Beneficial effect of vactosertib combined with nal-IRI/5-FU/LV in pancreatic cancer treatment

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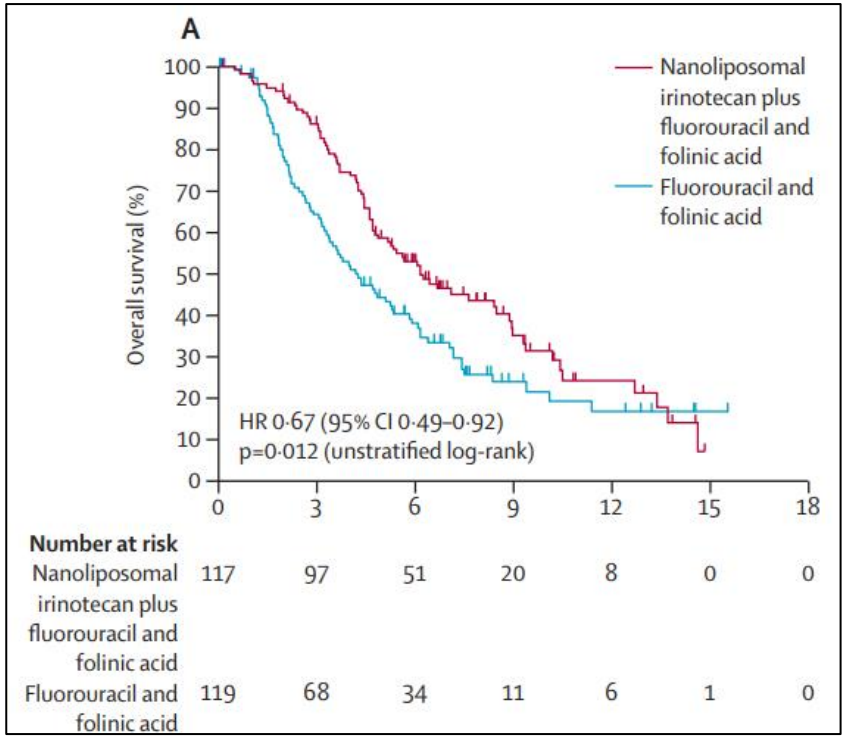
Introduction

- Combination treatment of pancreatic cancer

Table 3 Key trials for palliative chemotherapy in pancreatic cancer (1,2,4,5,12,15-17)

Line	Trial	n	Arms	OS (months)	P, HR
1	PRODIGE-4/ACCORD-11	342	FOLFIRINOX *vs.* gemcitabine	11.1 *vs.* 6.8	<0.001, 0.57
1	MPACT	861	Gemcitabine/nab-paclitaxel *vs.* gemcitabine	8.5 *vs.* 6.7	<0.001, 0.72
1	Moore *et al.*	569	Gemcitabine/erlotinib *vs.* gemcitabine	6.24 *vs.* 5.91	0.038, 0.82
1	Burris *et al.*	126	Gemcitabine *vs.* 5-FU	5.65 *vs.* 4.41	0.0025, –
2	CONKO-003	168	OFF *vs.* 5-FU	5.9 *vs.* 3.3	0.01, 0.66
2	Portal *et al.*	57	Gemcitabine/nab-paclitaxel after FOLFIRINOX	8.8	–
2/3	NAPOLI-1	417	Lip-irinotecan/5-FU/LV *vs.* 5-FU/LV	6.1 *vs.* 4.2	0.012, 0.67

