

SUPPLEMENTARY MATERIALS AND METHODS

Primers and siRNAs

Real-time PCR primers:

CTGF-up: 5'-CAGGCTAGAGAAGCAGAGCC-3';
CTGF-dn: 5'-TGGAGATTTTGGGAGTACGG-3';
BIRC5-up: 5'-TTGGTGAATTTTGAAGCTGGA-3';
BIRC5-dn: 5'-CTTCTCCGCAGTTTCCTCA-3';
BCL2L1-up: 5'-TTCAGTGACCTGACATCCCA-3';
BCL2L1-dn: 5'-CTGCTGCATTGTTCCCATAG-3';
GAPDH-up: 5'-ATTCCACCCATGGCAAATTC-3';
GAPDH-dn:
5'-TGGGATTTCCATTGATGACAAG-3'.

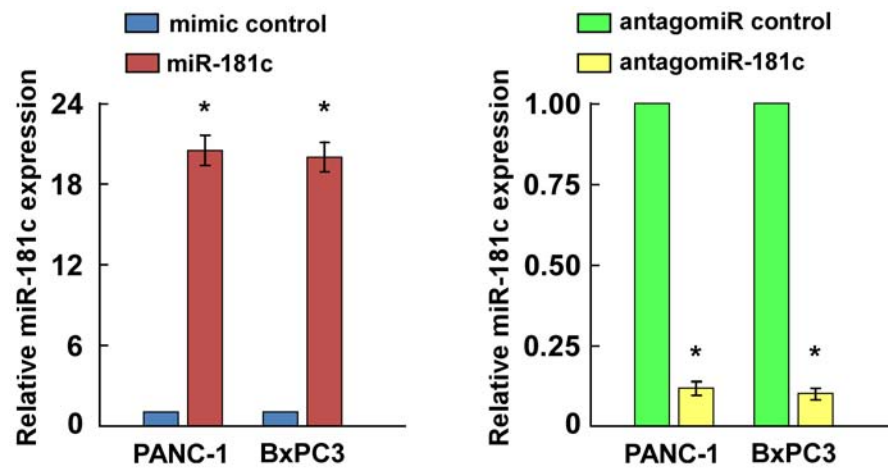
miRNP primers:

GAPDH-up: 5'-TCCCCACCACACTGAATCT-3';
GAPDH-dn: 5'-GCCCTCCCCTCTTCAAG-3';
MST1-up: 5'-GTGGCTAGAATATGGCAGAG-3';

MST1-dn: 5'-GTAGATTGGGCAGGAACA-3';
LATS2-up: 5'-ATATCTAGGCAACAACAC-3';
LATS2-dn: 5'-AATAGCGAGAATACTGAC-3';
MOB1-up: 5'-CCGATGGATTGGTTTCTG-3';
MOB1-dn: 5'-TTGTTTCAAGCCCTGTTC-3';
SAV1-up: 5'-ATTGTCTGGAAAGGGATG-3';
SAV1-dn: 5'-GCTGGGATTACAGGAGTG-3';

siRNA sequences:

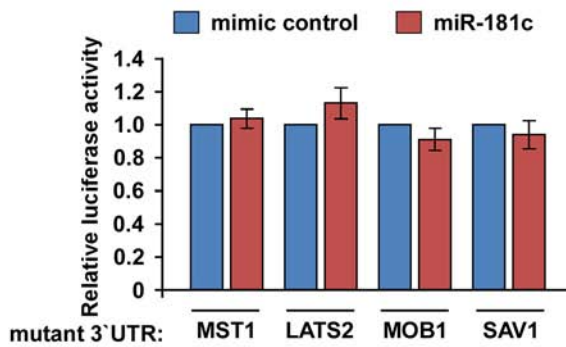
YAP siRNA:
5'-CAGGUGAUACUAUCAACCAAA-3';
TAZ siRNA:
5'-CCUGCCGGAGUCUUUCUUUAA-3';
MST1 siRNA:
5'-CCGGCCAGAUUGUUGCUAUUA-3';
LATS2 siRNA:
5'-CUACUCGCCAUACGCCUUUAA-3'.



