Transcriptional Deregulation by FOXM1–JUP Signaling Confers Dual Oncogenic Drivers for Pancreatic Tumorigenesis and Therapeutic Resistance

Kailing Yang[1,2#], Zhihong He[1,2#], Tingting Jiang[1,2], Tian Cai[1,2,3], Xiaojia Li[1,2 ✉], and Keping Xie[1,2✉]

[1]Center for Pancreatic Cancer Research, The South China University of Technology School of Medicine, Guangzhou, China

[2]The South China University of Technology Comprehensive Cancer Center, Guangdong, China

[3]Department of Laboratory Medicine, The First People's Hospital of Foshan, Foshan, China

✉ *Correspondence*: Dr. Keping Xie or Dr. Xiaojia Li, Center for Pancreatic Cancer Research, South China University of Technology School of Medicine, Guangzhou 510006; Phone: 020-3938-0579; E-mail: scutmedicine@scut.edu.cn or xiaojia0424@scut.edu.cn

[#]KY and ZH contribute equally to this work and are considered co-first authors. The work represents partial fulfillment of their PhD requirements and integral parts of their doctoral dissertations.

Supplementary Information

1. **Supplementary Tables**
2. **Supplementary Figures**

1. Supplementary Tables

Table S1. Detailed information of antibodies

Antibody	Company	Cat.	Application
Plakoglobin Polyclonal antibody	Proteintech	A303-718A	IHC,IF,WB
Anti-Desmocolin1 antibody	Abcam	Ab198904	WB
Desmocolin3 antibody	Invitrogen	PA5-84943	WB
Plakophilin-2 antibody	Invitrogen	PA5-102795	WB
Plakophilin-3 antibody	Abcam	Ab-109441	WB
Desmoglein1	Invitrogen	32-6000	WB
FOXM1 Polyclonal antibody	Proteintech	13147-1-AP	WB
FOXM1 antibody (G-5)	Santa	sc-376471	IHC, ChIP
Amylase antibody	Santa	sc-46657	IF
CK19 antibody	DSHB	TROMA-III	IF
Anti-Ki67 antibody	Abcam	Ab-15580	IHC
Caspase3 antibody	CST	9664S	IHC, IF, WB
P53 antibody	Beyotime	AF1402	IHC, IF, WB
P21 antibody	Beyotime	AF5252	IHC, WB
pAKT antibody	Abcam	Ab-38449	IHC, WB
AKT antibody	Abcam	Ab-32505	IHC, IF, WB
β-catenin antibody	Abcam	Ab-32572	IHC, IF, WB
CD44 antibody	CST	3570T	IHC, IF, WB
c-JUN antibody	CST	9165T	IHC, IF, WB
c-Myc antibody	CST	5605T	WB
E-cadherin antibody	CST	3195S	IHC, IF, WB
N-cadherin antibody	CST	3195S	IHC, IF, WB
Vimentin antibody	CST	5741S	IHC, IF, WB
CA199 antibody	Abcam	Ab-3982	IHC

Table S2. PCR primer and DNA sequences

Gene	Sense (5´ to 3´)	Antisense (5´ to 3´)
JUP	TCGCCATCTTCAAGTCGGG	AGGGGCACCATCTTTTGCAG
FOXM1	TGCAGCTAGGGATGTGAATCTTC	GGAGCCCAGTCCATCAGAACT
GAPDH	CAGGAGGCATTGCTGATGAT	GAAGGCTGGGGCTCATTT
CDH1	TTACTGCTGCGTTTTATGTTGGG	GCTGGCCGATGTGATGACTA
CDH2	GAGCTGACAGGCGACATTCAT	CCACGCTGCCAATATAGGG
Vim	GACGCCATCAACACCGAGTT	CTTTGTCGTTGGTTAGCTGGT
#1 primer	AATCCCACCCCAGCACAAAG	CCAAAGAAGCATCATGCCTGAC
#2 primer	TCCAATCAGGAGGAAGTAACTTTC	GAAGAGGACTGGAGGCGAG

Table S3. Primer amplicon products sizes and chromosomal locations

Gene	Amplicon products sizes	Chromosomal locations
JUP	169bp	chr17:41755422 - 41755590
FOXM1	154bp	chr12:2858417 - 2858570
GAPDH	138bp	chr12:6535012 - 6535149
CDH1	109bp	chr16:68801709 - 68801817
CDH2	123bp	chr18:28009719 - 28009841
Vim	238bp	chr10:17229690 - 17229927
#1 primer	98bp	chr17:41787188 - 41787285
#2 primer	154bp	chr17:41786854 - 41787007

Table S4. PCR primer sequences for SiRNA

SEQ ID	Sense (5´ to 3´)	Antisense (5´ to 3´)
Si-NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
Si-JUP-1	CCAUUGUGCAUCUCAUCAATT	UUGAUGAGAUGCACAAUGGTT
Si-JUP-2	GCUGAAGAUUCUGGUGAAUTT	AUUCACCAGAAUCUUCAGCTT
Si-JUP-3	GGAGUCGGUCCUGUUCUAUTT	AUAGAACAGGACCGACUCCTT
Si-jup-1	GCAGACAGUACACACUCAATT	UUGAGUGUGUACUGUCUGCTT
Si-jup-2	GCAAGCACUUGACAAGCAATT	UUGCUUGUCAAGUGCUUGCTT
Si-jup-3	CCAUCGUCCAUCUCAUCAATT	UUGAUGAGAUGGACGAUGGTT
Si-FOXM1	GGACCACUUUCCCUACUUUTT	AAAGUAGGGAAAGUGGUCCTT

