ADVANCED SCIENCE
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Supporting Information

for *Adv. Sci.*, DOI 10.1002/advs.202300548

A T Cell-Engaging Tumor Organoid Platform for Pancreatic Cancer Immunotherapy

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Supplementary Information

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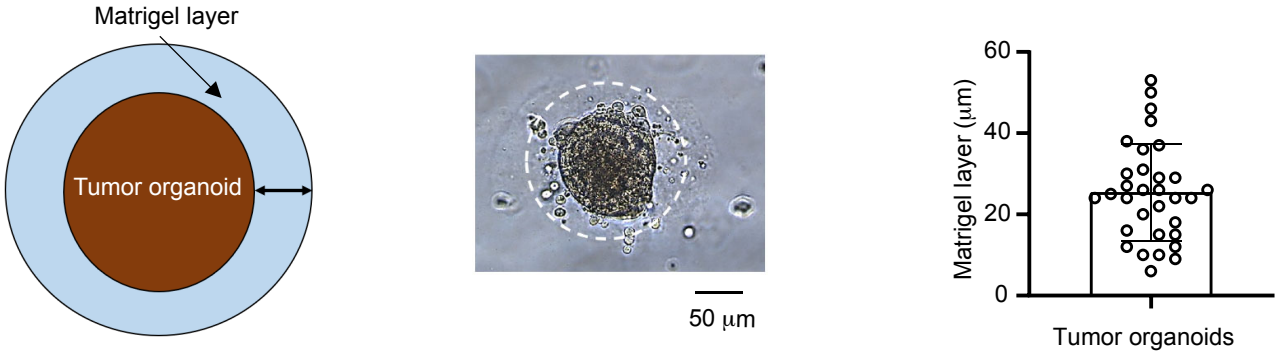
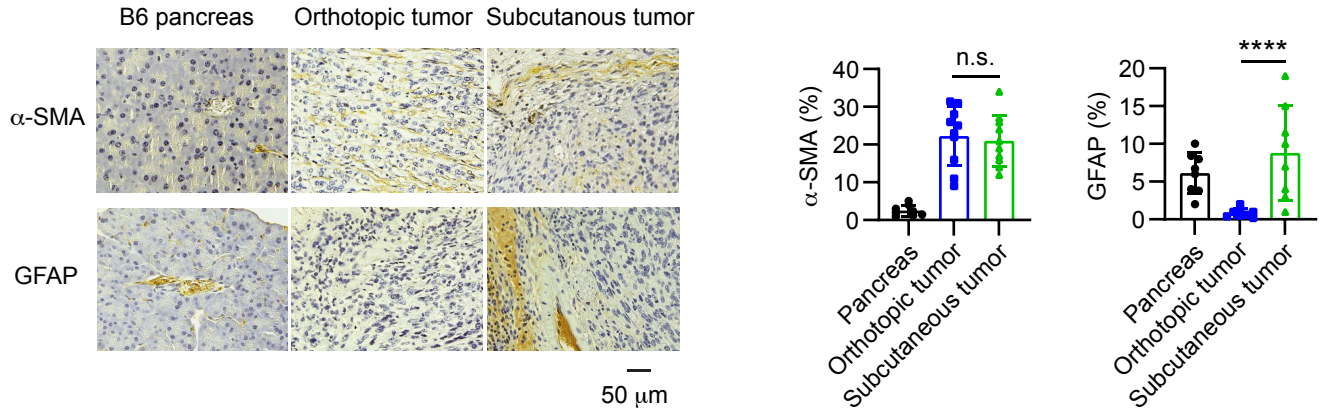
Inventory of Supplementary Information

Fig. S1

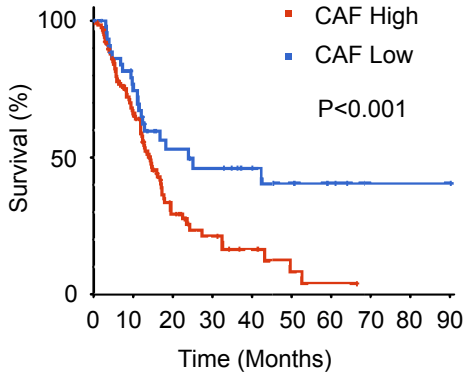
A


Matrigel layer
Tumor organoid
50 μm
Matrigel layer (μm)
60
40
20
0
Tumor organoids

B


B6 pancreas
Orthotopic tumor
Subcutanous tumor
α-SMA
GFAP
50 μm
α-SMA (%)
n.s.
Pancreas
Orthotopic tumor
Subcutaneous tumor
GFAP (%)
****

C


CAF High
CAF Low
P<0.001
Survival (%)
Time (Months)

D

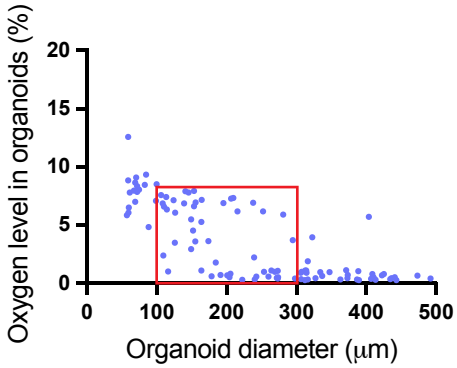

Oxygen level in organoids (%)
Organoid diameter (μm)

Figure S1. Characterization of mouse KPC tumors and derived tumor organoids

(A), T cell-incorporated KPC tumor organoids. Images were captured under a phase-contrast light microscope (Leica DMi1). Graphic illustration of a tumor organoid with a thin layer of Matrigel and quantification of the Matrigel layer thickness is shown.

(B), Immunohistochemistry (IHC) staining of C57BL/6 mouse pancreas, mouse orthotopic and subcutaneous KPC tumors, and histogram analysis of the αSMA or GAFP positive cells from the IHC images (sample size per group: n=8). The data were presented as mean ± SD by One-way ANOVA test. ****, $p < 0.0001$; ns, no significance. Scale bar = 50 μm.

(C), Progression free survival of human PDA with high and low CAFs, plotted from the cBioPortal database (PDA TCGA, Firehose Legacy). Expression levels of CAF genes (ACTA2, S100A4, PDGFRA, PDGFRB) were used to determine the CAF status in PDA tumors.

(D), Oxygen levels in mouse T cell-incorporated KPC tumor organoids with different sizes.

