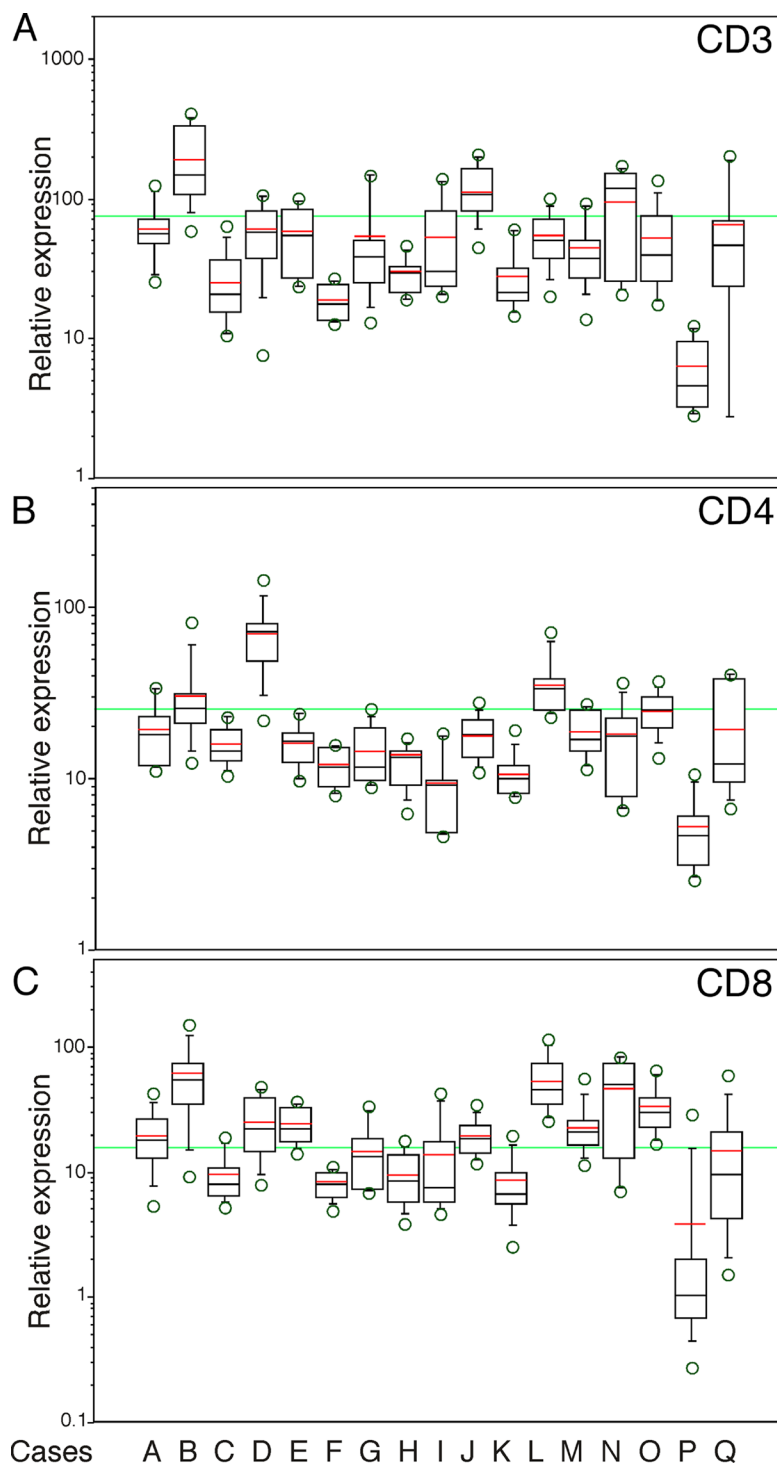