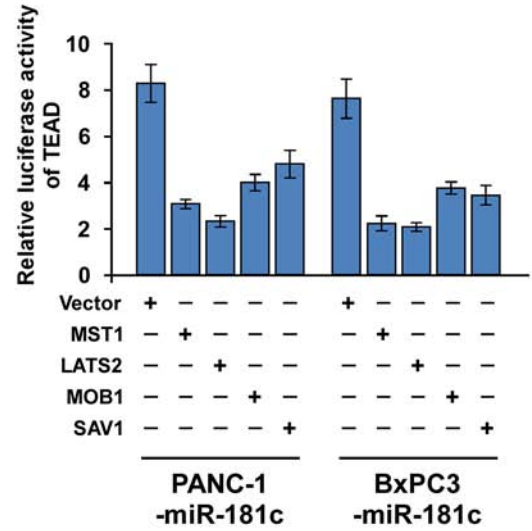
Supplementary Figure S1: Real-time PCR analysis of miR-181c expression in PANC-1 and BxPC3 cells transfected with miR-181c mimic or antagomiR-181c as compared to the controls. Transcript levels were normalized by *U6* expression. Error bars represent the mean \pm s.d. of three independent experiments. * $P < 0.05$.

A

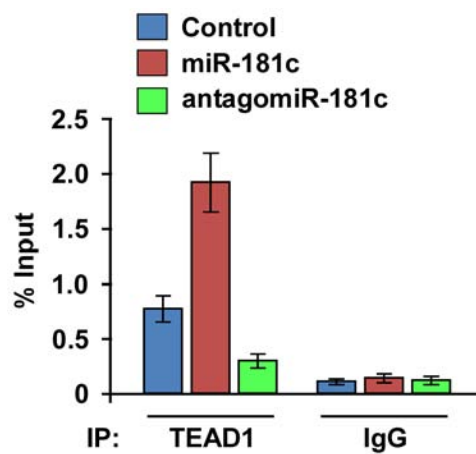
MST1-3'UTR-mut 5' CACCAAUUGGAUGGAUGACACAUUU 3'
 LATS2-3'UTR-mut 5' CACUGAAAGCUUCAGUGACACAUUU 3'
 MOB1-3'UTR-mut 5' AAGAGAAGGGGAUAGGACACAUUA 3'
 SAV1-3'UTR-mut 5' UUUGGUCAUUAAAUCGACACAUUA 3'