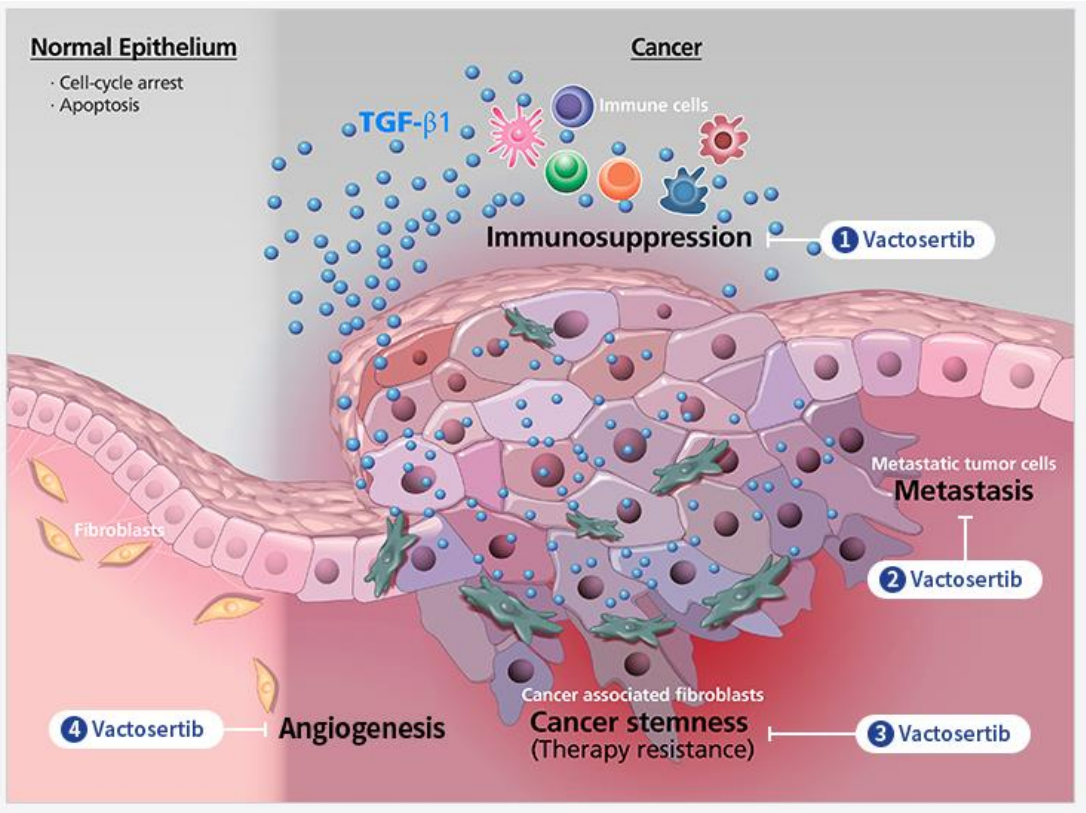
Abbassi R, Algül H. **Palliative chemotherapy in pancreatic cancer—treatment sequences**. Transl Gastroenterol Hepatol 2019;4:56.



Wang-Gillam, AndreaAdoo, Clarence et al. **Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial.** The Lancet, Volume 387, Issue 10018, 545 - 557

Introduction

➢ TGF-beta signaling inhibitor- Vactosertib



Clinical trial list

	Type	Indication	Combination Agent	Country
Solid Tumor	Chemotherapy Combination	Gastric Cancer	Paclitaxel	KOR
		Gastric Cancer	Paclitaxel, Ramucirumab	KOR
		Pancreatic Cancer	FOLFOX	KOR
		Pancreatic Cancer	5FU/LV/Onivyde	KOR
	Targeted therapy combination	Desmoid sarcoma	Imatinib	KOR
	Immunotherapy Combination	Colorectal & Gastric Cancer	Pembrolizumab	KOR
		NSCLC	Pembrolizumab	KOR
		NSCLC	Durvalumab	KOR
		Bladder Cancer	Durvalumab	USA
Hema	Immunomodulatory agent combination	Multiple myeloma	Pomalidomide	USA
	Targeted therapy combination	Myeloproliferative neoplasm	Ruxolitinib	USA

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TGF-β signaling inhibitor in pancreatic cancer

