Supplementary Information

Supplementary Figure Legends

Figure S1

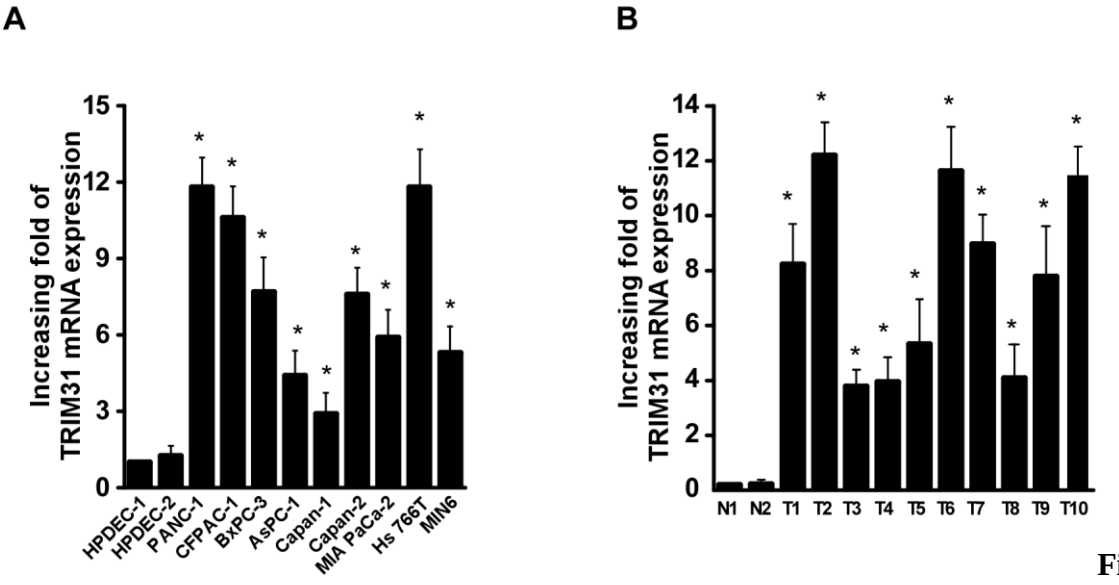

Figure S1. mRNA expression analysis shows that *TRIM31* is upregulated in pancreatic cancer cell lines and tissues. (A) Real-time PCR analysis of *TRIM31* expression in two primary normal human pancreatic duct epithelial cells (HPDEC) and in pancreatic cancer cell lines PANC-1, CFPAC-1, BxPC-3, AsPC-1, Capan-1, Capan-2, MIA PaCa-2, Hs 766T, and MIN6. Transcript levels were normalized to *GAPDH* expression. (B) Real-time PCR analysis of *TRIM31* expression in pancreatic cancer tissues (T) with matched adjacent non-tumor tissues from nine patients. Transcript levels were normalized to *GAPDH* expression. Each bar represents the mean ± SD of three independent experiments. *$P < 0.05$.