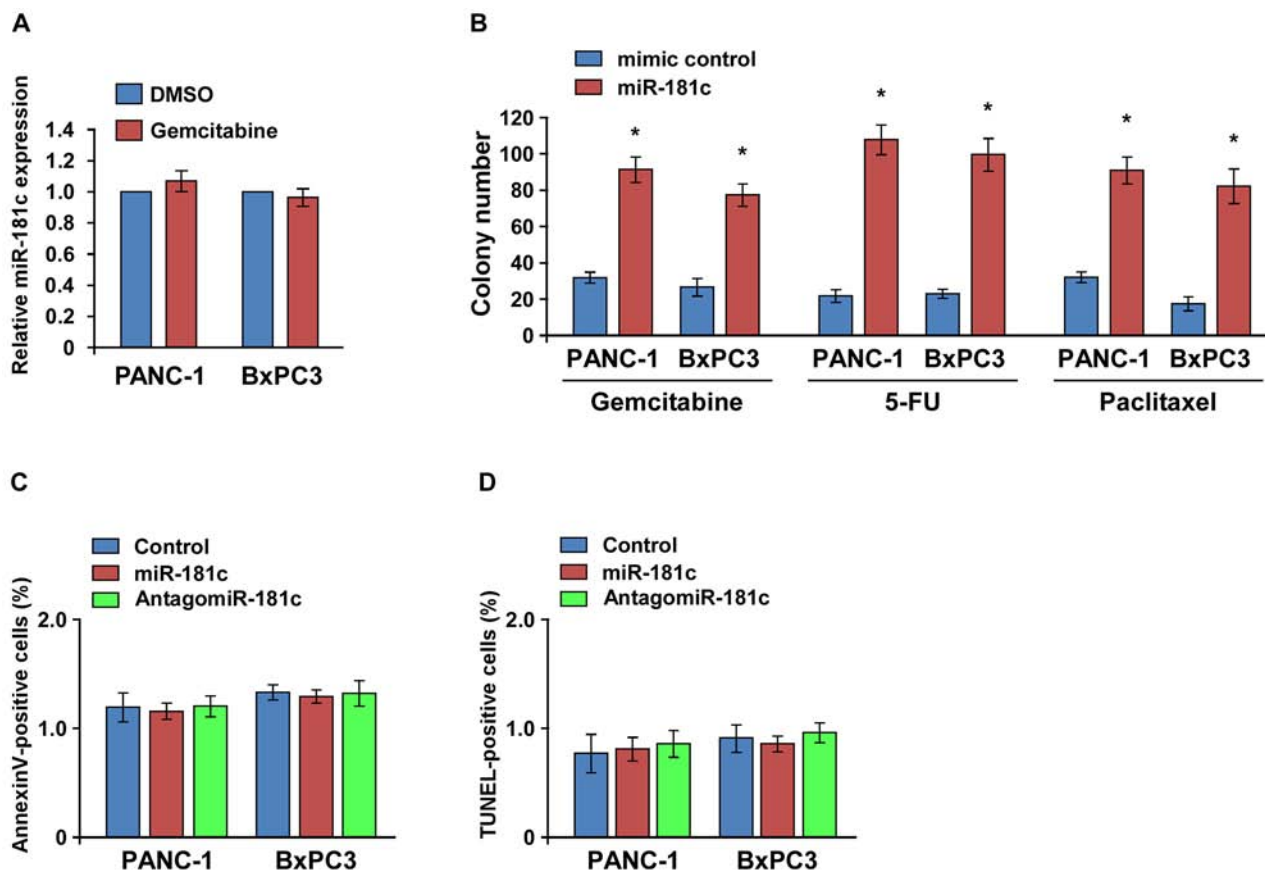
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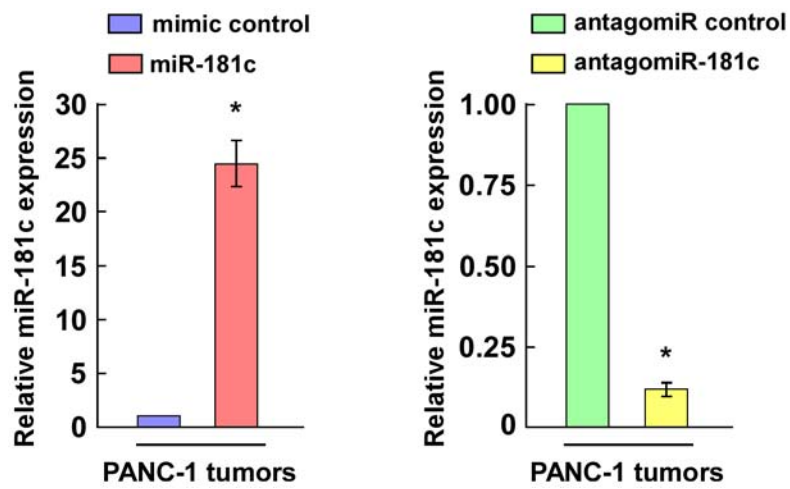
Supplementary Figure S2: A. Ectopic expression of the miR-181c did not exhibit repressive effects on the reporter activities driven by the mutant 3'UTRs of these transcripts within miR-181c-binding seed regions. B. Individual overexpression of MST1, LATS2, MOB1 or SAV1 potently inhibited the TEAD activity in miR-181c-overexpressing cells.



