Table S1. Clinicopathologic features of PDAC patients enrolled for analyses.

	Surgery Alone Group	Neoadjuvant Treatment Group		
Total	380	136		
Age				
≤60	179(47.1%)	73(53.7%)		
>60	201(52.9%)	63(46.3%)		
Gender				
Male	216(56.8%)	70(51.5%)		
Female	164(43.2%)	66(48.5%)		
TNM Stage				
Ι	108(28.4%)	33(24.3%)		
II	149(39.2%)	54(39.7%)		
III	119(31.3%)	47(34.6%)		
Unknown	4(1.1%)	2(1.5%)		
T				
T1/T2	199(52.4%)	78(57.4%)		
T3	181(47.6%)	56(41.2%)		
Unknown	0(1.1%)	2(1.5%)		
\mathbf{N}				
N0	184(48.4%)	65(47.8%)		
N1/N2	196(51.6%)	69(50.7%)		
Unknown	0(0.0%)	2(1.5%)		
Grade				
G1/2	221(58.2%)	83(61.0%)		
G3	159(41.8%)	50(36.8%)		
Unknown	0(0.0%)	3(2.2%)		
Tumor Location				
Head	199(52.4%)	49(36.0%)		
Body/Tail	171(45.0%)	86(63.2%)		
Unknown	10(2.6%)	1(0.7%)		
Nerve Invasion				
No	75(19.7%)	10(7.4%)		
Yes	301(79.2%)	125(91.9%)		
Unknown	4(1.1%)	1(0.7%)		
Vascular Invasion				
No	286(75.3%)	84(61.8%)		
Yes	81(21.3%)	51(37.5%)		
Unknown	13(3.4%)	1(0.7%)		

TNM stage: tumor-node-metastasis staging system (AJCC 8th edtion); presented as number (percentage).

Table S2. Detailed information of antibodies used in mIHC analyses.

Antibody	Source	Source Antigen retrieval condition	
CD4	Abcam(ab133616)	pH 9.0	1/200
CD8	Abcam(ab178089)	pH 9.0	1/100
CD20	Abcam(ab64088)	pH 6.0	1/100
CD21	Abcam(ab227668)	pH 6.0	1/100
CD23	Abcam(ab254162)	pH 9.0	1/100
FOXP3	Cell Signaling(98377s)	pH 9.0	1/100
CD68	Abcam(ab955)	pH 9.0	1/1000
CD11c	Abcam(ab52632)	pH 9.0	1/200
CD15	Abcam(ab241552)	pH 6.0	1/100
CD45RO	Abcam(ab23)	pH 6.0	1/100
CD69	Abcam(ab233396)	pH 9.0	1/500
CD30	Abcam(ab134080)	pH 9.0	1/1000
HLA-DR	Abcam(ab20181)	pH 6.0	1/100
CD25	Abcam(ab128955)	pH 9.0	1/100
CD28	Abcam(ab243228)	pH 9.0	1/1000
CD80	Abcam(ab134120)	pH 9.0	1/1000
CD86	Abcam(ab239075)	pH 9.0	1/100
OX40	Abcam(ab229021)	pH 9.0	1/1000
OX40L	Cell Signaling(59036)	pH 9.0	1/200
CTLA-4	Abcam(ab237712)	pH 9.0	1/500
PD-1	Abcam(ab52587)	pH 6.0	1/50
TIM-3	Abcam(ab241332)	pH 9.0	1/1000
LAG-3	Abcam(ab209236)	pH 9.0	1/1000

Table S3. Clinicopathologic features of PDAC patients in the external validation cohort.

	External validation cohort	Chi-Square test P-value (versus the SA group)			
Total	123				
Age					
≤60	56(45.5%)	0.761			
>60	67(54.5%)				
Gender					
Male	82(66.7%)	0.054			
Female	41(33.3%)				
TNM Stage					
I	42(34.1%)	0.055			
II	56(45.5%)				
III	25(20.3%)				
T					
T1/T2	71(57.7%)	0.300			
T3/T4	52(42.3%)				
N					
N0	73(59.3%)	0.035			
N1/N2	50(40.7%)				
Grade					
G1/2	73(59.3%)	0.816			
G3	50(40.7%)				
Tumor Location					
Head	68(55.3%)	0.772			
Body/Tail	55(44.7%)				
Nerve Invasion					
No	12(9.8%)	0.012			
Yes	109(88.6%)				
Unknown	2(1.6%)				
Vascular Invasion					
No	98(79.7%)	0.175			
Yes	19(15.4%)				
Unknown	6(4.9%)				

Table S4. Clinicopathologic features of PDAC patients with intratumoral TLSs in the SA and NAT group.