Fig. S2

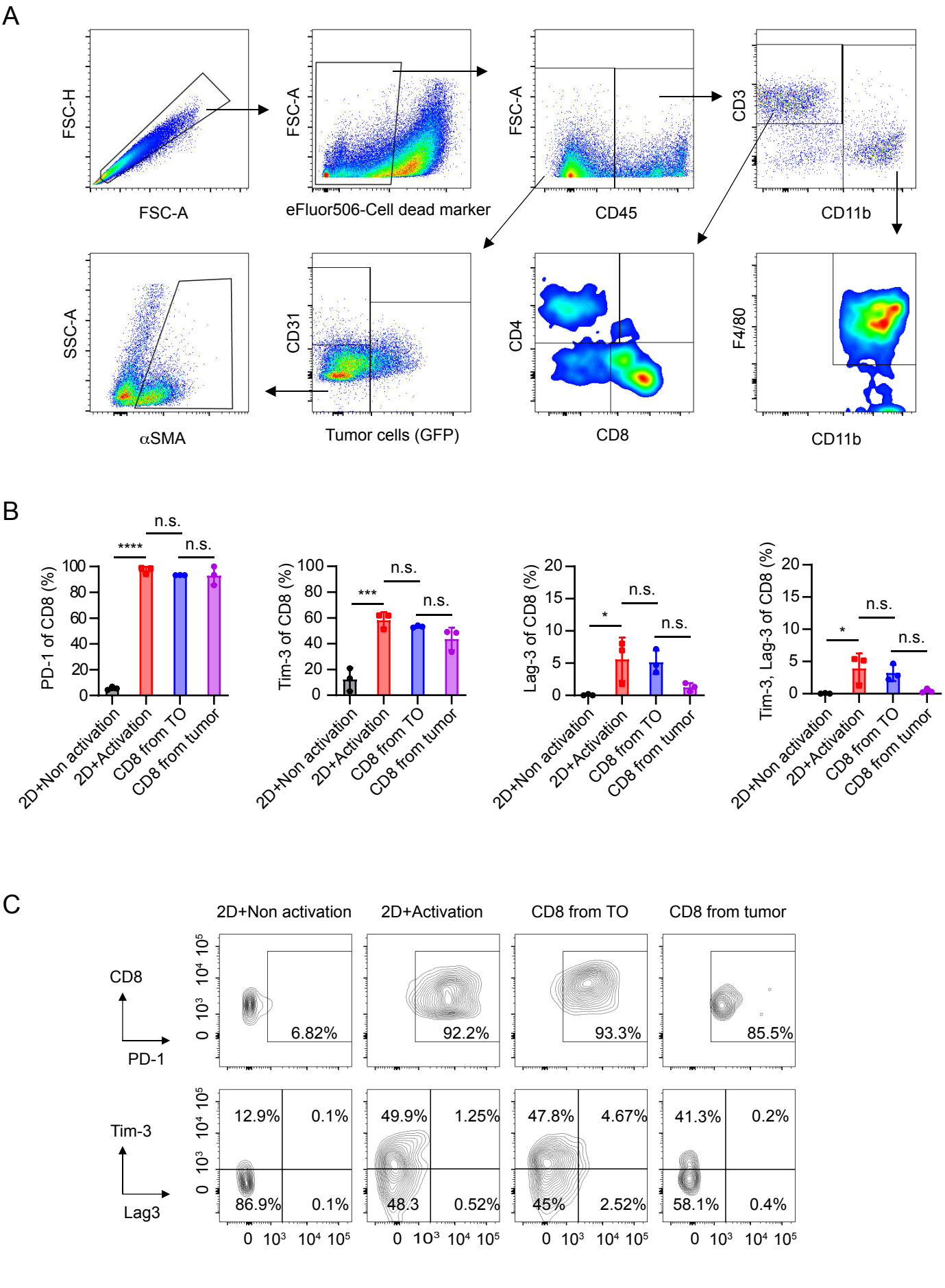

A
FSC-H
FSC-A
FSC-A
eFluor506-Cell dead marker
FSC-A
CD45
CD3
CD11b
SSC-A
αSMA
CD31
Tumor cells (GFP)
CD4
CD8
F4/80
CD11b
B
PD-1 of CD8 (%)
Tim-3 of CD8 (%)
Lag-3 of CD8 (%)
Tim-3, Lag-3 of CD8 (%)
n.s.
****
***
*
2D+Non activation
2D+Activation
CD8 from TO
CD8 from tumor
C
2D+Non activation
2D+Activation
CD8 from TO
CD8 from tumor
CD8
PD-1
Tim-3
Lag3
6.82%
92.2%
93.3%
85.5%
12.9%
0.1%
86.9%
0.1%
49.9%
1.25%
48.3
0.52%
47.8%
4.67%
45%
2.52%
41.3%
0.2%
58.1%
0.4%

Figure S2. Analysis of T cells incorporated in the KPC tumor organoids

(A), Flow cytometry gating strategy for cell subtypes in the T cell-incorporated KPC tumor organoids.

(B), Quantification of PD-1, Tim-3 or Lag-3 positive CD8 T cells, isolated from the spleen of OT-I mice or KPC tumor-bearing mice (sample size per group: n=3). Exhaustion (PD-1, Tim-3 or Lag-3) of CD8 T cells from OT-I mouse with or without CD3 and CD28 antibody activation in 2D culture, CD8 T cells from the tumor organoids, and CD8 T cells from the original KPC tumors was measured and analyzed. Data were presented as mean ± SD by One-way ANOVA test. *, $p < 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$; n.s., no significance.

(C), Flow cytometry analysis of the PD-1, Tim-3, or Lag-3 positive CD8 T cells.

Fig. S3

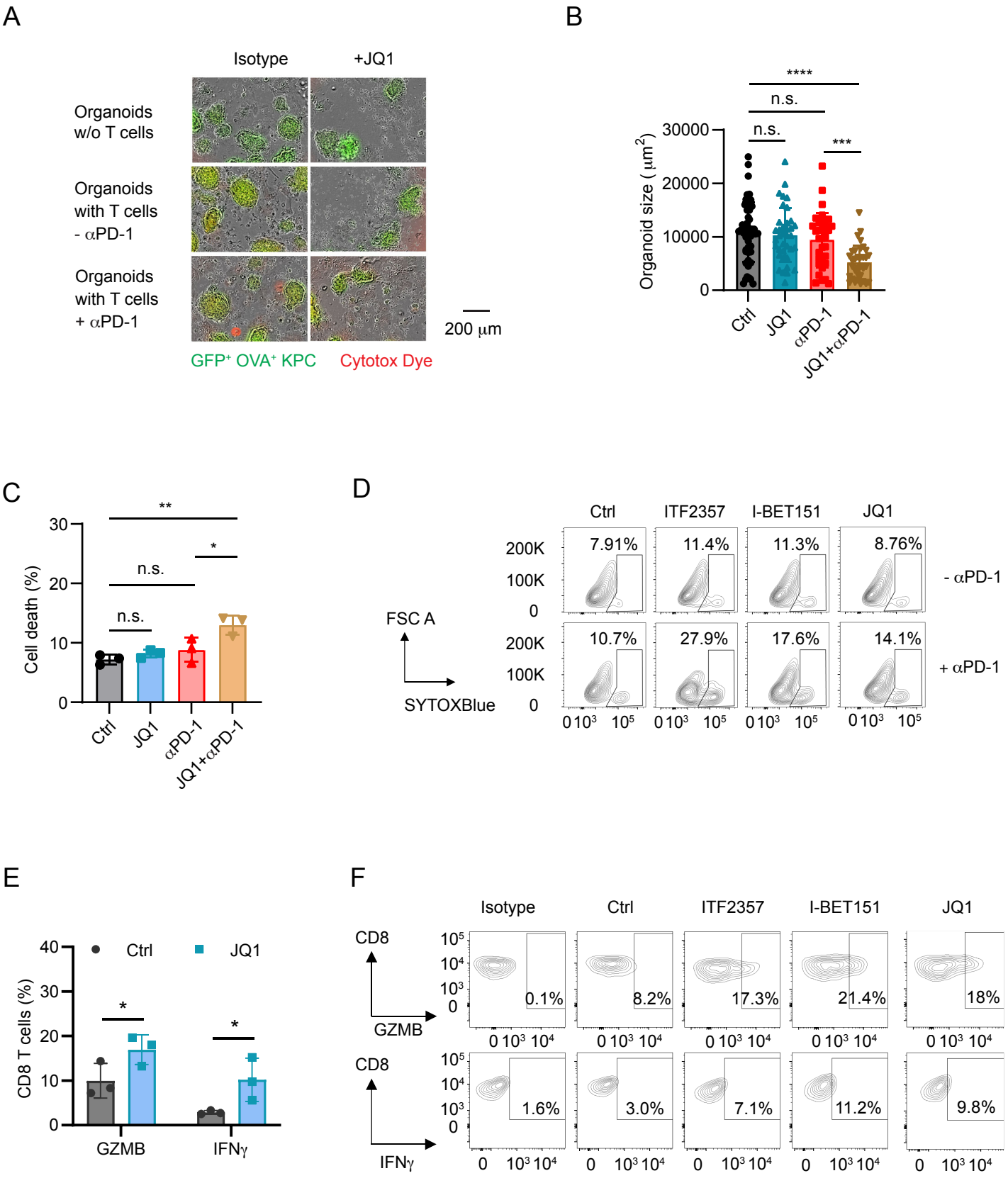

A
Isotype
+JQ1
Organoids w/o T cells
Organoids with T cells - αPD-1
Organoids with T cells + αPD-1
200 μm
GFP⁺ OVA⁺ KPC
Cytotox Dye
B
****
n.s.
n.s.
***
Organoid size (μm²)
30000
20000
10000
0
Ctrl
JQ1
αPD-1
JQ1+αPD-1
C
**
*
n.s.
n.s.
Cell death (%)
30
20
10
0
Ctrl
JQ1
αPD-1
JQ1+αPD-1
D
Ctrl
ITF2357
I-BET151
JQ1
FSC A
SYTOXBlue
200K
100K
0
7.91%
11.4%
11.3%
8.76%
- αPD-1
10.7%
27.9%
17.6%
14.1%
+ αPD-1
0 10³ 10⁵
E
Ctrl
JQ1
CD8 T cells (%)
40
30
20
10
0
*
*
GZMB
IFNγ
F
Isotype
Ctrl
ITF2357
I-BET151
JQ1
CD8
GZMB
IFNγ
0.1%
8.2%
17.3%
21.4%
18%
1.6%
3.0%
7.1%
11.2%
9.8%
0 10³ 10⁴

Figure S3. Epigenetic drugs enhance T cell activity in mouse KPC tumor organoids

(A), Optical and IF images of the $OVA^{+}GFP^{+}$KPC tumor organoids treated with isotype control, αPD-1, JQ1 or JQ1 + αPD-1, captured by the Incucyte S3 system.

(B), Size quantification of the tumor organoids in (A). Data were presented as mean ± SD by One-way ANOVA test. ***, $p < 0.001$; ****, $p < 0.0001$; n.s., no significance.

(C), The death rate of the $OVA^{+}GFP^{+}$KPC tumor cells in the T cell-incorporated tumor organoids treated with control, αPD-1, JQ1 or JQ1 + αPD-1 for 48h, measured by flow cytometry (sample size per group: n=3). Data were presented as mean ± SD by One-way ANOVA test. *, $p < 0.05$; **, $p < 0.01$; n.s., no significance.

(D), Representative flow cytometry data showing the $OVA^{+}GFP^{+}$ KPC cell death rate in the T cell-incorporated tumor organoids.

(E), GZMB and IFNγ positive CD8 T cells in the tumor organoids treated with control or JQ1 (sample size per group: n=3). Data were presented as mean ± SD by Two-way ANOVA test. *, $p < 0.05$.

(F), Representative flow cytometry data showing the GZMB and IFNγ positive CD8 T cells in the tumor organoids.

