Supplementary Tables

Supplementary Table 1. Clinical characteristics of 382 PC patients from 3 cohorts.

Characteristics	Verification samples (n = 382)		
	Cohort 1 (n = 58)	**Cohort 2 (n = 100)**	**Cohort 3 (n = 224)**
Follow-up time (OS)			
Years, Median (ranges)	N.A	10.0 (0.0-87.0)	10.1 (0.1-77.0)
Age			
Years, mean ± SD	64.8±7.2	61.7±11.3	66.9±9.9
<60 years, n (%)	18(31.0)	47(47.0)	54(24.1)
≥60 years, n (%)	40(69.0)	53(53.0)	170(75.9)
Gender			
Female, n (%)	24(41.4)	37(37.0)	95(42.4)
Male, n (%)	34(58.6)	63(63.0)	129(57.6)
Tumor location			
Head/neck, n (%)	42(72.4)	60(65.9)	153(68.3)
Body/tail, n (%)	16(27.6)	31(34.1)	71(31.7)
Pathologic stage			
I-II, n (%)	39(67.2)	69(69.0)	140(62.5)
III-IV, n (%)	19(32.8)	31(31.0)	84(37.5)
T classification			
T1-T2, n (%)	45(77.6)	78(79.6)	118(52.7)
T3-T4, n (%)	13(22.4)	20(20.4)	106(47.3)
Lymph node metastasis			
No, n (%)	32(55.2)	54(58.1)	153(68.3)
Yes, n (%)	26(44.8)	39(41.9)	71(31.7)
Distant metastasis			
No, n (%)	56(96.6)	98(98.0)	214(95.5)
Yes, n (%)	2(3.4)	2(2.0)	10(4.5)
AJCC TNM stage			
I, n (%)	23(39.7)	40(42.1)	85(37.9)
II-IV, n (%)	35(60.3)	55(57.9)	139(62.1)
Ki67$^+$ cell rate			
≤10%, n (%)	N.A	64(64.0)	144(64.3)
>10%, n (%)	N.A	36(36.0)	80(35.7)
NSUN6 expression			
Low, n (%)	28(50.0)	48(48.0)	111(49.6)
High, n (%)	28(50.0)	52(52.0)	113(50.4)

PC, pancreatic cancer; OS, overall survival; N.A, not applicable; SD, standard deviation.

Supplementary Table 2. Correlation analysis between clinical characteristics and NSUN6 expression of PC in verification cohort 2.

Characteristics	NSUN6 expression (n = 100)		*OR*(95%*CI*)	*P* value
	Low (n = 48)	High (n = 52)		
Age				
<60 years, n (%)	19(39.6)	28(53.8)	1.78(0.80-3.94)	0.153
≥60 years, n (%)	29(60.4)	24(46.2)		
Gender				
Female, n (%)	16(33.3)	21(40.4)	1.35(0.60-3.07)	0.466
Male, n (%)	32(66.7)	31(59.6)		
Tumor location				
Head/neck, n (%)	30(68.2)	30(63.8)	0.82(0.35-1.97)	0.662
Body/tail, n (%)	14(31.8)	17(36.2)		
Pathologic stage				
I-II, n (%)	32(66.7)	37(71.2)	1.23(0.53-2.88)	0.628
III-IV, n (%)	16(33.3)	15(28.8)		
T classification				
T1-T2, n (%)	32(69.6)	46(88.5)	3.35(1.17-9.66)	0.021
T3-T4, n (%)	14(30.4)	6(11.5)		
Lymph node metastasis				
No, n (%)	21(48.8)	33(66.0)	2.03(0.88-4.69)	0.094
Yes, n (%)	22(51.2)	17(34.0)		
Distant metastasis				
No, n (%)	46(95.8)	52(100.0)	/	/
Yes, n (%)	2(4.2)	0(0.0)		
AJCC TNM stage				
I, n (%)	10(22.7)	30(58.8)	4.86(1.98-11.93)	3.8×10^{-4}
II-IV, n (%)	34(77.3)	21(41.2)		
Ki67^{+} cell rate				
≤10%, n (%)	23(47.9)	41(78.8)	4.05(1.69-9.71)	0.001
>10%, n (%)	25(52.1)	11(21.2)		

PC, pancreatic cancer; OR, odds ratio; CI, confidence interval.

