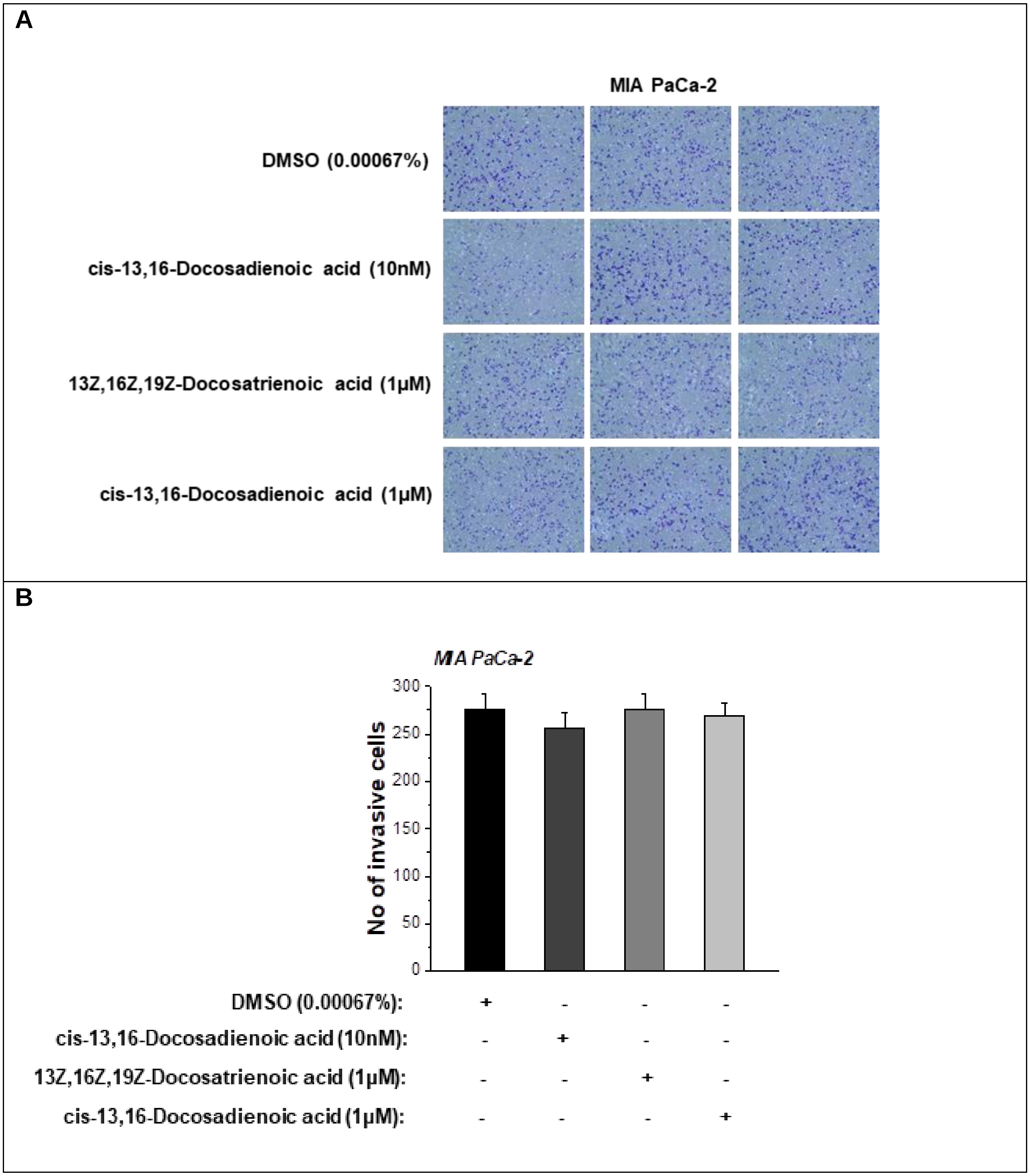
Supplementary Figure S12. Assessment of FASN expression levels upon KMT2D suppression.

(A) *Fatty Acid Synthase* (*FASN)* expression levels in *KMT2D* transiently-silenced pancreatic cancer cells, as assessed by RT-qPCR analysis. **(B)** FASN expression levels in KMT2D-transiently silenced pancreatic cancer cells, as assessed by IB analysis. **(C)** FASN mRNA levels in xenografts from mice injected with mock or shKMT2D#2-21a cells, as assessed by RT-qPCR analysis. Statistical analyses were performed using one-way ANOVA. Asterisks denote statistically significant differences, * *P*<.05*,* ** *P*<.01*,* *** *P*<.001



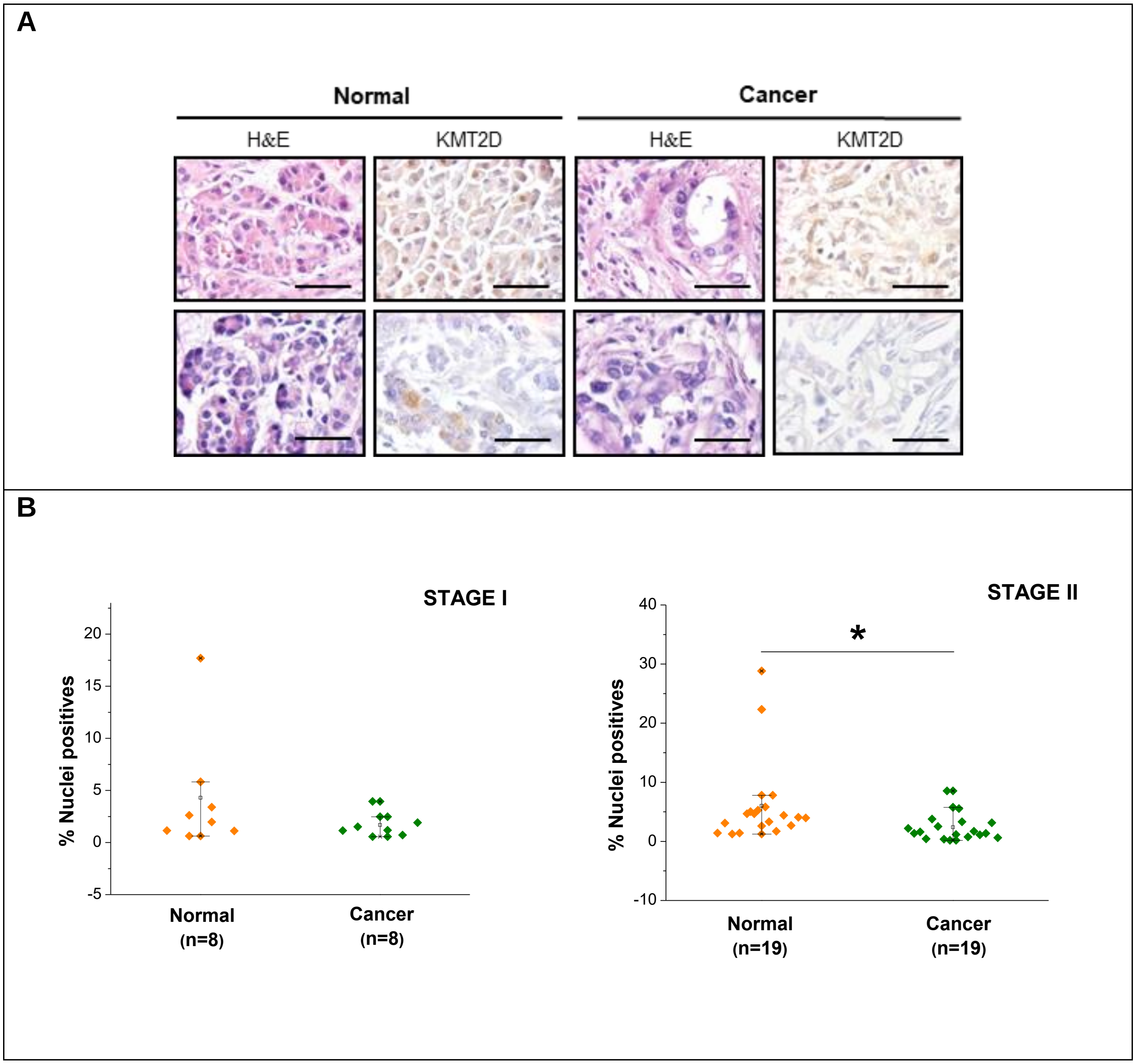
Supplementary Figure S13. Assessment of body weight changes in mice injected with control or KMT2D-lacking pancreatic cancer cells.

Body weight graphs of mouse xenografts bearing **(i)** MIA PaCa-2 or **(ii)** CAPAN-2 shKMT2D#2-21b clonal cell lines and mock-transfected cells (n=5 mice per group). Statistical analyses were performed using one-way ANOVA.


A
MIA PaCa-2
DMSO (0.00067%)
cis-13,16-Docosadienoic acid (10nM)
13Z,16Z,19Z-Docosatrienoic acid (1μM)
cis-13,16-Docosadienoic acid (1μM)
B
MIA PaCa-2
No of invasive cells
0
50
100
150
200
250
300
DMSO (0.00067%): + - - -
cis-13,16-Docosadienoic acid (10nM): - + - -
13Z,16Z,19Z-Docosatrienoic acid (1μM): - - + -
cis-13,16-Docosadienoic acid (1μM): - - - +

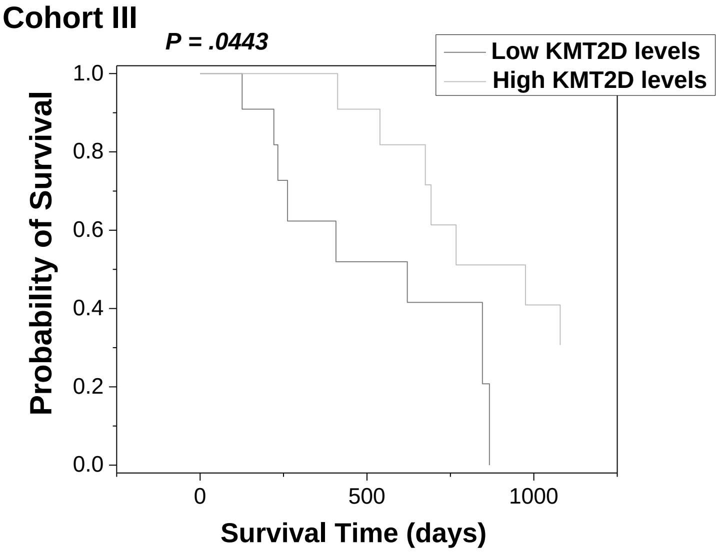
Supplementary Figure S14. Effects of docosadienoic, docosatrienoic and docosatretraenoic acid on pancreatic cancer cell invasiveness *in vitro*.

(A) Representative images of invading cells upon treatment with docosadienoic, docosatrienoic or docosatretraenoic acid for 22 h and **(B)** the respective quantification. Data are expressed as the mean number of invading cells per field ± SE.



Supplementary Figure S15. KMT2D staining patterns in matched pancreatic cancer and normal human tissues.

(A) Representative images (4x magnification) of KMT2D expression as assessed by IHC analysis. Scale bars represent 40 μm. **(B)** Graphs for nuclear staining quantification of KMT2D expression in normal pancreata and matched tumors derived from Stage I or Stage II pancreatic cancer patients (Cohort IV).



Supplementary Figure S16. Correlation of *KMT2D* expression with overall patient survival.

Kaplan-Meier survival curves of patients derived from Cohort III, harboring below median (<.25) and above median (>.25) *KMT2D* levels. 19 out of 22 cases were stratified as Stage III, 1 case as Stage IV pancreatic cancer and 1 case remains uncharacterized. r, Pearson correlation coefficient; Statistical analyses were performed using Pearson correlation.

2. SUPPLEMENTARY TABLES

Table S1

Gene symbols	Fold change	*P* value	q value	Probes
KMT2D	-1.741141121	.095383814	.253115	227527_at
KDM2A	1.717960305	.223050879	.444714	208988_at
KDM4C	-1.848905517	.002576512	.041733	1556493_a_at
KDM5B	1.825971671	.020177382	.086848	201548_s_at
KDM8	-2.020680417	.0000928	.001968	220070_at
SETDB2	-1.72662285	.006398742	.03863	235339_at
SETD6	-1.849110572	.001285416	.01239	1554555_a_at
SETD6	-1.785867564	.001087622	.011024	219751_at
SUV420H1	-1.705577585	.0073486	.042803	222566_at

Supplementary Table S1 Differential expression of chromatin regulators in pancreatic cancer.