Fig. S4

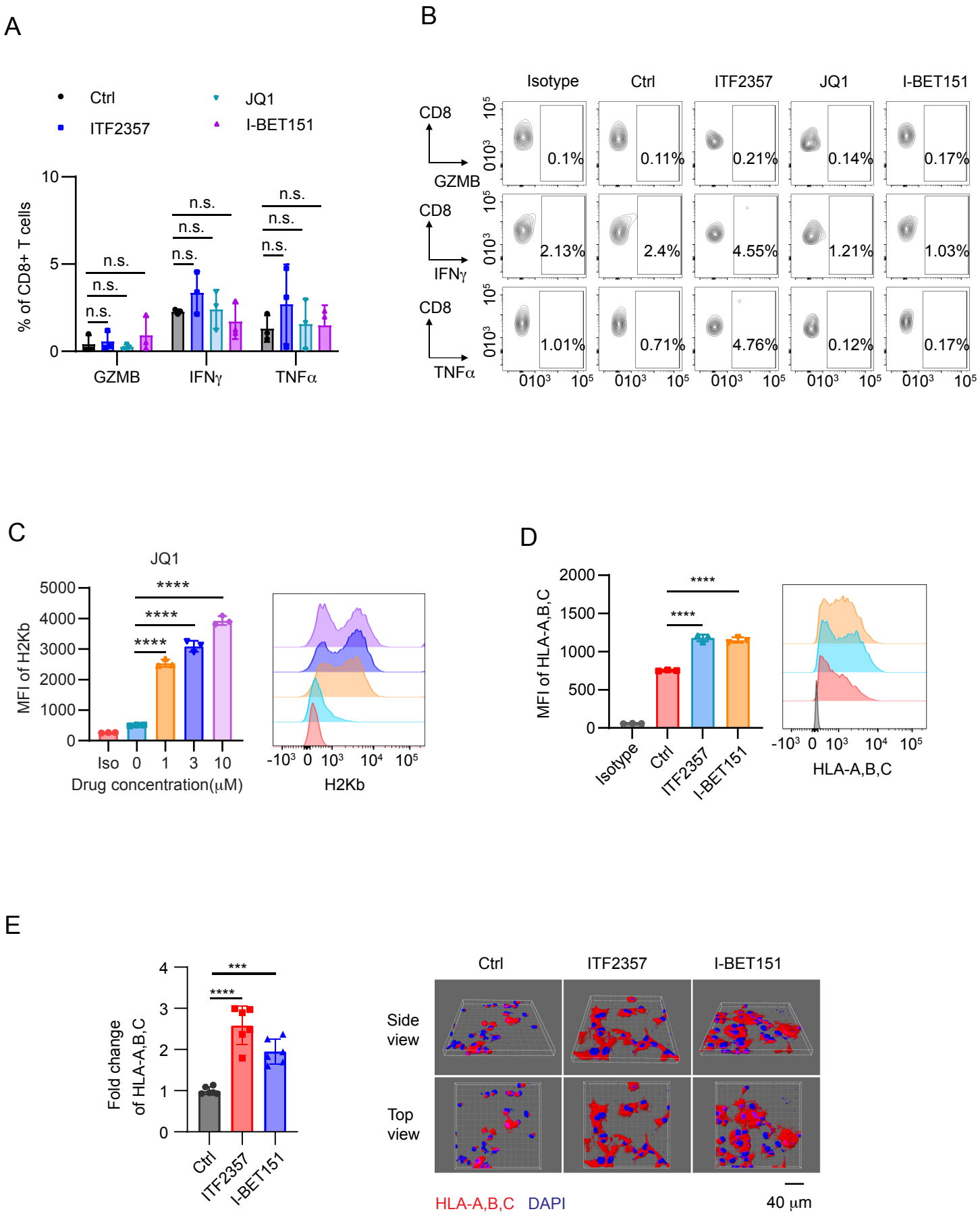

A
Ctrl
ITF2357
JQ1
I-BET151
% of CD8+ T cells
GZMB
IFNγ
TNFα
n.s.
B
Isotype
Ctrl
ITF2357
JQ1
I-BET151
CD8
GZMB
IFNγ
TNFα
0.1%
0.11%
0.21%
0.14%
0.17%
2.13%
2.4%
4.55%
1.21%
1.03%
1.01%
0.71%
4.76%
0.12%
0.17%
C
JQ1
MFI of H2Kb
Iso
Drug concentration(μM)
H2Kb
****
D
MFI of HLA-A,B,C
Isotype
Ctrl
ITF2357
I-BET151
HLA-A,B,C
E
Fold change of HLA-A,B,C
***
****
Side view
Top view
HLA-A,B,C DAPI
40 μm

Figure S4. The treatment effects of epigenetic drugs on mouse T cells

(A), GZMB, IFNγ and TNFα expression levels of the control, ITF2357, I-BET151 or JQ1 treated CD8 T cells that were isolated from the spleen of OT-I mouse (sample size per group: n=3). Data were presented as mean ± SD by Two-way ANOVA test. n.s., no significance.

(B), Representative flow cytometry data of (A).

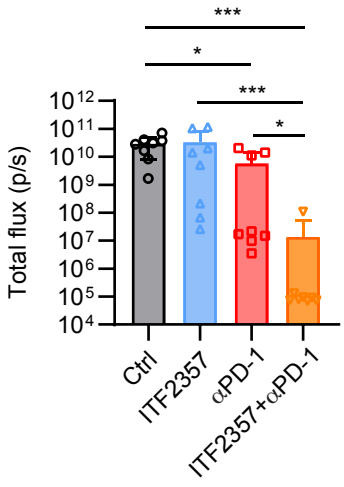
(C), The mean fluorescence intensity (MFI) of H-2Kb on the mouse $OVA^{+}GFP^{+}$ KPC cells treated with control or JQ1 and flow cytometry analysis (sample size per group: n=3). Data were presented as mean ± SD by One-way ANOVA test. ****, $p < 0.0001$.

(D), The mean fluorescence intensity (MFI) of HLA-A,B,C on the human PANC-1 cells treated with control, ITF2357, or I-BET151 (sample size per group: n=3). Data were presented as mean ± SD by One-way ANOVA test. ****, $p < 0.0001$.

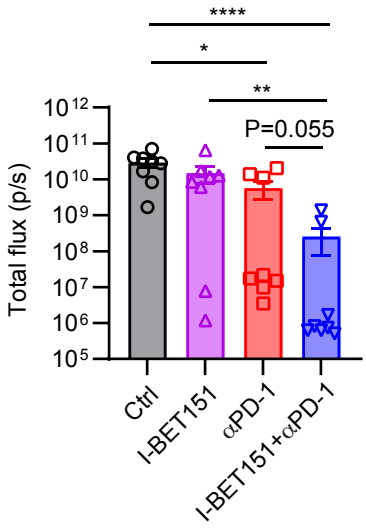
(E), The relative expression levels of HLA-A,B,C on the human PANC-1 cells treated with control, ITF2357, or I-BET151, analyzed with images captured by confocal microscope (sample size per group: n=6). Data were presented as mean ± SD by One-way ANOVA test. ***, $p < 0.001$; ****, $p < 0.0001$.

Fig. S5

A


***
*
***
*
Total flux (p/s)
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
Ctrl
ITF2357
αPD-1
ITF2357+αPD-1

B


****
*
**
P=0.055
Total flux (p/s)
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
Ctrl
I-BET151
αPD-1
I-BET151+αPD-1

C

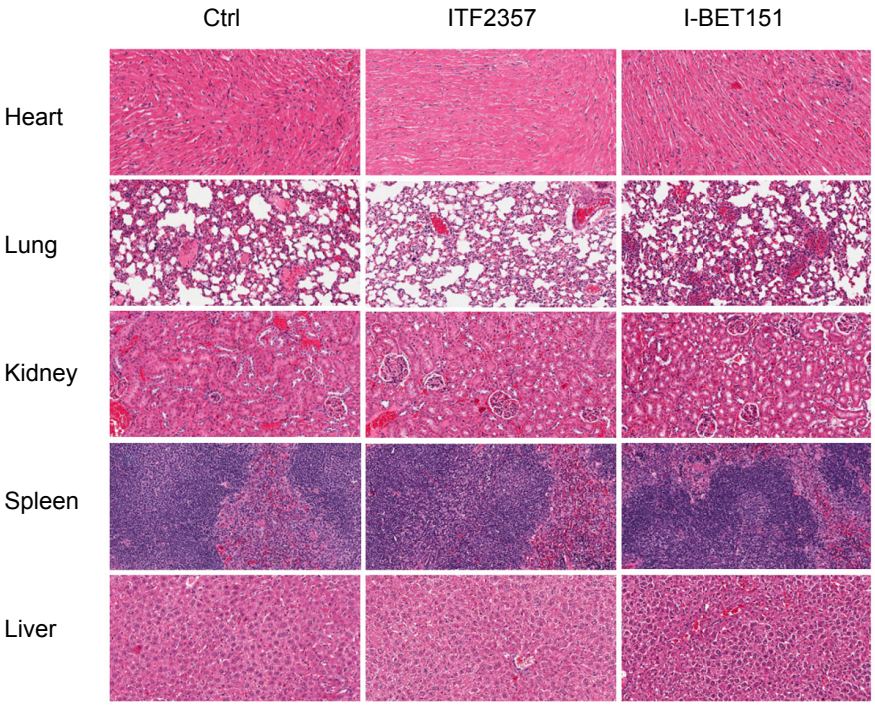

Ctrl
ITF2357
I-BET151
Heart
Lung
Kidney
Spleen
Liver

Figure S5. ITF2357 or I-BET151 augments anti-tumor activity of immune checkpoint blockade in KPC tumor-bearing mice

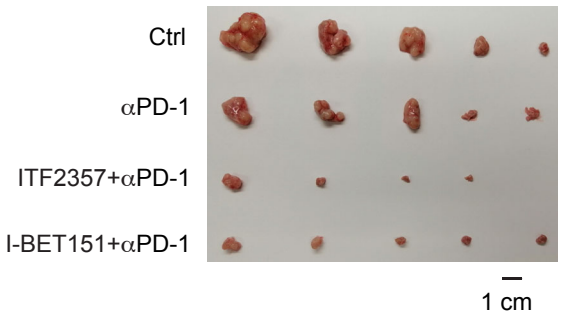
(A), The total flux of KPC tumors from C57BL/6 mice treated with isotype control, αPD-1, ITF2357 or ITF2357 + αPD-1, measured at day 43 post orthotopic injection of KPC cells into C57BL/6 mice (sample size per group: n=8). Data were presented as mean ± SD by One-way ANOVA test. *, $p < 0.05$; ***, $p < 0.001$.

