Supplementary tables and figures

Table S1. Patient and tumor characteristics

Characteristics	N (%). Patients
Age, y [mean (SD)]	61 (39-83)
Gender	
Male	132 (55.7)
Female	105 (44.3)
Serum CA19-9	
~37 U/mL	99 (41.8)
>37 U/mL	138 (58.2)
Location	
Head	99 (41.8)
Body and tail	126 (53.2)
Other	12 (5.0)
Size, mm [mean (SD)]	42 (1-97)
Lymph node metastasis	
Negative	129 (54.4)
Positive	108 (45.6)
Venous invasion	
Negative	195 (82.3)
Positive	42 (17.7)
Perineural invasion	
Negative	45 (19.0)
Positive	192 (81.0)
Histologic grading	
Well -Moderate	165 (69.6)
Poor	72 (30.4)
UICC T	
T1	33 (13.9)
T2	97 (40.9)
T3	89 (37.6)
T4	18 (8.0)
UICC N	
N0	129 (54.4)
N1	75 (31.6)
N2	33 (13.9)
UICC stage	
I	81 (34.2)
II	105 (44.3)
III	51 (21.5)

Data are expressed as percentages unless otherwise stated

SD: standard deviation.

Table S2. Immunohistochemistry of common driven genes and Shh protein expression

Variables	N (%). Patients
Driver gene	
Smad4	
Lost	168 (70.9)
Intact	69 (29.1)
p53	
Normal	90 (38.0)
Abnormal	147 (62.0)
P16	
Lost	177 (74.7)
Intact	60 (25.3)
Kras	237 (100.0)
Sonic hedgehog pathway	
Shh	
Low	135 (57.0)
High	102 (43.0)
Gli1	
Low	123 (51.9)
High	114 (48.1)
Gli2	
Low	102 (43.0)
High	135 (57.0)
SMO	
Low	90 (38.0)
High	147 (62.0)

SMO: smoothened; Gli: glioma-associated oncogene homolog.

Table S3. Relationship between Smad4, Gli1 and SMO protein expression and clinicopathologic characteristics using $\chi 2$ test

Characteristics	Smad4 immunoreactivity			Gli1 immunoreactivity			SMO immunoreactivity		
	Intact	Lost	*p* value	Low	High	*p* value	Low	High	*p* value
Age, y			0.421			0.100			0.250
～60	36	78		69	45		39	75	
>60	33	90		54	69		51	72	
Gender			0.015*			0.897			0.001*
Male	30	102		69	63		63	69	
Female	39	66		54	51		27	78	
Serum CA19-9			0.003*			0.490			0.329
~37 U/ml	39	60		54	45		34	65	
>37 U/ml	30	108		69	69		56	82	
Location			0.000*			0.006*			0.115
Head	42	57		63	36		30	69	
Body and tail	24	102		57	69		51	75	
Other	3	9	NA	3	9	NA	9	3	NA
Largest Size			0.028*			0.054			0.171
~2 cm	16	20		24	12		10	26	
>2 cm	53	148		99	102		80	121	
Lymph node metastasis			0.655			0.187			0.178
Negative	36	93		72	57		54	75	
Positive	33	75		51	57		36	72	
Venous invasion			0.227			0.196			0.739
Negative	60	135		105	90		75	120	
Positive	9	33		18	24		15	27	
Perineural invasion			0.489			0.227			0.476
Negative	15	30		27	18		15	30	
Positive	54	138		96	96		75	117	
Histologic grading			0.064			0.001*			0.100
Well -Moderate	54	111		99	66		57	108	
Poor	15	57		24	48		33	39	
UICC T			0.040*			0.026*			0.921
T1+2	45	85		76	54		49	81	
T3+4	24	83		47	60		41	66	
UICC N			0.614			0.252			0.001*
N0	36	93		72	57		54	75	
N1	21	54		33	42		36	39	
N2	12	21		18	15		0	33	
UICC Stage			0.569			0.162			0.003*
I	26	55		49	32		35	46	
II	31	74		50	55		46	59	
III	12	39		24	27		9	42	

*, $p<0.05$; UICC: International Union against Cancer. SMO: smoothened; Gli: glioma-associated oncogene homolog; NA: non-adoption.

Table S4. Relation between Smsd4 and Sonic Hedgehog Pathway Using $\chi 2$ Test

Variable	**Smad4 Immunoreactivity**			
	Intact（n）	Loss（n）	*p* value	Pearson's R
Shh			0.000	0.343
Low	21	114		
High	48	54		
Gli1			0.000	0.505
Low	63	60		
High	6	108		
Gli2			0.340	
Low	33	69		
High	36	99		
SMO			0.516	
Low	24	66		
High	45	102		

SMO: smoothened; Gli: glioma-associated oncogene homolog.