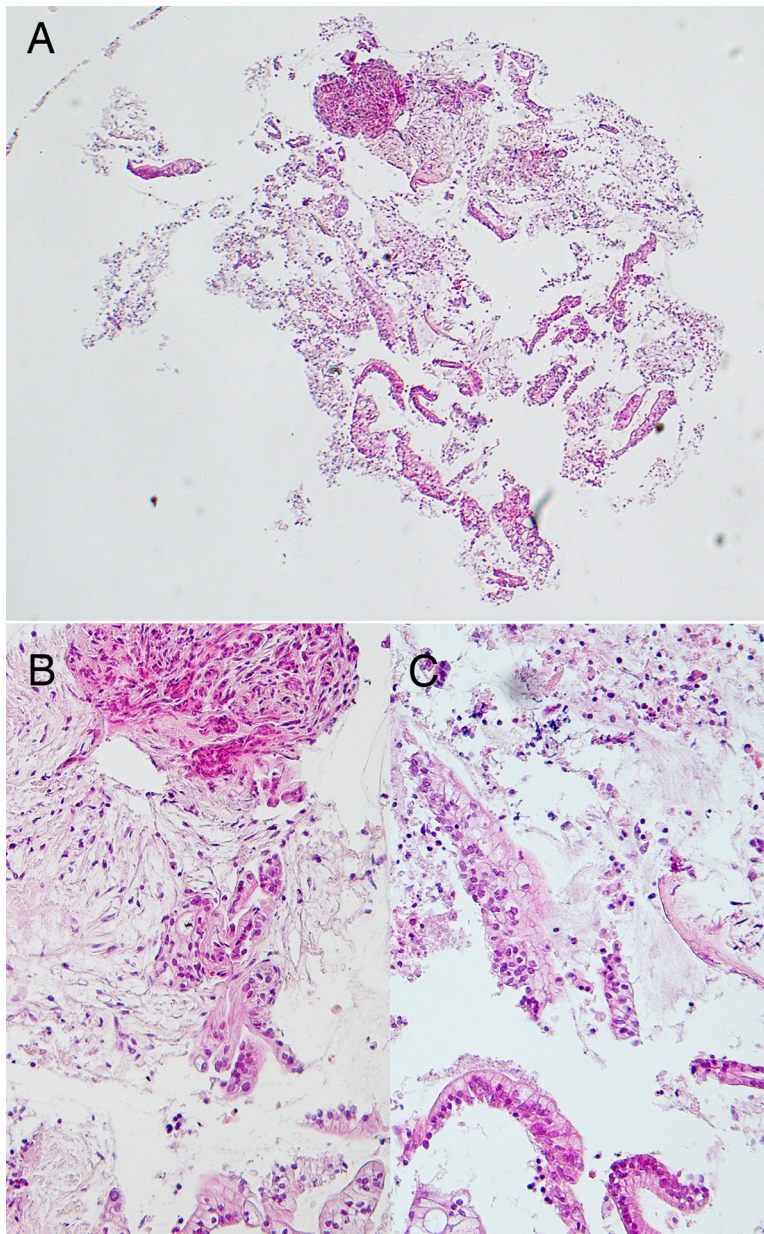