Figure S2

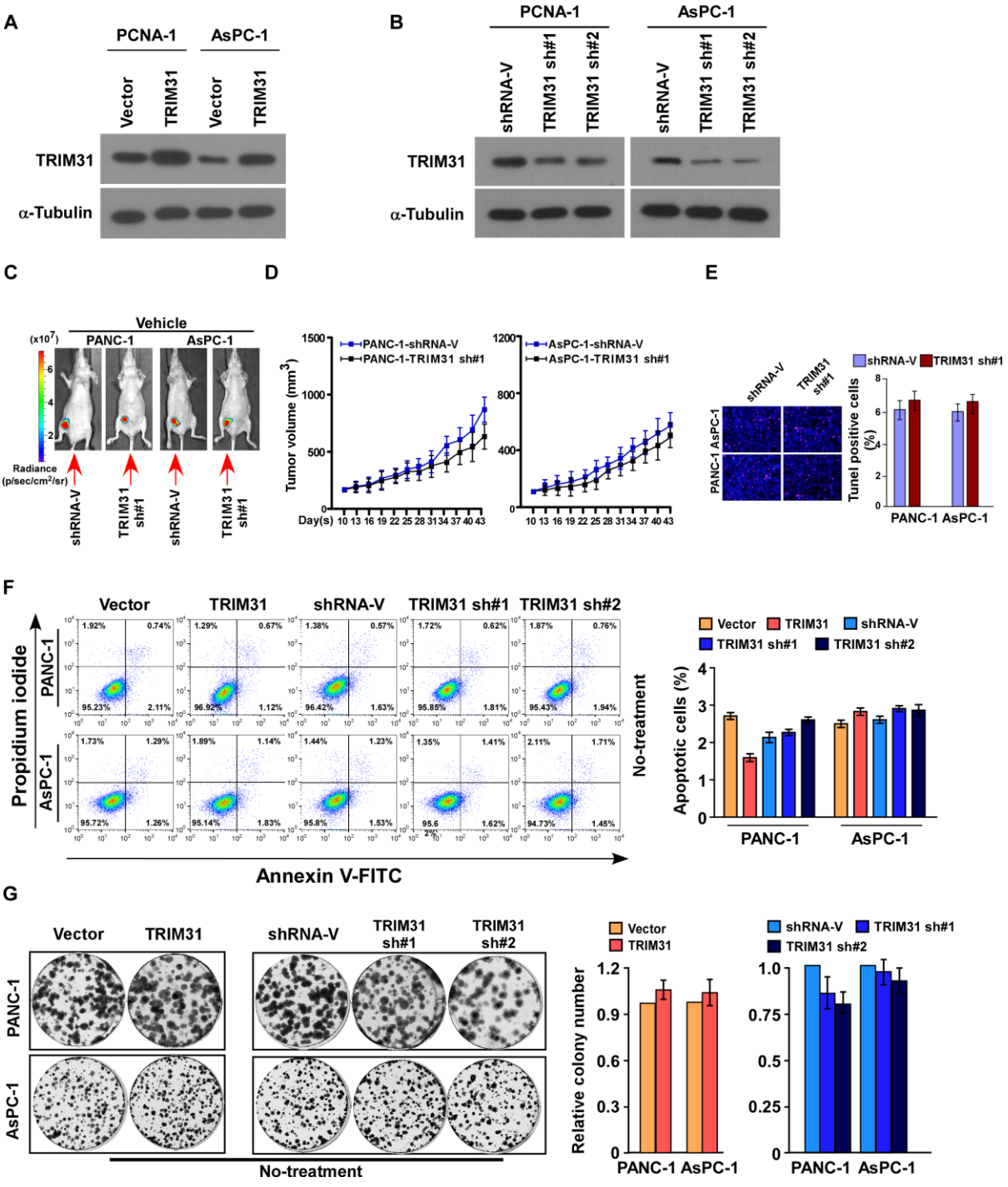

Figure S2. (A-B) Western blotting analysis of TRIM31 levels in the indicated cells; α-Tubulin was used as a loading control. (B). Representative images of tumor-bearing mice in the indicated cells treated with gemcitabine (100 mg/kg). (C). The mean tumor weights (right). (D). Tumor volumes were measured on the indicated days. (E) Staining demonstrating the expression of TUNEL-positive cells in the indicated tissues. Each bar

represents the mean ± SD of three independent experiments. * P <0.05. (F) Annexin V-FITC and PI staining of the indicated cells treated with gemcitabine (50 μM) for 48 h. Each bar represents the mean ± SD of three independent experiments. (G). Representative images (left panel) and quantification (right panel) of the indicated cells after crystal violet staining. Each bar represents the mean ± SD of three independent experiments.