Table of standardized (Z-scores) expression of the corresponding histone methyltransferases (KMTs) and demethylases (KDMs) in pancreatic cancer as well as adjacent normal tissues originating from Cohort I, as assessed by DNA microarray analysis (Affymetrix U133 Gene ChIP Set). Multi-array analysis followed by filtering of uninformative and low variance probes revealed 8 epigenetic factors to be up- or down-regulated ≥1.5 fold relatively to normal samples. Array fold change is generally reported as log value but has been converted to an arithmetic value for comparison purposes.

Table S2

	MIA PaCa-2								
Time (hours)	**Cell Index siC#1** (n=4)				**Cell Index siKMT2D#2** (n=4)				***P* value siKMT2D#2 vs siC#1**
0	0	0	0	0	0	0	0	0	
1.556944444	0.01993	0.00307	0.0161	0.02633	0.01218	0.01417	0.04009	0.04838	0.2787
3.306944444	0.17238	0.10747	0.13113	0.17127	0.16564	0.14392	0.15754	0.16934	0.45282
4.806944444	0.23896	0.17123	0.188	0.23339	0.2298	0.19187	0.20576	0.21341	0.90455
6.556944444	0.28382	0.21657	0.23608	0.28436	0.27899	0.2319	0.23863	0.24487	0.75331
8.056944444	0.31366	0.24275	0.26066	0.31161	0.31102	0.25793	0.26227	0.26644	0.73415
9.806944444	0.34585	0.26983	0.29308	0.34035	0.34456	0.28638	0.285	0.29172	0.67265
11.55694444	0.37777	0.29253	0.31851	0.36801	0.38798	0.31856	0.3136	0.31742	0.86442
13.05694444	0.40391	0.32055	0.34242	0.39765	0.4221	0.34908	0.33966	0.34518	0.94257
14.80694444	0.43768	0.34307	0.37141	0.42657	0.4706	0.39153	0.38318	0.38488	0.69096
16.30694444	0.46548	0.36034	0.39357	0.44251	0.51015	0.42361	0.42656	0.4204	0.39104
18.05694444	0.48411	0.37485	0.40839	0.46096	0.55653	0.46225	0.46366	0.45651	0.17771
19.55694444	0.49563	0.38574	0.4169	0.46539	0.58461	0.49454	0.49707	0.47523	0.08277
21.30694444	0.51023	0.39866	0.42816	0.47528	0.62531	0.51577	0.51739	0.5098	0.0539
22.80694444	0.53428	0.42219	0.4468	0.49818	0.65059	0.54778	0.54651	0.53385	0.04379
24.55694444	0.57582	0.45676	0.48193	0.53862	0.68852	0.58323	0.5879	0.56766	0.0515
26.05694444	0.61471	0.49414	0.52977	0.57605	0.73841	0.62387	0.62212	0.60624	0.05856
27.80694444	0.66688	0.55229	0.58403	0.6156	0.79959	0.67146	0.68054	0.65628	0.05531
29.55694444	0.71799	0.58852	0.62445	0.66588	0.8643	0.74643	0.7338	0.69781	0.0502
31.05694444	0.76111	0.6173	0.65519	0.69779	0.91719	0.78763	0.78436	0.74619	0.04052
32.80694444	0.79299	0.65679	0.68778	0.72991	0.97381	0.86092	0.82865	0.80414	0.01992
34.30694444	0.82135	0.6842	0.71556	0.75847	1.02194	0.90791	0.88465	0.83918	0.01372
36.05694444	0.84057	0.7155	0.73749	0.78684	1.07253	0.94268	0.9306	0.88986	0.00792
37.55694444	0.87557	0.73416	0.77224	0.82506	1.11671	0.9901	0.97506	0.91518	0.00931
39.30694444	0.92721	0.77848	0.81946	0.86033	1.15278	1.04075	1.0271	0.95036	0.00954
40.80694444	0.97444	0.82619	0.8596	0.90322	1.20265	1.06349	1.06287	0.99585	0.01243
42.55694444	1.03478	0.87915	0.92693	0.9688	1.25158	1.13502	1.13759	1.05507	0.01024

44.05694444	1.08756	0.91975	0.98197	1.02528	1.30741	1.18536	1.19222	1.10988	0.01116
45.80694444	1.14759	0.97162	1.03087	1.0803	1.38722	1.24957	1.26116	1.17628	0.0105
47.55694444	1.20711	1.0233	1.078	1.13413	1.44859	1.32177	1.33128	1.24172	0.00813
49.05694444	1.24837	1.07129	1.1352	1.19125	1.51097	1.38801	1.3996	1.30525	0.00558
50.80694444	1.30284	1.12654	1.19836	1.24521	1.57876	1.45095	1.47003	1.37221	0.0045

Supplementary Table S2 Xcelligence Cell Index and *P* values of siKMT2D#2-treated versus control MIA PaCa-2 cells.

List of Cell Index values derived from the measured impedances and continuously displayed on the Xcelligence Software user interface. Each experimental condition was performed in quadruplicates. Statistical analyses were performed using one-way ANOVA and the *P* values corresponding to the comparison of siRNA-treated versus control cells are shown at every single time point. *P* values ≤.05 are marked in red. Differences in Cell Index measurements are significant after 31 hours of monitoring MIA PaCa-2 cells.

Table S3

	CAPAN-2								
Time (hours)	**Cell Index siC#1** (n=4)				**Cell Index siKMT2D#2** (n=4)				***P* value siKMT2D#2 vs siC#1**
0	0	0	0	0	0	0	0	0	
1.556944444	-0.020073	0.034304	0.050577	0.05816	0.06867	0.059427	0.083027	0.085488	0.05928
3.306944444	0.037766	0.079534	0.099732	0.094963	0.141033	0.122201	0.144497	0.157935	0.00721
4.806944444	0.068535	0.10916	0.125919	0.113986	0.179764	0.165437	0.179974	0.200587	0.00175
6.556944444	0.08927	0.128111	0.139508	0.129772	0.205867	0.190239	0.200832	0.231135	9.12692E-4
8.056944444	0.099004	0.1331	0.155176	0.1359	0.219105	0.201924	0.214894	0.244162	8.90077E-4
9.806944444	0.101722	0.127247	0.158144	0.137542	0.229755	0.212719	0.219634	0.253497	5.65592E-4
11.55694444	0.098911	0.13078	0.15315	0.137105	0.230603	0.214544	0.22037	0.258289	5.14131E-4
13.05694444	0.098725	0.125915	0.146574	0.134241	0.233402	0.220184	0.221467	0.260783	2.389E-4

14.80694444	0.097222	0.124272	0.145863	0.134348	0.238026	0.220155	0.222833	0.264834	2.67765E-4
16.30694444	0.096102	0.122436	0.143388	0.131518	0.239895	0.219975	0.226634	0.263344	1.746E-4
18.05694444	0.094791	0.12158	0.140609	0.130565	0.241798	0.215101	0.232633	0.262699	1.63212E-4
19.55694444	0.087017	0.122059	0.139195	0.126579	0.241056	0.216168	0.227237	0.2572	1.79476E-4
21.30694444	0.082258	0.119865	0.137669	0.128171	0.241253	0.21935	0.225254	0.259881	2.21228E-4
22.80694444	0.080472	0.123005	0.136547	0.128523	0.243548	0.218359	0.230869	0.259986	2.22074E-4
24.55694444	0.075172	0.121803	0.132296	0.132782	0.243325	0.221071	0.22565	0.262633	3.19085E-4
26.05694444	0.074455	0.122927	0.134319	0.129331	0.244887	0.22282	0.226275	0.266265	3.30394E-4
27.80694444	0.0737	0.119391	0.131049	0.125469	0.242504	0.223553	0.223904	0.271749	3.16723E-4
29.55694444	0.072791	0.11973	0.12722	0.125928	0.242003	0.229328	0.218143	0.263189	2.28539E-4
31.05694444	0.072043	0.120048	0.124046	0.119541	0.24577	0.22249	0.225408	0.258103	1.34237E-4
32.80694444	0.066999	0.11541	0.12315	0.11772	0.244751	0.227915	0.220318	0.259354	1.52409E-4
34.30694444	0.062007	0.116527	0.121774	0.12072	0.244203	0.231497	0.223175	0.263073	2.01022E-4
36.05694444	0.061464	0.112372	0.116357	0.120599	0.24808	0.225763	0.225829	0.241364	1.1443E-4
37.55694444	0.065181	0.11423	0.102967	0.115519	0.245032	0.227805	0.22235	0.239662	4.57436E-5
39.30694444	0.06347	0.117356	0.09523	0.116264	0.245516	0.233652	0.219364	0.232157	6.4013E-5
40.80694444	0.063181	0.113218	0.090033	0.112343	0.244225	0.236082	0.223486	0.232254	3.19248E-5
42.55694444	0.063229	0.11298	0.080251	0.116045	0.250371	0.236341	0.229732	0.235528	3.98296E-5
44.05694444	0.066098	0.116247	0.079267	0.117831	0.249681	0.240117	0.232087	0.238075	3.97361E-5
45.80694444	0.064277	0.121713	0.084177	0.124887	0.248549	0.242266	0.234827	0.24359	7.50008E-5
47.55694444	0.06762	0.124783	0.082257	0.123198	0.252865	0.243433	0.241608	0.247328	5.8002E-5
49.05694444	0.070556	0.125857	0.083957	0.124076	0.249066	0.247302	0.249124	0.245079	4.609E-5
50.80694444	0.071493	0.12713	0.078742	0.124957	0.250745	0.24804	0.252373	0.25484	5.28337E-5

Supplementary Table S3 Xcelligence Cell Index and *P* values of siKMT2D#2-treated versus control CAPAN-2 cells.

List of Cell Index values derived from the measured impedances and continuously displayed on the Xcelligence Software user interface. Each experimental condition was performed in quadruplicates. Statistical analyses were performed using one-way ANOVA and the *P* values corresponding to the comparison of siRNA-treated versus control cells are shown at every single time point. *P* values ≤.05 are marked in red. Differences in Cell Index measurements are significant after 3.3 hours of monitoring CAPAN-2 cells.

Table S4

	MIA PaCa-2						
Time (hours)	**Cell Index mock** (n=3)			**Cell Index shKMT2D#2-21a** (n=3)			***P* value shKMT2D#2-21a vs mock**
0	0	0	0	0	0	0	
1.64611	-0.03548	-6.7E-4	0.04437	0.02274	0.03943	0.06906	0.20064
3.39611	0.02795	0.05192	0.11561	0.18392	0.17674	0.21105	0.01122
4.89611	0.08816	0.105	0.17799	0.27335	0.25557	0.29962	0.0074
6.64611	0.15481	0.16696	0.25148	0.35875	0.32387	0.3673	0.00872
8.14611	0.20299	0.20718	0.29803	0.40881	0.36791	0.41359	0.00934
9.89611	0.2414	0.24242	0.3348	0.45946	0.41235	0.45553	0.00791
11.39611	0.27765	0.27711	0.37328	0.51275	0.45182	0.50053	0.0084
13.14611	0.32252	0.31923	0.41425	0.57925	0.51468	0.56412	0.00546
14.64611	0.36765	0.36132	0.46573	0.63069	0.56235	0.60952	0.00676
16.39611	0.41964	0.3977	0.5132	0.67095	0.6043	0.65111	0.00806
18.14611	0.45588	0.42176	0.5516	0.69757	0.62371	0.67342	0.01334
19.64611	0.48118	0.43805	0.57682	0.71668	0.64357	0.68661	0.01645
21.39611	0.49712	0.46084	0.59246	0.76491	0.67262	0.72969	0.01243
22.89611	0.51328	0.47107	0.61206	0.82401	0.73223	0.78193	0.00751
24.64611	0.54207	0.50643	0.65696	0.92238	0.82223	0.879	0.00474
26.14611	0.59431	0.56295	0.69114	1.00921	0.90233	0.96789	0.00227
27.89611	0.66012	0.62616	0.7551	1.1097	1.01301	1.06958	0.0013
29.64444	0.74236	0.68291	0.82783	1.1898	1.11702	1.1598	0.00101
31.14444	0.78123	0.73267	0.88596	1.26025	1.19066	1.23467	9.85588E-4
32.89444	0.82113	0.78133	0.9338	1.32393	1.26901	1.29878	7.2956E-4
34.39444	0.86096	0.81316	0.96802	1.38432	1.32704	1.34736	6.35982E-4
36.14444	0.90486	0.85244	1.01082	1.46964	1.41646	1.44576	4.44133E-4

