

**Title:**

Inhibition of renalase expression and signaling has antitumor activity in pancreatic cancer

**Authors:**

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**INVENTORY OF SUPPLEMENTAL INFORMATION**

Figure 1S-9S

Table 1

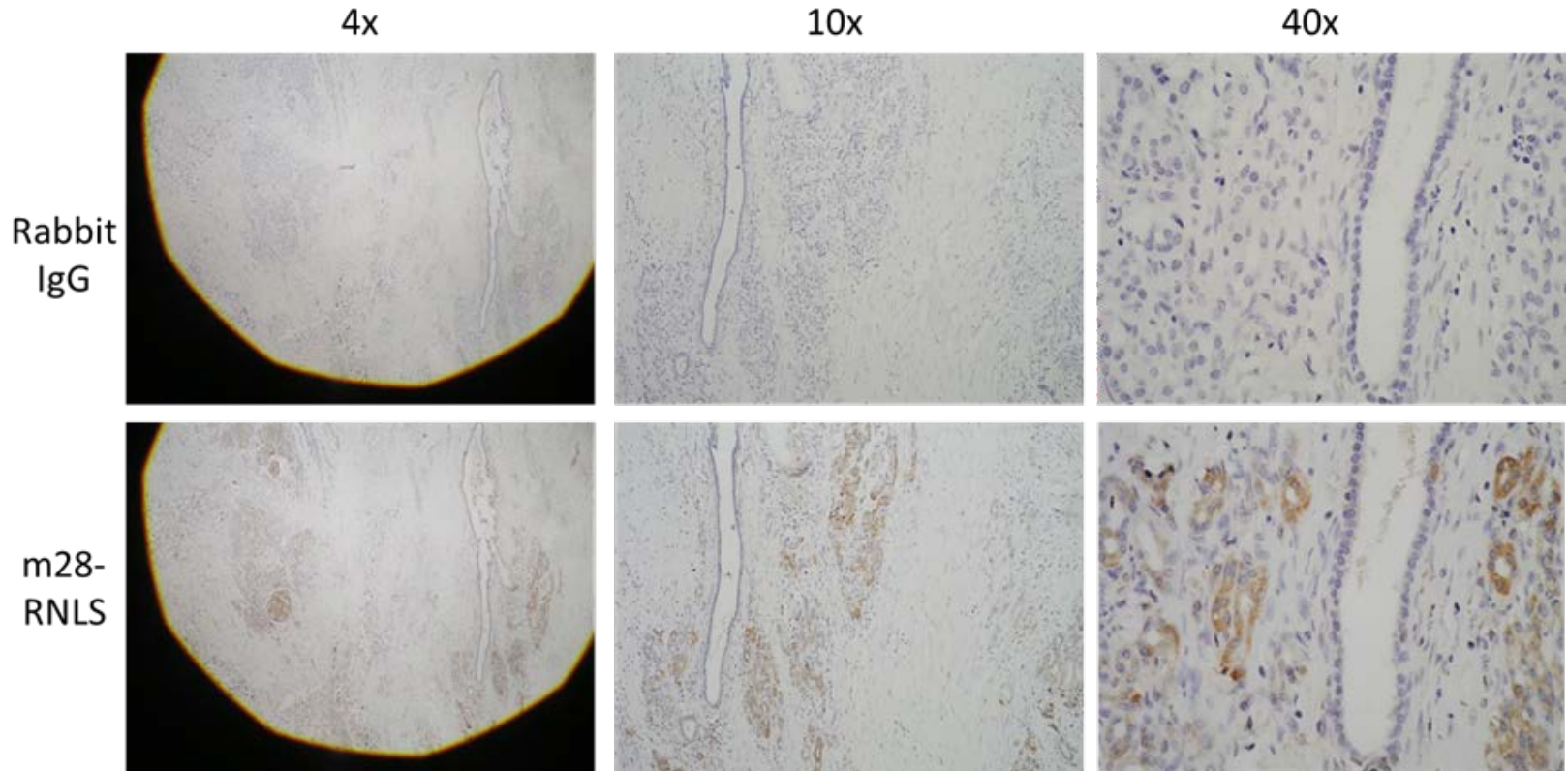


Figure 1S: Localization of RNLs in Grade II PDAC. Representative immunohistochemistry staining for RNLs protein expression (brown) using m28-RNLS in a Grade II human pancreatic ductal adenocarcinoma.