(B), The total flux of KPC tumors in C57BL/6 mice treated with isotype control, αPD-1, I-BET151 or I-BET151 + αPD-1, measured at day 43 (sample size per group: n=8). Data were presented as mean ± SD by One-way ANOVA test. *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$.

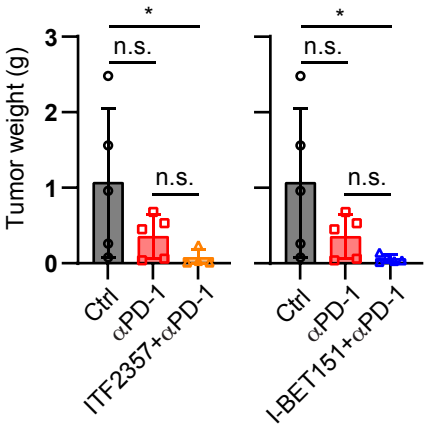
(C), Histological evaluation on the indicated organs of C57BL/6 mice treated with control, ITF2357 or I-BET151.

Fig. S6

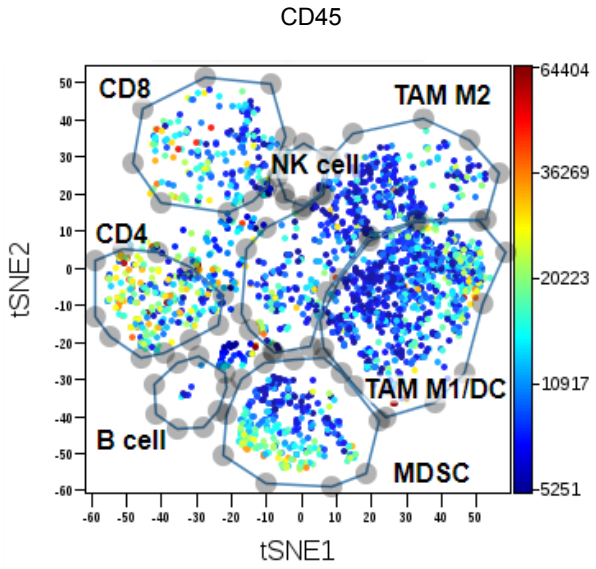
A


Ctrl
αPD-1
ITF2357+αPD-1
I-BET151+αPD-1
1 cm

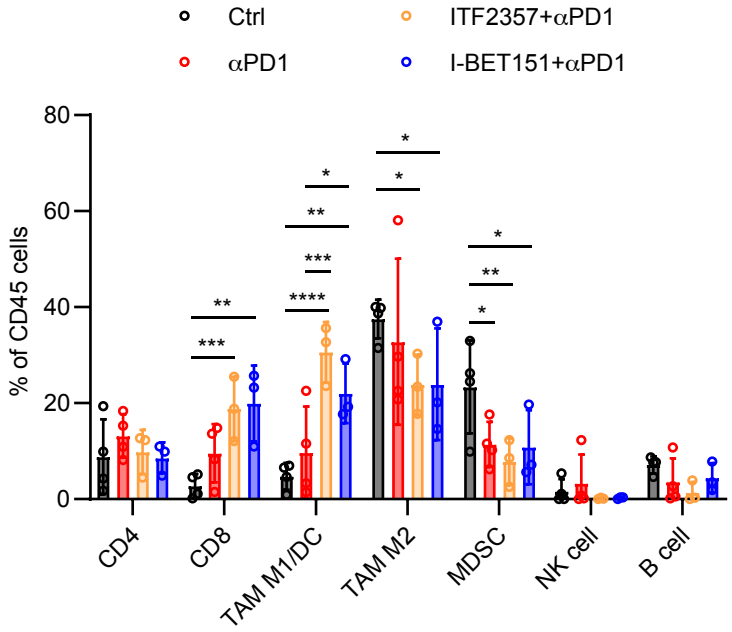
B


Tumor weight (g)
n.s.
n.s.
n.s.
n.s.
Ctrl
αPD-1
ITF2357+αPD-1
I-BET151+αPD-1

C


CD45
CD8
TAM M2
NK cell
CD4
TAM M1/DC
B cell
MDSC
tSNE2
tSNE1
64404
36269
20223
10917
5251

D


Ctrl
αPD1
ITF2357+αPD1
I-BET151+αPD1
% of CD45 cells
CD4
CD8
TAM M1/DC
TAM M2
MDSC
NK cell
B cell

E

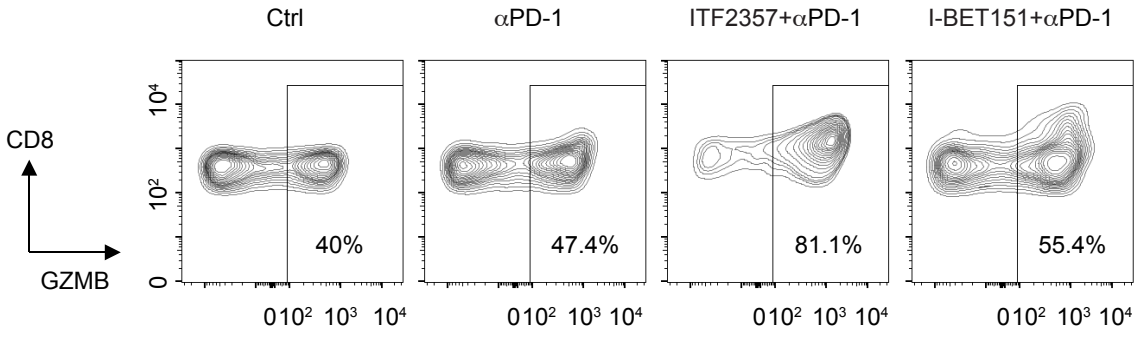

Ctrl
αPD-1
ITF2357+αPD-1
I-BET151+αPD-1
CD8
GZMB
40%
47.4%
81.1%
55.4%

Figure S6. Immune profiling analysis of orthotopic KPC tumors

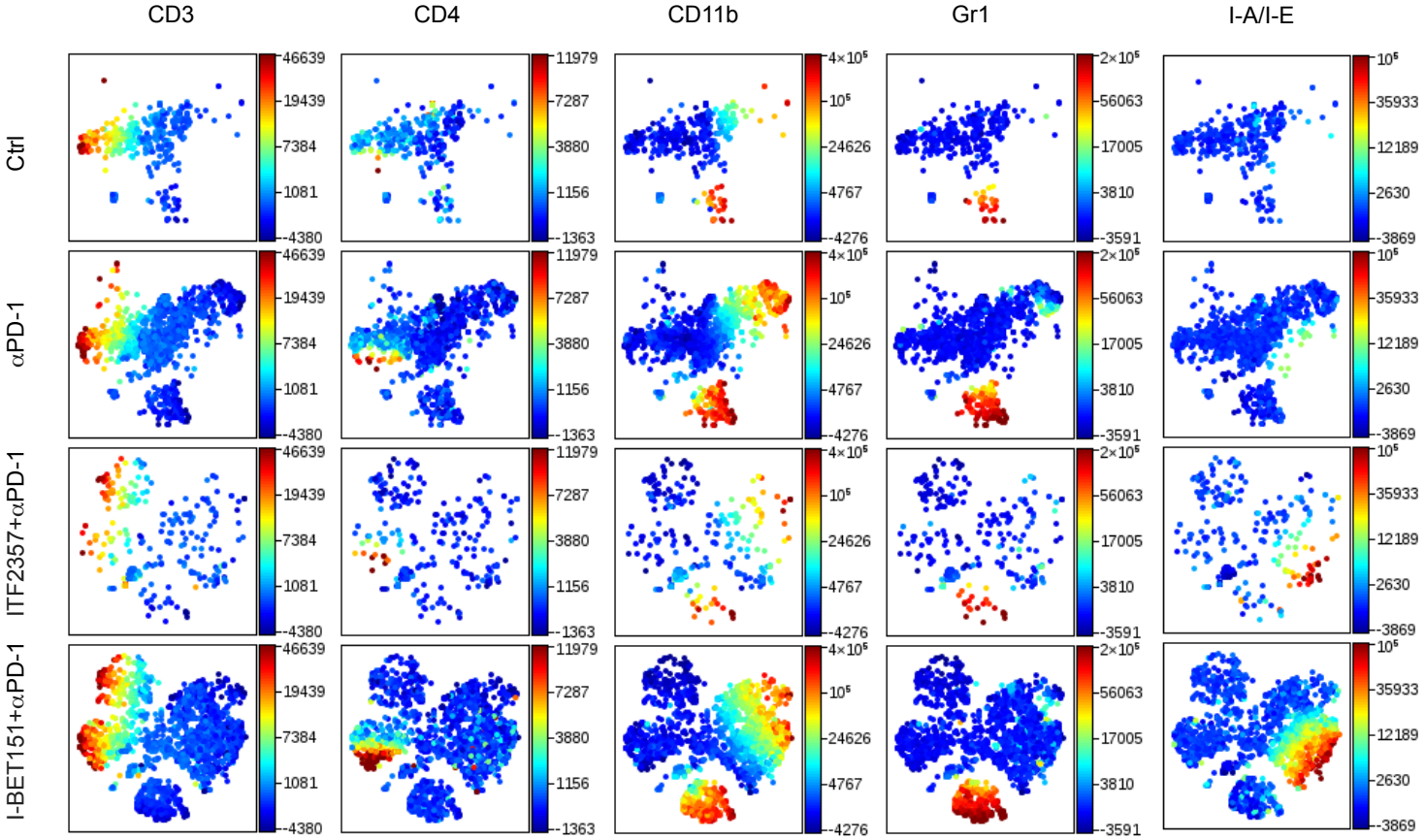
(A and B), Gross tumor images (A) and tumor weight (B) of the KPC tumors harvested from the C57BL/6 mice treated with control, αPD-1, ITF2357 + αPD-1 or I-BET151 + αPD-1 (sample size per group: n=5). The tumors were collected on day 30 post-orthotopic injection. Data shown as mean ± SD by One-way ANOVA test. *, $p < 0.05$; n.s., no significance.