37.64444	0.93605	0.88429	1.0517	1.55654	1.52442	1.54173	3.166E-4
39.39444	0.98666	0.94622	1.1091	1.68043	1.66193	1.6784	1.80687E-4
40.89444	1.04982	0.9958	1.172	1.79348	1.78453	1.81634	1.64543E-4
42.64444	1.10853	1.06787	1.24812	1.91062	1.96863	1.96647	1.52504E-4
44.39444	1.16738	1.12633	1.3259	2.01828	2.11814	2.1126	2.20452E-4
45.89333	1.21602	1.1799	1.38965	2.12459	2.24856	2.21155	2.33143E-4
47.64333	1.26615	1.24707	1.46187	2.21607	2.3618	2.33057	2.79254E-4
49.14167	1.31626	1.28497	1.50996	2.32416	2.48603	2.44008	2.53257E-4
50.88972	1.37807	1.34948	1.57186	2.45744	2.59205	2.58472	1.74688E-4
52.38972	1.43097	1.40787	1.63916	2.55202	2.71498	2.70421	2.09778E-4
54.13972	1.50776	1.4887	1.70548	2.70808	2.85097	2.89939	1.54796E-4
55.88972	1.61186	1.58677	1.79364	2.84836	2.98378	3.06371	1.36266E-4
57.38972	1.67382	1.66723	1.86846	2.96868	3.13848	3.19889	1.38547E-4
59.13972	1.76102	1.75566	1.96147	3.13693	3.29247	3.29779	7.90511E-5
60.63972	1.83668	1.84181	2.03916	3.27238	3.39747	3.41639	5.49798E-5
62.38972	1.92252	1.93019	2.12654	3.42067	3.51613	3.55384	4.18829E-5
63.88972	1.98447	2.00046	2.20477	3.52858	3.63979	3.66438	4.71E-5
65.63972	2.081	2.10635	2.31714	3.69513	3.75112	3.79362	3.91071E-5
67.13972	2.16031	2.20805	2.37155	3.81512	3.85113	3.88427	1.80775E-5
68.88972	2.27924	2.32252	2.45216	3.9797	3.95671	4.00217	6.98761E-6
70.63972	2.3875	2.41904	2.59425	4.08349	4.03866	4.14571	2.22305E-5
72.13972	2.48343	2.50893	2.70639	4.21164	4.11882	4.21704	3.09696E-5
73.88972	2.58672	2.61139	2.80666	4.32744	4.22684	4.2726	2.86289E-5
75.38972	2.72494	2.71189	2.88812	4.45171	4.32179	4.37495	1.90687E-5
77.13944	2.8011	2.83126	3.01627	4.59121	4.37858	4.4798	6.16085E-5
78.63944	2.91401	2.90089	3.0763	4.69108	4.42792	4.56312	7.22673E-5
80.38944	3.02859	3.00459	3.17597	4.83053	4.48886	4.5994	1.61295E-4
81.88944	3.13508	3.08395	3.24635	4.87581	4.53915	4.64591	1.55821E-4
83.63944	3.26149	3.17539	3.31704	4.98727	4.6286	4.68768	2.14344E-4
85.38944	3.39014	3.26267	3.42184	5.10971	4.70364	4.74254	4.15676E-4
86.88944	3.49607	3.32432	3.48201	5.156	4.74067	4.76751	5.56886E-4
88.63944	3.55179	3.40723	3.55229	5.20273	4.78268	4.80914	5.83157E-4

90.13944	3.68251	3.47698	3.62795	5.22825	4.83796	4.83767	6.76022E-4
91.88944	3.80046	3.55814	3.72993	5.40447	4.8729	4.88635	0.00199
93.38944	3.87641	3.6527	3.81331	5.46153	4.88317	4.93434	0.00261
95.13944	3.98026	3.72709	3.87598	5.55389	4.93473	4.9804	0.00365
96.88944	4.09917	3.79225	3.97422	5.58058	4.95065	5.0155	0.00498
98.38944	4.20116	3.86002	4.01449	5.60487	4.95606	5.02309	0.00689
100.13917	4.31632	3.92505	4.10531	5.61392	4.95159	5.04212	0.01001
101.63917	4.43169	3.98702	4.17207	5.68448	4.98647	5.00756	0.01735
103.38917	4.54915	4.03133	4.20871	5.70852	4.99687	5.00298	0.02571
104.88917	4.65601	4.05039	4.27636	5.75427	4.98478	4.98693	0.04245
106.63917	4.71997	4.08675	4.32626	5.76795	4.94804	4.93416	0.06472
108.13917	4.81067	4.13599	4.35874	5.79617	4.90208	4.91447	0.09709
109.88917	4.88043	4.13423	4.38586	5.80425	4.8672	4.86941	0.13465
111.63917	4.98009	4.16329	4.4206	5.82269	4.80838	4.8519	0.19337
113.13917	5.03647	4.18176	4.41413	5.83088	4.74924	4.80327	0.25046
114.88917	5.13655	4.20232	4.42149	5.7937	4.66986	4.75638	0.34815
116.38917	5.18354	4.19483	4.42152	5.77905	4.6451	4.7042	0.40361
118.13917	5.23074	4.18789	4.45534	5.76533	4.57388	4.6051	0.51611
119.63917	5.25751	4.19731	4.42753	5.72818	4.5083	4.57409	0.57714
121.38917	5.35591	4.17537	4.41114	5.70622	4.44587	4.49845	0.68849

Supplementary Table S4 Xcelligence Cell Index and *P* values of shKMT2D#2-21a versus mock MIA PaCa-2 cells.

List of Cell Index values derived from the measured impedances and continuously displayed on the Xcelligence Software user interface. Each experimental condition was performed in triplicates. Statistical analyses were performed using one-way ANOVA and the *P* values corresponding to the comparison of shRNA-stably transfected versus control cells are shown at every single time point. *P* values ≤.05 are marked in red. Differences in Cell Index measurements are significant after 3.3 hours of monitoring.

Table S5

	CAPAN-2								
Time (hours)	**Cell Index mock** (n=4)				**Cell Index shKMT2D#2-21a** (n=4)				***P* value shKMT2D#2-21a vs mock**
0	0	0	0	0	0	0	0	0	
1.64611	0.01462	0.01218	0.03884	0.06201	0.07853	0.04418	0.02288	0.03539	0.45515
3.39611	0.07544	0.05766	0.07975	0.10362	0.1246	0.08982	0.07047	0.0966	0.31017
4.89611	0.11267	0.08142	0.10833	0.13384	0.15458	0.11595	0.10411	0.13383	0.2854
6.64611	0.13282	0.09975	0.13087	0.15106	0.17986	0.13594	0.12981	0.1606	0.19468
8.14611	0.14139	0.10637	0.13721	0.15648	0.19189	0.14788	0.13682	0.17371	0.14612
9.89611	0.1525	0.10688	0.1374	0.1588	0.20485	0.15644	0.14623	0.18281	0.10382
11.39611	0.15252	0.10846	0.13751	0.15606	0.21631	0.16728	0.15531	0.19419	0.04309
13.14611	0.15796	0.10767	0.13622	0.15512	0.22899	0.1757	0.16183	0.20175	0.03085
14.64611	0.15662	0.10242	0.13536	0.15155	0.23498	0.18659	0.17484	0.20937	0.01139
16.39611	0.15975	0.09829	0.13219	0.14733	0.24868	0.19501	0.18465	0.21619	0.00748
18.14611	0.15871	0.09698	0.13278	0.14415	0.25905	0.21019	0.18786	0.22294	0.00473
19.64611	0.15639	0.09199	0.13041	0.1444	0.27136	0.22274	0.1972	0.23074	0.00301
21.39611	0.15989	0.09242	0.13139	0.13816	0.28585	0.23164	0.1939	0.23603	0.00402
22.89611	0.16406	0.08835	0.13004	0.13723	0.29695	0.24713	0.20966	0.23996	0.00258
24.64611	0.1619	0.08548	0.12986	0.13359	0.30964	0.25627	0.21183	0.25081	0.0023
26.14611	0.1678	0.0876	0.12719	0.1307	0.32135	0.27093	0.23042	0.2566	0.00136
27.89611	0.16923	0.08459	0.12604	0.13104	0.3342	0.28139	0.2374	0.26743	0.00124
29.64444	0.17416	0.08085	0.12452	0.12672	0.34964	0.29744	0.24991	0.27925	0.00104
31.14444	0.1824	0.07759	0.126	0.12553	0.35925	0.3033	0.25879	0.28762	0.00116
32.89444	0.18152	0.07712	0.12331	0.12159	0.36834	0.31755	0.2705	0.29826	7.34904E-4
34.39444	0.18649	0.07346	0.11996	0.11769	0.37439	0.32594	0.27598	0.30492	7.57403E-4
36.14444	0.18978	0.07419	0.12101	0.11213	0.37185	0.33697	0.28399	0.3099	5.85979E-4
37.64444	0.19512	0.06979	0.11535	0.11125	0.38244	0.35255	0.29787	0.32168	5.18973E-4
39.39444	0.19763	0.06798	0.10924	0.10578	0.39243	0.36354	0.30484	0.32708	5.16712E-4
40.89444	0.19881	0.06364	0.11474	0.09785	0.39526	0.37742	0.3182	0.33605	4.11666E-4
42.64444	0.20051	0.0574	0.10941	0.09803	0.40709	0.39498	0.33898	0.34726	3.17093E-4

44.39444	0.20842	0.05262	0.10969	0.09621	0.42697	0.41296	0.35257	0.35588	3.92894E-4
45.89333	0.21108	0.03697	0.11015	0.09583	0.44859	0.42364	0.36593	0.36544	4.67138E-4
47.64333	0.21688	0.03923	0.11628	0.09659	0.46535	0.44283	0.39169	0.3835	3.54637E-4
49.14167	0.22108	0.04193	0.11735	0.09839	0.48161	0.47117	0.40379	0.40015	3.13406E-4
50.88972	0.23155	0.04425	0.11829	0.09668	0.50937	0.48388	0.42265	0.41374	3.36406E-4
52.38972	0.23578	0.04409	0.12151	0.09185	0.52924	0.49896	0.44429	0.42949	2.90154E-4
54.13972	0.24363	0.04808	0.12219	0.08561	0.54634	0.5207	0.45773	0.4489	2.73627E-4
55.88972	0.253	0.05448	0.12579	0.08556	0.57381	0.54568	0.46975	0.46185	3.10678E-4
57.38972	0.25673	0.05762	0.13062	0.08629	0.58611	0.56827	0.49624	0.48122	2.27898E-4
59.13972	0.26142	0.06335	0.13304	0.08675	0.60869	0.60007	0.50725	0.49634	2.2984E-4
60.63972	0.26829	0.06477	0.13566	0.09055	0.62504	0.61133	0.53163	0.51868	1.79323E-4
62.38972	0.27364	0.06557	0.13821	0.089	0.64896	0.63632	0.54708	0.53735	1.76477E-4
63.88972	0.27408	0.07054	0.14284	0.0914	0.67956	0.66286	0.55842	0.54902	1.77675E-4
65.63972	0.28038	0.07291	0.14651	0.09407	0.7024	0.68778	0.57252	0.56675	1.79125E-4
67.13972	0.28963	0.07739	0.14796	0.09153	0.72935	0.71003	0.58763	0.58466	1.92732E-4
68.88972	0.29532	0.07692	0.15511	0.08452	0.75266	0.72862	0.60422	0.60104	1.99467E-4
70.63972	0.29942	0.08279	0.15458	0.08815	0.7841	0.76832	0.63173	0.62832	1.65234E-4
72.13972	0.30537	0.08631	0.1581	0.08772	0.80715	0.79405	0.65885	0.64177	1.55946E-4
73.88972	0.31327	0.09277	0.16368	0.08601	0.83972	0.81829	0.68513	0.67534	1.3085E-4
75.38972	0.3182	0.08969	0.16533	0.08903	0.85848	0.85382	0.71023	0.7047	1.09467E-4
77.13944	0.33027	0.09882	0.16565	0.09587	0.89576	0.90812	0.73604	0.72969	1.22138E-4
78.63944	0.33924	0.10003	0.16911	0.09508	0.94837	0.94157	0.77601	0.76276	1.10465E-4
80.38944	0.3487	0.10577	0.17359	0.0987	1.00222	0.98485	0.80443	0.79951	1.11257E-4
81.88944	0.35949	0.11137	0.18091	0.09913	1.03302	1.03145	0.84508	0.82332	1.06542E-4
83.63944	0.36418	0.11618	0.18941	0.10619	1.06645	1.07785	0.86758	0.8391	1.17714E-4
85.38944	0.37339	0.12079	0.19537	0.10567	1.11719	1.12588	0.89975	0.8771	1.16753E-4
86.88944	0.38011	0.12661	0.19735	0.10701	1.1595	1.18502	0.92108	0.90369	1.36251E-4
88.63944	0.39188	0.13656	0.20597	0.11024	1.2158	1.23839	0.96176	0.94011	1.36401E-4
90.13944	0.3994	0.14292	0.21497	0.10799	1.28016	1.3008	0.99606	0.97683	1.51674E-4
91.88944	0.4124	0.14896	0.22115	0.11206	1.35973	1.35436	1.02401	1.01358	1.84004E-4
93.38944	0.41764	0.15343	0.2324	0.11258	1.42202	1.43345	1.06019	1.0492	2.09515E-4
95.13944	0.42643	0.16578	0.23832	0.11547	1.52347	1.56719	1.07862	1.09339	3.56668E-4