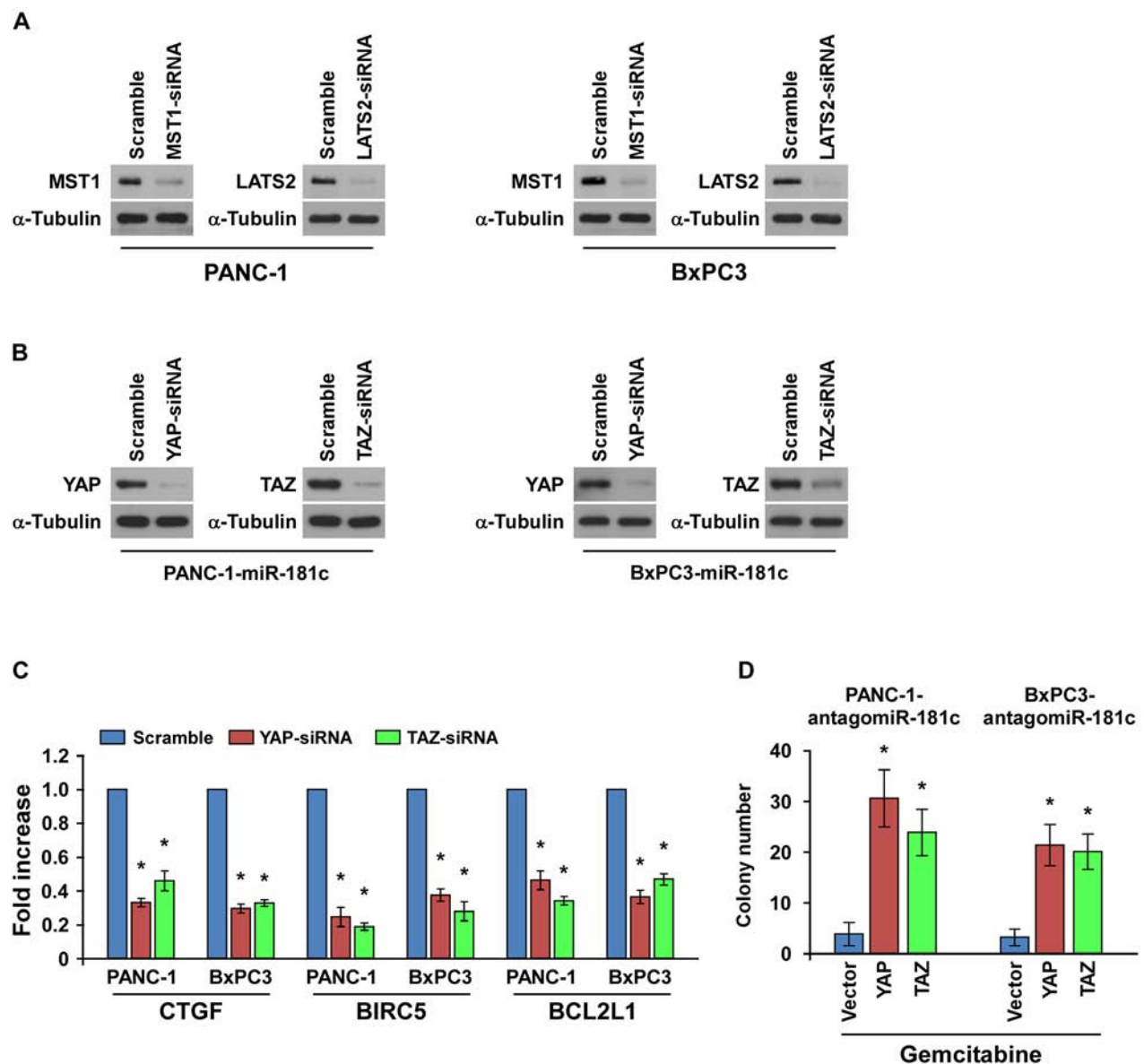
Supplementary Figure S3: Chip assay showing that overexpression of miR-181c increased, while silencing miR-181c reduced the binding capability of TEAD1 with CTGF promoter.