Reliable evaluation of tumor-infiltrating lymphocytes in pancreatic cancer tissue biopsies

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Box plots for expression of *CD3* (A), *CD4* (B), and *CD8* (C) genes in PDAC biopsy tissues. The red line indicates the gene expression level in the corresponding surgically-resected tissue sample. The green line indicates the gene expression cut-off value set at the median of 241 cases of surgically-resected PDAC.



Supplementary Figure 2: Representative biopsied materials stained with hematoxylin and eosin. Only very small-sized and fragmented tissues and cells are obtained. Very low magnification (A) and low magnification (B, C).

Supplementary Table 1: Median survival time, one-, two-, and five-year survival rate

Variables	Group	Overall survival				Disease-free survival			
		Median survival time ^a (months)	Survival rate (%)			Median survival time ^a (months)	Survival rate (%)		
			1-yr	2-yr	5-yr		1-yr	2-yr	5-yr
Expression of <i>CD3</i>	Low	24.3 ± 4.4	100	53.6 ± 20.0	53.6 ± 20.0	24.3 ± 4.4	100	53.6 ± 20.0	53.6 ± 20.0
	High	NA	100	83.3 ± 15.2	83.3 ± 15.2	NA	100	83.3 ± 15.2	83.3 ± 15.2
Expression of <i>CD4</i>	Low	24.3 ± 4.0	100	51.4 ± 20.4	51.4 ± 20.4	24.3 ± 4.0	100	51.4 ± 20.4	51.4 ± 20.4
	High	NA	100	85.7 ± 13.2	85.7 ± 13.2	NA	100	85.7 ± 13.2	85.7 ± 13.2
Expression of <i>CD8</i>	Low	24.3 ± 3.8	100	60.0 ± 21.9	60.0 ± 21.9	24.3 ± 3.8	100	60.0 ± 21.9	60.0 ± 21.9
	High	NA	100	75.0 ± 15.3	75.0 ± 15.3	NA	100	75.0 ± 15.3	75.0 ± 15.3
Expression of <i>FOXP3</i>	Low	24.3 ± 4.5	100	58.3 ± 18.6	58.3 ± 18.6	24.3 ± 4.5	100	58.3 ± 18.6	58.3 ± 18.6
	High	NA	100	83.3 ± 15.2	83.3 ± 15.2	NA	100	83.3 ± 15.2	83.3 ± 15.2
Ratio of <i>FOXP3/CD4</i>	Low	NA	100	72.9 ± 16.5	72.9 ± 16.5	NA	100	72.9 ± 16.5	72.9 ± 16.5
	High	24.3 ± 3.7	100	62.5 ± 21.4	62.5 ± 21.4	24.3 ± 3.7	100	62.5 ± 21.4	62.5 ± 21.4

Abbreviation: NA: not available, ^amedian ± standard error.

Supplementary Table 2: Clinicopathological characters of PDAC using biopsy model analysis

Age, years	<65	8
	≥65	9
Gender	Male	9
	Female	8
Pathologic tumor status	T1a/1b/1c	0/0/0
	T2	14
	T3	3
	T4	0
Pathologic node status	N0	6
	N1	5
	N2	6
Pathologic metastasis status	M0	15
	M1	2
Stage	IA	0
	IB	5
	IIA	1
	IIB	5
	III	4
	IV	2
Tumor grade	Grade 1	0
	Grade 2	13
	Grade 3	4
Nerve plexus invasion ^a	Absence	4
	Presence	13
Lymphatic invasion ^a	0, 1	1
	2, 3	16
Venous invasion ^a	0, 1	1
	2, 3	16
Intrapancreatic neural invasion ^a	0, 1	3
	2, 3	13
Tumor margin status	Negative	13
	Positive	4
Adjuvant chemotherapy	Negative	3
	Positive	14
Total		17

^aClassified according to the classification of pancreatic carcinoma of Japan Pancreas Society.

Supplementary Table 3: Primer sequences and Universal Probe Library probes for quantitative RT-PCR

Name of genes	Universal Probe Library Probe	Left primer	Right primer
<i>ACTB</i>	#64	CCAACCgCgAgAAgATgA	TCCATCACgATgCCAgTg
<i>CD3</i>	#58	TCCTTgCTgTTggggTCTAC	CgAgTCTgCTTgTCTgAAgCTC
<i>CD4</i>	#18	ggCAgTgTCTgCTgAgTgAC	gACCATgTgggCAgAACCT
<i>CD8</i>	#51	TCATggCCTTACCAgTgACC	AggTTCCAaggTCCgATCC
<i>FOXP3</i>	#20	gAgAAgCTgAgTgCCATgC	AgCCCTTgTCggATgATg