96.88944	0.43567	0.17158	0.24716	0.11923	1.60117	1.63223	1.12655	1.1347	3.58733E-4
98.38944	0.44724	0.17464	0.25659	0.12718	1.68784	1.75962	1.18409	1.18649	4.1616E-4
100.13917	0.46331	0.17956	0.26242	0.13036	1.77026	1.84637	1.23422	1.22194	4.72084E-4
101.63917	0.46731	0.18361	0.27419	0.1322	1.86374	1.9746	1.27434	1.27042	5.86156E-4
103.38917	0.48137	0.18737	0.28146	0.13378	1.95985	2.08392	1.32145	1.31949	6.71452E-4
104.88917	0.49534	0.19351	0.29001	0.13492	2.07408	2.28446	1.37379	1.37304	9.59862E-4
106.63917	0.51044	0.19558	0.29743	0.13514	2.18489	2.39506	1.40685	1.41617	0.00115
108.13917	0.52609	0.21009	0.3009	0.1425	2.32248	2.58844	1.46406	1.47154	0.00149
109.88917	0.53772	0.21542	0.31296	0.14786	2.45081	2.78011	1.51131	1.51776	0.00193
111.63917	0.54959	0.22733	0.32882	0.14885	2.67416	2.93264	1.56531	1.60026	0.00217
113.13917	0.56386	0.23495	0.33118	0.15368	2.86434	3.1599	1.62501	1.65958	0.00272
114.88917	0.57123	0.24506	0.34073	0.15369	3.10717	3.39293	1.70519	1.75151	0.00306
116.38917	0.58829	0.25	0.35437	0.16194	3.29853	3.66115	1.7787	1.82755	0.00361
118.13917	0.60761	0.2572	0.36414	0.16934	3.511	4.04172	1.85226	1.90569	0.00469
119.63917	0.62744	0.26197	0.36525	0.17169	3.77049	4.463	1.9252	2.01165	0.00587
121.38917	0.638784	0.27081	0.376880	0.177430	4.07119	5.0249788	2.02605	2.09975	0.00781

Supplementary Table S5 Xcelligence Cell Index and *P* values of shKMT2D#2-21a versus mock CAPAN-2 cells.

List of Cell Index values derived from the measured impedances and continuously displayed on the Xcelligence Software user interface. Each experimental condition was performed in triplicates. Statistical analyses were performed using one-way ANOVA and the *P* values corresponding to the comparison of shRNA-stably transfected versus control cells are shown at every single time point. *P* values ≤.05 are marked in red. Differences in Cell Index measurements are significant after 11 hours of monitoring.

Table S6

Gene symbols	Mean difference	*P* value	q value	Probes
SETD3	0.264457	.0000027	.000353114	cg16694837
KMT2D	0.293902	.000394	.003574961	cg13007988
KMT2D	0.276747	.0000201	.000762928	cg00522588
KDM3A	0.254105	.000000979	.000258094	cg01878308
KDM2B	-0.26654	.0000164	.000694	cg15234492
SETDB2	-0.31333	.0000133	.002972	cg05743713

Supplementary Table S6 Differential methylation of chromatin regulators in pancreatic cancer.

Table of standardized (Z-scores) expression of the corresponding KMTs and KDMs in pancreatic cancer as well as adjacent normal tissues, as assessed by the Infinium Human Methylation 450 Bead ChIP Array. Wilcoxon rank-sum tests were conducted to compare methylation array data between pancreatic cancer patients and healthy controls. Genes shown are up- or down-regulated ≥1.5 fold relatively to normal samples and with statistical significance *P*≤.001

Table S7

(i)

Chr	Start	End	Ref	Alt	Func. refGene	ExonicFunc. refGene	AAChange.refGene
12	49421179	49421179	G	C	intronic	na	na
12	49422795	49422795	G	A	intronic	na	na
12	49427919	49427919	T	C	exonic	synonymous SNV	KMT2D:NM_003482:exon38:c.A10671G:p.P3557P
12	49439659	49439659	C	T	intronic	na	na
12	49445447	49445447	T	A	exonic	synonymous SNV	KMT2D:NM_003482:exon10:c.A2019T:p.P673P

12	49445536	49445536	T	G	exonic	nonsynonymous SNV	KMT2D:NM_003482:exon10:c.A1930C:p.M644L
12	49445540	49445540	T	A	exonic	synonymous SNV	KMT2D:NM_003482:exon10:c.A1926T:p.S642S
12	49447819	49447819	T	C	exonic	synonymous SNV	KMT2D:NM_003482:exon5:c.A615G:p.L205L

(ii)

Chr	Start	End	Ref	Alt	Func. refGene	ExonicFunc. refGene	AAChange.refGene
12	49413208	49413208	-	A	UTR3	na	na
12	49415026	49415026	G	A	UTR3	na	na
12	49416048	49416048	C	-	intronic	na	na
12	49419677	49419677	G	C	intronic	na	na
12	49421179	49421179	G	C	intronic	na	na
12	49422094	49422094	A	G	intronic	na	na
12	49424616	49424616	G	A	intronic	na	na
12	49424878	49424881	TCTG	-	intronic	na	na
12	49425978	49425978	T	C	exonic	synonymous SNV	KMT2D:NM_003482:exon39:c.A12510G:p.P4170P
12	49427652	49427652	C	T	exonic	synonymous SNV	KMT2D:NM_003482:exon39:c.G10836A:p.Q3612Q
12	49434074	49434074	C	A	exonic	synonymous SNV	KMT2D:NM_003482:exon31:c.G7479T:p.G2493G
12	49436724	49436724	A	G	intronic	na	na
12	49439521	49439521	A	G	intronic	na	na
12	49439659	49439659	C	T	intronic	na	na
12	49441382	49441382	T	-	intronic	na	na
12	49442359	49442359	-	A	intronic	na	na
12	49442813	49442813	T	C	intronic	na	na
12	49444545	49444545	G	A	exonic	synonymous SNV	KMT2D:NM_003482:exon11:c.C2826T:p.I942I

12	49448881	49448881	T	G	intronic	na	na

Table S7 *KMT2D* mutational status.

Sequence alterations in *KMT2D* gene, as assessed by Whole Exome Sequencng, in **(i)** MIA PaCa-2 and **(ii)** CAPAN-2 cell lines. Chr, Chromosome number; Start, Start position; End, End position; Ref, Reference base(s); Alt, Alternate non-reference alleles called on at least one of the samples; Func.refGene, Regions (e.g., exonic, intronic, non-coding RNA)) that one variant hits; ExonicFunc.refGene, Exonic variant function, e.g., nonsynonymous, synonymous, frameshift insertion; AAChange.refGene, Amino acid change. For example, KMT2D:NM_003482:exon38:c.A10671G:p.P3557P stands for gene name, Known RefSeq accession, region, cDNA level change, protein level change.

Table S8

(i)

Chr	Start	End	Ref	Alt	Func. refGene	ExonicFunc. refGene	AAChange.refGene
12	8072008	8072008	T	C	UTR3	na	na
12	8073496	8073496	T	-	UTR3	na	na
12	8074192	8074192	G	A	exonic	synonymous SNV	SLC2A3:NM_006931:exon10:c.C1308T:p.T436T
12	8075117	8075117	A	G	intronic	na	na
12	8075286	8075286	C	T	intronic	na	na
12	8083541	8083541	C	T	intronic	na	na
12	8085547	8085547	T	C	intronic	na	na
12	8086083	8086083	C	A	intronic	na	na

	8088227	8088227	T	C	intronic	na	na
	8088766	8088766	T	C	UTR5	na	na

(ii)

Chr	Start	End	Ref	Alt	Func. refGene	ExonicFunc. refGene	AAChange.refGene
12	8072562	8072562	A	G	UTR3	na	na
12	8073496	8073496	T	-	UTR3	na	na
12	8074192	8074192	G	A	exonic	synonymous SNV	SLC2A3:NM_006931:exon10:c.C1308T:p.T436T
12	8075117	8075117	A	G	intronic	na	na
12	8075286	8075286	C	T	intronic	na	na
12	8086062	8086062	G	C	intronic	na	na
12	8088766	8088766	T	C	UTR5	na	na

Table S8 *SLC2A3* mutational status.

Sequence alterations in *SLC2A3* gene, as assessed by Whole Exome Sequencng, in **(i)** MIA PaCa-2 and **(ii)** CAPAN-2 cell lines.

Table S9

	MIA PaCa-2								
Time (hours)	Cell Index shKMT2D#2-21a+siC#1 (n=4)				Cell Index shKMT2D#2-21a+siSLC2A3 (n=4)				***P* value shKMT2D#2-21a+siSLC2A3 vs shKMT2D#2-21a+siC#1**
0	0	0	0	0	0	0	0	0	
1.64611	-0.09461	-0.10544	-0.10934	-0.09085	-0.06418	-0.08382	-0.1064	-0.09298	0.2297
3.39611	-0.07183	-0.08472	-0.08991	-0.06829	-0.04211	-0.06424	-0.08378	-0.0497	0.12519

4.89611	-0.03959	-0.05402	-0.05856	-0.0265	-0.01929	-0.04226	-0.05887	-0.01414	0.41835
6.64611	-0.00333	-0.02005	-0.02692	0.00252	-0.00723	-0.02847	-0.04887	0.00368	0.5636
8.14611	0.03037	0.01308	0.00741	0.02951	0.00352	-0.01821	-0.03962	0.0122	0.05644
9.89611	0.06093	0.03873	0.03554	0.05915	0.01221	-0.00894	-0.02679	0.02	0.00747
11.39611	0.08987	0.06699	0.06234	0.08699	0.02703	0.00411	-0.0171	0.03304	0.00291
13.14611	0.11748	0.09022	0.08578	0.1108	0.03469	0.0146	-0.00307	0.04192	8.19E-04
14.64611	0.14544	0.11846	0.11708	0.13531	0.04826	0.02964	0.0146	0.05787	2.46E-04
16.39611	0.17055	0.14461	0.13905	0.15488	0.06123	0.04342	0.03029	0.07208	1.29E-04
18.14611	0.19574	0.17415	0.1595	0.18154	0.07805	0.05888	0.04607	0.08932	1.09E-04
19.64611	0.21736	0.19735	0.17909	0.20363	0.09044	0.07096	0.05506	0.10093	8.85E-05
21.39611	0.24085	0.21538	0.20297	0.22107	0.1039	0.08493	0.06319	0.11479	8.77E-05
22.89611	0.26035	0.23619	0.21879	0.23155	0.10422	0.09528	0.07271	0.12445	5.91E-05
24.64611	0.28008	0.2552	0.23872	0.24416	0.11326	0.10717	0.08278	0.13412	4.69E-05
26.14611	0.29911	0.26855	0.2621	0.25453	0.12754	0.11606	0.09111	0.14491	5.39E-05
27.89611	0.32114	0.28338	0.27851	0.2651	0.14007	0.12484	0.09979	0.15369	8.00E-05
29.64444	0.34657	0.30347	0.2998	0.27632	0.15044	0.13389	0.11386	0.1615	8.82E-05
31.14444	0.35513	0.32492	0.3265	0.28211	0.16899	0.1474	0.13319	0.17644	9.34E-05
32.89444	0.38004	0.34996	0.34583	0.30357	0.18761	0.16214	0.15399	0.18929	7.82E-05
34.39444	0.42083	0.38988	0.37766	0.32976	0.21553	0.16931	0.17833	0.20924	1.50E-04
36.14444	0.451	0.42665	0.41102	0.34823	0.23936	0.18943	0.19445	0.23121	2.47E-04
37.64444	0.49111	0.46056	0.45351	0.38222	0.27224	0.21294	0.21425	0.25569	2.74E-04
39.39444	0.51728	0.4972	0.4868	0.40949	0.29648	0.23014	0.22787	0.27045	2.56E-04
40.89444	0.5545	0.52714	0.51567	0.43311	0.31419	0.24799	0.24713	0.29207	2.92E-04
42.64444	0.59323	0.54773	0.54368	0.44909	0.32836	0.26515	0.25549	0.30506	4.09E-04
44.39444	0.6212	0.58563	0.5737	0.4755	0.34719	0.28024	0.27408	0.31986	3.47E-04
45.89333	0.65597	0.62375	0.60767	0.48826	0.3702	0.28844	0.29087	0.3374	6.08E-04
47.64333	0.70713	0.67223	0.64871	0.51336	0.39095	0.2897	0.31313	0.36012	8.35E-04
49.14167	0.75288	0.71725	0.69244	0.53825	0.41959	0.31031	0.33353	0.38414	0.00108
50.88972	0.82426	0.77215	0.75003	0.57887	0.4602	0.33434	0.36341	0.41361	0.00133
52.38972	0.87652	0.83089	0.79804	0.61428	0.48923	0.35649	0.38867	0.44307	0.0014
54.13972	0.94527	0.89607	0.87458	0.66318	0.5208	0.38831	0.41366	0.4755	0.00124
55.88972	0.98306	0.96064	0.93668	0.70036	0.5501	0.41436	0.42933	0.49565	0.00114