Supplementary Table 3. Correlation analysis between clinical characteristics and NSUN6 expression of PC in verification cohort 3.

Characteristics	NSUN6 expression (n = 224)		*OR*(95%*CI*)	*P* value
	Low (n = 111)	High (n = 113)		
Age				
<60 years, n (%)	26(23.4)	28(24.8)	1.08(0.58-1.99)	0.813
≥60 years, n (%)	85(76.6)	85(75.2)		
Gender				
Female, n (%)	49(44.1)	46(40.7)	0.87(0.51-1.48)	0.603
Male, n (%)	62(55.9)	67(59.3)		
Tumor location				
Head/neck, n (%)	74(66.7)	79(69.9)	1.16(0.66-2.04)	0.602
Body/tail, n (%)	37(33.3)	34(30.1)		
Pathologic stage				
I-II, n (%)	65(58.6)	75(66.4)	1.4(0.81-2.4)	0.227
III-IV, n (%)	46(41.4)	38(33.6)		
T classification				
T1-T2, n (%)	48(43.2)	70(61.9)	2.14(1.25-3.64)	0.005
T3-T4, n (%)	63(56.8)	43(38.1)		
Lymph node metastasis				
No, n (%)	76(68.5)	77(68.1)	0.99(0.56-1.73)	0.958
Yes, n (%)	35(31.5)	36(31.9)		
Distant metastasis				
No, n (%)	105(94.6)	109(96.5)	1.56(0.43-5.68)	0.499
Yes, n (%)	6(5.4)	4(3.5)		
AJCC TNM stage				
I, n (%)	33(29.7)	52(46)	2.01(1.16-3.49)	0.012
II-IV, n (%)	78(70.3)	61(54)		
Ki67+ cell rate				
≤10%, n (%)	59(53.2)	85(75.2)	2.68(1.52-4.72)	5.7×10^{-4}
>10%, n (%)	52(46.8)	28(24.8)		

PC, pancreatic cancer; OR, odds ratio; CI, confidence interval.

Supplementary Table 4. Correlation analysis between clinical characteristics and NSUN6 expression of PC in verification cohort 2 combined 3.