Figure S3

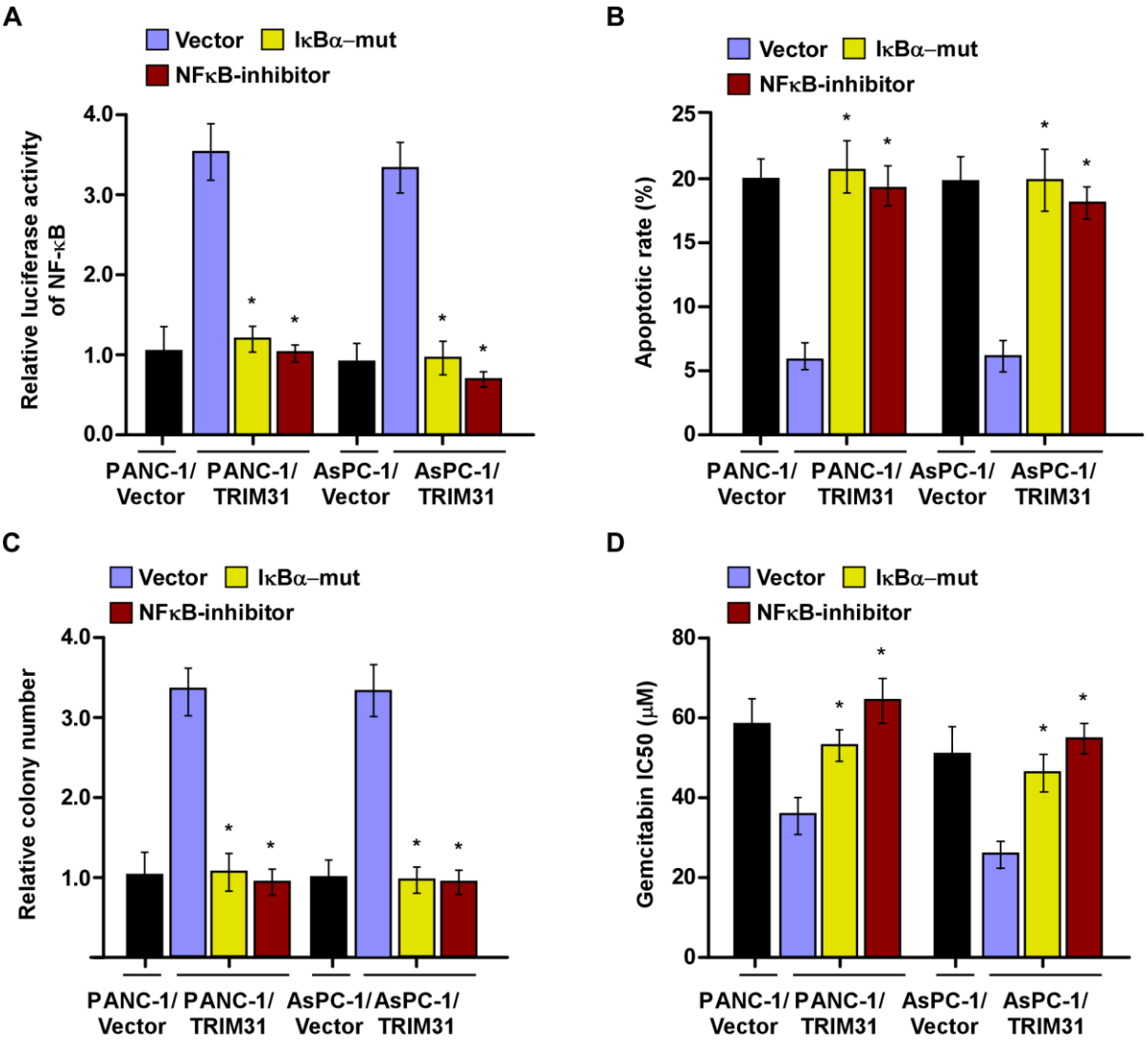

Figure S3. (A). Relative luciferase reporter activity in the indicated cells transfected with vector, IκBα-mut, or treated with an NF-κB inhibitor (JSH-23). Each bar represents the mean ± SD of three independent experiments. * P <0.05. (B) Quantification of indicated cells after crystal violet staining. Each bar represents the mean ± SD of three independent experiments.

* *P* <0.05. (C). Annexin V-FITC and PI staining of the indicated cells treated with gemcitabine (50 μM) for 48 h. Each bar represents the mean ± SD of three independent experiments. * *P* <0.05. (D). IC_{50} of Gemcitabine in the indicated cells. Each bar represents the mean ± SD of three independent experiments. * *P* <0.05.

Figure S4

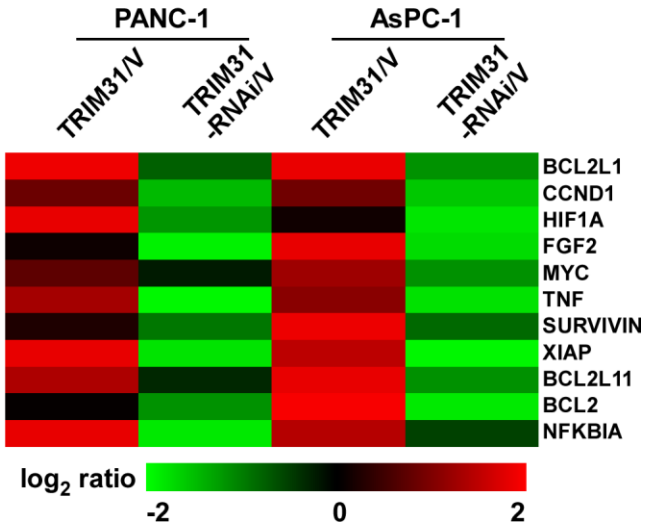

Figure S4. Upregulation of *TRIM31* activates the NF-κB signaling pathway in pancreatic cancer. Real-time PCR analysis demonstrating an apparent overlap between NF-κB-dependent gene expression and TRIM31-regulated gene expression.