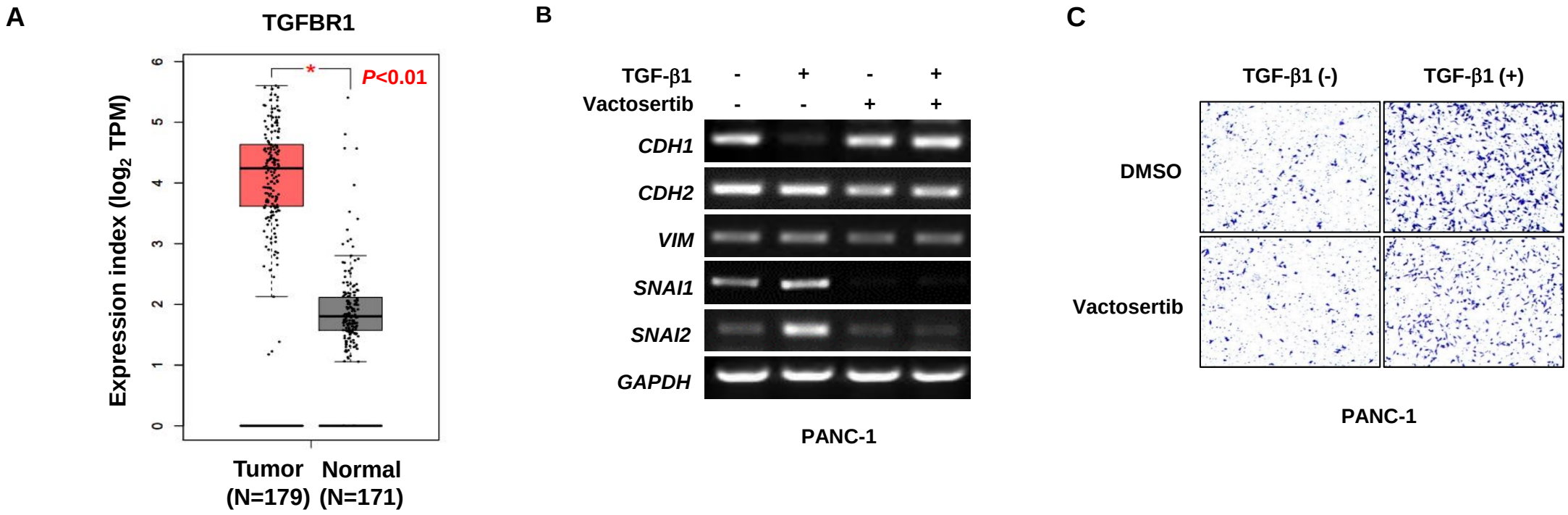


Figure 1. Overexpressed TGFBR1 in human pancreatic tumor and inhibition of the TGF-β1-induced migration and EMT response by vactosertib in pancreatic cancer cell

(A) TCGA analysis of TGFBR1 expression in pancreatic cancer patient tissue. Tumor (red box) has overexpressed level of TGFBR1 compared to normal tissue (grey box). (B) RT-PCR showing EMT marker expression and (C) migration assay of PANC-1 cells treated with vactosertib and TGF-β1. For *in vitro* experiments, PANC-1 cells were pre-treated with vactosertib for 2 hours and incubated with TGF-β1 for 48 hours.

Improvement of survival rate by Vac+nal-IRI/5-FU/LV combination

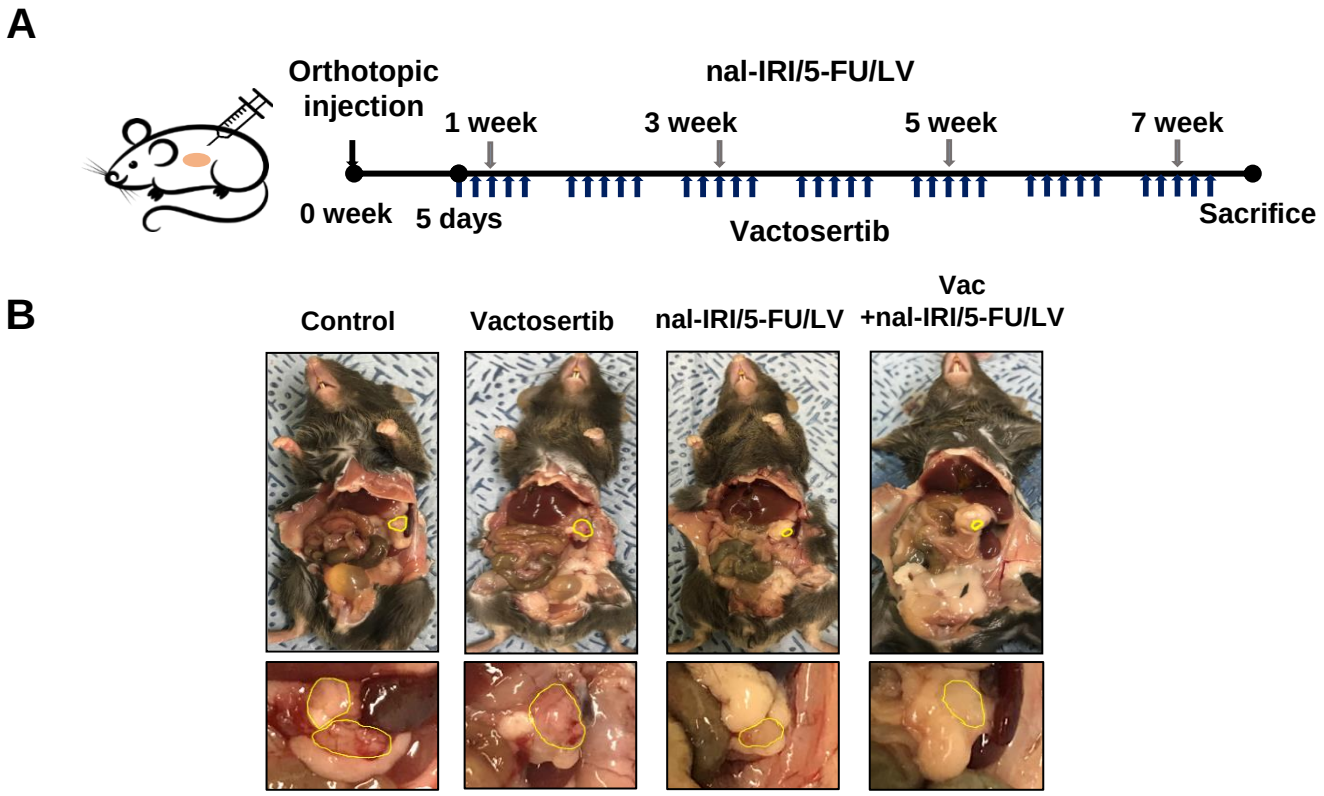


Figure 2. Survival improvement in response to combination treatment of vactosertib with nal-IRI/5-FU/LV in the orthotopic pancreatic cancer mouse model