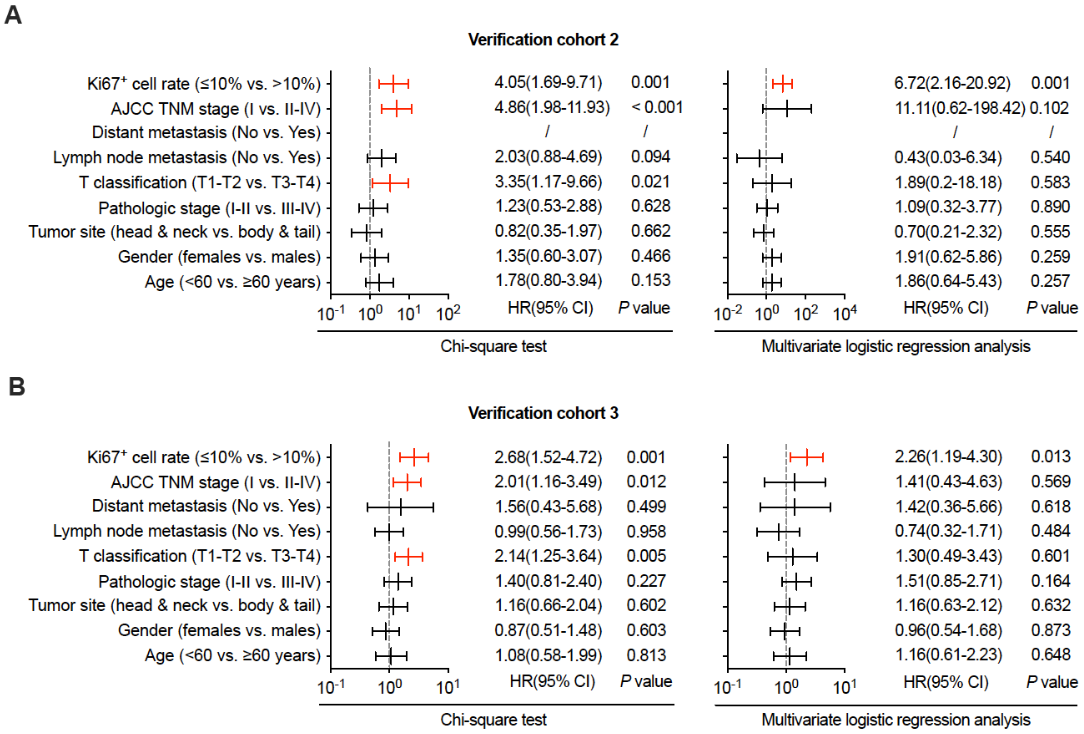
Characteristics	NSUN6 expression (n = 324)		*OR*(95%*CI*)	*P* value
	Low (n = 159)	High (n = 165)		
Age				
<60 years, n (%)	45(28.3)	56(33.9)	1.3(0.81-2.09)	0.273
≥60 years, n (%)	114(71.7)	109(66.1)		
Gender				
Female, n (%)	65(40.9)	67(40.6)	0.99(0.63-1.54)	0.960
Male, n (%)	94(59.1)	98(59.4)		
Tumor location				
Head/neck, n (%)	104(67.1)	109(68.1)	1.05(0.65-1.68)	0.845
Body/tail, n (%)	51(32.9)	51(31.9)		
Pathologic stage				
I-II, n (%)	97(61.0)	112(67.9)	1.35(0.86-2.13)	0.196
III-IV, n (%)	62(39.0)	53(32.1)		
T classification				
T1-T2, n (%)	80(51.0)	116(70.3)	2.28(1.44-3.6)	3.7×10^{-4}
T3-T4, n (%)	77(49.0)	49(29.7)		
Lymph node metastasis				
No, n (%)	97(63.0)	110(67.5)	1.22(0.77-1.94)	0.400
Yes, n (%)	57(37.0)	53(32.5)		
Distant metastasis				
No, n (%)	151(95.0)	161(97.6)	2.13(0.63-7.23)	0.214
Yes, n (%)	8(5.0)	4(2.4)		
AJCC TNM stage				
I, n (%)	43(27.7)	82(50.0)	2.6(1.63-4.15)	4.7×10^{-5}
II-IV, n (%)	112(72.3)	82(50.0)		
Ki67$^+$ cell rate				
≤10%, n (%)	82(51.6)	126(76.4)	3.03(1.89-4.88)	3.3×10^{-6}
>10%, n (%)	77(48.4)	39(23.6)		

PC, pancreatic cancer; OR, odds ratio; CI, confidence interval.

Supplementary Table 5. Sequence of shRNA and qPCR primers

Name		**Primer sequence (5'-3')**
NSUN6-shRNA		CCGGCGTCTGGCTAATAAGGACTCTCTCG AGAGAGTCCTTATTAGCCAGACGTTTTTG
DNMT1	Forward	ATCGGATGAGTCCATCAAGG
	Reverse	TGGGTGTTGGTTCTTTGGTT
NSUN2	Forward	GGTATCCTGAAGAACTTGCC
	Reverse	ATCTTATGATGAGGCCGCA
NSUN3	Forward	TCCAAAGAACTCGGAGATGC
	Reverse	TGGGTAAAGATCCCTGAGAGAG
NSUN5	Forward	GGAATGAGGACCTGTTGGAA
	Reverse	AAGGGGTCCAGGAGAAAATG
NSUN6	Forward	GAAAGGCATGGGCATAAGAA
	Reverse	TGTGTGTTGTTTTCCCTCCA
NSUN7	Forward	TATGAGCTGGCTTTCAGTGC
	Reverse	TGCTGCAGCCAATTTTATCTT
CDK10	Forward	CCTGAAGGTTTCCAACTTGC
	Reverse	GGTTCCCAACAGCAGTTCA
MKI67	Forward	CTGCTTGTTTGGAAGGGGTA
	Reverse	AGCCGTACAGGCTCATCAAT
GAPDH	Forward	TGCACCACCAACTGCTTAGC
	Reverse	GGCATGGACTGTGGTCATGAG