Figure S5

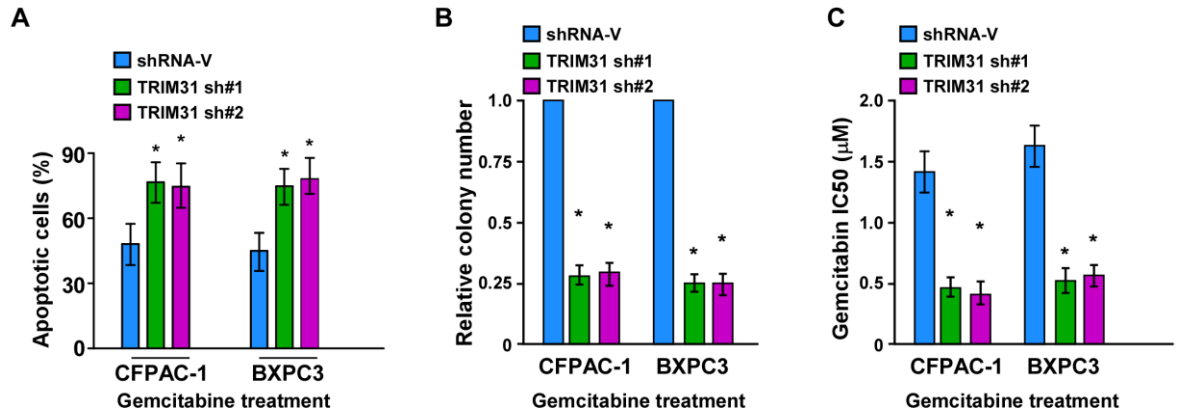

Figure S5 (only for Reviewer). (A) Quantification of indicated cells after crystal violet staining. Each bar represents the mean ± SD of three independent experiments. * P <0.05. (B). Annexin V-FITC and PI staining of the indicated cells treated with gemcitabine (50 μM) for 48 h. Each bar represents the mean ± SD of three independent experiments. * P <0.05. (C). IC_{50} of Gemcitabine in the indicated cells. Each bar represents the mean ± SD of three independent experiments. * P <0.05.

Figure S6

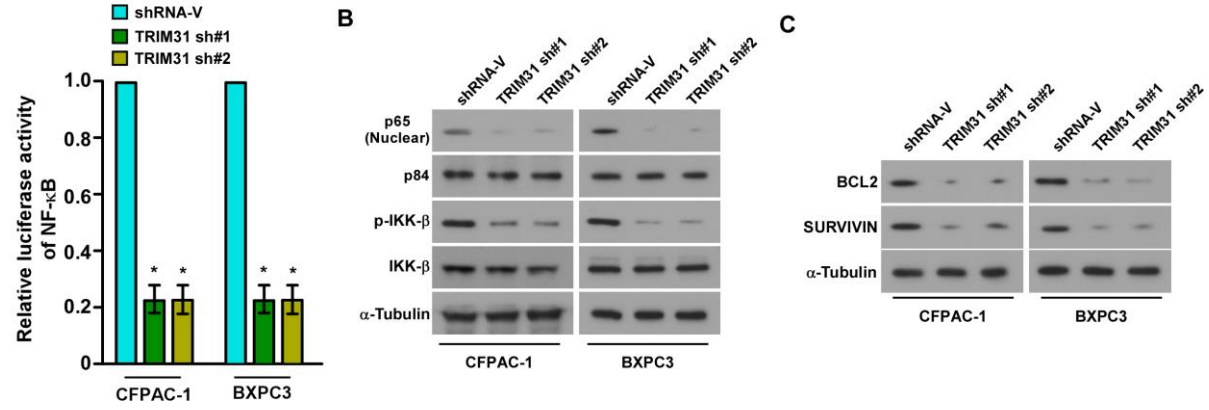

Figure S6 (only for Reviewer). (A). Relative luciferase reporter activity in the indicated cells. Each bar represents the mean ± SD of three independent experiments. * P <0.05. (B) Western blotting analysis of p65 and p-IKK- β levels in the indicated cells; α-tubulin was used as a loading control. (C) Western blotting analysis of BCL2 and SURVIVIN protein levels in the indicated cells; α-tubulin was used as a loading control.