(A) Experimental design of C57BL/6 syngeneic orthotopic mouse model using Panc02 murine pancreatic cancer cells. Vactosertib was administered orally for 5 consecutive days followed by 2 days of resting period starting from day 6 post cell injection. nal-IRI/5-FU/LV was injected intraperitoneally every 2 weeks starting from day 8 post cell injection. (B) Representative pictures of mice in each group (above) and enlarged pictures of the pancreatic tumor tissues outlined by yellow lines (bottom). (C) Relative survival rates of the control and vactosertib, nal-IRI/5-FU/LV, and combined treatment groups (***P<0.0005 and **P<0.005 compared to the control group)

Inhibition of tumor cell invasion and EMT responses in mouse tissues

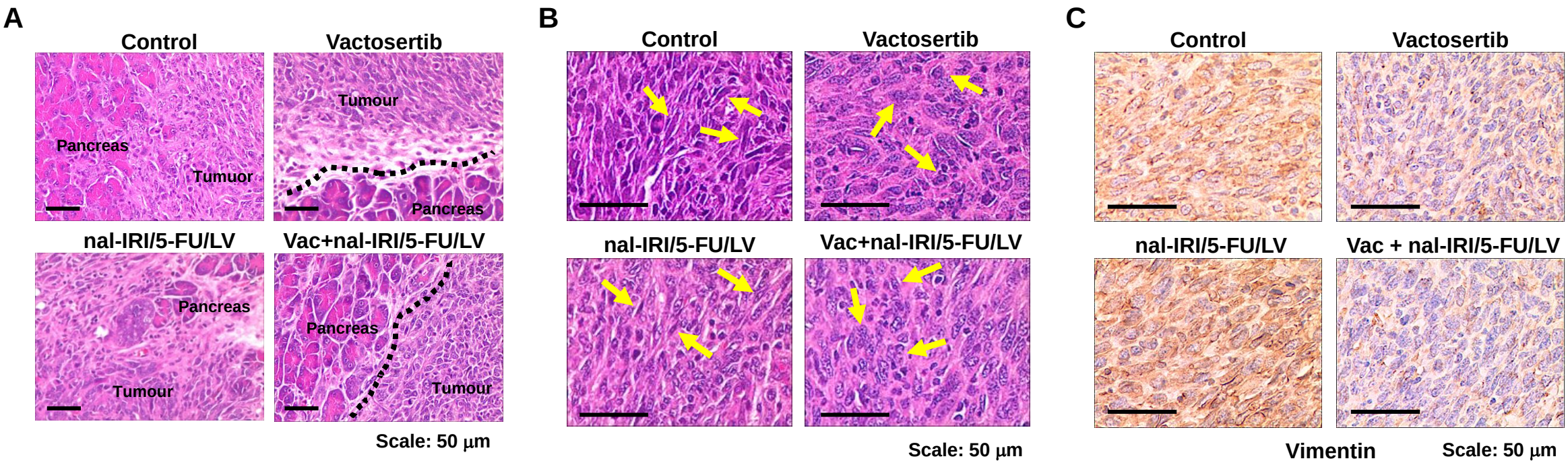


Figure 3. Cancer cell invasion is decreased by combination treatment of vactosertib with nal-IRI/5-FU/LV in the orthotopic pancreatic cancer mouse model

(A) Haematoxylin and eosin (H&E) staining of pancreatic tumor tissues showing tumor cell invasion to adjacent pancreas tissues. Black dotted lines indicate the borders between tumorous and normal pancreas without invading tumor cells. (B) The morphologies of tumor cells presented by H&E staining. Yellow arrows point out the representative mesenchymal (control and nal-IRI/5-FU/LV) and epithelial (vactosertib and Vac+nal-IRI/5-FU/LV) cell morphologies in the images. (C) The immunohistochemical staining of vimentin in tumor tissues of each group.