2. Supplementary Figures

Fig. S1

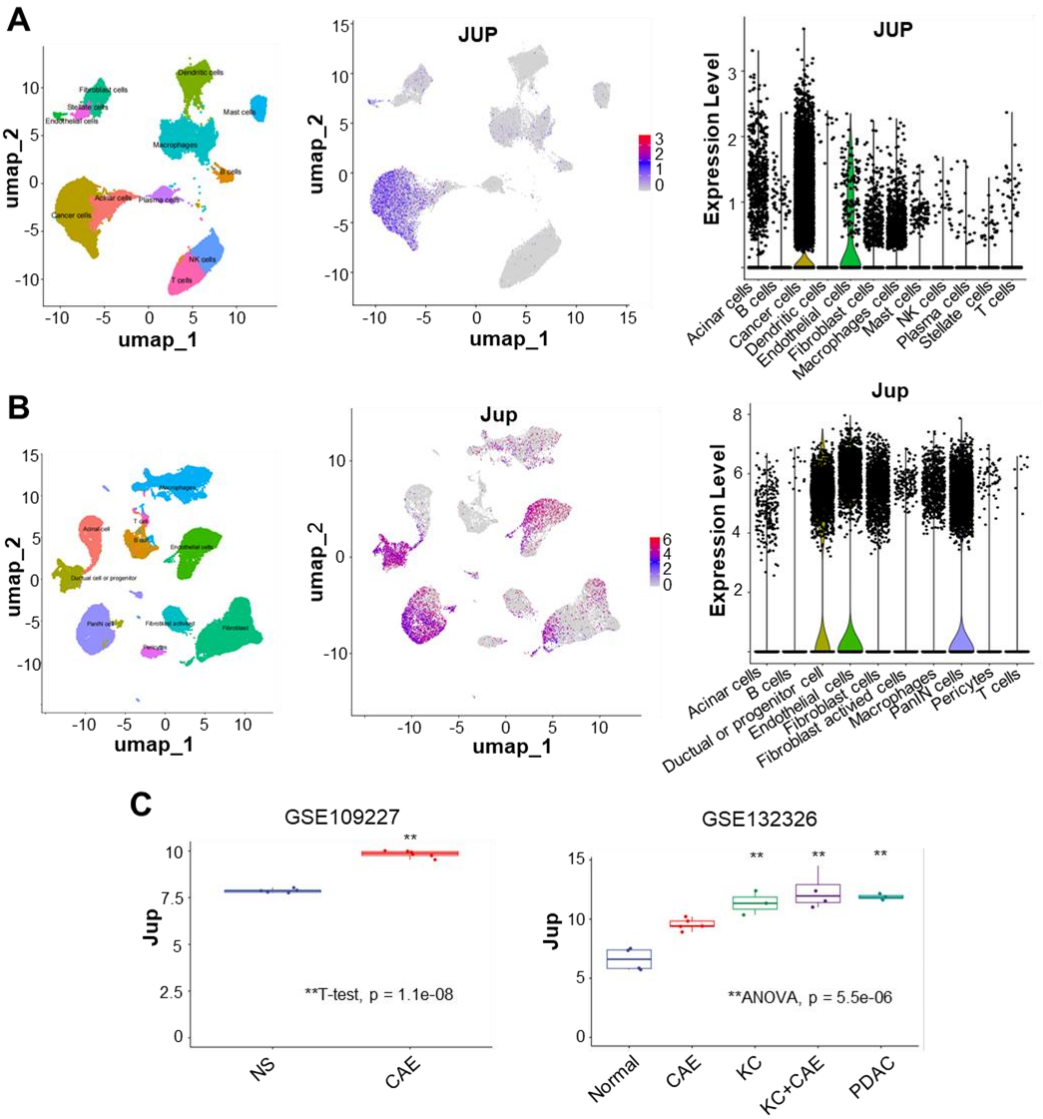

A
JUP
umap_1
umap_2
Expression Level
Acinar cells
B cells
Cancer cells
Dendritic cells
Endothelial cells
Fibroblast cells
Macrophages cells
Mast cells
NK cells
Plasma cells
Stellate cells
T cells
B
Jup
Ductual or progenitor cell
Fibroblast activated cells
Macrophages
PanIN cells
Pericytes
C
GSE109227
Jup
**T-test, p = 1.1e-08
NS
CAE
GSE132326
**ANOVA, p = 5.5e-06
Normal
KC
KC+CAE
PDAC

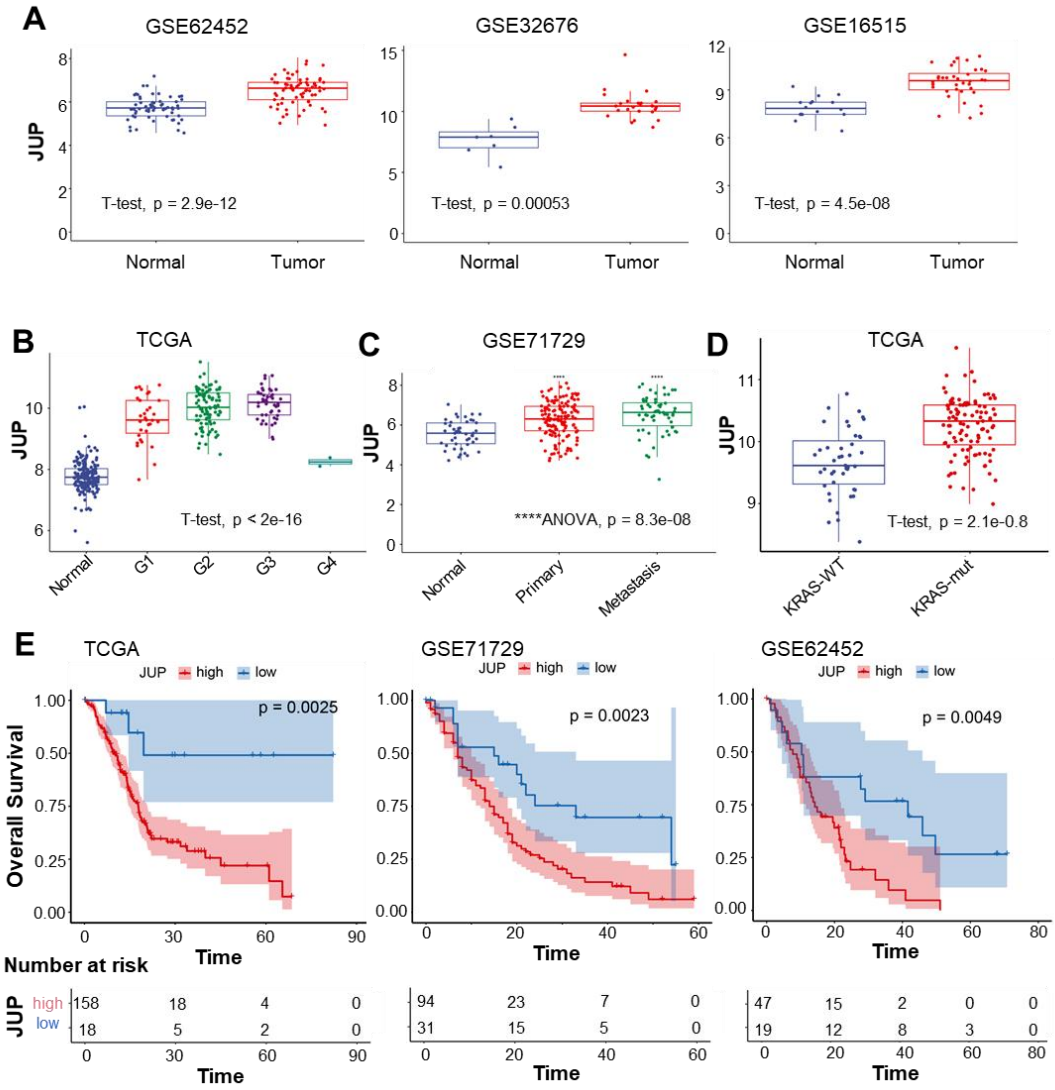
Fig. S1. JUP was upregulated in the tissues of pancreatitis, PanIN and PDAC.

(**A**) From single-cell gene expression database of human PDAC tissues (We integrated two single-cell transcriptomic datasets of pancreatic cancer (GSE154778 and GSE155698) using the "Harmony" algorithm), Uniform Manifold Approximation and Projection (UMAP) analyses show that JUP was predominantly expressed in human PDAC cells.

(**B**) From single-cell gene expression database of KC mouse tissues (GSE141017, https://www.ncbi.nlm.nih.gov/geo/), UMAP analysis show that with the clustering of different cell clusters during the disease progression of KC mice, JUP expression level was high in ductal or ductal-like cells from ADM and PanIN.

(**C**) Analysis of the GEO databases (https://www.ncbi.nlm.nih.gov/geo/) of mouse models of pancreatic injury found that JUP expression was increased in pancreatitis (caerulein, CAE; GSE109227), and pancreas with “injury” (CAE treatment), “Kras” (KC mouse model), “injury_Kras” (KC+CAE), and “PDAC” (KPC mouse model) (GSE132326).