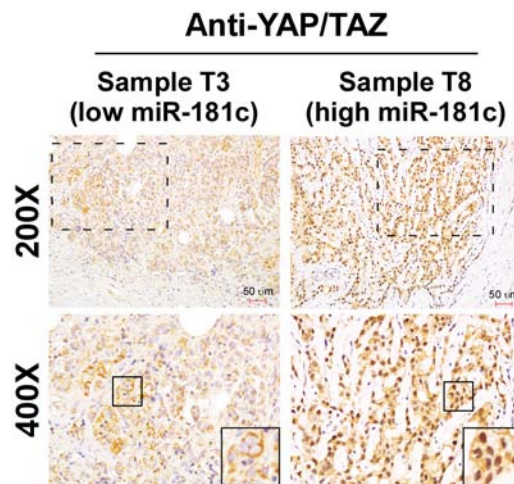
Supplementary Figure S4: A. Real-time PCR analysis of miR-181c expression under gemcitabine treatment. Transcript levels were normalized to *U6* expression. **B.** Quantification of crystal violet-stained PANC-1 and BxPC3 cell colonies overexpressing miR-181c in the presence of gemcitabine (5 μM), 5-FU (5 μM), or paclitaxel (10 μM). **C and D.** Annexin V-FITC/PI staining assay (C) and TUNEL analysis (D) revealed that neither overexpression of miR-181c nor silencing of miR-181c had an effect on the apoptotic percentage of PANC-1 and BxPC3 cell lines without chemo-drug treatment. Error bars represent the mean ± s.d. of three independent experiments. **P* < 0.05.



Supplementary Figure S5: Real-time PCR analysis of miR-181c expression in PANC-1 xenograft tumors injected with miR-181c mimic or antagomiR-181c as compared to controls. Transcript levels were normalized by *U6* expression. Error bars represent the mean \pm s.d. of three independent experiments. * $P < 0.05$.



Supplementary Figure S6: A. MST1 or LATS2 was silenced by siRNA transfection in pancreatic cancer cell lines, and their expression levels were examined by western blotting. α -Tubulin served as the loading control. **B. YAP or TAZ was silenced by siRNA transfection in miR-181-overexpressing cells, and their expression levels were examined by western blotting.** α -Tubulin served as the loading control. **C. Real-time PCR analysis revealed that the stimulatory effect of miR-181c on the expression of CTGF, BIRC5 and BCL2L1 were inhibited by YAP or TAZ silencing.** **D. Colony formation assay showed that overexpression of either YAP or TAZ significantly rendered resistance of miR-181c-silenced cells to gemcitabine.** Error bars represent the mean \pm s.d. of three independent experiments. * $P < 0.05$.



Supplementary Figure S7: IHC staining of YAP/TAZ protein in pancreatic tissues. Two representative cases (T3 with low miR-181c, T8 with high miR-181c) are shown. The results revealed that nuclear YAP/TAZ was strongly expressed in pancreatic cancer cells but not in the surrounding stromal cells, and correlated with miR-181c expression.

Supplementary Table S1: Clinicopathological characteristics of studied patients and expression of miR-181c in pancreatic cancer

Factor	NO.	(%)
Age (years)		
≥60	52	41.9
<60	72	58.1
Sex		
male	84	67.7
female	40	32.3
Histological differentiation		
Grade 1/2	91	73.4
Grade 3/4	33	26.6
T stage		
T1-2	82	66.1
T3	42	33.9
N stage		
N0	69	55.6
N1	55	44.4
M stage		
No	46	37.1
Yes	78	62.9
Expression of miR-181c		
Low expression	62	50.0
High expression	62	50.0

Supplementary Table S2: Correlation between the clinicopathological features and expression of miR-181c

Patient characteristics		miR-181c expression		P value
		Low	High	
Age (years)	≥60	31	21	0.069
	<60	31	41	
Sex	male	44	40	0.442
	female	18	22	
Histological differentiation	Grade 1/2	52	39	0.008
	Grade 3	10	23	
T Stage	T1-2	48	34	0.008
	T3	14	28	
N Stage	N0	43	26	0.002
	N1	19	36	
M Stage	No	31	15	0.003
	Yes	31	47	