## Supplement 2S

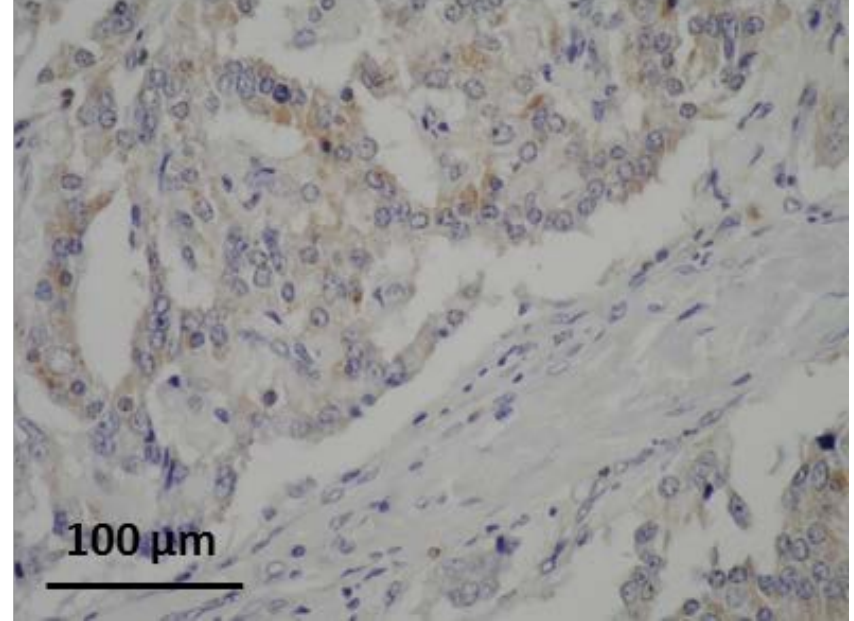
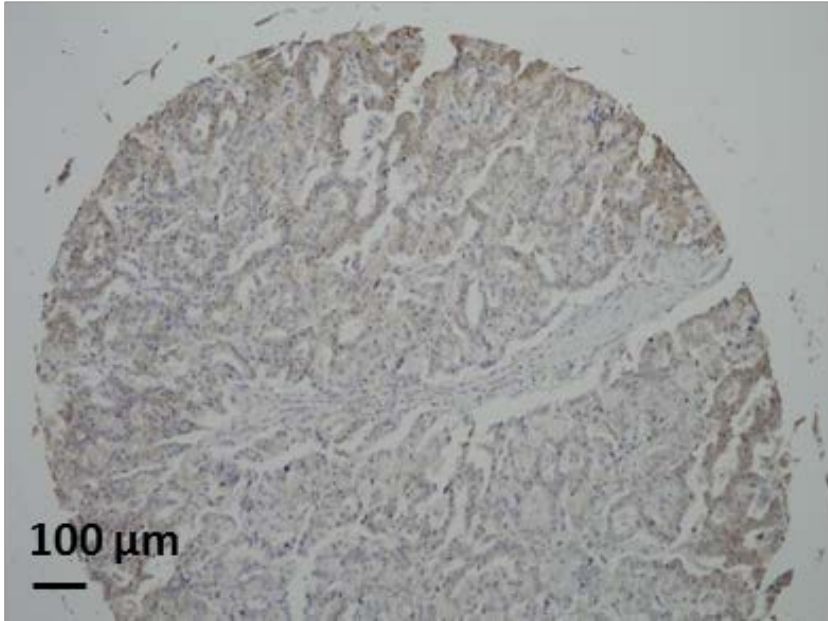