Fig. S2


A
GSE62452
GSE32676
GSE16515
JUP
T-test, p = 2.9e-12
T-test, p = 0.00053
T-test, p = 4.5e-08
Normal
Tumor
B
TCGA
T-test, p < 2e-16
Normal
G1
G2
G3
G4
C
GSE71729
****ANOVA, p = 8.3e-08
Normal
Primary
Metastasis
D
TCGA
T-test, p = 2.1e-0.8
KRAS-WT
KRAS-mut
E
TCGA
GSE71729
GSE62452
JUP high low
p = 0.0025
p = 0.0023
p = 0.0049
Overall Survival
Time
Number at risk
high
low
158 18 4 0
18 5 2 0
94 23 7 0
31 15 5 0
47 15 2 0 0
19 12 8 3 0

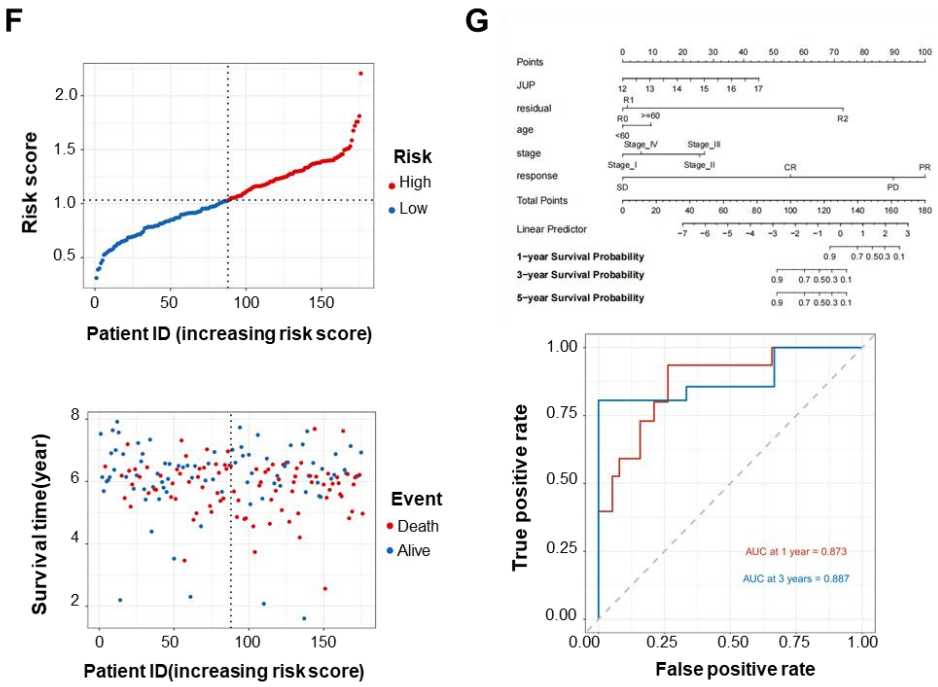


Fig. S2. JUP expression correlated with poor prognosis in PDAC patients.

(**A**) Analysis of JUP expression levels in PDAC ("tumor") and adjacent normal pancreatic tissues ("normal") using GSE62452, GSE32676 and GSE16515 datasets: the expression of JUP was higher in PDAC than that in non-cancer pancreatic tissues.

(**B**) Analysis of the expression levels of JUP in different pathological grades of PDAC using TCGA dataset: increased expression of JUP was associated with pathological grades of PDAC.

(**C**) Analysis of the expression levels of JUP in primary tumors *vs* metastases using GSE71729 dataset: higher expression of JUP was observed in metastases than that in primary tumors.

(**D**) Analysis of the expression levels of JUP in KRAS-WT PDAC versus KRAS-mutant PDAC using TCGA dataset: increased expression of JUP was associated with KRAS mutation in PDAC.

(**E**) Analysis of the effect of JUP expression levels (high or low) on overall survival of patients using TCGA, GSE71729 and GSE62452 datasets: high expression of JUP was associated with low survival in PDAC patients.

(**F**) The ggrisk risk score molecular plot for JUP: Patients with high JUP levels was associated with elevated risk stratification and poorer survival outcomes.

(G) Cox regression model was constructed to obtain nomogram and ROC curves of JUP expression in combination with clinical information: Patients with high expression of JUP demonstrate a significantly lower 3-year survival rate.