	SA group			NAT group				
	TLS (+)	TLS (-)	Total	Chi-square test P-value	TLS (+)	TLS (-)	Total	Chi-square test P-value
Number	80(21.1%)	300(78.9%)	380		21(15.4%)	115(84.6%)	136	
Age				0.353				0.897
≤60	34(8.9%)	145(38.2%)	179		11(8.1%)	62(45.6%)	73	
>60	46(12.1%)	155(40.8%)	201		10(7.4%)	53(39.0%)	63	
Gender				0.894				0.928
Male	46(12.1%)	170(44.7%)	216		11(8.1%)	59(43.4%)	70	
Female	34(8.9%)	130(34.2%)	164		10(7.4%)	56(41.2%)	66	
TNM Stage				0.688				0.689
I	26(6.8%)	82(21.6%)	108		7(5.1%)	26(19.1%)	33	
II	32(8.4%)	117(30.8%)	149		7(5.1%)	47(34.6%)	54	
III	21(5.5%)	98(25.8%)	119		7(5.1%)	40(29.4%)	47	
Unknown	1(0.3%)	3(0.8%)	4		0(0.0%)	2(1.5%)	2	
T				0.079				0.697
T1/T2	49(12.9%)	150(39.5%)	199		11(8.1%)	67(49.3%)	78	
Т3	31(8.2%)	150(39.5%)	181		10(7.4%)	46(33.8%)	56	
Unknown	0(0.0%)	0(0.0%)	0		0(0.0%)	2(1.5%)	2	
\mathbf{N}				0.947				0.770
N0	39(10.3%)	145(38.2%)	184		11(8.1%)	54(39.7%)	65	
N1/N2	41(10.8%)	155(40.8%)	196		10(7.4%)	59(43.4%)	69	
Unknown	0(0.0%)	0(0.0%)	0		0(0.0%)	2(1.5%)	2	
Grade				0.308				0.755
G1/2	51(13.4%)	170(44.7%)	221		13(9.6%)	70(51.5%)	83	
G3	29(7.6%)	130(34.2%)	159		8(5.9%)	42(30.9%)	50	
Unknown	0(0.0%)	0(0.0%)	0		0(0.0%)	3(2.2%)	3	
Tumor Location				0.224				0.660
Head	45(11.8%)	154(40.5%)	199		6(4.4%)	43(31.6%)	49	
Body/Tail	35(9.2%)	136(35.8%)	171		15(11.0%)	71(52.2%)	86	
Unknown	0(0.0%)	10(2.6%)	10		0(0.0%)	1(0.7%)	1	
Nerve Invasion				0.163				0.335
No	21(5.5%)	54(14.2%)	75		0(0.0%)	10(7.4%)	10	
Yes	59(15.5%)	242(63.7%)	301		21(15.4%)	104(76.5%)	125	
Unknown	0(0.0%)	4(1.1%)	4		0(0.0%)	1(0.7%)	1	
Vascular Invasion				0.514				0.578
No	62(16.3%)	224(58.9%)	286		13(9.6%)	71(52.2%)	84	
Yes	17(4.5%)	64(16.8%)	81		8(5.9%)	43(31.6%)	51	
Unknown	1(0.3%)	12(3.2%)	13		0(0.0%)	1(0.7%)	1	
Therapy								0.143

AG	12(8.8%)	75(55.1%)	87
GS	6(4.4%)	16(11.8%)	22
GEMOX	1(0.7%)	20(14.7%)	21
FOLFIRINOX	2(1.5%)	3(2.2%)	5
Other	0(0.0%)	1(0.7%)	1

TNM stage: tumor-node-metastasis staging system (AJCC 8th edtion); T: primary tumor, N: regional lymph node, M: distant metastasis.

Figure S1. Representative H&E staining images of intratumoural (left) and peritumoural (right) TLS in PDAC; TLSs are indicated by red arrows.