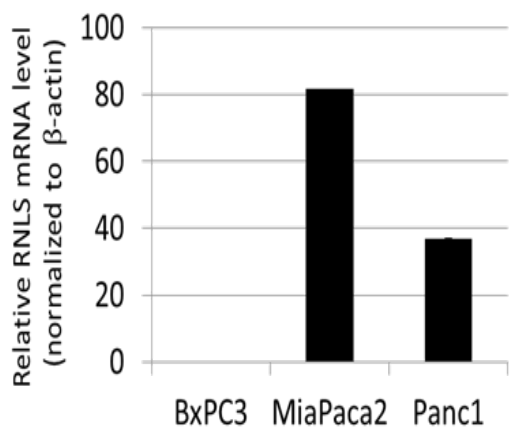
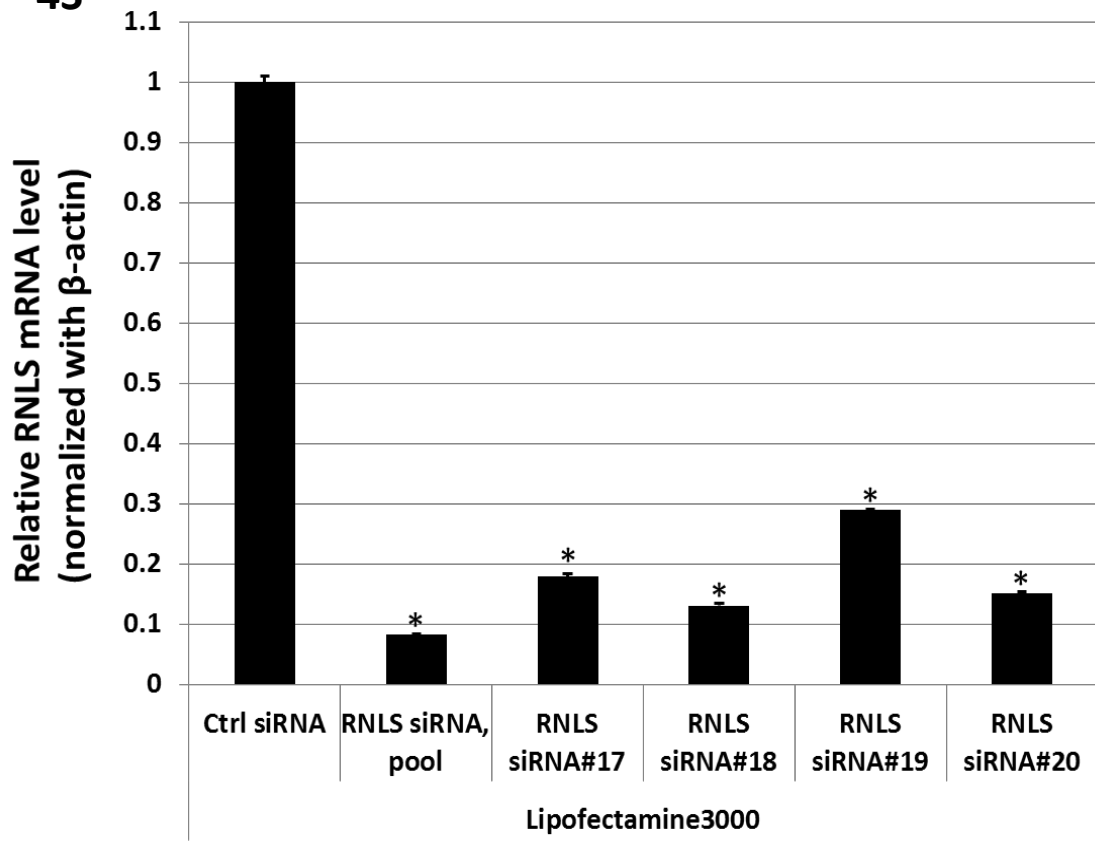