(C), Gating strategy for immune cells in the KPC tumors harvested from the tumor-bearing C57BL/6 mice. The data were analyzed using t-SNE in the Cytobank platform.

(D), The immune cell subtype populations in the KPC tumors treated with isotype antibody control, αPD-1, ITF2357 + αPD-1 or I-BET151 + αPD-1, determined by t-SNE analysis (sample size per group: n=3 or 4). Data were analyzed using two-way ANOVA and shown as mean ± SD. *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

(E), The flow cytometry analysis of the GZMB expression levels on CD8 T cells in the KPC tumors.

Fig. S7


CD3
CD4
CD11b
Gr1
I-A/I-E
Ctrl
αPD-1
ITF2357+αPD-1
I-BET151+αPD-1
46639
19439
7384
1081
-4380
11979
7287
3880
1156
-1363
4×10⁵
10⁵
24626
4767
-4276
2×10⁵
56063
17005
3810
-3591
10⁵
35933
12189
2630
-3869

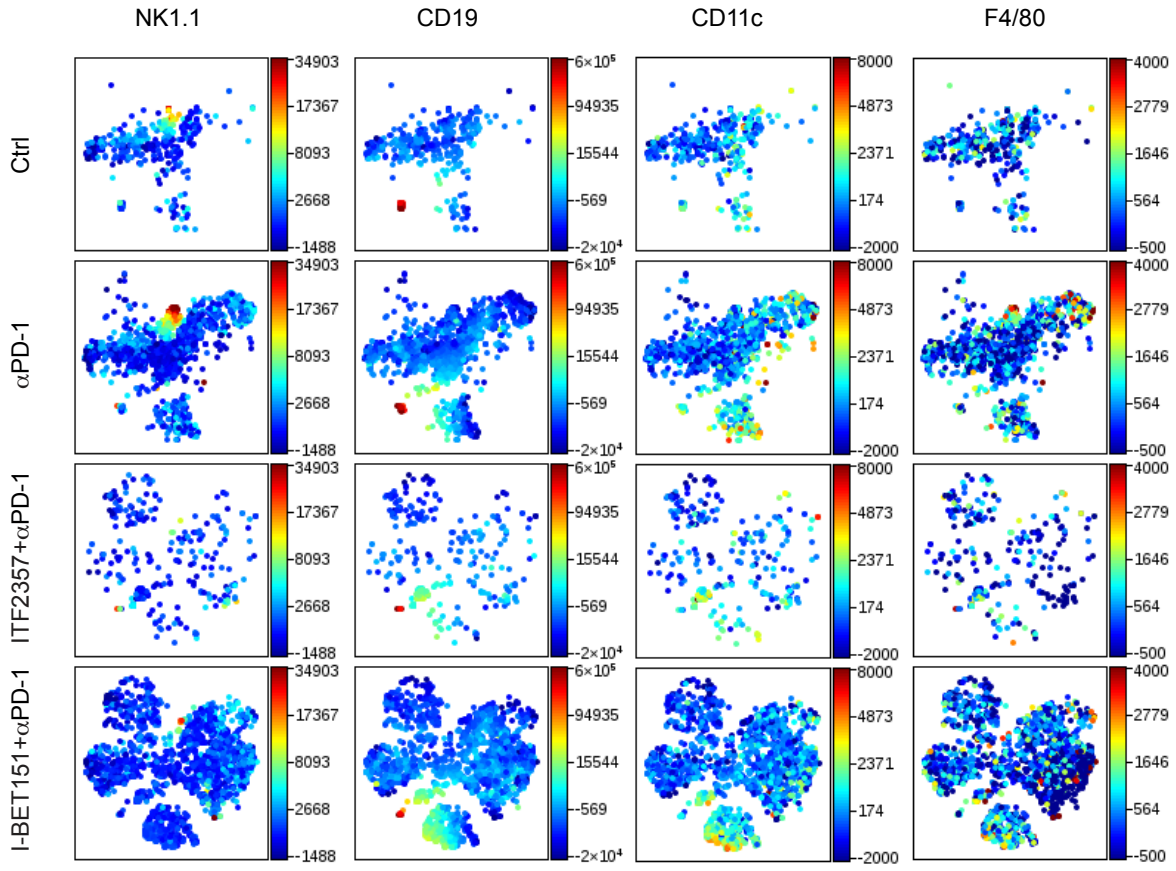
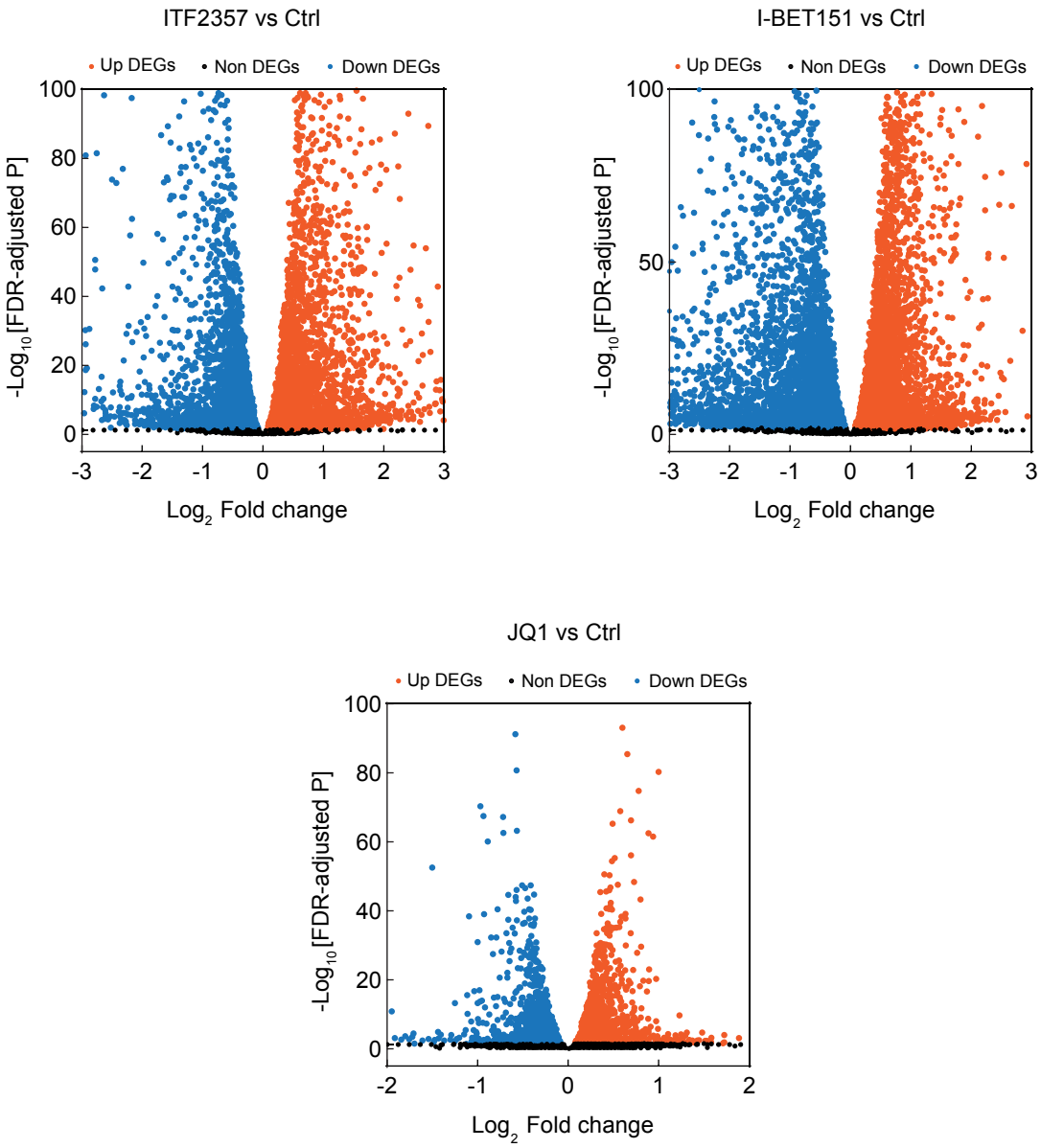

NK1.1
CD19
CD11c
F4/80
Ctrl
αPD-1
ITF2357+αPD-1
I-BET151+αPD-1
34903
17367
8093
2668
-1488
6×10⁵
94935
15544
-569
-2×10⁴
8000
4873
2371
174
-2000
4000
2779
1646
564
-500