Fig. S3

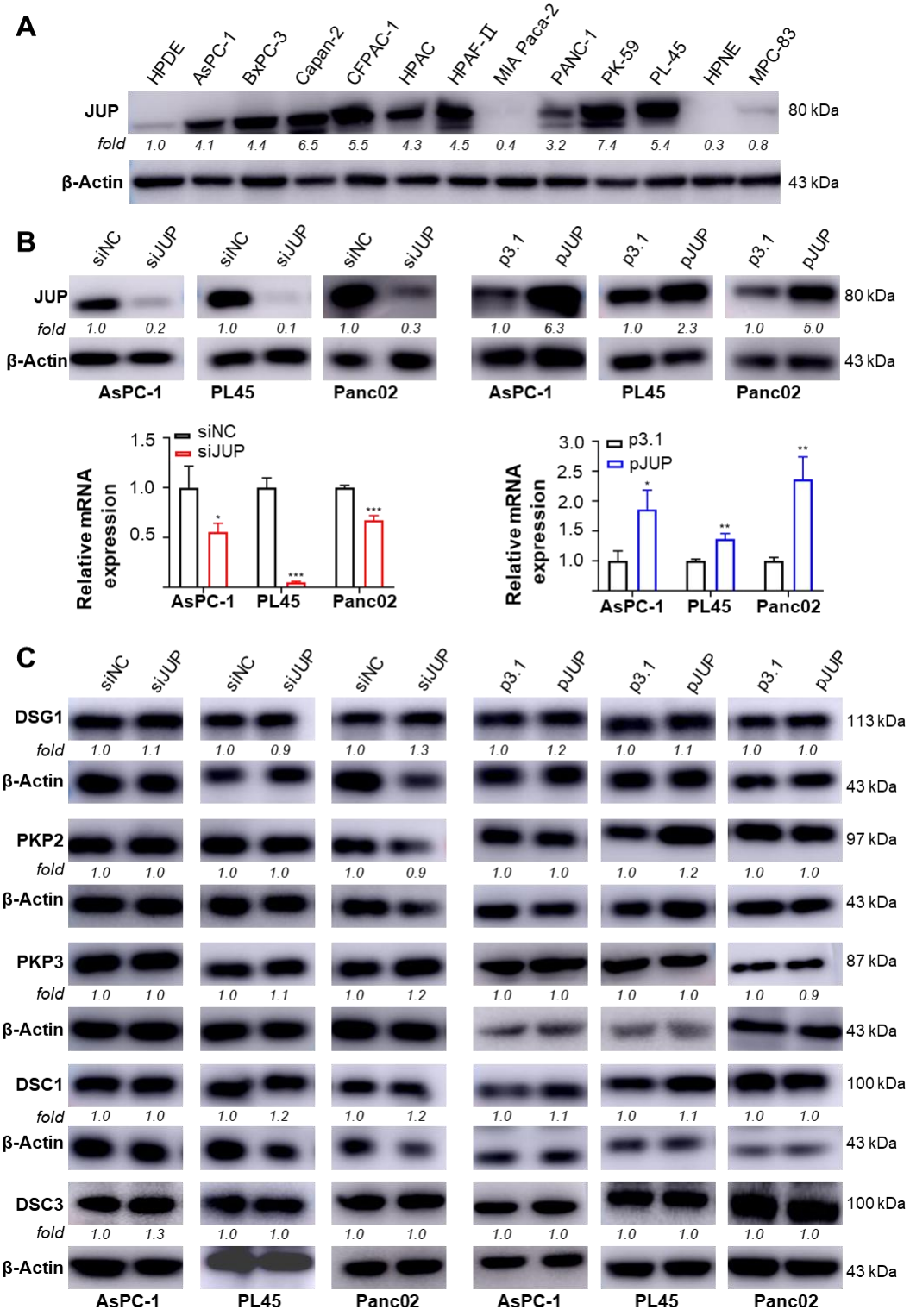

A
HPDE
AsPC-1
BxPC-3
Capan-2
CFPAC-1
HPAC
HPAF-II
MIA Paca-2
PANC-1
PK-59
PL-45
HPNE
MPC-83
JUP
80 kDa
fold 1.0 4.1 4.4 6.5 5.5 4.3 4.5 0.4 3.2 7.4 5.4 0.3 0.8
β-Actin
43 kDa
B
siNC siJUP siNC siJUP siNC siJUP
p3.1 pJUP p3.1 pJUP p3.1 pJUP
JUP
80 kDa
fold 1.0 0.2 1.0 0.1 1.0 0.3 1.0 6.3 1.0 2.3 1.0 5.0
β-Actin
43 kDa
AsPC-1 PL45 Panc02 AsPC-1 PL45 Panc02
Relative mRNA expression
siNC
siJUP
1.5
1.0
0.5
AsPC-1 PL45 Panc02
Relative mRNA expression
p3.1
pJUP
3.0
2.5
2.0
1.5
1.0
AsPC-1 PL45 Panc02
C
siNC siJUP siNC siJUP siNC siJUP p3.1 pJUP p3.1 pJUP p3.1 pJUP
DSG1
113 kDa
fold 1.0 1.1 1.0 0.9 1.0 1.3 1.0 1.2 1.0 1.1 1.0 1.0
β-Actin
43 kDa
PKP2
97 kDa
fold 1.0 1.0 1.0 1.0 1.0 0.9 1.0 1.0 1.0 1.2 1.0 1.0
β-Actin
43 kDa
PKP3
87 kDa
fold 1.0 1.0 1.0 1.1 1.0 1.2 1.0 1.0 1.0 1.0 1.0 0.9
β-Actin
43 kDa
DSC1
100 kDa
fold 1.0 1.0 1.0 1.2 1.0 1.2 1.0 1.1 1.0 1.1 1.0 1.0
β-Actin
43 kDa
DSC3
100 kDa
fold 1.0 1.3 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0
β-Actin
43 kDa
AsPC-1 PL45 Panc02 AsPC-1 PL45 Panc02

Fig. S3. JUP expression was high in PDAC cells and did not affect the expression of DSG1, PKP2, PKP3, DSC1 and DSC3.

(**A**) JUP protein expression was determined using Western blot analysis in HPDE pancreatic ductal epithelial cells, and PDAC cell lines including AsPC-1, BxPC-3, CaPan-2, HPAF-Ⅱ, PANC-1, PK-59 and PL45: JUP was highly expressed in most PDAC cell lines.

(**B**) JUP-knockdown or JUP overexpression were achieved by using JUP siRNA or JUP expression vector in AsPC-1, PL45 and Panc02 cells. The protein levels of JUP were detected by Western blot, and JUP mRNA levels were detected by RT-qPCR.

(**C**) The effects of altered expression of JUP on the expression of DSG1, PKP2, PKP3, DSC1 and DSC3 were determined by using Western blot analyses in AsPC-1, PL45 and Panc02 cells: altered expression of JUP did not affect the expression of DSG1, PKP2, PKP3, DSC1 and DSC3 in PDAC cells.

All data are expressed as mean ± SD, n = 3. Statistical analysis: Student t-test or ANOVA, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 as compared with control group.