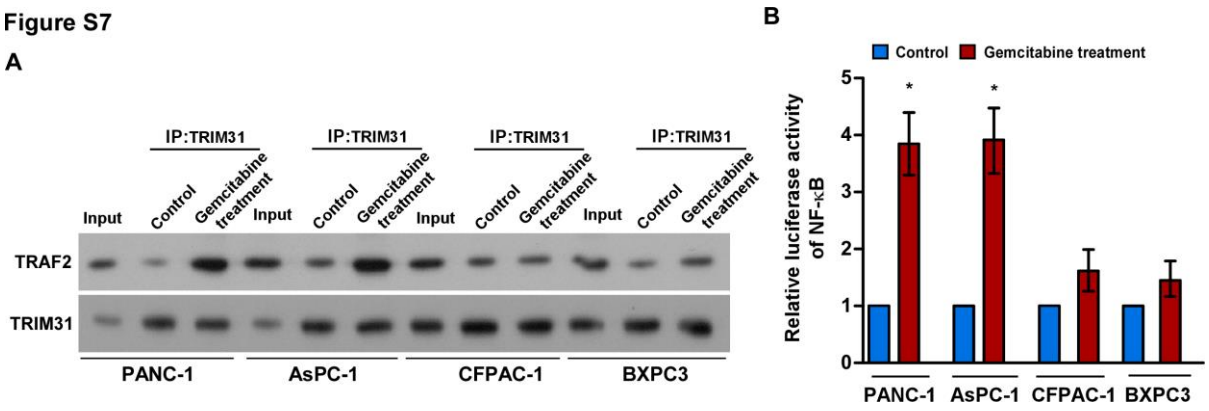

Figure S7 (only for Reviewer). (A) Immunoprecipitation assay indicating that TRIM31 interacts with TRAF2 in the indicated cells (endogenous). (B). Relative luciferase reporter activity in the indicated cells. Each bar represents the mean ± SD of three independent experiments. * P <0.05.

Table S1. The relationship between TRIM31 and the clinicopathological characteristics of 89 patients with pancreatic cancer.

Parameters	Number of cases	TRIM31 expression		*P* values
		Low	High	
Sex				
Male	53	24	29	0.736
Female	36	15	21	
Age (years)				
< 60	38	19	19	0.311
≥ 60	51	20	31	
Location				
Head of pancreas	66	27	39	0.348
Other	23	12	11	
Tumor size (cm)				
< 2	19	10	9	0.383
≥ 2	70	29	41	
Histological grade				
High/moderate	31	17	14	0.126
Poor	58	22	36	
Clinical stage				
I–II	12	2	10	0.042
III–IV	77	37	40	
Local and distant recurrence				
Negative	8	0	8	0.009
Positive	81	39	42	
Status				
Alive	11	1	10	0.013
Dead	78	38	40	
IHC status of p65				
Cytoplasm	43	27	16	0.001
Nuclear	46	12	34	

Supplementary Table 2.Interaction effect analysis in combination of gemcitabine treatment and TRIM31 inhibition via 2x2 ANOVA analysis.

Tests of Between-Subjects Effects

Dependent Variable: Apoptosis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6276.123[a]	3	2092.041	796.968	.000
	5355.188	1	5355.188	2.040E3	.000
TRIM31 inhibition	906.541	1	906.541	345.349	.000
Gemcitabine	4590.341	1	4590.341	1.749E3	.000
Gemcitabine * TRIM31 inhibition	779.241	1	779.241	296.854	.000
Error	21.000	8	2.625		
Total	11652.310	12			
Corrected Total	6297.123	11			

a. R Squared = .997 (Adjusted R Squared = .995)

Gemcitabine * TRIM31 inhibition

Dependent Variable: Apoptosis

Gemcitabine	TRIM31 inhibition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	0	.933	.935	-1.224	3.090
	1	23.933	.935	21.776	26.090
1	0	2.200	.935	.043	4.357
	1	57.433	.935	55.276	59.590