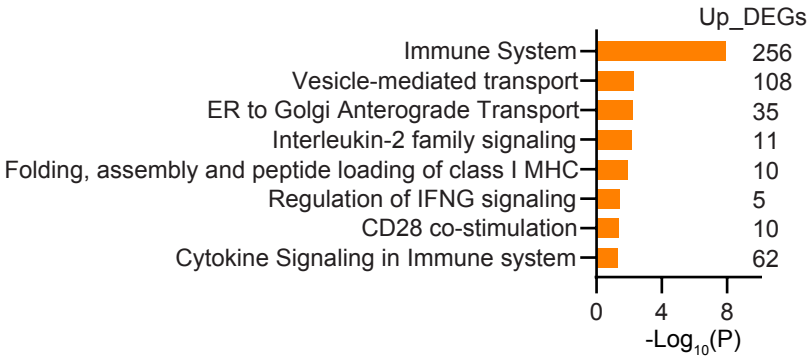
Figure S7. t-SNE representation of immune cell subtypes

Fig. S8

A


ITF2357 vs Ctrl
Up DEGs
Non DEGs
Down DEGs
-Log10[FDR-adjusted P]
Log2 Fold change
I-BET151 vs Ctrl
JQ1 vs Ctrl

B


Up_DEGs
Immune System 256
Vesicle-mediated transport 108
ER to Golgi Anterograde Transport 35
Interleukin-2 family signaling 11
Folding, assembly and peptide loading of class I MHC 10
Regulation of IFNG signaling 5
CD28 co-stimulation 10
Cytokine Signaling in Immune system 62
-Log10(P)

C

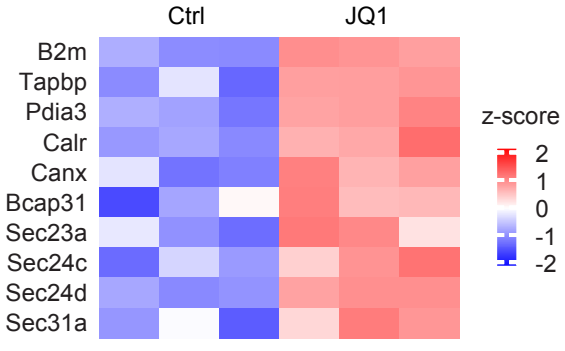

Ctrl
JQ1
B2m
Tapbp
Pdia3
Calr
Canx
Bcap31
Sec23a
Sec24c
Sec24d
Sec31a
z-score

Figure S8. RNA-seq data analysis of mouse KPC cells treated with epigenetic drugs

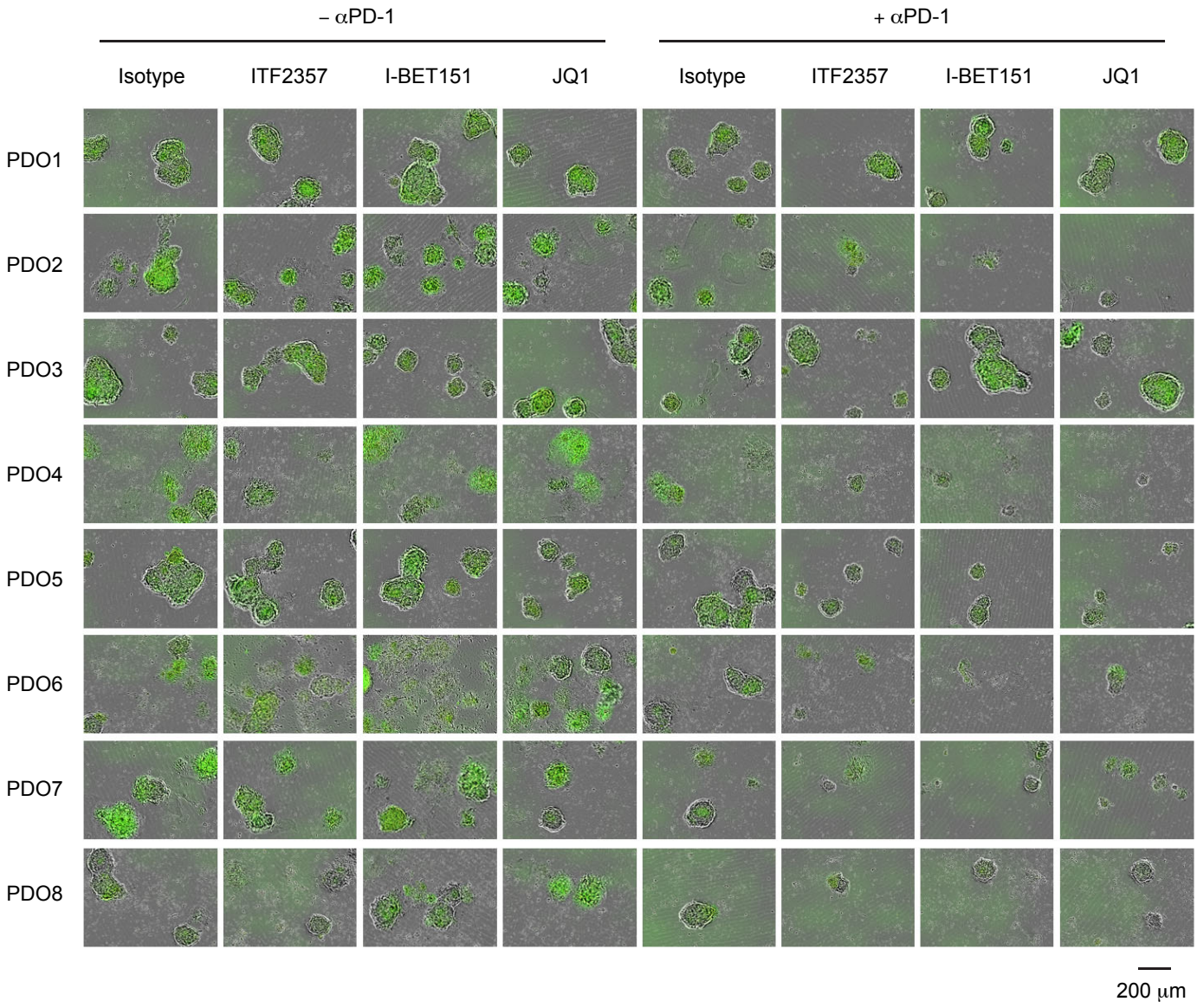
(A), Volcano plots of RNA-seq data showing the DEGs from the ITF2357, I-BET151 or JQ1 treated group in comparison with the control group.

(B), Analysis of up-regulated DEGs pathways of the control and JQ1 treatment groups from the RNA-seq data.

(C), Heatmap of the DEGs pathway of Folding, Assembly and Peptide loading of class I MHC-I of the control and JQ1 treatment groups.

Fig. S9

A


– αPD-1
+ αPD-1
Isotype
ITF2357
I-BET151
JQ1
Isotype
ITF2357
I-BET151
JQ1
PDO1
PDO2
PDO3
PDO4
PDO5
PDO6
PDO7
PDO8
200 μm

B

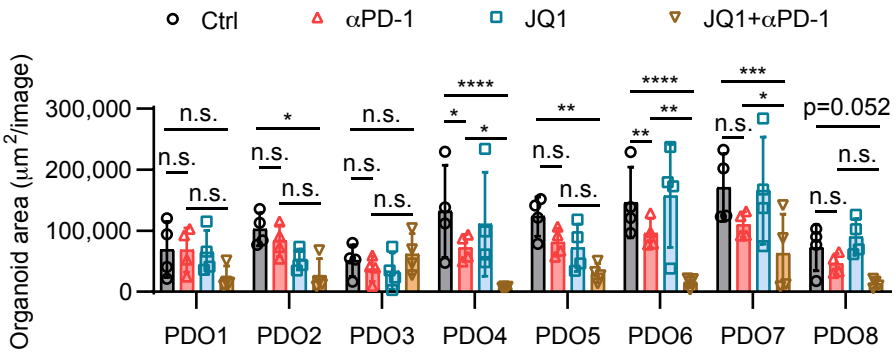

Ctrl
αPD-1
JQ1
JQ1+αPD-1
Organoid area (μm²/image)
300,000
200,000
100,000
0
n.s.
*
**
***
****
p=0.052
PDO1
PDO2
PDO3
PDO4
PDO5
PDO6
PDO7
PDO8

Figure S9. Epigenetic drugs enhance the cytotoxicity of autologous T cells incorporated in the PDOs

(A), Optical and fluorescence images of T cell-incorporated PDOs, treated with isotype control, αPD-1, ITF2357, I-BET151, JQ1, ITF2357 + αPD-1, I-BET151 + αPD-1 or JQ1 + αPD-1 for 48h, captured by the Incucyte S3 system. The Cytolight Green dye stains live cells.

(B), Quantification of T cell-mediated cytotoxicity in the PDOs treated with control, JQ1, αPD-1 or JQ1 + αPD-1. Smaller areas of PDOs indicate higher cytotoxicity (sample size per group: n=4). Note that the data of control and αPD-1 groups are shared with those in Figure 8C from the same set of experiments. Data were presented as mean ± SD by Two-way ANOVA test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, no significance.