Fig. S4

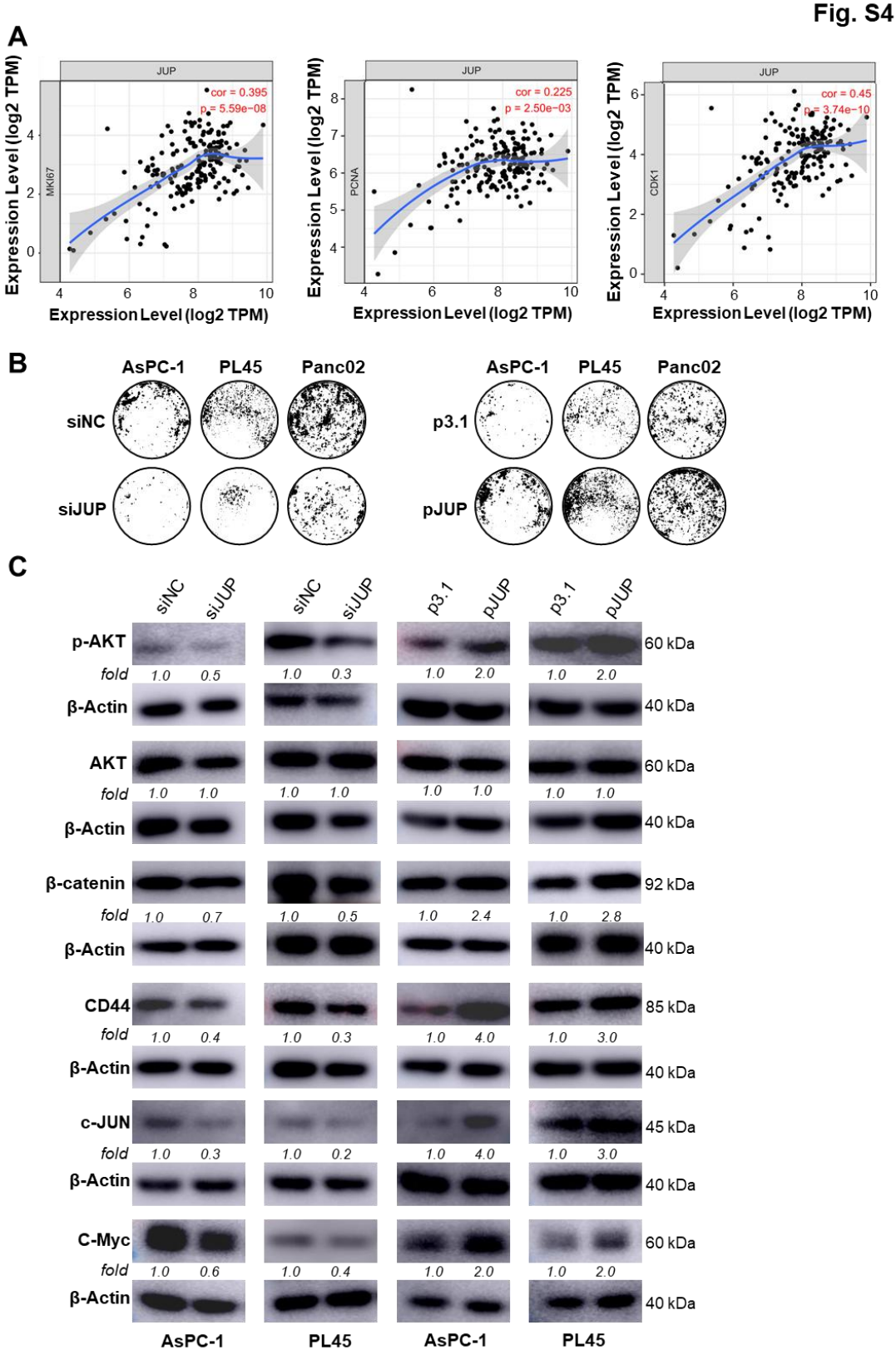

A
JUP
MKI67
cor = 0.395
p = 5.59e−08
PCNA
cor = 0.225
p = 2.50e−03
CDK1
cor = 0.45
p = 3.74e−10
Expression Level (log2 TPM)
B
AsPC-1
PL45
Panc02
siNC
siJUP
p3.1
pJUP
C
siNC
siJUP
p3.1
pJUP
p-AKT
AKT
β-catenin
CD44
c-JUN
C-Myc
β-Actin
fold
60 kDa
40 kDa
92 kDa
85 kDa
45 kDa
AsPC-1
PL45

Fig. S4. JUP expression levels correlated with that of cell proliferation genes and AKT and Wnt-β-catenin signaling pathways.

(**A**) Single cell transcriptome data analysis on the correlation between the expression levels of cell proliferation-related genes *MKI67, PCNA, CDK1* with the expression of *JUP* gene in human PDAC (TCGA database).

(**B**) Image of colony formation processed by software.

(**C**) The effects of altered expression of JUP on the expression of p-AKT, AKT, β-catenin, CD44, C-JUN and C-Myc were determined by using Western blot: JUP knockdown inhibited p-AKT and Wnt-β-catenin signaling pathways, while JUP overexpression promoted those signaling pathways.

All data are expressed as mean ± SD, n = 3. Statistical analysis: Student t-test or ANOVA, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 as compared with control group.

Fig. S5

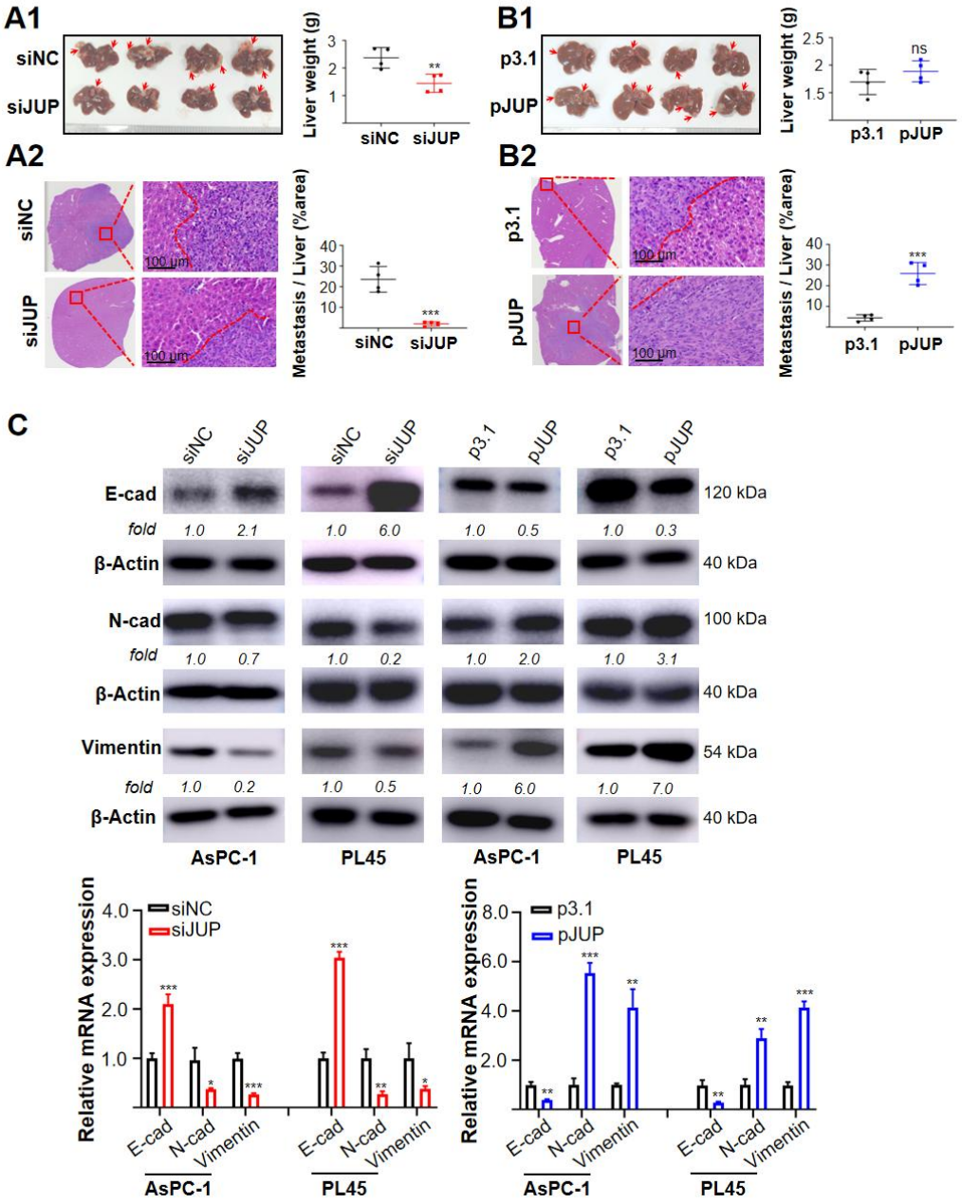

A1
siNC
siJUP
Liver weight (g)
siNC siJUP
B1
p3.1
pJUP
Liver weight (g)
ns
p3.1 pJUP
A2
siNC
siJUP
100 μm
Metastasis / Liver (%area)
siNC siJUP
B2
p3.1
pJUP
100 μm
Metastasis / Liver (%area)
p3.1 pJUP
C
siNC siJUP siNC siJUP p3.1 pJUP p3.1 pJUP
E-cad
fold 1.0 2.1 1.0 6.0 1.0 0.5 1.0 0.3
120 kDa
β-Actin
40 kDa
N-cad
100 kDa
fold 1.0 0.7 1.0 0.2 1.0 2.0 1.0 3.1
β-Actin
40 kDa
Vimentin
54 kDa
fold 1.0 0.2 1.0 0.5 1.0 6.0 1.0 7.0
β-Actin
40 kDa
AsPC-1 PL45 AsPC-1 PL45
Relative mRNA expression
siNC
siJUP
E-cad N-cad Vimentin E-cad N-cad Vimentin
AsPC-1 PL45
Relative mRNA expression
p3.1
pJUP
E-cad N-cad Vimentin E-cad N-cad Vimentin
AsPC-1 PL45

Fig. S5. JUP expression affected PDAC cells EMT

(**A1**) Representative images of metastatic tumors formed by JUP-silenced Panc02 cells in the livers of mice and analyses of the liver weight. (**A2**) Representative HE staining of liver tissues and analyses of tumor metastatic areas. Shown were gross livers with metastasis formed by JUP-overexpressing PDAC cells (**B1**) and representative HE staining images (**B2**).