57.38972	1.06	1.03367	1.00026	0.74032	0.58465	0.43986	0.44855	0.51596	0.00128
59.13972	1.12301	1.09276	1.07692	0.7769	0.60392	0.46201	0.46392	0.53602	0.00124
60.63972	1.20626	1.14633	1.13268	0.81675	0.63557	0.49094	0.48428	0.56445	0.00136
62.38972	1.28918	1.20455	1.18868	0.84646	0.65848	0.50778	0.50738	0.58652	0.0016
63.88972	1.37471	1.28074	1.27408	0.89532	0.69283	0.53255	0.53213	0.61546	0.00162
65.63972	1.44241	1.35446	1.32066	0.92819	0.72384	0.5516	0.55807	0.64672	0.00184
67.13972	1.54195	1.44198	1.41084	0.97504	0.76773	0.58073	0.59579	0.68441	0.0021
68.88972	1.62172	1.51839	1.47243	1.03179	0.80514	0.6037	0.62269	0.7066	0.0019
70.63972	1.72848	1.62262	1.55557	1.0837	0.85393	0.6376	0.65647	0.7417	0.00213
72.13972	1.80786	1.69168	1.62729	1.13608	0.89613	0.66294	0.68323	0.77687	0.0021
73.88972	1.88601	1.77459	1.70508	1.18451	0.93812	0.70083	0.71112	0.80651	0.00213
75.38972	1.9323	1.84716	1.77276	1.23341	0.97532	0.73422	0.73653	0.83511	0.00196
77.13944	2.00516	1.93677	1.84752	1.27405	1.02062	0.77242	0.75711	0.87031	0.00217
78.63944	2.0589	1.98732	1.91977	1.31567	1.05548	0.80312	0.77064	0.8978	0.00211
80.38944	2.1331	2.01494	2.02523	1.36859	1.08688	0.83444	0.80255	0.93638	0.00197
81.88944	2.1557	2.07281	2.05688	1.41857	1.11514	0.85874	0.822	0.96617	0.00168
83.63944	2.19256	2.13124	2.05782	1.47013	1.16515	0.89392	0.85701	1.00212	0.00158
85.38944	2.23593	2.17164	2.10071	1.5153	1.19504	0.9209	0.88714	1.03901	0.00147
86.88944	2.2729	2.1846	2.13259	1.577	1.23163	0.96151	0.92578	1.06566	0.00116
88.63944	2.27382	2.20528	2.13942	1.61148	1.26288	0.99543	0.95666	1.09706	0.00105
90.13944	2.27634	2.21071	2.12413	1.66209	1.30815	1.02607	0.99548	1.12739	8.67E-04
91.88944	2.26027	2.19989	2.13127	1.70817	1.34063	1.04828	1.02661	1.16042	6.55E-04
93.38944	2.22563	2.17577	2.08407	1.74883	1.3773	1.07453	1.05412	1.19905	5.11E-04
95.13944	2.1836	2.15322	2.07722	1.77086	1.38817	1.09131	1.07173	1.22476	3.82E-04
96.88944	2.13645	2.12463	1.99197	1.77793	1.42416	1.12489	1.09436	1.26069	4.36E-04

Supplementary Table S9 Xcelligence Cell Index and *P* values of shKMT2D#2-21a+siSLC2A3 versus shKMT2D#2-21a+siC#1 MIA PaCa-2 cells.

List of Cell Index values derived from the measured impedances and continuously displayed on the Xcelligence Software user interface. Each experimental condition was performed in triplicates. Statistical analyses were performed using one-way ANOVA and the *P* values corresponding to the comparison of siRNA- transfected versus control cells are shown at every single time point. *P* values ≤.05 are marked in red. Differences in Cell Index measurements are significant after 9.9 hours of monitoring.

Table S10

	CAPAN-2								
Time (hours)	**Cell Index shKMT2D#2-21a+siC#1** (n=4)				**Cell Index shKMT2D#2-21a+siSLC2A3** (n=4)				***P* value shKMT2D#2-21a+siSLC2A3 vs shKMT2D#2-21a+siC#1**
0	0	0	0	0	0	0	0	0	
1.64611	-0.00518	0.02887	0.02781	0.05786	0.0328	0.05089	0.05253	0.08391	0.14817
3.39611	0.04612	0.07247	0.03966	0.09728	0.12267	0.11334	0.1014	0.14091	0.01182
4.89611	0.08429	0.10152	0.05783	0.13086	0.1671	0.14826	0.12715	0.16902	0.01723
6.64611	0.09141	0.12081	0.07221	0.15163	0.18629	0.1777	0.14465	0.19019	0.01749
8.14611	0.10227	0.13077	0.08522	0.16305	0.19681	0.19102	0.15491	0.19491	0.0175
9.89611	0.10822	0.14479	0.10014	0.17095	0.20304	0.19155	0.15909	0.20452	0.0243
11.39611	0.11602	0.14908	0.10879	0.18075	0.20815	0.19342	0.15649	0.20683	0.04243
13.14611	0.12673	0.15749	0.11432	0.19297	0.20311	0.19383	0.15725	0.20882	0.08766
14.64611	0.13734	0.16688	0.12238	0.20425	0.20318	0.1959	0.16033	0.20762	0.15637
16.39611	0.13679	0.1788	0.12986	0.21527	0.20663	0.19367	0.16094	0.20704	0.28
18.14611	0.13963	0.18715	0.13867	0.22552	0.20577	0.19182	0.15731	0.21143	0.46563
19.64611	0.13908	0.20014	0.15055	0.24238	0.20772	0.18509	0.15691	0.21236	0.79081
21.39611	0.15199	0.20827	0.16008	0.25841	0.21072	0.18474	0.15826	0.21277	0.91564
22.89611	0.16439	0.22046	0.16757	0.26948	0.20681	0.18091	0.15888	0.2217	0.65516
24.64611	0.17178	0.22879	0.17618	0.27906	0.21022	0.17862	0.15943	0.22131	0.4854
26.14611	0.18233	0.24194	0.18778	0.28877	0.20975	0.17247	0.16625	0.22506	0.31264
27.89611	0.19079	0.25002	0.19631	0.29901	0.21337	0.16567	0.16833	0.22944	0.23377
29.64444	0.19871	0.26096	0.20498	0.31507	0.21382	0.16072	0.16708	0.22533	0.14486
31.14444	0.20416	0.26983	0.20815	0.32568	0.2169	0.15077	0.17228	0.22927	0.13198
32.89444	0.21547	0.27794	0.21468	0.33238	0.21841	0.14868	0.17111	0.22952	0.09304

34.39444	0.2292	0.2868	0.22262	0.32484	0.22026	0.155	0.17275	0.22971	0.05689
36.14444	0.23987	0.29629	0.23187	0.32977	0.21612	0.153	0.17661	0.2296	0.03314
37.64444	0.25174	0.31045	0.24004	0.33863	0.21891	0.14798	0.16968	0.23424	0.02475
39.39444	0.25973	0.3197	0.24715	0.3491	0.21942	0.14642	0.17331	0.23079	0.01765
40.89444	0.27946	0.33161	0.25708	0.36537	0.21993	0.14594	0.17611	0.23741	0.01242
42.64444	0.29606	0.34571	0.26887	0.37698	0.22579	0.14306	0.17781	0.23661	0.00828
44.39444	0.30998	0.35912	0.28344	0.39855	0.22584	0.14444	0.1766	0.24021	0.00591
45.89333	0.32379	0.36613	0.29789	0.40662	0.23146	0.14379	0.1792	0.2435	0.00422
47.64333	0.34235	0.38699	0.31054	0.42459	0.23412	0.14304	0.17697	0.2435	0.00293
49.14167	0.36082	0.39765	0.32663	0.44115	0.23574	0.14884	0.18105	0.24735	0.00187
50.88972	0.37941	0.42018	0.34334	0.46218	0.2378	0.14949	0.18381	0.25057	0.00136
52.38972	0.39413	0.41995	0.36094	0.48011	0.24189	0.15193	0.18888	0.25398	0.00104
54.13972	0.41497	0.43158	0.37869	0.49187	0.24582	0.14865	0.19262	0.25755	7.32891E-4
55.88972	0.43311	0.44867	0.39118	0.50838	0.24916	0.15321	0.19742	0.2592	5.46944E-4
57.38972	0.45302	0.47498	0.41281	0.53264	0.25755	0.15661	0.19852	0.2666	4.56875E-4
59.13972	0.46446	0.49226	0.4216	0.54882	0.26464	0.15728	0.20195	0.26656	4.56939E-4
60.63972	0.47616	0.50906	0.44211	0.5688	0.26744	0.15994	0.20132	0.27022	3.60677E-4
62.38972	0.49577	0.53169	0.46098	0.59144	0.27397	0.15731	0.20344	0.27539	3.3748E-4
63.88972	0.50828	0.54829	0.47664	0.60875	0.27296	0.15572	0.20593	0.27813	2.84032E-4
65.63972	0.5195	0.56537	0.48394	0.63331	0.27788	0.15846	0.21138	0.28111	3.37041E-4
67.13972	0.53669	0.58838	0.49631	0.66112	0.2775	0.1593	0.21224	0.28775	3.56313E-4
68.88972	0.5662	0.61035	0.51694	0.69972	0.28775	0.15971	0.21376	0.28993	3.59497E-4
70.63972	0.584	0.62813	0.54239	0.73745	0.29622	0.16154	0.21927	0.29396	3.72348E-4
72.13972	0.59859	0.65662	0.56239	0.74433	0.30039	0.1621	0.22218	0.29927	2.69419E-4
73.88972	0.61695	0.68457	0.59003	0.79462	0.31094	0.16276	0.22757	0.29996	3.21261E-4
75.38972	0.64572	0.71715	0.62019	0.82155	0.31558	0.16979	0.22811	0.30751	2.27107E-4
77.13944	0.67032	0.75109	0.64926	0.87338	0.32503	0.16878	0.22842	0.30924	2.60778E-4
78.63944	0.70091	0.77414	0.68272	0.91828	0.33496	0.17422	0.23751	0.31654	2.47164E-4
80.38944	0.719	0.817	0.71987	0.96488	0.34224	0.1776	0.24023	0.32514	2.55432E-4
81.88944	0.73772	0.84741	0.76302	0.99968	0.35463	0.17843	0.24233	0.33096	2.3224E-4
83.63944	0.76381	0.88565	0.79464	1.03528	0.36315	0.18018	0.2539	0.33904	2.13786E-4
85.38944	0.79156	0.92192	0.84333	1.08365	0.37281	0.18552	0.25962	0.34019	1.86516E-4

86.88944	0.82089	0.95469	0.89068	1.14278	0.3834	0.18351	0.26606	0.34651	2.03876E-4
88.63944	0.86111	0.98606	0.93542	1.22052	0.39078	0.19116	0.27524	0.35727	2.36421E-4
90.13944	0.88858	1.02288	0.98232	1.2608	0.40177	0.19443	0.27683	0.36444	2.11559E-4
91.88944	0.92279	1.07905	1.0497	1.32095	0.40469	0.19637	0.28283	0.36852	1.79287E-4
93.38944	0.94733	1.12063	1.10369	1.37082	0.41868	0.19939	0.28276	0.37743	1.86388E-4
95.13944	0.98508	1.15215	1.17704	1.46032	0.43141	0.20711	0.28999	0.38536	2.29796E-4
96.88944	1.01497	1.19294	1.25461	1.51927	0.44505	0.21306	0.3005	0.39972	2.39577E-4

Supplementary Table S10 Xcelligence Cell Index and *P* values of shKMT2D#2-21a+siSLC2A3 versus shKMT2D#2-21a+siC#1 CAPAN-2 cells.