Co-treatment of vactosertib with nal-IRI/5-FU suppresses cell migration, invasion, and EMT

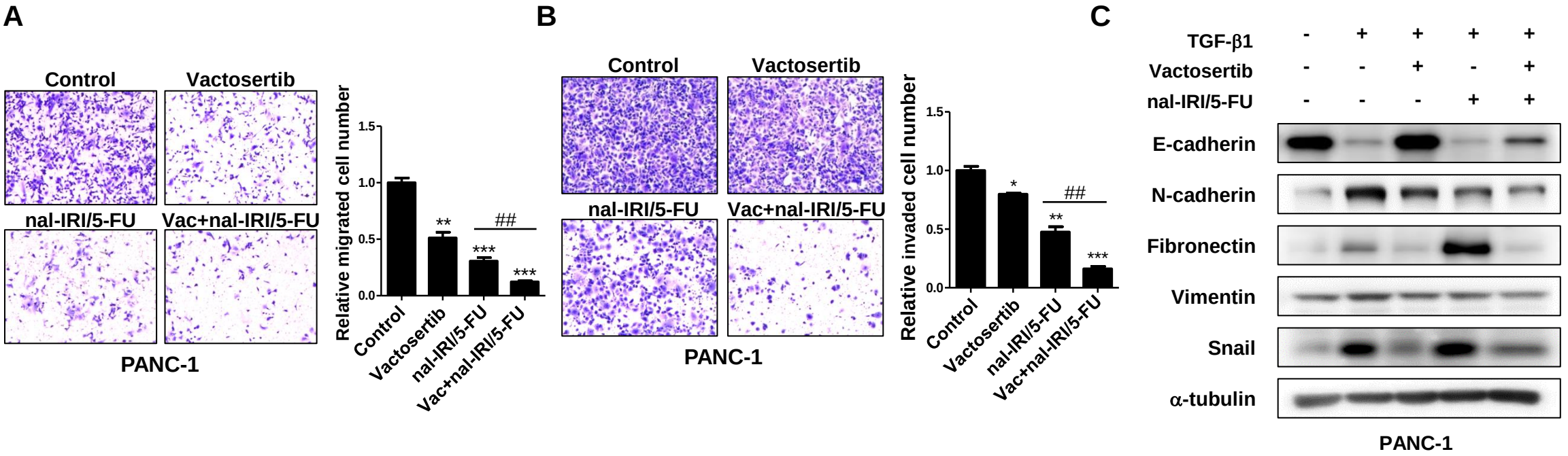


Figure 2. Synergistic effect of vactosertib with nal-IRI/5-FU on migration, invasion, and EMT of pancreatic cancer cells

(A) Transwell cell migration assay. Cells were treated with the indicated reagent(s) and incubated for 48 hours before placed in a migration chamber. The relative number of migrated cells in each group was counted. (B) Cell invasion assay. Cells were treated with the indicated reagent(s) and incubated for 48 hours before placed in an invasion chamber. The number of invaded cells in each group was counted. (A-B) The values for migrated/invaded number of cells represent the mean ± SD of triplicate data. ***P<0.0005, **P<0.005, and *P<0.05 compared to the control group; ##P<0.005 and #P<0.05 compared to the nal-IRI-treated group. (C) Western blot analysis for EMT markers.

Transcriptome profiling of pancreatic tumor tissues following combination treatment of Vactosertib with nal-IRI/5-FU/LV