Supplementary Figures



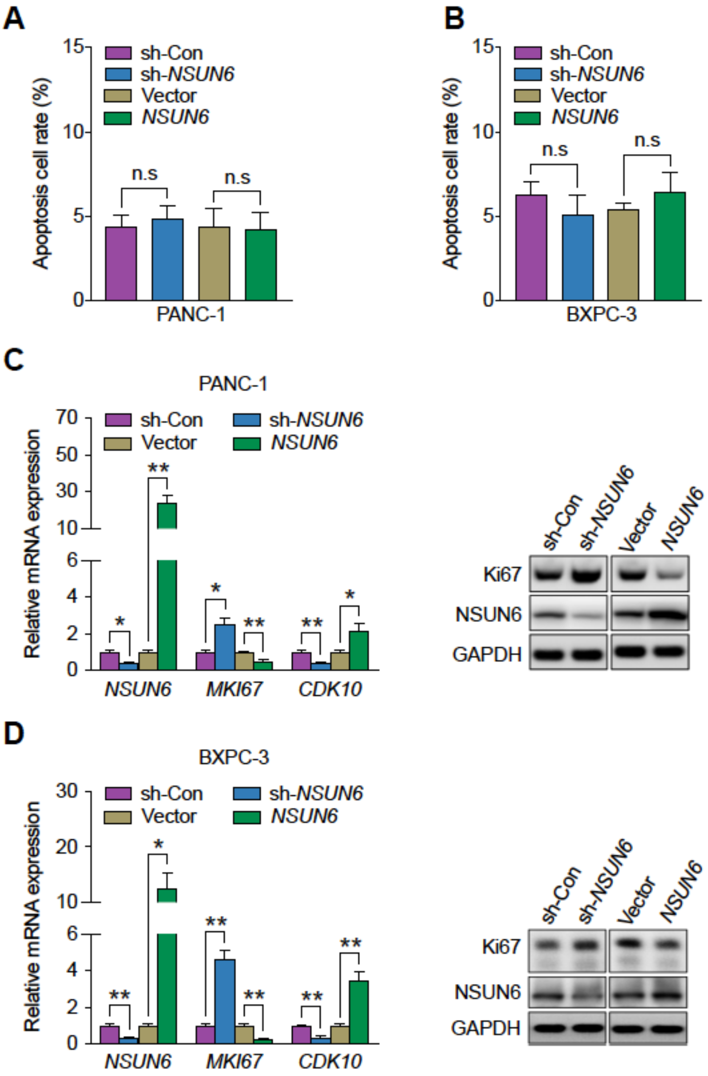
Verification cohort 2

	Chi-square test HR(95% CI)	*P* value	Multivariate logistic regression analysis HR(95% CI)	*P* value
Ki67^{+} cell rate (≤10% vs. >10%)	4.05(1.69-9.71)	0.001	6.72(2.16-20.92)	0.001
AJCC TNM stage (I vs. II-IV)	4.86(1.98-11.93)	< 0.001	11.11(0.62-198.42)	0.102
Distant metastasis (No vs. Yes)	/	/	/	/
Lymph node metastasis (No vs. Yes)	2.03(0.88-4.69)	0.094	0.43(0.03-6.34)	0.540
T classification (T1-T2 vs. T3-T4)	3.35(1.17-9.66)	0.021	1.89(0.2-18.18)	0.583
Pathologic stage (I-II vs. III-IV)	1.23(0.53-2.88)	0.628	1.09(0.32-3.77)	0.890
Tumor site (head & neck vs. body & tail)	0.82(0.35-1.97)	0.662	0.70(0.21-2.32)	0.555
Gender (females vs. males)	1.35(0.60-3.07)	0.466	1.91(0.62-5.86)	0.259
Age (<60 vs. ≥60 years)	1.78(0.80-3.94)	0.153	1.86(0.64-5.43)	0.257