Table S5. Multivariate Cox Regression for Survival Analysis Using the Combination of The Status of *SMAD4* and the SHH Pathway

Variables		**RFS**		**OS**	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value
Gender	**Famale**	1	0.000*	1	0.000*
	Male	2.436 (1.793–3.309)		2.296 (1.689–3.122)	
Serum CA19–9	**~37 U/ml**	1	0.000*	1	0.000*
	>37 U/ml	2.854 (1.967–4.141)		3.312 (2.318–4.732)	
Location	**Head**	1	0.000*	1	0.000*
	Body and Tail	2.101 (1.551–2.846)		2.044 (1.516–2.756)	
Largest tumor size	**~ 2 cm**	NA		NS	0.327
	> 2 cm				
Lymph node metastasis	**Negative**	NA		1	0.005*
	Positive			1.521(1.138–2.032)	
Venous invasion	**Negative**	NS	0.692	1	0.002*
	Positive			1.869 (1.218–2.851)	
Histologic grading	**Well -Moderate**	NS	0.985	NS	0.800
	Poor				
TP53	**Normal**	NA		NS	0.088
	Abnormal				
Group	**I**	1	0.000*	1	0.000*
	II	1.813 (1.172–2.806)	0.008*	1.864 (1.218–2.851)	0.004*
	III	2.853 (1.755–4.639)	0.000*	3.309 (2.035–5.382)	0.000*

OS: overall survival; RFS: recurrence-free survival; HR: hazard ratio; CI: confidence interval; NS: non-significant; NA: non-adoption.

Table S6. Comparison of the Prognositic Accuracies of *SMAD4*, Activated SHH Pathway and the Combined Model

Model	**OS**				**RFS**			
	C-Index	AIC	BIC	*p* value	C-Index	AIC	BIC	*p* value
SMAD4	0.5862	2052	2056	0.000	0.5830	1927	1930	0.000
Activated SHH Pathway	0.5684	2045	2049	0.000	0.5548	1922	1926	0.000
***SMAD4*+activated SHH Pathway**	0.6220	2033	2037	0.000	0.6076	1914	1918	0.000

Table S7. Antibodies

Antibodies	Company	Dilution
Rabbit anti-Kras polyclonal antibody ab180772	Abcam	1:100
Rabbit anti-p16 monoclonal antibody ab108349	Abcam	1:50
Rabbit anti-p53 polyclonal antibody ab31333	Abcam	1:50
Mouse anti-Smad4 monoclonal antibody sc-7966 B	Santa Cruz Biotechnology	1:200
Rabbit anti-Shh polyclonal antibody sc-9024	Santa Cruz Biotechnology	1:100
Rabbit anti-Gli1 polyclonal antibody #43926	SAB	1:100
Rabbit anti-SMO polyclonal antibody 20787-1-AP	Proteintech	1:100
Mouse anti-Gli2 monoclonal antibody sc-271786	Santa Cruz Biotechnology	1:200

Table S8. Relation between other driver genes and Sonic Hedgehog Pathway Using $\chi 2$ Test

	TP53			***P16***		
	Abnormal	Normal	*p* value	Lost	Intact	*p* value
Shh			0.090			0.079
Low	90	45		95	40	
High	57	45		82	20	
Gli1			0.647			0.797
Low	78	45		91	32	
High	69	45		86	28	
Gli2			0.018			0.582
Low	72	30		78	24	
High	75	60		99	36	
SMO			0.615			0.194
Low	54	36		63	27	
High	93	54		114	33	

SMO: smoothened; Gli: glioma-associated oncogene homolog.

Fig. S1 Immunohistochemical Labeling of Driver Genes

A1) Smad4 were scored as intact (positive), indicating the presence of an intact gene, **A2)** Smad4 were scored as lost (negative), indicating a deletion or inactivating mutation of the gene had occurred. **bB** Immunohistochemical labeling of Kras was all scored as intact (positive), indicating the presence of an intact gene. **C1)** Pancreatic ductal adenocarcinoma (PDAC) showing a "normal" pattern of p53 immunohistochemical labeling. **C2)** Example of PDAC with diffusely positive nuclear labeling for p53, namely robust nuclear accumulation of immunolabeled protein in ≥30% of neoplastic cells compared with adjacent normal cells. **C3)** Example of PDAC with loss of nuclear labeling for p53, namely a virtual absence of immunolabeling compared with adjacent normal tissue (immunolabeling in <5% of neoplastic cells), suggesting the presence of an intragenic deletion, nonsense or frameshift mutation. **D1)** Positive p16 im- munolabeling indicating an intact CDKN2A/p16 gene. **D2)** Negative p16 immunolabeling indicating alteration of the CDKN2A/p16 gene.