Figure 2S: RNL5 expression in neuroendocrine tumor of the pancreas. Representative immunohistochemistry staining for RNL5 protein expression (brown) using m28-RNL5 in a human neuroendocrine tumor.

3S



4S



5S

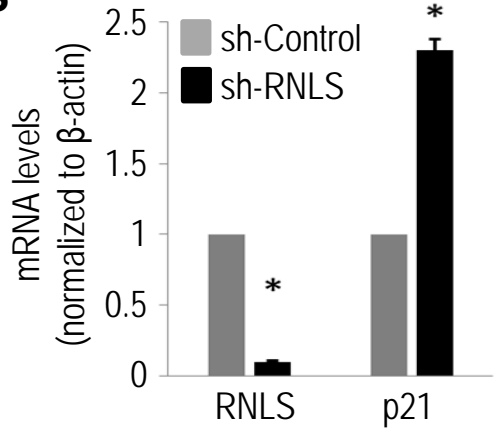
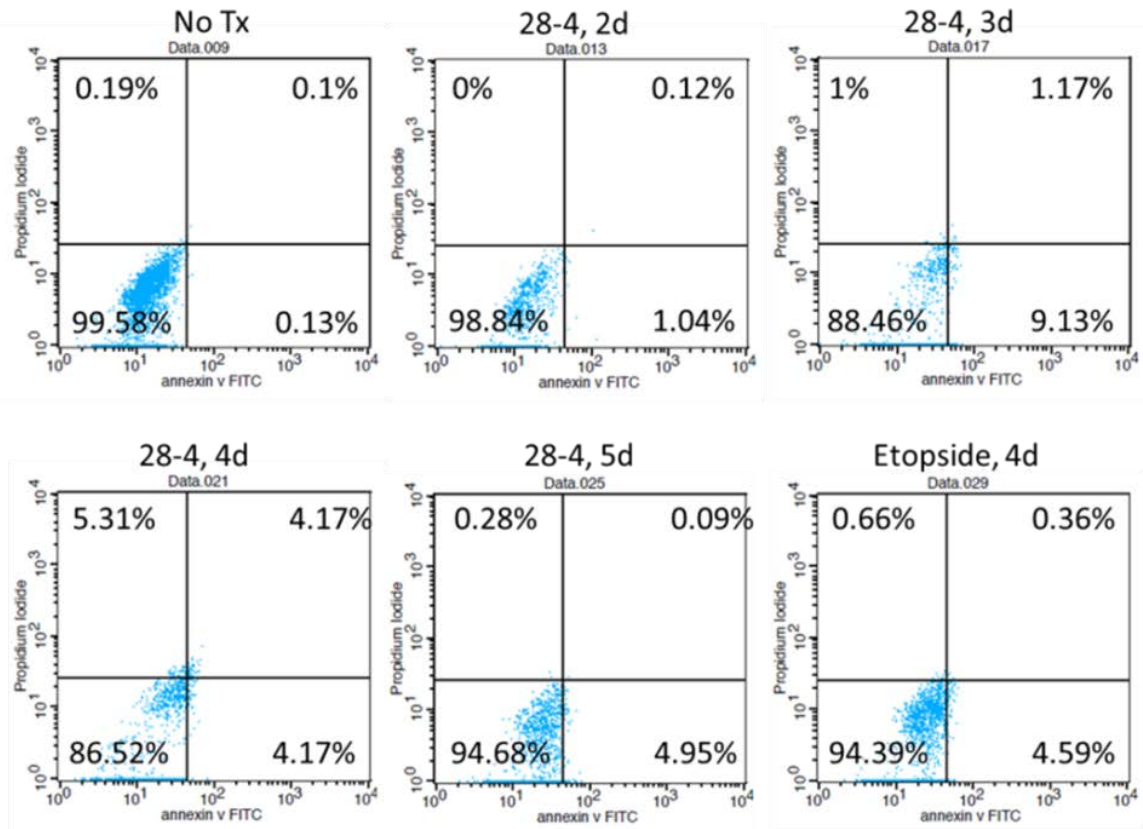


Figure 3S: Renalase mRNA level in pancreatic cancer cell lines. RNL5 mRNA level measured by RT-qPCR.  
Figure 4S: Knockdown of RNL5 expression using siRNA. MiaPaCa2 cells were transfected with indicated siRNA for 3days and RNL5 mRNA levels were measured by RTqPCR.  
Figure 5S: Knockdown of RNL5 expression using shRNA. Panc1 cells were stably transfected with shRNA construct to specifically knockdown RNL5. The RNL5 and p21 mRNA levels were measured by RT-qPCR.

Supplement 6S

Figure 6S: Inhibition of RNLS by monoclonal antibody (clone 28-4) induces PDAC cell apoptosis. Panc1 cells were treated with 28-4 for days followed by flow cytometry analysis. Numbers in the upper left quadrants of dot plots indicate the percentage of necrotic cells; numbers in the upper right quadrants, late apoptotic cells; numbers in the lower left quadrants, viable cells; numbers in the lower right quadrants, early apoptotic cells.