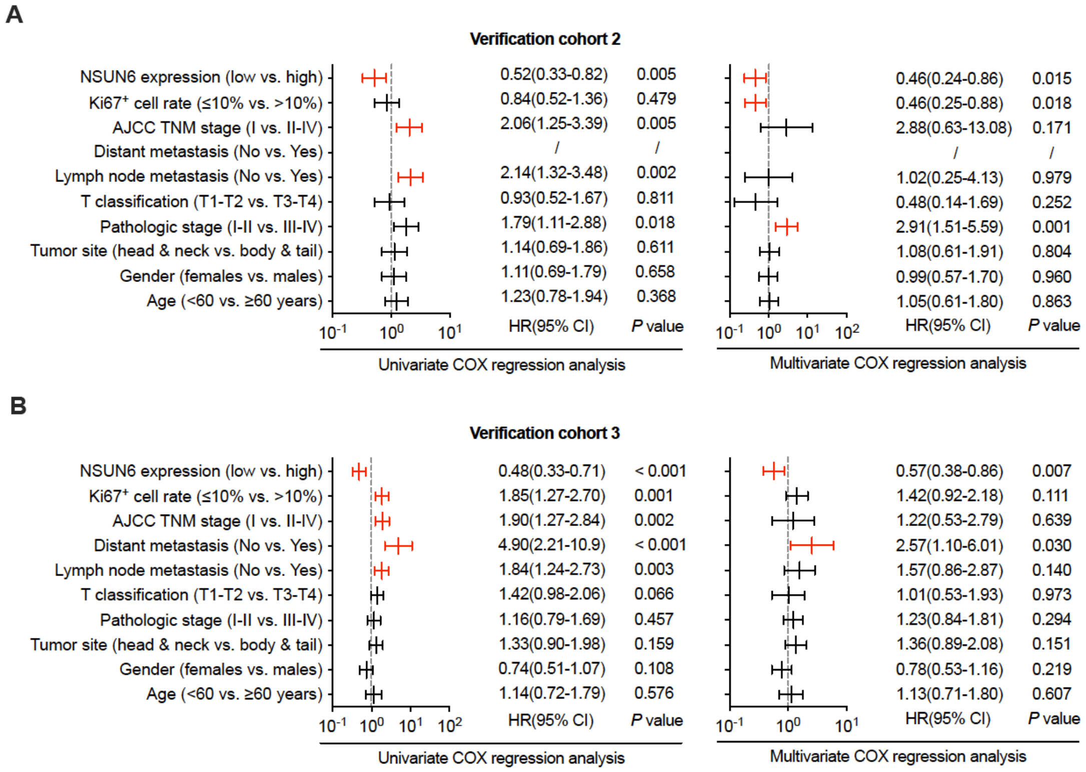
Verification cohort 3

	Chi-square test HR(95% CI)	*P* value	Multivariate logistic regression analysis HR(95% CI)	*P* value
Ki67^{+} cell rate (≤10% vs. >10%)	2.68(1.52-4.72)	0.001	2.26(1.19-4.30)	0.013
AJCC TNM stage (I vs. II-IV)	2.01(1.16-3.49)	0.012	1.41(0.43-4.63)	0.569
Distant metastasis (No vs. Yes)	1.56(0.43-5.68)	0.499	1.42(0.36-5.66)	0.618
Lymph node metastasis (No vs. Yes)	0.99(0.56-1.73)	0.958	0.74(0.32-1.71)	0.484
T classification (T1-T2 vs. T3-T4)	2.14(1.25-3.64)	0.005	1.30(0.49-3.43)	0.601
Pathologic stage (I-II vs. III-IV)	1.40(0.81-2.40)	0.227	1.51(0.85-2.71)	0.164
Tumor site (head & neck vs. body & tail)	1.16(0.66-2.04)	0.602	1.16(0.63-2.12)	0.632
Gender (females vs. males)	0.87(0.51-1.48)	0.603	0.96(0.54-1.68)	0.873
Age (<60 vs. ≥60 years)	1.08(0.58-1.99)	0.813	1.16(0.61-2.23)	0.648

Supplementary Figure 1. Univariate and multivariate analysis were used to explore the association between the NSUN6 protein levels and the clinicopathologic features of PC patients from verification cohort 2 (A) and 3 (B). Red lines represent NSUN6 significantly related clinicopathologic features with $P < 0.05$.