Table S1. Epigenetic Compounds (Cayman Chemical)

Item No.11076; Batch No. 0522205

Number	Drug Name	Catalog#
1	(+)-Abscisic Acid	10073
2	3-amino Benzamide	10397
3	SB939	10443
4	PCI 34051	10444
5	4-iodo-SAHA	10495
6	Sirtinol	10523
7	C646	10549
8	Tubastatin A (trifluoroacetate salt)	10559
9	Garcinol	10566
10	Ellagic Acid	10569
11	Scriptaid	10572
12	Apicidin	10575
13	HC Toxin	10576
14	UNC0321 (trifluoroacetate salt)	10582
15	(−)-Neplanocin A	10584
16	Cl-Amidine (trifluoroacetate salt)	10599
17	F-Amidine (trifluoroacetate salt)	10610
18	JGB1741	10641
19	coumarin-SAHA	10671
20	I-BET762	10676
21	UNC0638	10734
22	Phthalazinone pyrazole	10735
23	Isoliquiritigenin	10739
24	CCG-100602	10787
25	CAY10669	10974
26	Zebularine	10975
27	Delphinidin (chloride)	11012
28	ITF 2357	11045
29	UNC0631	11084
30	UNC0646	11085
31	Methylstat (hydrate)	11091
32	3-Deazaneplanocin A (hydrochloride)	11102
33	Suramin (sodium salt)	11126
34	Nicotinamide	11127
35	2,4-Pyridinedicarboxylic Acid	11138
36	PFI-1	11155
37	5-Azacytidine	11164
38	SGI-1027	11165
39	Decitabine	11166
40	I-BET151	11181

41	(+)-JQ1	11187
42	(−)-JQ1	11232
43	BSI-201	11304
44	1-Naphthoic Acid	11322
45	Sodium 4-Phenylbutyrate	11323
46	Rucaparib (phosphate)	11570
47	IOX1	11572
48	MI-2 (hydrochloride)	11620
49	MI-nc (hydrochloride)	11621
50	Gemcitabine	11690
51	Lomeguatrib	11732
52	GSK4112	11931
53	Octyl-α-ketoglutarate	11970
54	Daminozide	12033
55	GSK-J1 (sodium salt)	12054
56	GSK-J2 (sodium salt)	12056
57	GSK-J4 (hydrochloride)	12073
58	CI-994	12084
59	CPTH2 (hydrochloride)	12086
60	Etoposide	12092
61	Lestaurtinib	12094
62	Butyrolactone 3	12095
63	Valproic Acid (sodium salt)	13033
64	Tenovin-1	13085
65	Tenovin-6	13086
66	Sodium Butyrate	13121
67	BIX01294 (hydrochloride hydrate)	13124
68	Anacardic Acid	13144
69	AGK2	13145
70	CAY10603	13146
71	Splitomicin	13168
72	CBHA	13172
73	M 344	13174
74	Oxamflatin	13176
75	Salermide	13178
76	Mirin	13208
77	Pimelic Diphenylamide 106	13212
78	KD 5170	13214
79	Panobinostat	13280
80	MS-275	13284
81	HNHA	13295
82	RG-108	13302
83	2’,3’,5’-triacetyl-5-Azacytidine	13373
84	S-Adenosylhomocysteine	13603

85	UNC0224	13631
86	Chidamide	13686
87	Tubacin	13691
88	3-Deazaneplanocin A	13828
89	Sinefungin	13829
90	Pyroxamide	13870
91	N-Oxalylglycine	13944
92	WDR5-0103	13945
93	EPZ005687	13966
94	SGC0946	13967
95	UNC1215	13968
96	AK-7	14004
97	GSK343	14094
98	Bromosporine	14119
99	GSK2801	14120
100	SIRT1/2 Inhibitor IV	14407
101	I-CBP112 (hydrochloride)	14468
102	SGC-CBP30	14469
103	UNC0642	14604
104	UNC1999	14621
105	I-PFI-2 (hydrochloride)	14678
106	HPOB	15066
107	2-hexyl-4-Pentynoic Acid	15205
108	PFI-3	15267
109	JIB-04	15338
110	CAY10683	15403
111	GSK126	15415
112	CPI-203	15479
113	6-Thioguanine	15774
114	Tubastatin A	15785
115	3,3’-Diindolylmethane	15927
116	OTX015	15947
117	5-Methylcytidine	16111
118	AGK7	16152
119	5-Methyl-2’-deoxycytidine	16166
120	EPZ5676	16175
121	MC 1568	16265
122	α-Hydroxyglutaric Acid (sodium salt)	16374
123	S-(5’-Adenosyl)-L-methionine (tosylate)	16376
124	RVX-208	16424
125	CUDC-101	16426
126	LAQ824	16427
127	Nullscript	16433
128	GSK-LSD1 (hydrochloride)	16439

129	RGFP966	16917
130	BRD73954	16919
131	*trans*-Resveratrol	70675
132	DMOG	71210
133	Trichostatin A	89730
134	CAY10398	89740
135	RSC-133	9001839
136	BML-210	10005019
137	Piceatannol	10009366
138	CAY10591	10009797
139	EX-527	10009798
140	SAHA	10009929
141	2-PCPA (hydrochloride)	10010494

Table S2. The antibody panel for Cytek Aurora analysis

Number	Channel	Antibody name	Dye	Company	Catalog#
1	V1	PD-1	BV421	BioLegend	135218
2	V2	CD25	Superbright436	Invitrogen	2470828
3	V3	CD11b	eFluor 450	Invitrogen	2403283
4	V4	EpCAM	BV480	BD Biosciences	746367
5	V5	Viable	eFluor 506	ThermoFisher	65-0866-14
6	V7	CD69	BV510	BioLegend	104532
7	V8	CD11c	BV570	BioLegend	117331
8	V10	Gr1	BV605	BioLegend	108440
9	V11	Ly108	BV650	BioLegend	740628
10	V13	CD3	BV711	BioLegend	100241
11	V14	CD103	BV750	BioLegend	747478
12	V15	CD62L	BV785	BioLegend	104440
13	B1	CD19	BB515	BioLegend	564509
14	B2	GFP^+ KPC cell	GFP		
15	B3	CD45	AF532	Invitrogen	58-0451-82
16	B8	IAIE	PerCP	BioLegend	107624
17	B9	F4/80	PerCPCy5.5	BioLegend	123128
18	B10	GZMB	PerCP-eFluor™ 710	Invitrogen	46-8898-80
19	YG1	TNFα	PE	BioLegend	506306
20	YG3	CD8	PE-eFluor 610	Invitrogen	61-0081-80
21	YG5	CD44	PE/Cy5	BioLegend	103010
22	YG9	CD31	PE/Cy7	Invitrogen	25-0311-81
23	R1	IFNγ	APC	BioLegend	505810
24	R2	α-SMA	AF647	Cell Signaling Technology	76113S
25	R4	NK1.1	AF700	BioLegend	108730
26	R7	CD4	APC/CY7	BioLegend	100414

Table S3. Clinical information of human pancreatic cancer samples.

Sample	Sex	Surgical information	Diagnosis
1	M	Whipple	Pancreatic adenocarcinoma
2	M	Pancreatectomy	Anaplastic undifferentiated carcinoma
3	F	Whipple	Pancreatic adenocarcinoma
4	F	Whipple	Pancreatic adenocarcinoma
5	M	Whipple	Pancreatic adenocarcinoma
6	F	Whipple	Pancreatic adenocarcinoma
7	F	Distal pancreatectomy and splenectomy	Pancreatic adenocarcinoma
8	F	Pancreaticoduodenectomy	Pancreatic adenocarcinoma

Table S4. Components in the culture medium for mouse pancreatic tumor organoids

Products	Name	Company	Catalog	Concentration
1	GlutaMAX Supplement	ThermoFisher	35050061	1x
2	B-27™ Supplement (50X)	Sigma	17504044	1x
3	N-acetyl-L-cysteine	Sigma	A7250	1 mM
4	Recombinant murine EGF	PeproTech	315-09	5 μg/ml
5	Wnt-3A, R-spondin 3, and noggin conditioned medium	ATCC	CRL-3276	10%
6	FGF10	BioLegend	751004	10 ng/ml
7	Recombinant murine FGF-10	Sigma	PMG0034	5 ng/ml
8	A83-01	Tocris	2939	500 nM
9	Nicotinamide	Sigma	N3376	10 mM

Table S5. Key resources

Reagent or resource	Source	Identifier
Antibodies		
Recombinant-EPCAM antibody	Abcam	Cat# ab32392
AF647-conjugated Donkey anti-rabbit IgG	BioLegend	Cat# 406414
AF 647-conjugated anti-Cytokeratin 19	BioLegend	Cat# 628506
Spark YG 570-conjugated anti-mouse CD31	BioLegend	Cat# 102531
PE-conjugated anti-human CD31	BioLegend	Cat# 303106
AF488 -conjugated anti-human CD68	BioLegend	Cat# 333811
Spark YG 570-conjugated anti-human CD8a	BioLegend	Cat# 372910
Spark YG 570-conjugated anti-mouse F4/80	BioLegend	Cat# 123159
AF488-conjugated anti-mouse CD8a	BioLegend	Cat# 100723
FITC-conjugated Mouse α-Smooth Muscle Actin	GeneTex	Cat# GTX72531
AF647-conjugated anti-mouse CD8a	BioLegend	Cat# 100724
AF647-conjugated anti-mouse CD4	BioLegend	Cat# 100533
anti-mouse CD8α antibody, rabbit monoclonal	Cell Signaling Technology	Cat# 98941S; Clone: D4W2Z
CD4 Rabbit mAb	Cell Signaling Technology	Cat# 25229; Clone: D7D2Z
α-Smooth Muscle Actin XP Rabbit mAb	Cell Signaling Technology	Cat# 19245S; Clone: D4K9N
GFAP Mouse mAb	Cell Signaling Technology	Cat# 3670S; Clone:GA5
AF647-conjugated anti-mouse CD326	BioLegend	Cat# 118212
AF647-conjugated α-Smooth Muscle Actin XP Rabbit mAb	Cell Signaling Technology	Cat# 76113S; Clone: D4K9N
AF594-conjugated anti-mouse CD31	BioLegend	Cat# 102520
BV605-conjugated anti-mouse CD45	BioLegend	Cat# 103139
PE/Cy7-conjugated anti-mouse CD3	BioLegend	Cat# 100220
AF700-conjugated anti-mouse CD4	BioLegend	Cat# 100430
APC/Cy7-conjugated anti-mouse CD8	BioLegend	Cat# 100714
PE-conjugated anti-mouse/human CD11b	BioLegend	Cat# 101208
AF647-conjugated anti-mouse CD11c	BioLegend	Cat# 117312