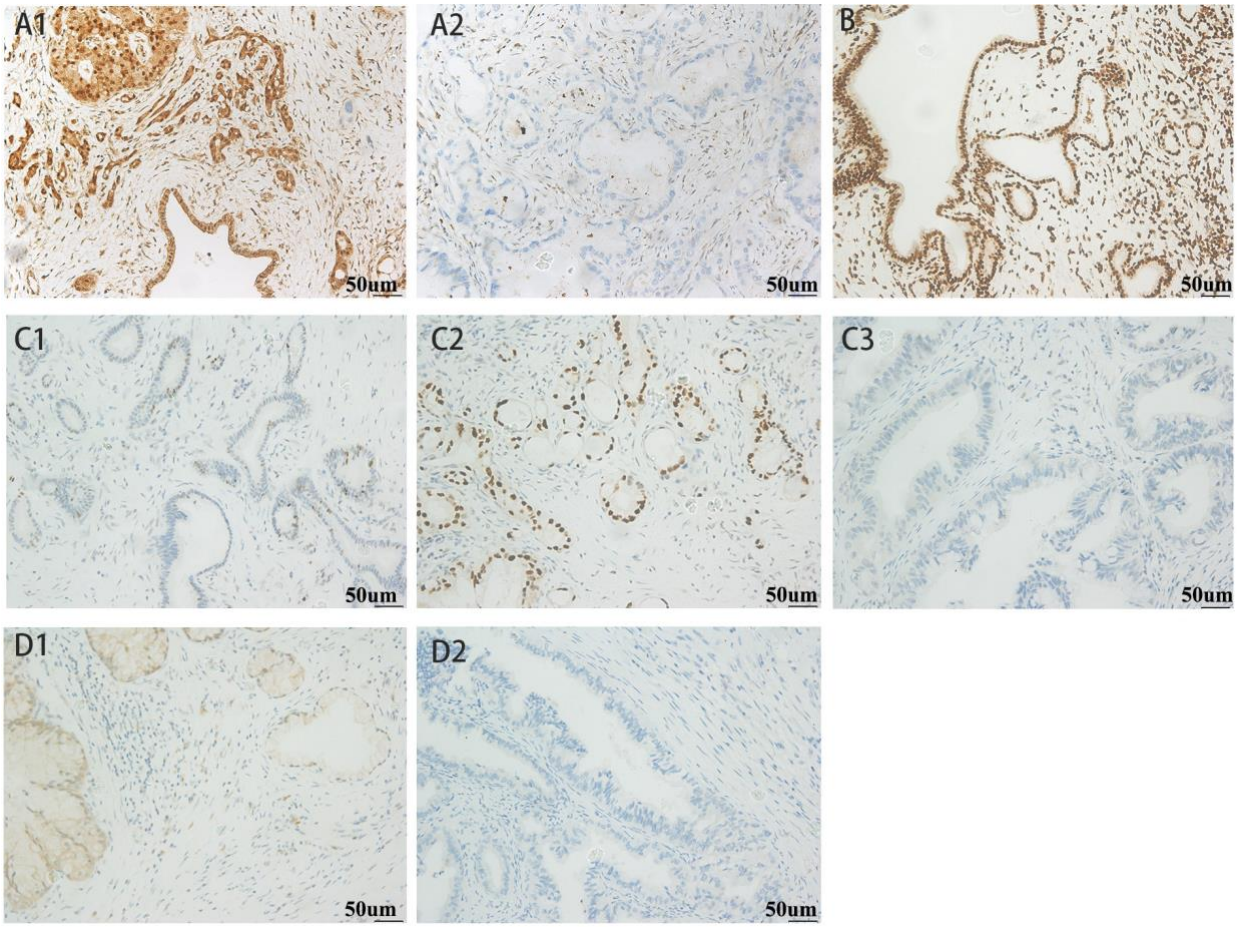

Fig. S2 Immunohistochemical Labeling of Shh and Gli2 Expression in Pancreatic Cancer

Shh: **A1)** weak expression, **A2)** moderate expression, and **A3)** intense expression in tumor cells. Gli2: **B1)** weak expression, **B2)** moderate expression, and **B3)** intense expression in tumor cells and stroma. All magnification = 400×. Positive staining appears brown.