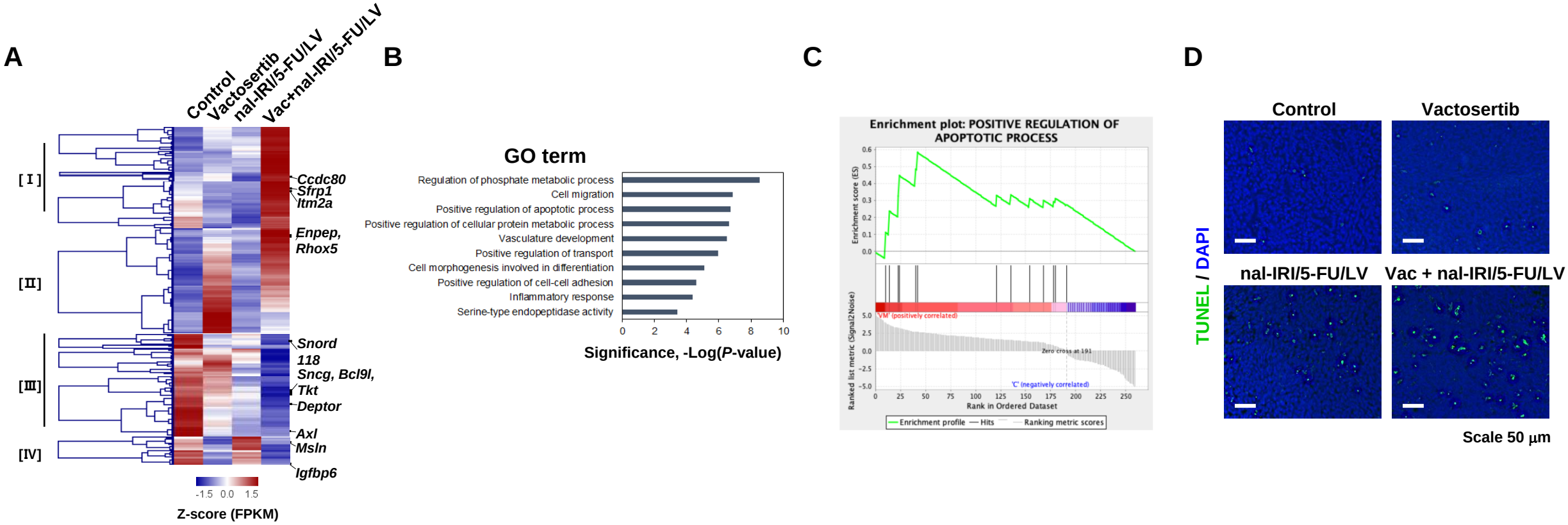


Figure 3. RNA-sequencing analysis of pancreatic tumor tissues obtained from orthotopic mouse model administered with vactosertib, nal-IRI/5-FU/LV, and the combination

(A) Heatmap plot of DEGs using the hierarchical clustering method. (B) Top 10 pathways characterized by from GO enrichment analysis of DEGs (cutoff of P<0.001). (C) GO enrichment plot for positive regulation of apoptotic process plotted by Gene Set Enrichment Analysis (GSEA). (D) TUNEL assay of pancreatic tumor tissues used for RNA sequencing. Note that more apoptotic cells were shown in the tumor tissues obtained from mice administered with combination of vactosertib with nal-IRI/5-FU/LV.

Identification of *Ccdc80* regulated by combination treatment

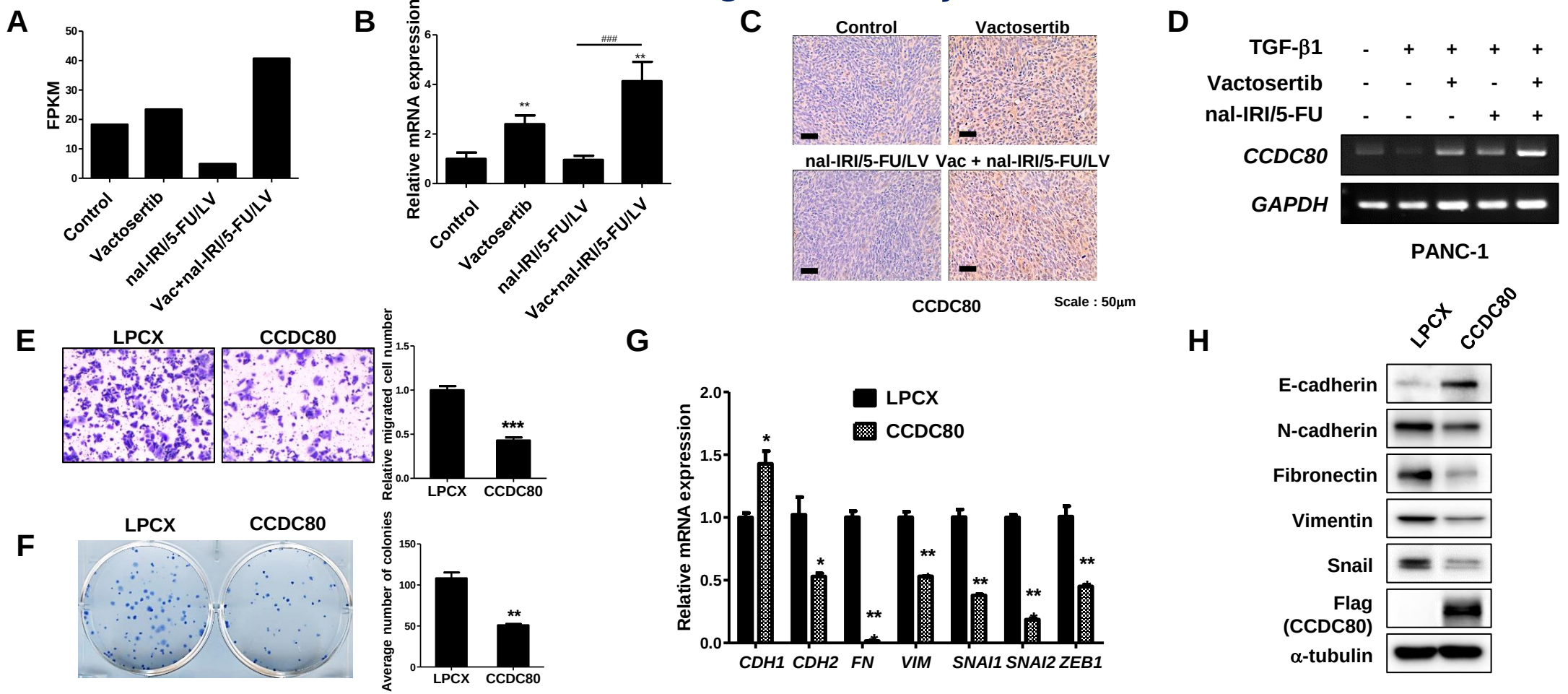
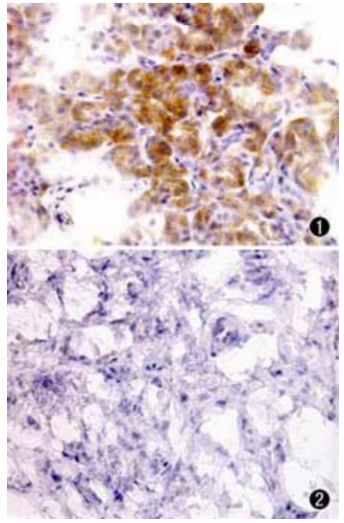


