Supplementary methods 1. For peripheral immune cell analysis, 15 mL blood was collected at baseline and at the time of each disease evaluation during treatment, and PBMCs were purified by standard density gradient centrifugation. Multicolor flow cytometry was performed using the CytoFLEX flow cytometry platform (Beckman Coulter, Brea, CA) to determine the peripheral immune cell proportions levels. The panel for multicolor flow cytometry to evaluate peripheral immune cell proportions (immune cell panel) included CD3 (UCHT1; BioLegend, San Diego, CA), CD4 (OKT4; BioLegend), CD8 (SK1; BioLegend), CD14 (63D3; BioLegend), CD11c (3.9; BioLegend), CD56 (5.1H11; BioLegend), γδ-TCR (B1; BioLegend), HLA-DR (L243; BioLegend), CD69 (FN50; BioLegend), and FoxP3 (236A-E7; eBioscience, San Diego, CA). The proportion of each immune cell population, including $CD4^+$ T cells, $CD4^+Foxp3^+$ regulatory T cells, $CD8^+$ T cells, $CD56^+$ NK cells, γδ T cells, HLA-DR^+ myeloid cells, $CD14^+$ $CD11c^+$HLA-DR^+ monocytes, and $CD14^-$ $CD11c^+$HLA-DR^+ dendritic cells was estimated based on the total number of live cells. The proportions of activated $CD4^+$ T cells, $CD8^+$ T cells, $CD56^+$ NK cells, and γδ T cells were measured by gating on $CD69^+$ cells. The proportions of $CD4^+$ and $CD8^+$ T cells with checkpoint expression were measured using a multicolor flow cytometry panel (T cell checkpoint panel) that included PD-1 (EH12.2H7; BioLegend), LAG-3 (11C3C65; BioLegend), CTLA-4 (L3D10; BioLegend), and TIGIT (A15153G; BioLegend).

Supplementary methods 2. Total RNA was extracted and purified from formalin-fixed paraffin embedded (FFPE) tissue from each patient who underwent surgical resection. The Denovix DS 11 AATI Fragment Analyzer was used to evaluate the quantity and quality of each isolated RNA sample before analysis. Approximately 500–1000 ng total RNA was used for gene expression analysis, and the input amount of total RNA wa

s increased for samples with excess fragmentation of the RNA strands. The samples w ere thawed right before the analysis and mixed with the reporter code set and probe s et in hybridization buffer. The samples were hybridized to the probes at 65°C for 16–24 hours. Using the Nanostring nCounter prep station, inadequately hybridized probes w ere removed, and properly hybridized transcript-probe complexes were immobilized ont o Nanostring cartridges. Finally, the fixed samples on the cartridges were scanned and read using the Nanostring nCounter Digital Analyzer (NCT-DIGT-120) and were recorde d as reporter code count (RCC) files. RCC files were analyzed using nSolver softwar e (Nanostring Technologies) for data QC, including imaging QC, binding density QC, a nd positive and negative control QC. The expression levels of each gene in samples with adequate data QC results were normalized to the expression of positive control a nd housekeeping genes using nSolver. Differential gene expression analyses were perfo rmed using ROSALIND (https://rosalind.onramp.bio/), with a HyperScale architecture d eveloped by OnRamp BioInformatics (San Diego, CA). Fold changes and p-values we re calculated using criteria provided by Nanostring Technologies. The nominal p-value s were adjusted for the false discovery rate, and genes with a differential expression of absolute $\log_2$ fold change ($\log_2$FC) > 1.0 with an adjusted p-value < 0.15 were co nsidered significant.

Supplementary methods 3. From the differential expression analysis, t-statistics of ea ch gene against each covariate in the model is calculated. The directed global signific ance scores of each gene set were calculated as the square root of the mean squared signed t-statistics of each genes, which represent the relative over- or under-expression of the genes relative to a set of genes annotated as belonging to the specific immun ologic pathway.

$$U = \left(\frac{1}{p}\sum_{i=1}^{p} sign(t_i \cdot t_i^2\right)$$

$$Directed\ global\ significance\ score = sign(U)|U|^{\frac{1}{2}},$$

$$sign(U)\ equals\ 1 \text{ if } U > 0 \text{ and } -1 \text{ if } U < 0$$

Supplementary methods 4. Four markers (CD8, CD68, CD163, and myeloperoxidase [MPO]) were selected for evaluation of cytotoxic T cells, macrophages, M2-like macr ophages, and neutrophils, respectively. Formalin-fixed paraffin-embedded (FFPE) tissue sections (4 μm thick) were stained with and automatic immunohistochemical staining device (Benchmark XT; Ventana Medical Systems, Tucson, AZ) for pan-cytokeratin (1: 400, mouse monoclonal, clone AE1/AE3, catalog no. NCL-L-AE1/AE3; Novocastra, C A), CD8 (1:400, clone POLY, catalog no. 108M-96; Cell Marque, Rocklin, CA), CD1 63 (1:400, clone 10D6, catalog no. NCL-CD163; Novo, Newcastle, UK), CD68 (1:200 0, clone KP1, catalog no. M0814; DAKO, Glostrup, Denmark), MPO (1:2000, clone POLY, catalog no. A0398; DAKO) and MARCO (1:75, Rabbit polyclonal, clone POL Y, catalog No.AB231046, Abcam, Cambridge, Uk).

Supplementary figure 1. Detailed method of the tumor microenvironment immune cell analysis. “Invasive tumor margin/desmoplastic” area annotation, “Peritumoral” area annotation (25 μm from cytokeratin-positive cells), and “Immune cell counting” were merged to count stained immune cells in the two regions (peritumoral and stromal). The cell counts per mm^2 of each region were than obtained and compared between the two recurrence groups (DFS10 Achieved vs. DFS10 Failed).