(**C**) The effects of altered expression of JUP on the expression of E-cadherin, N-cadherin and Vimentin were determined by using Western blot and RT-qPCR. JUP knockdown inhibited EMT, but JUP overexpression promoted EMT.

All data were expressed as mean ± SD, n = 3. Statistical analysis: Student t-test or ANOVA, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 vs. control group.

.

Fig. S6

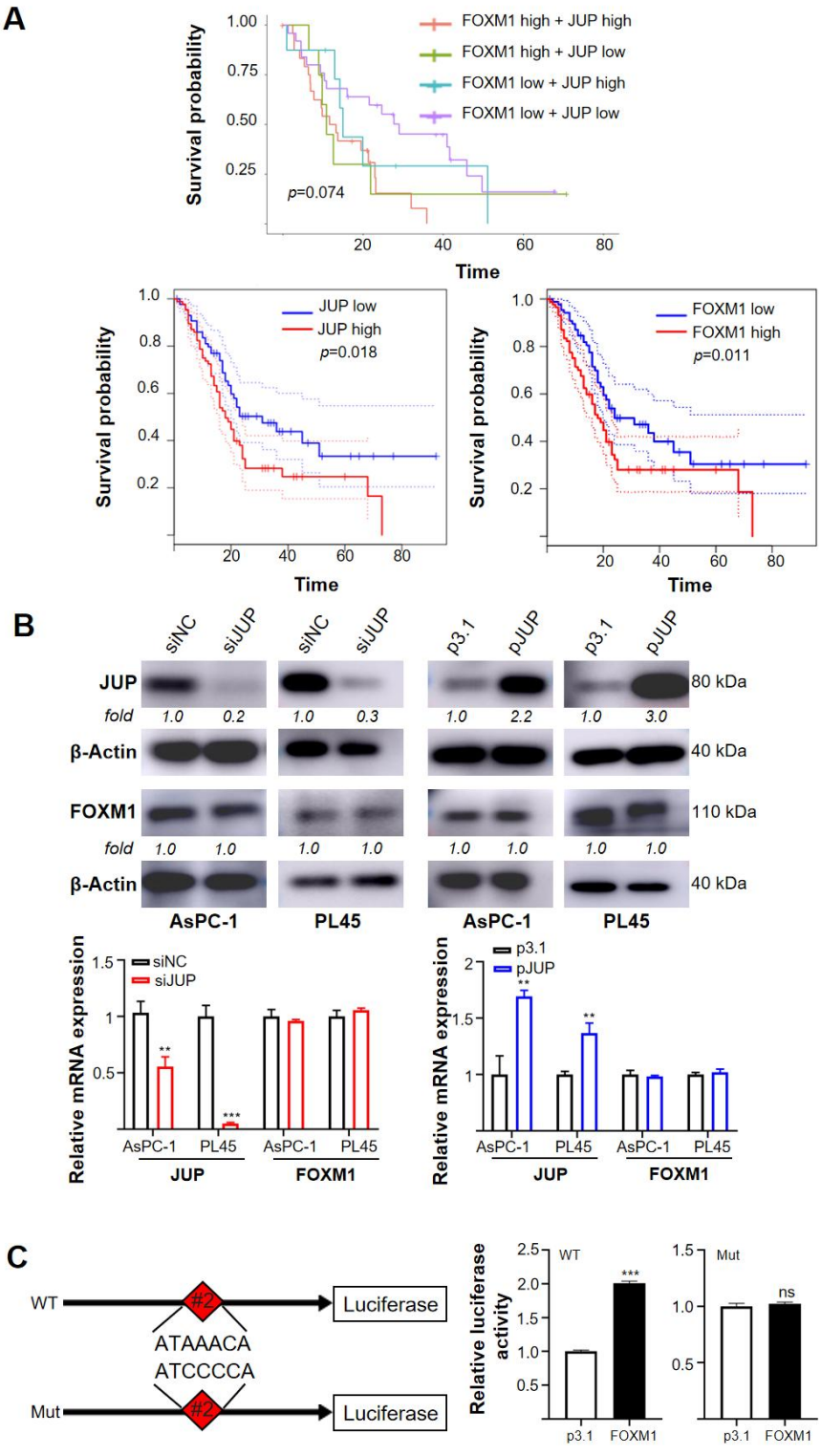

A
Survival probability
1.00
0.75
0.50
0.25
FOXM1 high + JUP high
FOXM1 high + JUP low
FOXM1 low + JUP high
FOXM1 low + JUP low
p=0.074
20
40
60
80
Time
JUP low
JUP high
p=0.018
FOXM1 low
FOXM1 high
p=0.011
B
siNC
siJUP
p3.1
pJUP
JUP
fold
1.0
0.2
0.3
2.2
3.0
β-Actin
FOXM1
80 kDa
40 kDa
110 kDa
AsPC-1
PL45
Relative mRNA expression
JUP
FOXM1
C
WT
Mut
#2
ATAAACA
ATCCCCA
Luciferase
Relative luciferase activity
ns

Fig. S6. FOXM1–JUP signaling axis and its aberrant activation informed poor prognosis in PDAC patients.

(**A**) Correlation between the expression of FOXM1 and JUP and overall survival of PDAC patients from different database (TCGA): high expression of both FOXM1 and JUP predicted poorest survival in PDAC patients.

(**B**) Western blot Analysis: altered expression of JUP by gene knockdown or overexpression did not impact FOXM1 protein expression in PDAC cells. RT-qPCR: altered expression of JUP by gene knockdown or overexpression did not impact FOXM1 mRNA expression in PDAC cells.

(**C**) Luciferase reporter assay with elimination of FOXM1 binding sites in the promoter of JUP by point mutation.

All data were expressed as mean ± SD, n = 3. Statistical analysis: Student t-test or ANOVA, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 *vs*. control group.