Figure 4. Up-regulation of CCDC80 expression by vactosertib and its combination with nal-IRI/5-FU/LV, and ectopic expression of CCDC80

(A) FPKM value of Ccdc80 in the tumor tissues from RNA sequencing. (B) qRT-PCR result validating Ccdc80 mRNA expression in mouse tumor tissues. The values represent the mean ± SD of triplicate samples. **P<0.005 compared to the control group. ###P<0.0005 compared to the nal-IRI/5-FU/LV group. (C) Immunohistochemical staining of mouse tumor tissues with CCDC80. Note that CCDC80 expression is increased in the tumor tissues from the mice administered with vactosertib or its combination with nal-IRI/5-FU/LV. (D) RT-PCR analysis revealing up-regulation of CCDC80 by vactosertib and its combination with nal-IRI/5-FU in PANC-1. (E) Transwell migration assay measuring migration abilities of PANC-1 expressing LPCX or CCDC80. (F) Representative images of colonies stained with methylene blue in PANC-1 stably expressing CCDC80. (G) qRT-PCR results showing down-regulation of EMT marker expression in PANC-1 by ectopic expression of CCDC80. All the data is represented as the mean of three repeated values. ***P<0.0005, **P<0.005, and *P<0.05 compared to the control. (H) Western blot analysis showing reduction of EMT markers in PANC-1 with stably expressing CCDC80.

Sfrp1 – target of Vac+nal-IRI/5-FU/LV combination treatment

- SFRP1
  - Secreted Frizzled Related Protein 1
  - Known as Wnt antagonist
  - Loss of SFRP1 is detected in pancreatic cancer
  - *SFRP1* is hyper-methylated in pancreatic tumor tissues