PerCP/Cy5.5-conjugated anti-mouse F4/80	BioLegend	Cat# 123128
BV421-conjugated anti-mouse I-A/I-E	BioLegend	Cat# 107631
BV650-conjugated anti-mouse CD19	BioLegend	Cat# 115541
BV605-conjugated anti-mouse Gr1	BioLegend	Cat# 108440
AF700-conjugated anti-mouse NK1.1	BioLegend	Cat# 108730
APC-conjugated anti-mouse IFNγ	BioLegend	Cat# 505810
PE-conjugated anti-mouse TNFα	BioLegend	Cat# 506306
PerCP/Cyanine5.5-conjugated anti-human/mouse Granzyme B	BioLegend	Cat# 372212
AF647-conjugated anti-mouse H-2Kb	BioLegend	Cat# 116512
APC-conjugated anti-mouse H-2Kb bound to SIINFEKL antibody	BioLegend	Cat# 141606
PE/Cyanine7-conjugated anti-human CD45	BioLegend	Cat# 368532
AF647-conjugated anti-human CD3	BioLegend	Cat# 300422
AF700-conjugated anti-human CD4	BioLegend	Cat# 357418
APC/Cy7-conjugated anti-human CD8a	BioLegend	Cat# 300926
AF647-conjugated Anti-Human HLA-A,B,C	BioLegend	Cat# 311414
anti-mouse PD-1	Bioxcell	Cat# BE0146; Clone: RMP1-14
Rat IgG2a isotype control	Bioxcell	Cat# BE0089
Beta-2 microglobulin antibody	Abcam	Cat# Ab175031
Beta-Actin antibody	Abgent	Cat# AM1829b
Chemicals, Peptides, and Proteins		
Mouse IL-2	BioLegend	Cat# 575402
Human IL-2	Peprotech	Cat# 200-02
penicillin/streptomycin solution	HyClone	Cat# SV30010
Matrigel	Corning	Cat# 356255
Image-iT^{TM} Red Hypoxia reagent	Invitrogen	Cat# H10498
phorbol 12-myristate 13-acetate	Sigma	Cat# 16561-29-8
Ionomycin	Sigma	Cat# 56092-82-1
Brefeldin A	BioLegend	Cat# 420601
Lipofectamine 3000 reagent	ThermoFisher Scientific	Cat# 25530049
TRIzol	Ambion	Cat# 15596018

Citrate buffer antigen retriever	Sigma	Cat# C9999-1000ml
Tween 20	ThermoFisher Scientific	Cat# BP337-500
eBioscience™ Fixable Viability Dye eFluor™ 506	ThermoFisher Scientific	Cat# 65-0866-14
Incucyte Cytotox Red Dye	Sartorius	Cat# 4632
Incucyte Cytolight Rapid Dye (Green)	Sartorius	Cat# 4705
SYTOX™ Blue dead cells	Invitrogen	Cat# 2078615
DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride)	ThermoFisher Scientific	Cat# D1306
Commercial assays and Consumables		
Mouse tumor dissociation kit	Milenyi Biotec	Cat# 130-096-730
Human tumor dissociation kit	Milenyi Biotec	Cat# 130-095-929
Pan T cell isolation kit II (mouse)	Milenyi Biotec	Cat# 130-095-130
Pan T cell isolation kit II (human)	Milenyi Biotec	Cat# 130-096-535
RNeasy mini-isolation kit	QIAGEN	Cat# 157029493
QIAseq FastSelect rRNA Removal HMR Kit	QIAGEN	Cat# 334387
KAPA RNA Hyper Prep Kit	Roche Corporate	Cat# KK8541
QscriptXLT cDNA super Mix	Quantabio	Cat# 66141329
SYBR Green fastmix low Rox	TaKaRa	Cat# 4385610
Cytofix/cytoperm fixation/permeabilization kit	BD Biosciences	Cat# 554714
DAB substrate Kit Peroxidase	Vector	Cat# SK-4100
Epigenetic drug library	Cayman Chemical	Cat# 11071
Human CD8 magnetic beads	Milenyi Biotec	Cat# 130-045-201
Mouse CD8 magnetic beads	Milenyi Biotec	Cat# 130-117-044
BD comp Beads Negative Control	BD Biosciences	Cat# 51-90-9001291
BD comp Beads Anti-Mouse Ig,κ	BD Biosciences	Cat# 51-90-9001229
BD comp Beads Anti-Rat and Anti-Hamster Ig,κ	BD Biosciences	Cat# 51-90-9000949
Picro-Sirius Red Solution	Abcam	Cat# ab246832
Prolong Gold anti-fade medium	Invitrogen	Cat# P36930
RBC lysis buffer(10X)	BioLegend	Cat# 420301
MicroAmp Optical 96-well reaction plate	Applied biosystems	Cat# N8010560
PhenoPlate-384 ULA-Coated	PerkinElmer	Cat# 1780-22141

low-attachment surface 96-well microplate	Corning	Cat# 3474
low-attachment surface 6-well microplate	Corning	Cat# 3471
4-Well glass Millicell EZ Slide	Millipore	Cat# PEZGS0416
8-Well glass Millicell EZ Slide	Millipore	Cat# PEZGS0816
Cell strainers (40 μm)	Fisher Scientific	Cat# 07201430
Cell strainers (70 μm)	Fisher Scientific	Cat# 07201431
Cell strainers (100 μm)	Fisher Scientific	Cat# 07201432
Cell strainers (150 μm)	pluriStrainer	Cat# 43-50150-50
Cell strainers (200 μm)	pluriStrainer	Cat# 43-50200-03
Cell strainers (300 μm)	pluriStrainer	Cat# 43-50300-03
Cell strainers (500 μm)	pluriStrainer	Cat# 43-50500-03
Experimental models: Cell lines		
Mouse KPC cell lines	Dr. Ronald A. DePinho's Lab	N/A
Human Panc-1	ATCC	Cat# CRL-1469
Experimental models: Organisms/strains		
Mouse: C57BL/6	Jackson Laboratories	Stock#: 000664
Human pancreatic cancer samples (PDOs)	Tissue Procurement and Distribution Core of Indiana University Simon Cancer Center	Table S3
Software and Algorithms		
GraphPad	GraphPad Software Inc.	GraphPad Prism 9.0 software
Cytobank	Cytobank Inc.	https://premium.cytobank.org/
FlowJo	FlowJo LLC	FlowJo version 10.8.0
Matlab	MathWorks Inc.	Version 8.2.1, R2016b
IMARIS	Oxford instruments	IMARIS x64 8.1.2
ImageJ	N/A	ImageJ (64bit 1.50e)
Aperio ImageScope	Leica Biosystems	V12.4.3.5008
R software	R Software Inc.	https://www.R-project.org/

Table S6. Quantitative reverse transcription PCR Primers

Mouse primers			
Name	**Sequence 5' to 3'**	**Name**	**Sequence 5' to 3'**
B2m qF	TTCTGGTGCTTGTCTCACTGA	Tapbp qF	GGCCTGTCTAAGAAACCTGCC
B2m qR	CAGTATGTTCGGCTTCCCATTC	Tapbp qR	CCACCTTGAAGTATAGCTTTGGG
Calr qF	TGGCTGCTCCCAATAATGTCT	Pdia3 qF	CGCCTCCGATGTGTTGGAA
Calr qR	GAGGGTAGTGACCAAAAGATGG	Pdia3 qR	CAGTGCAATCCACCTTTGCTAA
Erap1 qF	TAATGGAGACTCATTCCCTTGGA	Canx qF	ATGGAAGGGAAGTGGTTACTGT
Erap1 qR	AAAGTCAGAGTGCTGAGGTTTG	Canx qR	GCTTTGTAGGTGACCTTTGGAG
Sec23a qF	AGATGGGGTCCGGTTCAGTT	Hspa5 qF	ACTTGGGGACCACCTATTCCT
Sec23a qR	GGTAGGTCGGGTCTCTCCTT	Hspa5 qR	ATCGCCAATCAGACGCTCC
Sec24a qF	TCCTGTCCACAATACACTGATGT	H2-T22 qF	TCCCTTTGGGTTCACACTCG
Sec24a qR	GAACCACCGTAGTTCGACTGT	H2-T22 qR	AGTCGTCCATGCTCTTGTTGT
Sec24c qF	CTGGCCGGAATGCAGATCAG	H2-T23 qF	ACAGTCCCGACCCAGAGTAG
Sec24c qR	TGAGGATAGGAGCCGTATGGA	H2-T23 qR	CCACGTAGCCGACAATGATGA
Sec24d qF	GGAGAGGTCTTTGTTCCTTTGTT	Bcap31 qF	GCCACCTTCCTCTACGCAG
Sec24d qR	GTCTCTGTTCTTGAGCTTCCC	Bcap31 qR	TGCCATAGGTCACTACCAACTC
Sec31a qF	CAGTCCTCACTTACGCTAAACC	Sec13 qF	GAACACTGTGGACACCTCTCA
Sec31a qR	ATTCCCTGCACAGATGTAGCA	Sec13 qR	CTCCATTCCGCACATCGAAAA