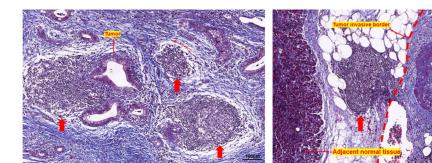
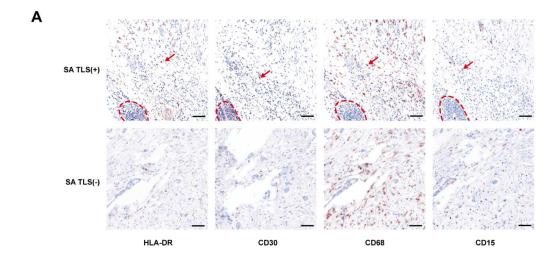


Figure S2. Representative brightfield mIHC images comparing infiltration levels of several other immune cells; TLSs are circled with dotted red lines; red arrows indicate the examples of positively stained cells; scale bar: $100 \mu m$.



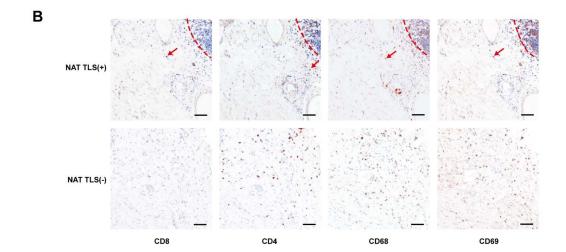


Figure S3. Kaplan–Meier curves comparing OS and PFS probabilities between TLS (+) and TLS (-) patients receiving different NAT therapies.

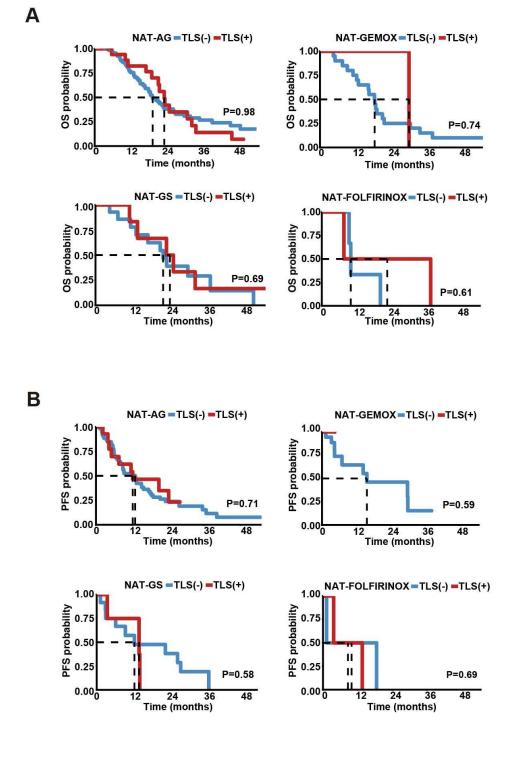


Figure S4. Frequency and prognostic value of TLS location and maturation in the SA cohort; peri-TLS: peritumoural TLS, intra-TLS: intratumoural TLS; eTLS group: eTLS(+) pTLS(-) sTLS(-) samples, pTLS group: pTLS(+) sTLS(-) samples, sTLS group: sTLS(+) samples.

Summary of peritumoral TLSs in the SA group							
C.1	peri-TLS(-)	peri-TLS(+)					
SA group	Total	Total	eTLS	pTLS	sTLS		
intra-TLS(-)	43	23	19	0	4		
intra-TLS(+)	7	22	8	6	8		
Total	50	45	27	6	12		