List of Cell Index values derived from the measured impedances and continuously displayed on the Xcelligence Software user interface. Each experimental condition was performed in triplicates. Statistical analyses were performed using one-way ANOVA and the *P* values corresponding to the comparison of siRNA- transfected versus control cells are shown at every single time point. *P* values ≤.05 are marked in red. Differences in Cell Index measurements are significant after 36.1 hours of monitoring.

Table S11

Top Networks	
ID Associated Network Functions Score	**Score**
Cell Cycle, Cell Death and Survival, RNA Post-Transcriptional Modification	47
Cell Morphology, Connective Tissue Disorders, Developmental Disorder	44
Cellular Assembly and Organization, DNA Replication, Recombination and Repair, Cell Cycle	43
Cancer, Endocrine System Disorders, Nervous System Development and Function	39
Lipid Metabolism, Small Molecule Biochemistry, Vitamin and Mineral Metabolism	36

Supplementary Table S11 Bioinformatics prediction of the top networks regulated by KMT2D.

The list of the top 5 networks derived from IPA GO algorithms for the KMT2D-regulated genes (Figure 3G). “Score” refers to the numerical value used to rank networks according to how relevant they are to the genes in the input dataset.

Table S12

Relative Hazard ($Surv_1/Surv_2$)	Median Survival Group 2	Total events needed
.90 (1.11)	667 (540)	2828
.85 (1.18)	706 (510)	1189
.80 (1.25)	750 (480)	631
.75 (1.33)	800 (450)	379
.70 (1.43)	857 (420)	247
.67 (1.50)	900 (400)	191
.65 (1.54)	923 (390)	169
.60 (1.67)	1000 (360)	120
.57 (1.75)	1050 (343)	100
.55 (1.82)	1090 (330)	88
.50 (2.00)	1200 (300)	65

Table S12 Sample Size Calculations.

2-tail test has been conducted by the Mayo Clinic Pancreatic Cancer SPORE Biostatistics Core, for sample size calculations (Cohort V). To correctly reject a false null hypothesis, critical values have been set as follows: α .05, Power=.80, 50% allocation per group.

Table S13

KMT2D Expression	Quartile 1 (≤1.743) (N=54)	Quartile 2 (>1.743, ≤3.917) (N=55)	Quartile 3 (>3.917, ≤26.986) (N=55)	Quartile 4 (>26.986) (N=56)	*P* value
Age at Diagnosis					.7052
N	41	45	48	54	
Mean (SD)	64.78 (12.12)	64.53 (11.48)	64.98 (12.61)	63.15 (10.51)	
Median	67.00	66.00	64.50	62.00	
Q1, Q3	55.00, 75.00	55.00, 74.00	53.50, 75.00	57.00, 72.00	
Range	(41.00-88.00)	(37.00-81.00)	(41.00-89.00)	(43.00-92.00)	
Vital Status					.2316
Missing	6	4	4	1	
Alive	8 (16.7%)	6 (11.8%)	2 (3.9%)	6 (10.9%)	
Deceased	40 (83.3%)	45 (88.2%)	49 (96.1%)	49 (89.1%)	
Survival (Days)					
N	30	39	39	50	
Events	25	35	37	44	
Median Survival Days	949.0 (588.0-1239.0)	602.0 (413.0-874.0)	788.0 (409.0-1127.0)	565.0 (487.0-751.0)	
5 Yr Survival Rate	19.1% (4.0%-34.2%)	6.0% (0.0%-14.1%)	17.9% (5.9%-30.0%)	21.4% (9.6%-33.3%)	
Year 5 N at Risk	4	2	7	8	
Sex					.2540
Missing	6	4	4	1	
Female	21 (43.8%)	31 (60.8%)	24 (47.1%)	24 (43.6%)	
Male	27 (56.3%)	20 (39.2%)	27 (52.9%)	31 (56.4%)	
Race					.6593
Missing	9	5	4	1	
1=American Indian/Alaskan Native	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.8%)	
2=Asian/Asian-American	1 (2.2%)	1 (2.0%)	0 (0.0%)	0 (0.0%)	
3=Black/African-American	0 (0.0%)	1 (2.0%)	0 (0.0%)	1 (1.8%)	
5=White	44 (97.8%)	48 (96.0%)	51 (100.0%)	53 (96.4%)	
Usual Adult BMI					.0252
N	35	39	44	51	
Mean (SD)	27.01 (5.12)	27.59 (5.69)	30.21 (6.09)	29.67 (5.27)	
Median	26.63	28.34	29.32	28.80	
Q1, Q3	24.24, 29.23	23.81, 31.46	26.24, 32.03	25.06, 33.66	
Range	(15.31-43.72)	(16.46-38.80)	(20.60-46.18)	(18.88-43.02)	
Usual Adult BMI (<30,30+)					.1108
Missing	19	16	11	5	
<30	28 (80.0%)	27 (69.2%)	24 (54.5%)	32 (62.7%)	
30+	7 (20.0%)	12 (30.8%)	20 (45.5%)	19 (37.3%)	
Weight Loss					.0060
Missing	6	4	4	1	
No	22 (45.8%)	21 (41.2%)	11 (21.6%)	11 (20.0%)	
Yes	26 (54.2%)	30 (58.8%)	40 (78.4%)	44 (80.0%)	
Pounds Lost					.0004
N	48	51	51	55	
Mean (SD)	8.63 (10.71)	13.78 (15.62)	20.10 (19.31)	21.16 (17.58)	

KMT2D Expression	**Quartile 1** (≤1.743) (N=54)	**Quartile 2** (>1.743, ≤3.917) (N=55)	**Quartile 3** (>3.917, ≤26.986) (N=55)	**Quartile 4** (>26.986) (N=56)	***P* value**
Median	5.00	11.00	15.00	20.00	
Q1, Q3	0.00, 13.50	0.00, 22.00	7.00, 30.00	10.00, 30.00	
Range	(0.00-40.00)	(0.00-60.00)	(0.00-85.00)	(0.00-70.00)	
Stage at Surgery					.7224
Missing	26	23	18	8	
IA	0 (0.0%)	0 (0.0%)	1 (2.7%)	0 (0.0%)	
IB	3 (10.7%)	3 (9.4%)	3 (8.1%)	3 (6.3%)	
IIA	5 (17.9%)	7 (21.9%)	13 (35.1%)	15 (31.3%)	
IIB	20 (71.4%)	22 (68.8%)	20 (54.1%)	29 (60.4%)	
IV	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.1%)	

Table S13 Quartile-based correlation of *KMT2D* expression with demographic and clinical characteristics of pancreatic cancer patients (Cohort V).

Pancreatic carcinomas were subdivided in 2 groups: carcinomas with below median (<3.917) *KMT2D* expression and carcinomas with above median (>3.917) *KMT2D* expression. N: number of patients with clinical information; BMI: Body Mass Index. Clinical correlations were examined using the SAS software.

Table S14

Primer	**Sequence**	**Tm (°C)**
FASN Forward	CTT GGC CTT GGG TGT GTA CT	57.4
FASN Reverse	CTG ATC ATC AAG AGC CAC CA	54.6
KMT2D Forward	ATG CAG CCA AGG ACC TAG AA	56.4
KMT2D Reverse	ATG CCT CGA TTC TGC TCT TC	56
KDM8 Forward	TCA GTG CAG AGA GCC AGA GA	54.1
KDM8 Reverse	ATC GGC CTC GTG TAA CAA GT	55.9
KDM4C Forward	TGG ATC CCA GAT AGC AAT GA	53.1
KDM4C Reverse	TGT CTT CAA ATC GCA TGT CA	52.3
KDM2A Forward	CCT CAG TGG CAT CAT CAA GA	54.5
KDM2A Reverse	TTT CAG TCC TGG CAG CCT AT	56.1
KDM5B Forward	CCT TGC CAA ATG GAA AG AAA	51.5
KDM5B Reverse	CTT CCC CAA GAG TTG CCA TA	54.6
KMT2B Forward	ACT TCG AGG ACA TGG AGG TG	55.6
KMT2B Reverse	GCG GCT ACA ATC TCT TCC TG	55.6
KMT2C Forward	GAA TCA CTT CCT GGG GTT GA	54.5
KMT2C Reverse	GGC AAG AGG AAG TTC CAT GA	54.8
RELA Forward	CCA GAC CAA CAA CAA CCC CT	57.6

RELA Reverse	TCA CTC GGC AGA TCT TGA GC	57.0
SETD6 Forward	GCT TTC AGG AAC CAC TGG AG	55.8
SETD6 Reverse	GGC GTT GTG ATT GGC TAA GT	55.8
SLC2A3 Forward	TCC ACG CTC ATG ACT GTT TC	55.1
SLC2A3 Reverse	GCC TGG TCC AAT TTC AAA GA	53.3
SLC2A1 Forward	GTG GAG ACT AAG CCC TGT CG	57.3
SLC2A1 Reverse	CAT AGC CAC CTC CTG GGA TA	55.8
STK11 Exon 6 Forward	TCG AAA TGA AGC TAC AAC ATC	50.7
STK11 Exon 6 Reverse	TTT CAG CAG GTC AGA GAG	51.3
SUV420H1 Forward	TCA ACT GGT CGA GAT ACA GCA	55.6
SUV420H1 Reverse	CTC CAA AGA ACC CAT CTC CA	54.3
TSC1 Forward	CTG GAG GAC TGC AGG AAC AT	56.9
TSC1 Reverse	GAG CAG CAG CTC AGT GTG AC	58.5
β-actin Forward	CCC AGC ACA ATG AAG ATC AA	57.1
β-actin Reverse	ACA TCT GCT GGA AGG TGG AC	53.0
GAPDH Forward	ATG TTC GTC ATG GGT GTG AA	54.4
GAPDH Reverse	GGT GCT AAG CAG TTG GTG GT	57.9

Supplementary Table S14 Primers used in qPCR analysis.

The list of all primer sequences used for real-time PCR analysis.

3. Supplementary Materials and Methods

Cell Cultures and Treatments

Pancreatic cancer cell lines (MIA PaCa-2, PANC-1, CAPAN-1, CAPAN-2, AsPC-1, BxPC-3, HPAF-II and CFPAC-1) were purchased from ATCC. MIA PaCa-2 and PANC-1 were grown in DMEM, CAPAN-1 and CFPAC-1 in IMDM, CAPAN-2 in McCoy’s 5A, AsPC-1 and BxPC-3 in RPMI 1640 (Gibco), HPAF-II in EMEM (ATCC), all supplemented with 10% FBS (Gemini Bioproducts). In the case of MIA PaCa-2, medium was also supplemented with 2.5% horse serum (Gibco). STR analysis has been performed as a method for human cell line authentication.

Pancreatic cancer cells were plated ($2.5*10^5$ cells in 35mm dishes) and after 24 h were treated with 1 or 2 µM of 5-AZA-CdR (A3656, Sigma-Aldrich). Cells were incubated for 0 to 4 days before DNA, RNA or protein extraction.

Around 80% confluent MIA PaCa-2 cells were pretreated with 100 nM rapamycin (#1292, TOCRIS) for 24 h. Cell lysates were analyzed by IB analysis.

KMT2D-silenced MIA PaCa-2 cells were treated with the inhibitors of NF-κB activation, Tanshinone IIA (4426) and RO 106-9920 (1778) purchased from TOCRIS, for 24 h before RNA extraction.

MIA PaCa-2 cells were plated in 96-well dishes ($1*10^3$ cells/well) and treated with a) different doses of lipids including: cis-13,16-Docosadienoic (Hebei Zhongzhuo Import and Export Trade Co, Ltd), 13Z,16Z,19Z-Docosatrienoic (SC-200782, Santa Cruz Biotechnology, Inc) or 4,10,13,16-Docosatetraenoic acid (D3659, Sigma-Aldrich) and b) the lipid synthesis inhibitors SC 26196 (4189, TOCRIS) and SB 204990 (4962, TOCRIS). Cells were incubated for 1, 3 or 5 days before measurement of cell viability and for 3 days before performing *in vitro* invasion assays.