6S



## Supplement 7S

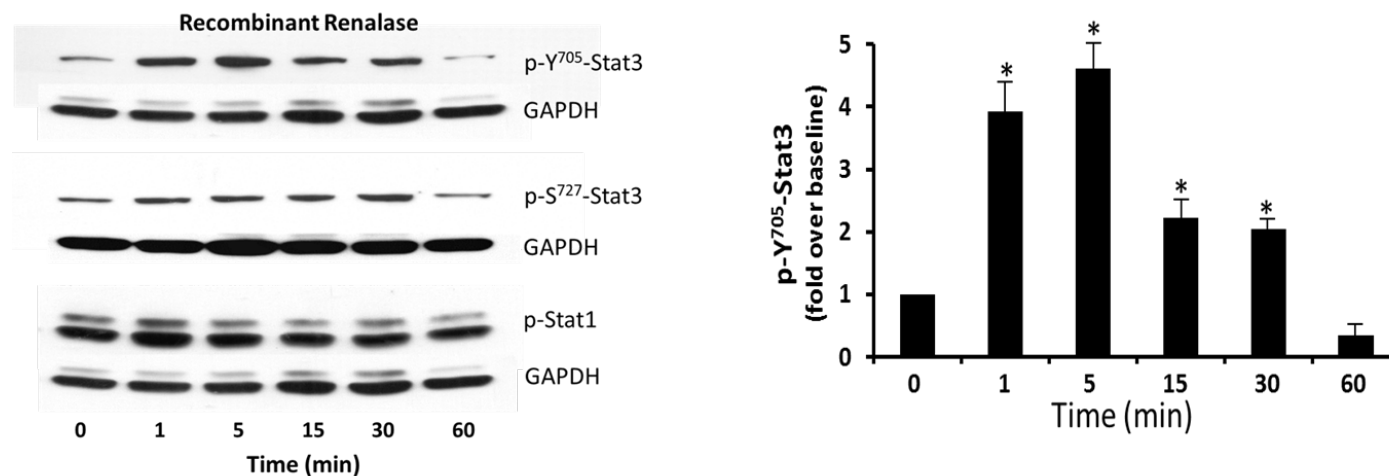
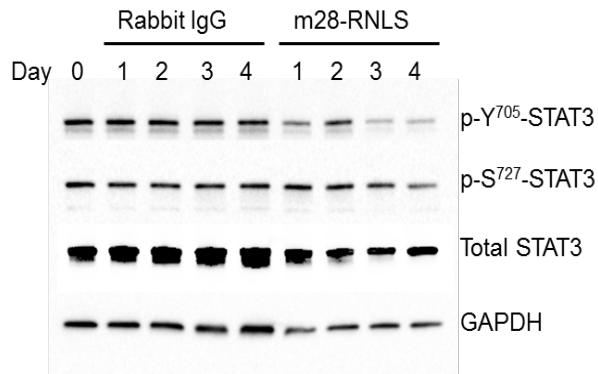
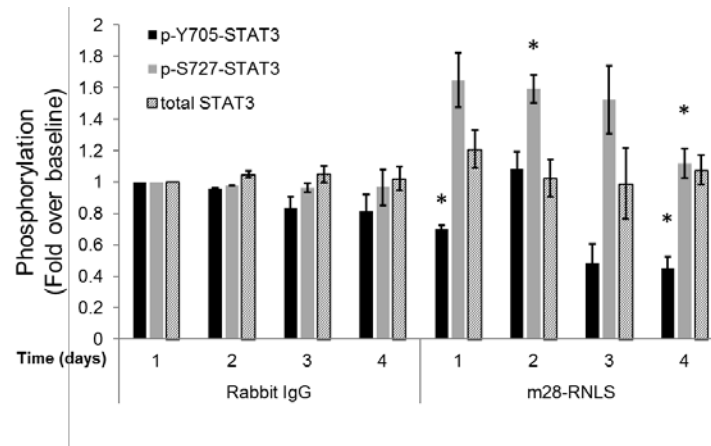


Figure 7S: RNLs activates STAT3 in human embryonic kidney cells (HK-2). *Left panel:* Cells were treated recombinant (30 mcg/ml) and STAT3 and STAT1 phosphorylation determined by Western blotting. *Right panel:* quantification of STAT3 phosphorylation at tyrosine 705 with normalization to GAPDH. n=5, \* indicates p<0.01.

## Supplement 8S, 9S



8S: m28-RNLS inhibits STAT3 phosphorylation; Panc1 cells in culture treated with either rabbit IgG or anti- RNLS monoclonal m28-RNLS for up to 4 days, and STAT3 phosphorylation assessed by western blot; p-Ser<sup>727</sup>-STAT3: phosphorylation at serine 727, p-Y<sup>705</sup>-STAT3: phosphorylation at tyrosine 705; GAPDH loading control; representative study.



9S: Quantification of STAT3 phosphorylation with m28-RNLS; signals normalized to GAPDH loading control; n=3, \*=P<0.05. m28-RNLS decreases STAT3 phosphorylation at tyrosine 705 by ~60%.

# Supplement Table 1S

**Table 1S.:** Characteristics of patient cohort with PDAC

Characteristics		Number /Total number
Gender	Female	24/69 (34.8%)
	Male	45/69 (65.2%)
Age (years)	Median	61 (36-85)
	36-50	14/69 (20.3%)
	51-69	39/69 (56.5%)
	70-85	16/69 (23.2%)
Tumor Grade	1	1/69 (1.4%)
	2	48/69 (69.6%)
	3	15/69 (21.7%)
	4	1/69 (1.4%)
	Unknown	4/69 (5.8%)
Survival (Months)	0-12	29/69 (42%)
	13-24	9/69 (13%)
	25-48	18/69 (26%)
	49-87	13/69 (19%)