Fig. S7

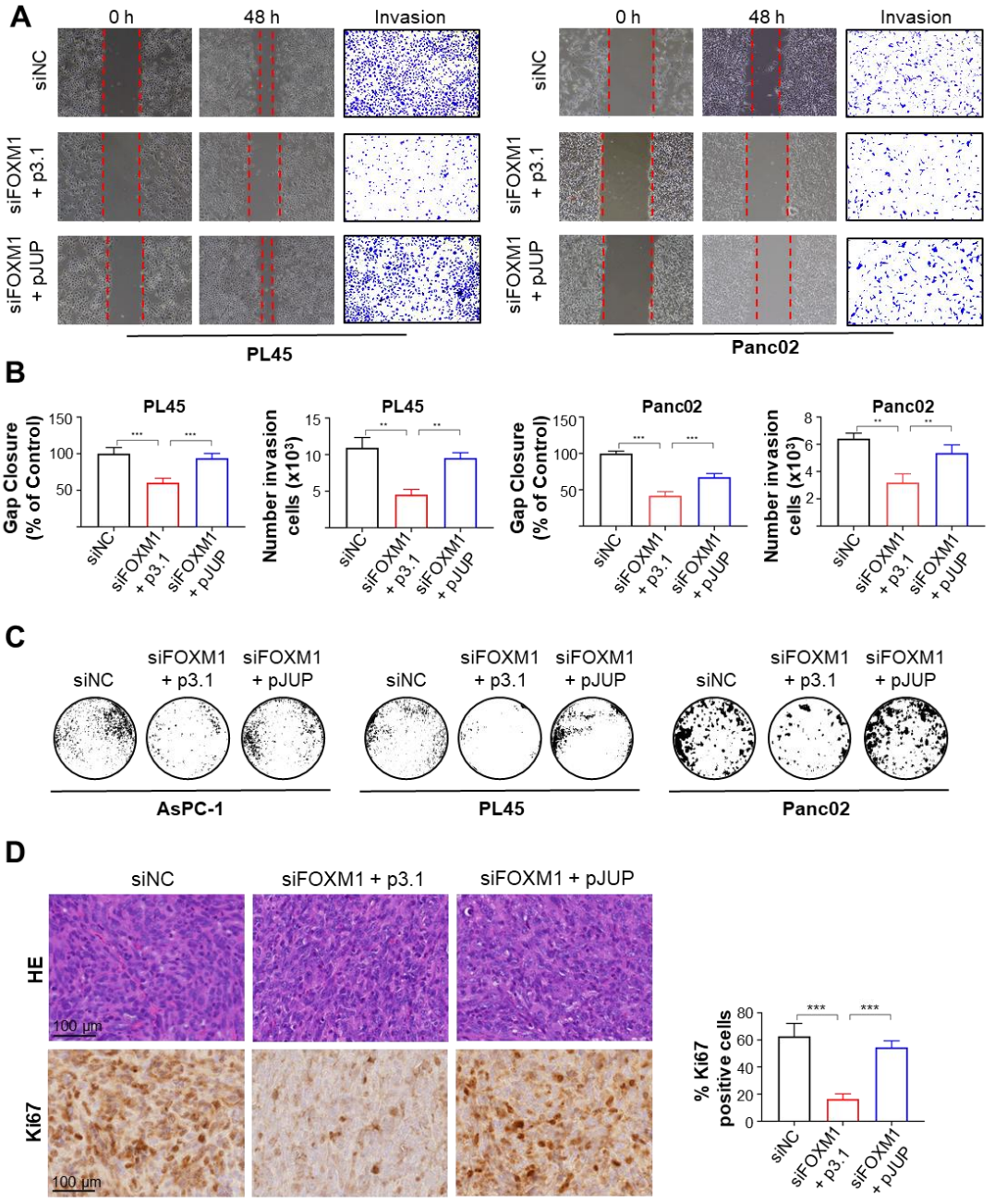

A
0 h
48 h
Invasion
siNC
siFOXM1 + p3.1
siFOXM1 + pJUP
PL45
Panc02
B
PL45
Panc02
Gap Closure (% of Control)
Number invasion cells (x10³)
siNC
siFOXM1 + p3.1
siFOXM1 + pJUP
C
AsPC-1
PL45
Panc02
D
HE
Ki67
100 μm
% Ki67 positive cells

Fig. S7. JUP expression was necessary for FOXM1-mediated promotion of cancer cell migration and invasion.

(**A**) Wound healing and Matrigel invasion assays: knockdown of FOXM1 inhibited the migration and invasion of PDAC cells, which attenuation effects were reversed by increased JUP expression.

(**B**) Quantification of data from (**A**).

(**C**) Image of colony formation processed by software.

(D) IHC: FOXM1 knockdown inhibited the expression of Ki67 in tumors, which was reversed by increased JUP expression.

All data were expressed as mean ± SD, n = 3. Statistical analysis: Student t-test or ANOVA, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ *vs*. control group. Scale bars, 100 μm.