Real-Time PCR Analysis

RNA purified from cells and tissues with TRIZOL (Life Technologies) was reverse-transcribed to form cDNA using the iSCRIPT RT Supermix (Bio-Rad), which was subjected to real-time PCR analysis using the iQ SYBR Green Supermix (Bio-Rad) on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad). The primer sequences used for real-time PCR were acquired from previous studies [3] or designed using the NCBI Nucleotide Database (http://www.ncbi.nlm.nih.gov/nuccore), Primer3 v.0.4.0

(http://bioinfo.ut.ee/primer3-0.4.0) and UCSC In-Silico PCR (http://genome.ucsc.edu/cgi-bin/hgPcr) and are included in Table S14. Gene expression levels were normalized to the levels of GAPDH and β-actin. Normalized gene expression levels were quantified to the respective control. Bars represent means ± SE; experiments were performed in quadruplicates for each condition.

For Kaplan-Meir studies in patients' Cohort V, transcript expression for human KMT2D was determined using PerfeCTa SYBR Green FastMix (Quanta BioSciences Inc.,Gaithersburg, MD) and the following primer sets: KMT2D, 5'- AACCATATCGGCCTGGCATT -3' (forward) and 5'- CAGCAGGTATCACCTCGTCG -3' (reverse); 18S, 5'- AACCCGTTGAACCCCATTCGTGAT -3' (forward) and 5'- AGTCAAGTTCGACCGTCTTCTCAG -3' (reverse). 500 ng RNA was reverse transcribed using High Capacity cDNA synthesis kit (Applied Biosystems). 10 ng cDNA from each sample was used for qPCR analysis. Amplification was performed using the C1000 Thermal Cycler (Bio-Rad). RNA levels were normalized by comparison with the corresponding housekeeping RNA level in the same sample. The results are calculated following the 2ΔCt (where ΔCt represents the difference in threshold cycles between the target and control gene).

Whole Exome Sequencing

Genomic DNA was isolated from human MIA PaCa-2 and CAPAN-2 pancreatic cancer cell lines using QIAamp DNA Mini Kit (51304) and used for Whole Exome Sequencing that was conducted at the UCLA Clinical Microarray Core. The library construction was performed using the SeqCap EZ System from NimbleGen according to the manufacturer's instructions. Briefly, genomic DNA was sheared, size selected to roughly 300 base pairs, and the ends were repaired and ligated to specific adapters and multiplexing indexes. Fragments were then incubated with SeqCap biotinylated DNA baits after LM-PCR and the hybrids were purified using streptavidin-coated magnetic beads. After amplification of 18 or less PCR cycles, the libraries were then sequenced on the HiSeq 3000 platform from Illumina, using 100-bp pair-ended reads. The sequence data were aligned to the GRCh37 human reference genome using BWA v0.7.7-r411. PCR duplicates were marked using MarkDuplicates program in Picard-tools-1.115 tool set. GATK v3.2-2 was used for INDEL (insertions and deletions) realignment and base quality recalibration. Exome coverage was calculated using the bedtools. Samtools was used to call the SNVs (single nucleotide variants) and small INDELs. Varscan2 was used to call the somatic SNVs. All variants were annotated using the Annovar program.

Immunoblot Analysis

Total cell extracts were separated by SDS-PAGE and transferred to PVDF membranes following standard procedures. Frozen tissue biopsies were homogenized using RIPA buffer (Cell Signaling Technology), followed by sonication. In the case of KMT2D, protein levels were monitored by 5% SDS-PAGE using modified long apparatus for extended running time. Transfer time reached 24 h at 4^0C and the buffering system contained 15% methanol. The following antibodies were used for immunoblot analysis: KMT2D (R0118-1, Abiocode), mono-methyl-Histone H3 (Lys4) (5326), di-methyl-Histone H3 (9725), tri-methyl-Histone H3 (Lys4) (9751), Histone H3 (14269), FASN

(3180), phospho-mTOR (Ser2448) (5536), mTOR (2983), RICTOR (2114), phospho-NF-κB p65 (Ser536) (3033), NF-κB p65 (8242), CREB (9104) (Cell Signaling Technology). The protein levels that corresponded to the immunoreactive bands were quantified using the Scion Image analysis software (Scion Corp., Frederick, MD).

Cell Viability Assay

MIA PaCa-2 cells were plated in quadruplicates and treated with exogenously added lipids in 96-well plate ($1*10^3$ cells/well) and cell growth was assessed 1, 3 or 5 days later using the CellTiter Glo Luminescence Cell Viability Assay (Promega). Data are expressed as mean fluorescence (arbitrary units) ± S.D.

Anchorage-Independent Cell Growth Assay

Triplicate samples of $25*10^3$ MIA PaCa-2 cells from each treatment were assayed in 48-well plates for colony formation using the CytoSelect Cell Transformation kit (Cell Biolabs, Inc). Colorimetric quantitation of colonies has been performed according to the manufacturer’s instructions. Data were expressed ± SE of the mean of at least 2 independent experiments.

Invasion assay

Invasion in matrigel has been conducted by using standardized conditions with BD BioCoat Matrigel invasion chambers (354480; BD Biosciences) according to the manufacturer's protocol. Assays for MIA PaCa-2 cells were conducted using 10% FBS-containing media as chemoattractant. Noninvading cells on the top side of the membrane were removed, whereas invading cells were fixed and stained with .1% crystal violet, 22 h after seeding. The cells that migrated through the filter were quantified by counting the entire area of each filter divided in four fields, using a grid and an Evos microscope at a X20 magnification. Data are expressed as the mean number of invading cells per field ± SD.

Dynamic Monitoring of Cell Proliferation

Real-time cell proliferation analysis based on the application of electrical cell substrate impedance changes (https://lifescience.roche.com/wcsstore/RASCatalogAssetStore/Articles/BIOCHEMICA_4_08_p14-16.pdf) was performed using the xCELLigence RTCA instrument (ACEA Biosciences). The presence of cells affects the local ionic environment at the electrode solution interface. Cell status is represented by a dimensionless parameter termed Cell Index, which is derived as the relative change in measured electrical impedance, after subtraction of the background measurements from media alone. Local ionic environment varies according to cell size, cell morphology and strength of adhesion of the cells to the surface of the electrode, resulting in changes of the electrode impedance. $5*10^3$ cells were seeded in quadruplicates of an E-Plate 96 with interdigitated microelectrode arrays integrated in the bottom of each well. Subsequently, the E-Plate 96 was mounted on the SP Station of the xCELLigence RTCA system which is placed in a standard temperature-controlled CO_2 incubator under humidity saturation. The RTCA Software preinstalled on the RTCA control unit allows automatic selection of wells for measurement and real-time data acquisition within preprogrammed 15 min time intervals. Bars represent means ± SD; experiments were performed in quadruplicates for each condition.

Immunohistochemistry and Digital Pathology Analysis

For immunohistochemical analysis of KMT2D in matched normal and cancer human tissues, deparaffinized 5-μm sections were incubated sequentially in accordance with the instructions of the LSAB kit (DAKO Corporation). For digital automated morphometry, the immunohistochemically stained sections were digitized at 40x magnification using an Aperio Scanscope CS (Aperio). The final immunohistochemical score was calculated from a combination of the intensity and percentage scores [4]. Antigen retrieval was performed by incubating the slides in boiling .01% sodium citrate pH 6.0 for 5 min. The endogenous peroxidase activity was inhibited by immersing the slides in 3% H_2O_2-methanol for 25 min and the background nonspecific binding was reduced by incubating with 1% BSA in PBS for 60 min. The slides were incubated overnight with antibody against KMT2D (1:200) (HPA035977, Sigma-Aldrich). In order to reduce the variability, all samples from each group were processed at the same time in a single experiment using a single batch of diluted antibody. The slides were then washed 5 times in PBS, followed by sequential incubations with biotinylated secondary antibody for 30 min at RT, streptavidin-HRP conjugate for 30 min at RT and 3,3'-diaminobenzidine tetra-hydrochloride (liquid DAB) for 3 min in the dark. The reaction was arrested with distilled water and the slides were counterstained with hematoxylin. Thereafter, the tissues were washed in tap water for 5 min, dehydrated through ethanol baths (70, 90 and 100%) and xylene. Slides were finally mounted with E-2 Mount medium (Shandon lab).

The Aperio Scanscope CS obtains 40X images with a spatial resolution of 0.45 μm/pixels. The images were reviewed using an ImageScope (Aperio). Once the areas were recorded (500 μm for each tissue), they were sent for automated image analysis using the Spectrum Software V11.1.2.752 (Aperio). For the within tissue intensity, an algorithm was developed to quantify the total or nuclear protein expression. The output from the algorithm gives a number of quantitative measurements, namely the intensity, concentration and percentage of positive staining. The quantitative scales for the intensity and percentage were categorized into 4 and 5 classes, respectively, after the cut-off values were determined. The staining intensity was categorized as 0 (no staining), 2+ (moderate) and 3+ (strong).

All other immunostainings of formalin fixed paraffin embedded (FFPE) sections have been performed by the Translational Pathology Core Laboratory, UCLA. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 min. Heat-induced antigen retrieval was carried out for all sections in .01M Citrate buffer, pH=6.0 by using a Biocare decloaker at 95^{o}C for 25 min. The slides were incubated for 1h with antibodies against KMT2D (1:200) (HPA035977, Sigma-Aldrich), SLC2A3 (1:100) (20403-1-AP, Proteintech), phosphorylated NF-κB p65 (536) (1:100) (ab86299, Abcam) and Ki-67 (1:100) (M7240, Agilent). The signal was detected using Mach3 rabbit, HRP conjugated polymer for 30 min (Biocare Medical) and visualized with the diaminobenzidine reaction. Images were further captured with an Axio Imager.Z1 upright microscope (Carl-Zeiss).

HM450 Methylation array

For global methylation profiling, the Illumina Infinium HumanMethylation450 (HM450) BeadChIP has been used (Illumina, San Diego, CA). Bisulfite conversion has been performed on 1 µg of genomic DNA from each sample using the EZ-96 DNA Methylation Kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. Bisulfite-converted DNA was whole genome amplified and enzymatically fragmented prior to hybridization to BeadChIP arrays. The oligomer probe designs of HM450 arrays follow the Infinium I and II chemistries, in which locus-specific base extension follows hybridization to a methylation-specific oligomer. The level of DNA methylation at each CpG locus was scored as beta (β) value calculated as (M/(M+U)), ranging from 0 to 1, with 0 indicating no DNA methylation and 1 indicating fully methylated DNA. The data were extracted using Illumina Genome Studio Methylation Module and quantile normalized using 'preprocesscore' R package (https://cran.r-project.org/). Of the 485,577 CpG probes on the array, we filtered out probes with high detection *P* values, probes with a SNP within 10 base pairs of the target CpG [5] and repeat regions and probes on X and Y chromosomes, leaving 371,478 probes. The term 'hyper-methylation' was used when there was an increased DNA methylation in patients compared to controls and, the term 'hypo-methylation' was used when we observed a decreased DNA methylation in patients compared to controls.

For statistical and bioinformatics analyses, Wilcoxon rank-sum tests were conducted to compare methylation array data between pancreatic cancer patients and healthy controls. Magnitude of DNA methylation changes was assessed using methylation beta values. Correction for multiple comparisons was performed using FDR (Benjamini-

Hochberg) approach. A corrected *P* value, denoted as, ‘q’≤.05 was considered significant. The mean difference in betas, associated *P* and q values for chromatin modifiers are presented in Table EV1.

Targeted Bisulfite Sequencing and Data Analysis

Next-generation sequencing for the evaluation of DNA methylation at single-nucleotide resolution has been conducted by Zymo Research Corporation, Irvine, CA. Assays were designed targeting CpG sites in the specified ROI using primers created with Rosefinch, Zymo Research’s proprietary sodium bisulfite converted DNA-specific primer design tool. The primer sequences used are as follows: (Forward: TTTAGTTTATGTTTTTGTGTTAGGATTAGAA, Reverse: AATAAACATATAAATCTCTTTCTTAACACCAA). Sequence reads were aligned back to the reference genome using Bismark (http://www.bioinformatics.babraham.ac.uk/projects/bismark/), an aligner optimized for bisulfite sequence data and methylation calling (Krueger & Andrews, 2011). The methylation level of each sampled cytosine was estimated as the number of reads reporting a C, divided by the total number of reads reporting a C or T. Following primer validation, provided samples were bisulfite converted using the EZ DNA Methylation-LightningTM Kit (D5030, Zymo Research) according to the manufacturer’s instructions. Multiplex amplification of all samples using ROI specific primer pair and the Fluidigm Access ArrayTM System was performed according the to the manufacturer’s instructions. The resulting amplicons were pooled for harvesting and subsequent barcoding according to the Fluidigm instrument’s guidelines. After barcoding, samples were purified using ZR-96 DNA Clean & Concentrator™-5 (D4023, Zymo Research) and then prepared for massively parallel sequencing using a MiSeq V2 300bp Reagent Kit and paired-end sequencing protocol according to the manufacturer's guidelines. Sequence reads were identified using standard Illumina base-calling software and then analyzed using a Zymo Research proprietary analysis pipeline. Low quality nucleotides and adapter sequences were trimmed off during analysis QC. Paired-end alignment was used as default. Index files were constructed using the *bismark_genome_preparation* command and the entire reference genome. The --*non_directional* parameter was applied while running Bismark. All other parameters were set to default. Nucleotides in primers were trimmed off from amplicons during methylation calling.