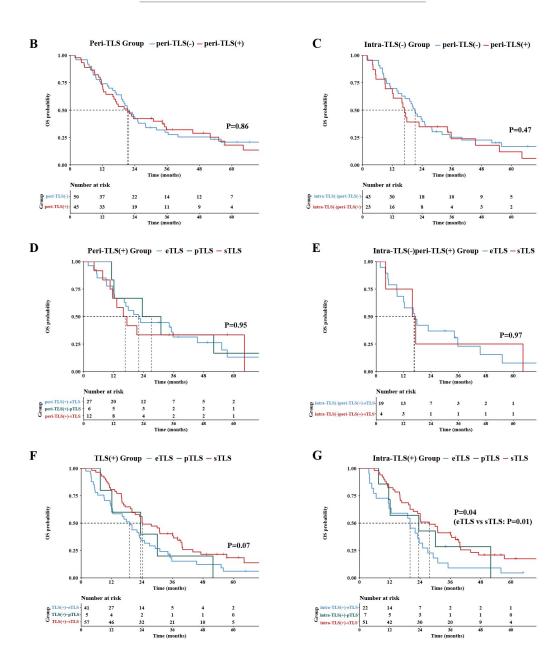


Figure S5. Frequency and prognostic value of TLS location and maturation in the NAT cohort; peri-TLS: peritumoural TLS, intra-TLS: intratumoural TLS; eTLS group: eTLS(+) pTLS(-) sTLS(-) samples, pTLS group: pTLS(+) sTLS(-) samples, sTLS group: sTLS(+) samples.

A	Summary of peritumoral TLSs in the NAT group							
	NAT group	peri-TLS(-)		peri-T				
		Total	Total	eTLS	pTLS	sTLS		
	intra-TLS (-)	13	7	5	1	1		
	intra-TLS (+) Total	1 14	1	1 6	0 1	0 1		
,	Total	17		- 0	1			
B Post TI S Coord	TIS()	: TI 5(1)	\mathbf{C}		TI 5() 4	S	IS() .	: TI S(1)
1.00	p — peri-TLS(-) — per	1-1 LS(+)		1.00 T	ntra-1LS(-)	Group — peri-TI -	_S(-) —]	peri-1LS(+)
					1			
0.75	· · ·			0.75	٦			
à l	1			ility		4		
OS probability				OS probability				
8	<u> </u>	→		so		П	<u> </u>	
0.25	<u> </u>	P=0.07		0.25				P=0.11
						_ L	-	
0.00	24 36 Time (months)	48		0.00	12	24 Time (months)	36	48
Number at risk	Time (months)			1	Number at risk	Time (montas)		
peri-TLS(-)- 14 8 8 9 9 peri-TLS(-)- 8 6	4 1	1.	d intr	ra-TLS(-)peri-TLS(-)- a-TLS(-)peri-TLS(+)		4	1	1
© peri-TLS(+) 8 6	3 2	48	G intri	a-TLS(-)peri-TLS(+) 0	7 5 12	3 24	36	48
	Time (months)					Time (months)		
D Peri-TLS(+) Group — eTLS — p1	TLS/sTLS	Е	Intra-	0.75	LS(+) Group — ε	TLS -	pTLS/sTLS
0.25	24 36 48 Time (months)	P=0.05		٥	0.25	12 24 36 Time (mc	i 48	P=0.08
Number at risk					Number		ntus)	
peri-TLS(+)-eTLS 6 4 D peri-TLS(+)-pTLS/sTLS 2 2	3 2 0	0	Group intra	intra-TLS(-)peri-TI		3 3 2	0	0
© peri-TLS(+)-pTLS/sTLS 2 2	0 0 0	60	O intra	a-TLS(-)peri-TLS(+)-p	oTLS/sTLS 2	2 0 0	48	60
F	Time (months)		G			Time (mo	nths)	
	oup — eTLS — pTLS -	- sTLS	J		Intra-TLS(+) Group — eTLS	– pTL	S - sTLS
0.75 - 0.75 - 0.50 - 0.25 - 0.00 0 12	24 26 48 Time (months)	P=0.91		0.75 - 1.00 0.75 - 1.00 0.50 - 1.00 0.50 - 1.00 0.50 - 1.00 0.50 0.50 0.50 0.50 0.50 0.50 0.50	12	24 36 Time (months)	48	P=0.40
Number at risk	6 3 0	0	9-	intra-TLS(+)-eTLS	umber at risk 8 7	3 1	0	0
E TLS(+)-eTLS 13 10 E TLS(+)-pTLS 8 6 TLS(+)-sTLS 7 6 0 12	4 1 0 4 2 2 24 36 48	0 1 60	Gro	intra-TLS(+)-pTLS intra-TLS(+)-sTLS	7 5 6 5 12	4 1 4 2 24 36	0 2 48	0 1 60
	Time (months)					Time (months)		

Figure S6. Representative mIHC images showing the expression levels of activation makers, costimulation molecules and inhibitory molecules within intratumoral TLSs in the SA and NAT group; TLSs are circled with dotted white lines; scale bar: $50 \mu m$.