Fig. S8

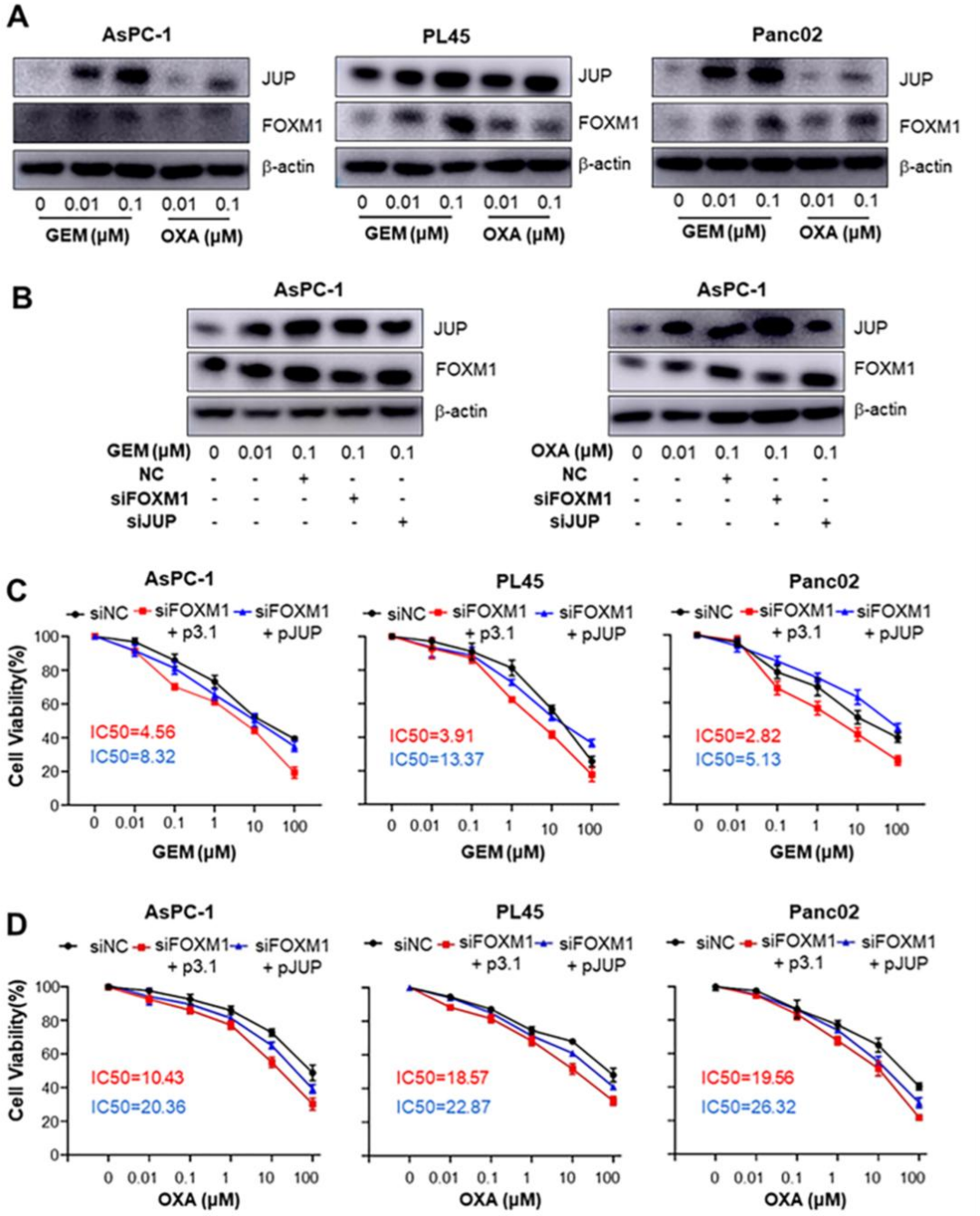

A
AsPC-1
PL45
Panc02
JUP
FOXM1
β-actin
0 0.01 0.1 0.01 0.1
GEM (μM)
OXA (μM)
B
AsPC-1
AsPC-1
GEM (μM) 0 0.01 0.1 0.1 0.1
NC - - + - -
siFOXM1 - - - + -
siJUP - - - - +
OXA (μM) 0 0.01 0.1 0.1 0.1
NC - - + - -
siFOXM1 - - - + -
siJUP - - - - +
C
AsPC-1
PL45
Panc02
siNC
siFOXM1 + p3.1
siFOXM1 + pJUP
Cell Viability(%)
IC50=4.56
IC50=8.32
IC50=3.91
IC50=13.37
IC50=2.82
IC50=5.13
GEM (μM)
D
AsPC-1
PL45
Panc02
IC50=10.43
IC50=20.36
IC50=18.57
IC50=22.87
IC50=19.56
IC50=26.32
OXA (μM)

Fig. S8. **FOXM1-mediated therapeutic resistance requires JUP expression.**

(A), AsPC-1, PL45 and Panc02 cells were incubated with different concentrations of gemcitabine ("GEM") or oxaliplatin ("OXA") for 48 h. Western blot analyses were performed to determine the expression of JUP and FOXM1. Note that both GEM and OXA induced FOXM1 and JUP expression, while GEM induced gene expression more effectively than OXA at the same concentration. (**B)**, AsPC-1 cells were transfected with either siJUP or siFOXM1 and treated with either GEM or OXA for 48 h, and Western blot analyses were performed to determine the expression of JUP and FOXM1. Note that both GEM and OXA induced FOXM1 and JUP expression, while knockdown of FOXM1 prevented the induction of JUP by treatment of either GEM or OXA, but not vice versa, suggesting that induction of JUP was dependent on FOXM1 expression. (**C)**, We then performed FOXM1 knockdown in AsPC-1, PL45 and Panc02 cells with or without JUP gene transfection to maintain the expression of JUP, and then treated the cells with different concentrations of GEN or OXA for 48 h. Cell viability was determined by using CCK8 assay. Note that FOXM1 knockdown sensitized PDAC cells to chemotherapy, while restoring the expression of JUP attenuated the effects of FOXM1 knockdown.

All data were expressed as mean ± SD, n = 3. Statistical analysis: Student t-test or ANOVA, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 *vs*. control group.

Fig. S9

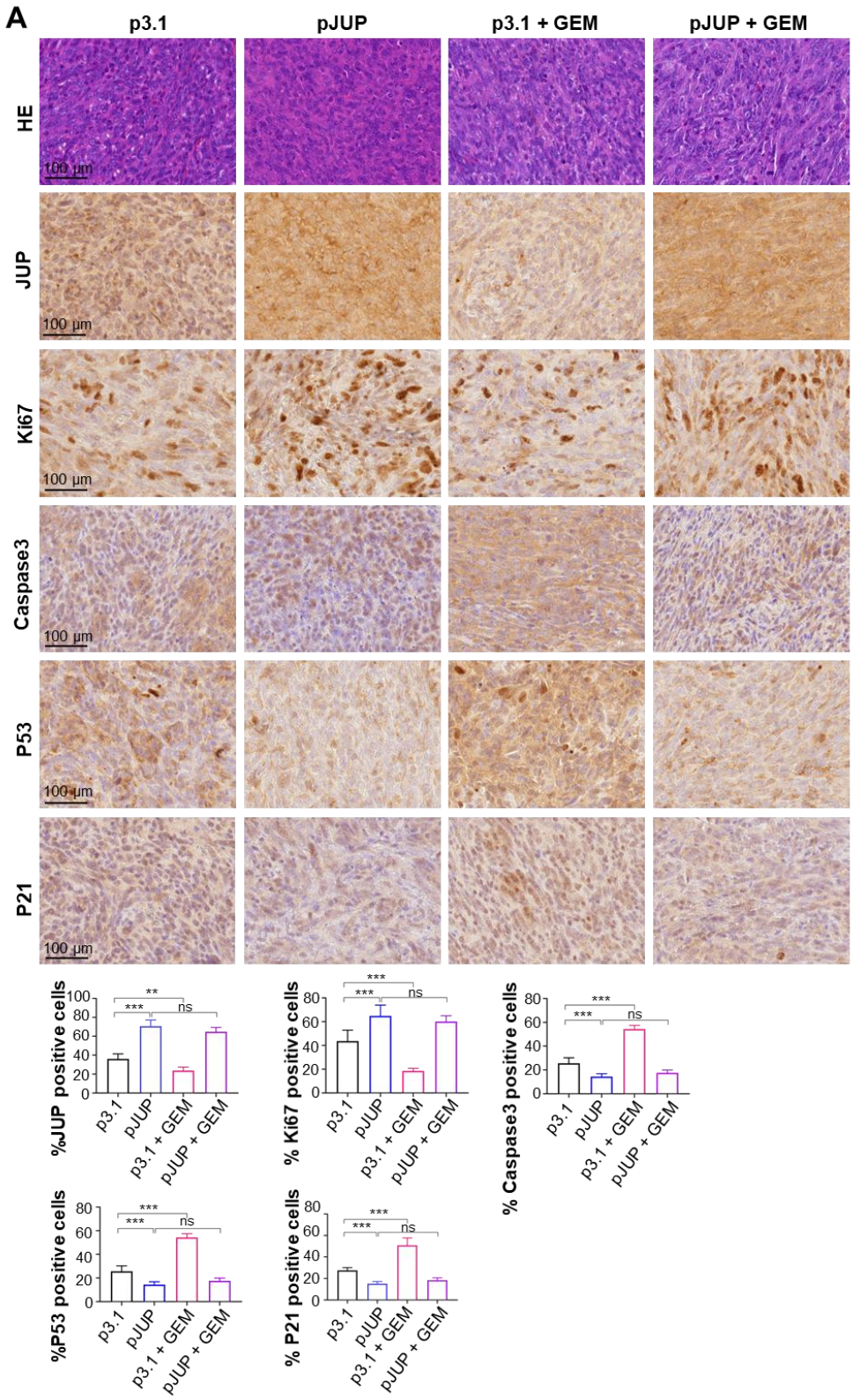

A
p3.1
pJUP
p3.1 + GEM
pJUP + GEM
HE
JUP
Ki67
Caspase3
P53
P21
100 μm
%JUP positive cells
% Ki67 positive cells
% Caspase3 positive cells
%P53 positive cells
% P21 positive cells
**
***
ns

Fig. S9. **JUP inhibited P53 and P21 and enhanced PDAC cells resistance to GEM.**

(A): IHC was employed to determine the expression of JUP, Ki67, Caspase3, P53 and P21. A decreased P53 and P21 expression correlated with JUP overexpression in tumor specimens. GEM induced the arrest of the cell cycle progression, while JUP overexpression attenuated the promotional effect of GEM on cell cycle arrest markers. All data were expressed as mean ± SD, n = 3. Statistical analysis: Student t-test or ANOVA, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 *vs*. control group. Scale bars, 100 μm.