Plasmid Construction and *in vitro* Methylation

Linear 300 bp DNA fragments between nt: -179 and +122 relatively to the transcription start site in the human *KMT2D* genomic region were constructed by Genewiz either in the wild type form (unmodified), or modified by a C to A mutation at CpG sites -29 (Mut 1) or +145 (Mut 2) or both (double Mut). Artificial SacI and HindIII restriction sites have been incorporated using Native Taq Polymerase (18038-018, Life Technologies) and the following primers: sense, 5'-GTAGATCAGAGCTCACTTTCTTG-3'; antisense, 5'-CTAGTCATAAGCTTTCCTTGTGC- 3'. The resulting PCR products were subsequently cloned into the pCR 2.1-TOPO vector (450641, Life Technologies) using the TOPO TA cloning kit (Life Technologies). The pCR 2.1 TOPO plasmids containing the *KMT2D* genomic inserts (hereafter referred to as TOPO*KMT2D*) were linearized with SacI

(R3156M, New England Biolabs) and *in vitro* methylated using SssI methylase (M0226L, New England Biolabs), which nonspecifically methylates all CpG dinucleotides. The efficiency of *in vitro* methylation was confirmed by resistance to cleavage by the methylation-sensitive restriction enzyme HpaII (R0171L, New England Biolabs) that has recognition site(s) in the analyzed regions and no sites within the sequence of pCR 2.1 TOPO vector. The linearized methylated and unmethylated TOPO*KMT2D* vectors were then digested with HindIII (R3104T, New England Biolabs) to excise the *KMT2D* inserts. After fractionation on a 1.8 % agarose gel, the DNA bands corresponding to 300 bp were cut from the gel, isolated using NucleoSpin® Gel and PCR Clean-up columns (Macherey-Nagel). To determine whether global or site-specific CpG methylation of the *KMT2D* genomic region affected gene expression in a reporter gene construct, the methylated and unmethylated control fragments were then ligated into the pGL4.82 [hRluc/Puro] Vector (E750A, Promega) between the SacI and HindIII restriction sites. The pGL4.82 [hRluc/Puro] plasmid is designed for high expression and reduced anomalous transcription. The vector encodes the luciferase reporter gene *hRluc* but lacks eukaryotic promoter and enhancer sequences. T4 DNA Ligase (M0202S, New England Biolabs) was used to perform the ligation reaction according to the manufacturer's instructions, at 1:3 vector to insert ratio. The efficiency of ligation and equivalence of incorporated DNA into the methylated and unmethylated constructs were confirmed by agarose gel electrophoresis. For further validation, DNA sequence analysis across the multiple cloning site region located upstream of the *hRluc* gene was performed using RVprimer3 clockwise primer (E448A, Promega) by Genewiz. The effect of total methylation on the transcriptional activity of the inserted *KMT2D* fragments was expressed as the relative change in reporter gene activity. Data were presented as the mean of Luminescence Units ± SE of 3 independent experiments performed in triplicates.

Chromatin Immunoprecipitation, Sequencing and Analysis

Chromatin immunoprecipitation was carried out using the SimpleChIP Plus Enzymatic Chromatin IP Kit (Cell Signaling Technology). Briefly, the chromatin fragments, derived from siC#1, siKMT2D#1 and siKMT2D#2 treated cells, were immunoprecipitated with antibody against tri-methyl-Histone H3 (Lys4) (9751, Cell Signaling Technology) at a ratio 1:50 or 1:100, respectively. After purification, libraries for next generation sequencing were prepared using NEBNext® ChIP-seq Library Prep Master Mix Set for Illumina® (E6240, New England BioLabs) and further analyzed using Illumina NextSeq500 system (single-end 75bp protocol). Both ChIP-seq and Bioinformatics Analyses were performed by the Center for Cancer Computational Biology, Dana-Farber Cancer Institute, Boston, MA. Sequencing reads in fastq-format were aligned to the UCSC hg19 reference genome using BWA (version 0.7.9a bwa mem with default options). Duplicate reads were removed with Picard tools (v. 1.115) MarkDuplicates and were filtered to retain only primary alignments with samtools (v0.1.19, view command with -F 0x100 flag). ChIP-seq peaks were called using HOMER (v4.7) findPeaks by selecting matched input samples with default settings (Poisson *P* value of <=1E-4 and fold change >=4.0). Histone option was employed. Further, peaks were annotated using HOMER's annotatePeaks utility using the hg19 annotations database provided with the software. Analysis of differentially bound peaks was performed using HOMER's getDifferentialPeaks command, which examines the distributions of reads to determine enrichment in a particular condition (Poisson *P* value <1E-4, fold-change >=4.0), suggesting a differentially bound peak. Plots of the distribution of distances to the annotated transcription start site were created directly from the annotated peak data provided as output by HOMER. Similar approach was applied for the pie charts representing the distribution of annotated peak features. Enrichment plots were constructed by comparing the read density (number of reads divided by the length of the peak region) in each of the experimental conditions for each set of peak regions. Read densities were found in both siC#1and siKMT2D conditions for all peak regions in the siKMT2D condition; enrichment values were calculated as the scaled difference between the densities. The increase in H3K4me3 read density (comparing siKMT2D to siC#1) was tested with a one-sided Wilcoxon signed-rank test (*P*<.001). Similarly, enrichment values were calculated in both conditions for the peak regions found in siC#1 condition (*P*<.001).

Luciferase Assay

MIA PaCa-2 cells were transfected with the untreated or CpG MSssl-treated *KMT2D*/*hRluc* constructs and the pGL4.51 [luc2/CMV/Neo] (E1320, Promega) containing synthetic firefly luciferase *luc2* gene. 48 h later luciferase activity was measured using the Dual Luciferase Reporter Assay System (E1960, Promega). Data were expressed ± SE of the mean of 3 independent experiments.

In order to assess whether *STK11* represents a direct transcriptional KMT2D target, MIA PaCa-2 cells were transfected with the LightSwitch RenSP reporter vector carrying human STK11 promoter (S714439, SwitchGear Genomics) and the pLenti CMV Puro LUC (w168-1) (17477, Addgene) containing Luc reporter gene. At 24 h, the cells were transfected with siC#1 or siKMT2D#2 and 48 h later luciferase activity was measured using the Dual Luciferase Reporter Assay System (Promega). Data were expressed ± SE of the mean of 3 independent experiments.

Metabolic Profiling

Cellular metabolic rates were measured using a XF24-3 Analyzer (Seahorse Biosciences) by the Cellular Bioenergetics Core, UCLA. Cells were plated as a confluent monolayer in the Seahorse plate and left undisturbed for 24 h. Bioenergetic parameters were obtained in basal and after sequential injection of an ATPase inhibitor oligomycin (oligo), a mitochondrial uncoupler (FCCP) and mitochondrial inhibitors rotenone and myxothiazol (RM) in pancreatic cancer cells. Bars represent means ± SD; experiments were performed in quadruplicates for each condition.

Lipids extraction from a) human cancer cells b) tumor tissues from mice bearing human pancreatic cancer xenografts and c) human biopsies, reconstitution in the solvent system suitable for analysis and quantitative evaluation of altered lipid profiles by LC-MS analysis were performed by the Lipidomics Core Facility, Wayne State University. Lipid classes currently analyzed: FAs and total cholesterol. Bars represent means ± SD; experiments were performed in triplicates for each condition.

NADP/NADPH-Glo™ Assay

The bioluminescent homogeneous NADP/NADPH-Glo™ Assay (G9081, Promega) was used for detecting total reduced nicotinamide adenine dinucleotides phosphates (NADPH) in cells pretreated with siC#1, siKMT2D#1 or siKMT2D#2, following the manufacturer's protocol. Bars represent means ± SD; experiments were performed in triplicates for each condition.

Lactate Assay

The Lactate Assay Kit (MAK064, Sigma) was used to determine the lactate production in cells pretreated with siC#1, siKMT2D#1 or siKMT2D#2 based on an enzymatic assay, which results in a colorimetric (570 nm) product, proportional to the lactate present, according to manufacturer's instructions. Bars represent means ± SD; experiments were performed in triplicates for each condition.

Glucose Uptake Assay

The Glucose Uptake Assay Kit (MAK083, Sigma) was used to determine the glucose uptake in cells pretreated with siC#1, siKMT2D#1 or siKMT2D#, according to manufacturer's instructions. The glucose analogue, 2-deoxyglucose (2-DG) used, is taken up by cells and phosphorylated by hexokinase to 2-DG6P. 2-DG6P cannot be further metabolized and accumulates in cells, directly proportional to the glucose uptake by cells. Briefly, 2-DG uptake is determined by a coupled enzymatic assay in which the 2-DG6P is oxidized, resulting in the generation of NADPH, which is then determined by a recycling amplification reaction in which the NADPH is utilized by glutathione reductase in a coupled enzymatic reaction that produces glutathione. Glutathione reacts with DTNB to colorimetric product TNB, which is detected at 412 nm. Bars represent means ± SD; experiments were performed in triplicates for each condition.

Cholesterol Uptake Cell-based Assay

Cholesterol Uptake Cell-based Assay Kit (Cayman Chemical) was used to study cellular cholesterol trafficking, following the manufacturer's protocol. Cells were treated with siC#1 or siKMT2D#2 in culture medium containing 20 μg/ml NBD Cholesterol and incubated for 72 h. Detection of cholesterol uptake was assessed by fluorescence microscopy using Axio Observer.D1 inverted microscope (Carl-Zeiss).

Patient Samples

RNA and DNA were extracted from 'normal' (adjacent non-tumoral) and PDAC tissues using TRIZOL (Life Technologies) and (QIAamp DNA Mini Kit, Qiagen), respectively. Samples originating from Cohort I were used for Gene expression profiling that was conducted at the UCLA Clinical Microarray Core, while DNA methylation analysis using Infinium HumanMethylation450 BeadChIP assay has been performed at the Translational Genomics Core, Cambridge, MA. For validation of the Gene expression array data, tissues originating from Cohorts II and III were subjected to RT-qPCR analysis., while FFPE tissues (Cohort IV) were subjected to immunohistochemical analysis. For correlation of *KMT2D* expression with overall patient survival, human pancreatic tumors (Cohort V) were approved by the institutional review boards of Mayo

School of Medicine and informed consent was obtained from all patients prior to tissue procurement and subsequent analysis.

Statistical Analyses

Quantitative data were expressed as means ± SD or SE of the mean, as indicated, or as boxes and whiskers (minimum-to-maximum), using Origin 9.1 Software. Statistical analyses were performed using one-way ANOVA or Pearson correlation. P values of <.05 were considered statistically significant.

Clinical correlations were examined using the SAS software. The Kaplan-Meier test was used for univariate survival analysis. The Cox proportional hazard model was used for multivariate analysis and for determining the 95% confidence interval.

4. Supplementary References

1. Badea L, Herlea V, Dima SO, Dumitrascu T, Popescu I. Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. Hepato-gastroenterology 2008;**55**(88):2016-27
2. Segara D, Biankin AV, Kench JG, et al. Expression of HOXB2, a retinoic acid signaling target in pancreatic cancer and pancreatic intraepithelial neoplasia. Clinical cancer research : an official journal of the American Association for Cancer Research 2005;**11**(9):3587-96 doi: 10.1158/1078-0432.ccr-04-1813[published Online First: Epub Date]|.
3. Vorvis C, Hatziapostolou M, Mahurkar-Joshi S, et al. Transcriptomic and CRISPR/Cas9 technologies reveal FOXA2 as a tumor suppressor gene in pancreatic cancer. American journal of physiology. Gastrointestinal and liver physiology 2016;**310**(11):G1124-37 doi: 10.1152/ajpgi.00035.2016[published Online First: Epub Date]|.
4. Kim BW, Cho H, Chung JY, et al. Prognostic assessment of hypoxia and metabolic markers in cervical cancer using automated digital image analysis of immunohistochemistry. Journal of translational medicine 2013;**11**:185 doi: 10.1186/1479-5876-11-185[published Online First: Epub Date]|.
5. Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer cell 2010;**17**(5):510-22 doi: 10.1016/j.ccr.2010.03.017[published Online First: Epub Date]|.