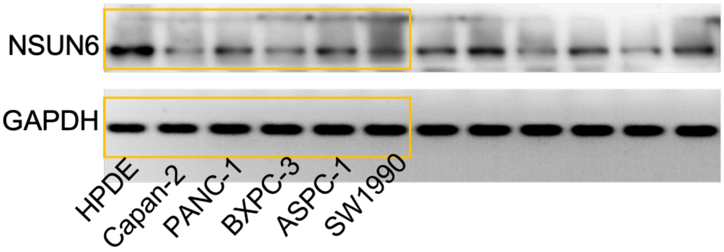


Supplementary Figure 2. Effects of NSUN6 on cell apoptosis and MKI67 expression. (A-B) Apoptosis rate analysis of PANC-1 and BXPC-3 cells transfected with *NSUN6* shRNA (sh-*NSUN6*), control shRNA (sh-Con), *NSUN6* overexpression vector (*NSUN6*), or control vector (Vector). (C-D) qPCR and western blot analysis of NSUN6, MKI67 and CDK10 expression in PANC-1 and BXPC-3 cells transfected with *NSUN6* shRNA (sh-*NSUN6*), control shRNA (sh-Con), *NSUN6* overexpression vector (*NSUN6*), or control vector (Vector). All experiment, n = 3; bar, S.E.M., **P* < 0.05; ** *P* < 0.01; n.s, not significant; Student's t-test

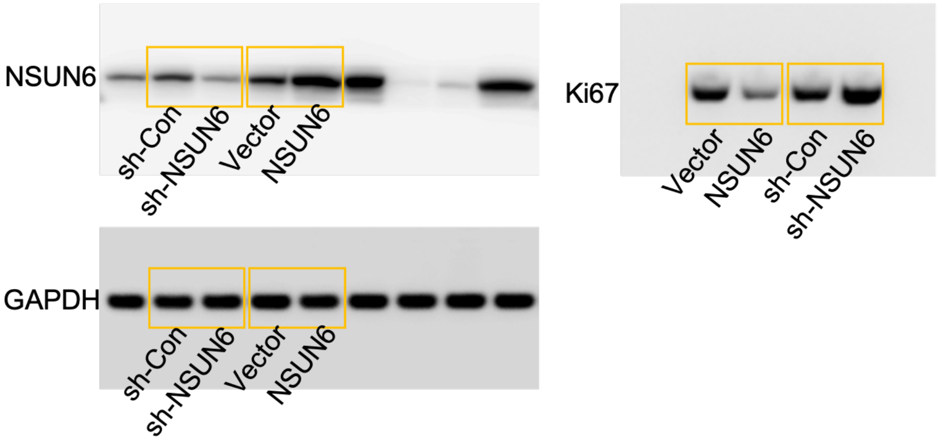


Supplementary Figure 3. Univariable and multivariable COX regression analysis of the associations between the prognostic parameters and OS among the PC patients in verification cohort 2 (A) and 3 (B). Red lines represent the factors significantly correlated with PC prognosis with $P < 0.05$.

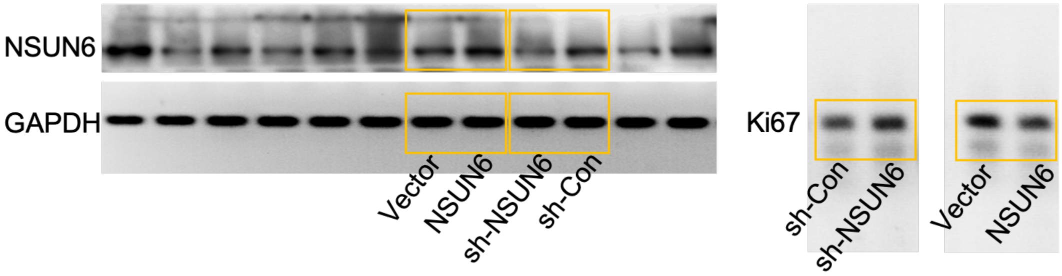
Figure 2f



Supplemental Figure 2c



Supplemental Figure 2d



Supplementary Figure 4. Full blots of figures.