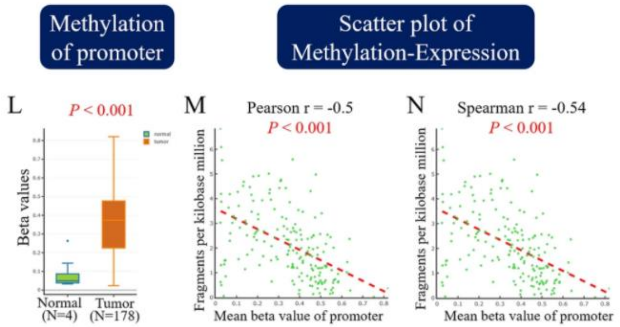
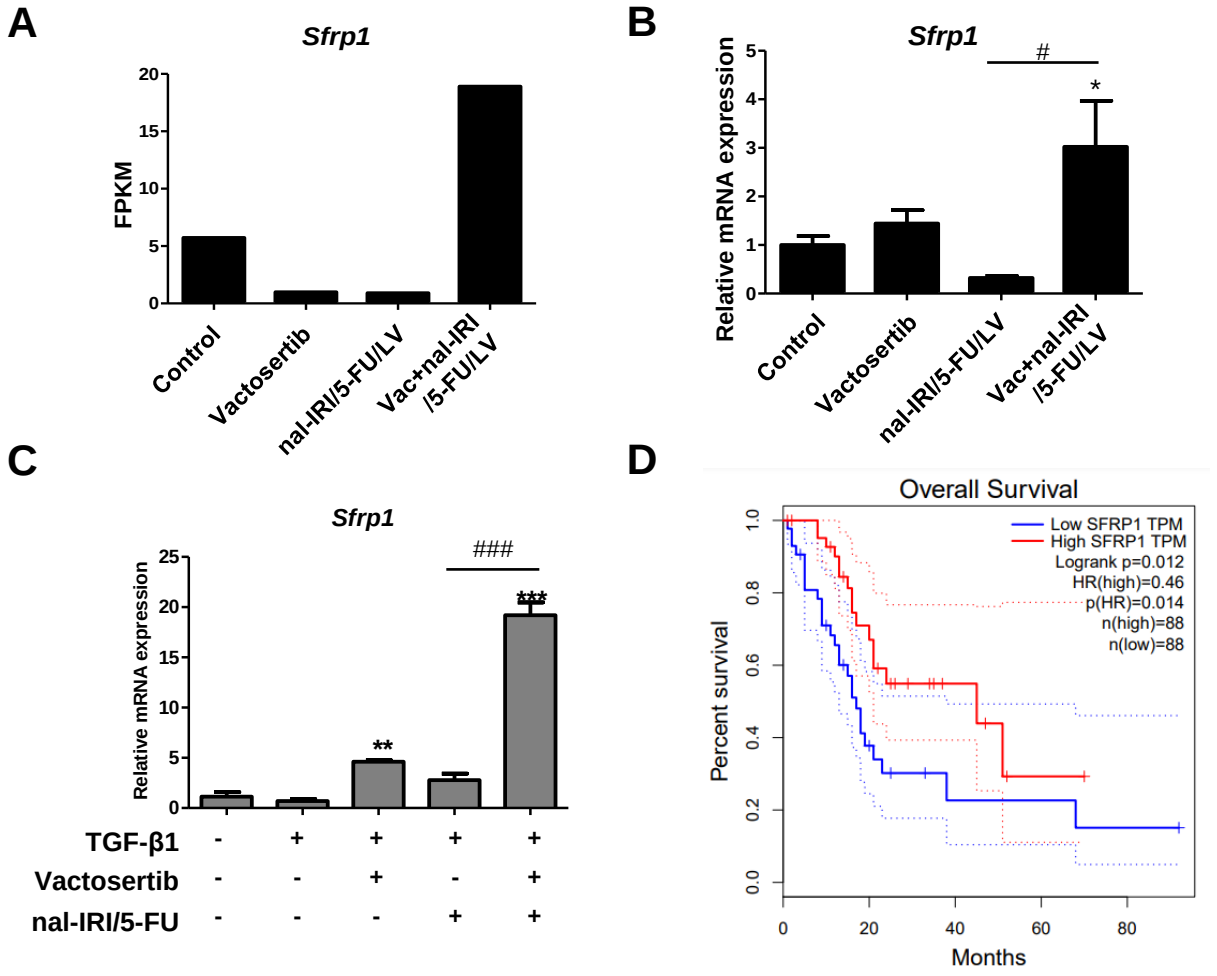


Figure 1. SFRP1 positive expression in normal pancreatic tissue (SABC, original magnification ×400)
Figure 2. SFRP1 loss of expression in primary pancreatic cancer (SABC, original magnification ×400).

Bu XM, Zhao CH, Dai XW. Aberrant expression of Wnt antagonist SFRP1 in pancreatic cancer. Chin Med J (Engl). 2008 May 20;121(10):952-5. PMID: 18706212.

Cheng, LC., Chao, Y.J., Overman, M.J. et al. Increased expression of secreted frizzled related protein 1 (SFRP1) predicts ampullary adenocarcinoma recurrence. Sci Rep 10, 13255 (2020). https://doi.org/10.1038/s41598-020-69899-8



Tang, Z. et al. (2017) GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res, 10.1093/nar/gkx247.

Figure 5. Tumor suppressor *Sfrp1* is up-regulated in vactosertib and nal-IRI/5-FU/LV combination treatment and the expression correlated with pancreatic cancer patient survival.

(A) FPKM value of *Sfrp1* from tumor RNA-sequencing. (B) Validation of *Sfrp1* expression in tumor tissue by qRT-PCR. (C) Relative mRNA expression of Sfrp1 in Vactosertib and nal-IRI/5-FU combination treated Panc02 cell. (D) Overall Survival in pancreatic adeonocarcinoma correlated with SFRP1 expression.

Conclusions

- Combination with Vactosertib significantly improves the survival of mouse pancreatic cancer model.
- The invasion of tumor tissue was inhibited by Vactosertib+nal-IRI/5-FU/LV treatment.
- EMT responses were reduced with Vactosertib both in mouse tissues and cancer cells.
- Moreover, the expressions of tumor suppressors were highly upregulated in combination treated tumor RNA
- These results may provide a basis for pursuing clinical trials of the combination therapy for pancreatic cancer patients.

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