Supplementary figure 2. Representative flow cytometry plots. A. $Foxp3^+CD4^+$ regulatory T cell. B. $CD14^+CD11c^+HLA\text{-}DR^+$ monocyte. C. $TIGIT^+CD4^+$ T cell. D. $PD\text{-}1^+CD8^+$ T cell.

Supplementary figure 3. Non-significant changes in the peripheral immune phenotype from baseline to completion of eight cycles of mFOLFIRINOX using the immune cell panel and the T cell checkpoint panel.

Supplementary figure 4. $CD68^+$ cells, $CD163^+$ cells, MPO^+ and $MARCO^+$ cells in the tumor microenvironment according to the recurrence group (DFS10 achieved vs. failed).

Supplementary figure 5. Comparison of immune cell scores between the two recurrence groups (DFS10 achieved vs. failed) estimated from the immune-related gene expression profile using the Nanostring annotation.

Table S1. Baseline clinical characteristics of patients treated with neoadjuvant mFOLFIRINOX.

Table S2. Availability of biomarker samples of patients treated in the phase 2 clinical trial (NCT02749136).

Table S3. Univariate analysis of survival outcomes according to baseline peripheral immune cell level (Cox proportional hazards model, continuous variable)

Table S4. Non-parametric bootstrapping of paired comparison of immune cell level pre- and post-chemotherapy.

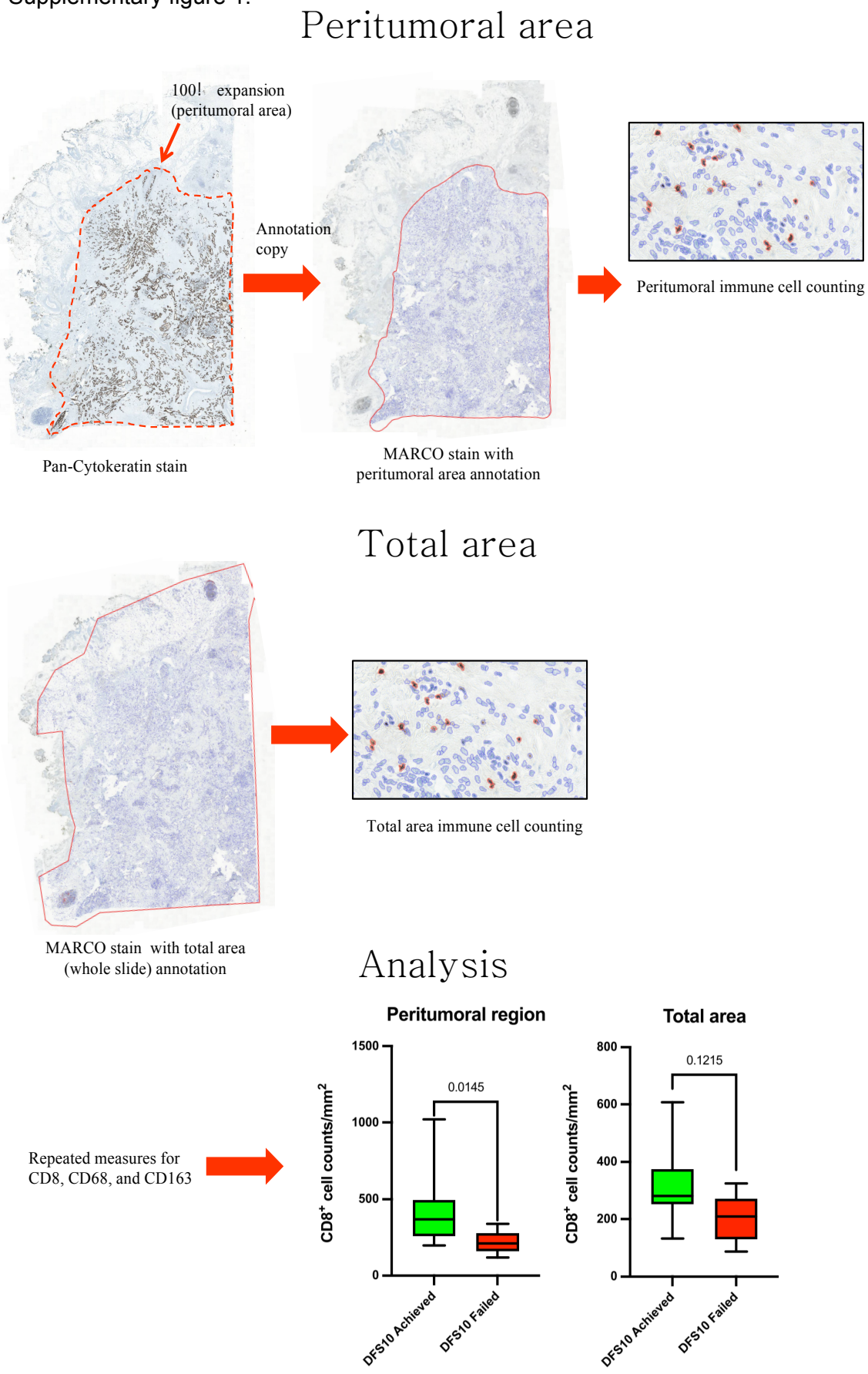
Table S5. Comparison of clinical variables between the two recurrence groups

Table S6. Gene list of significant differential expression between the recurrence groups.

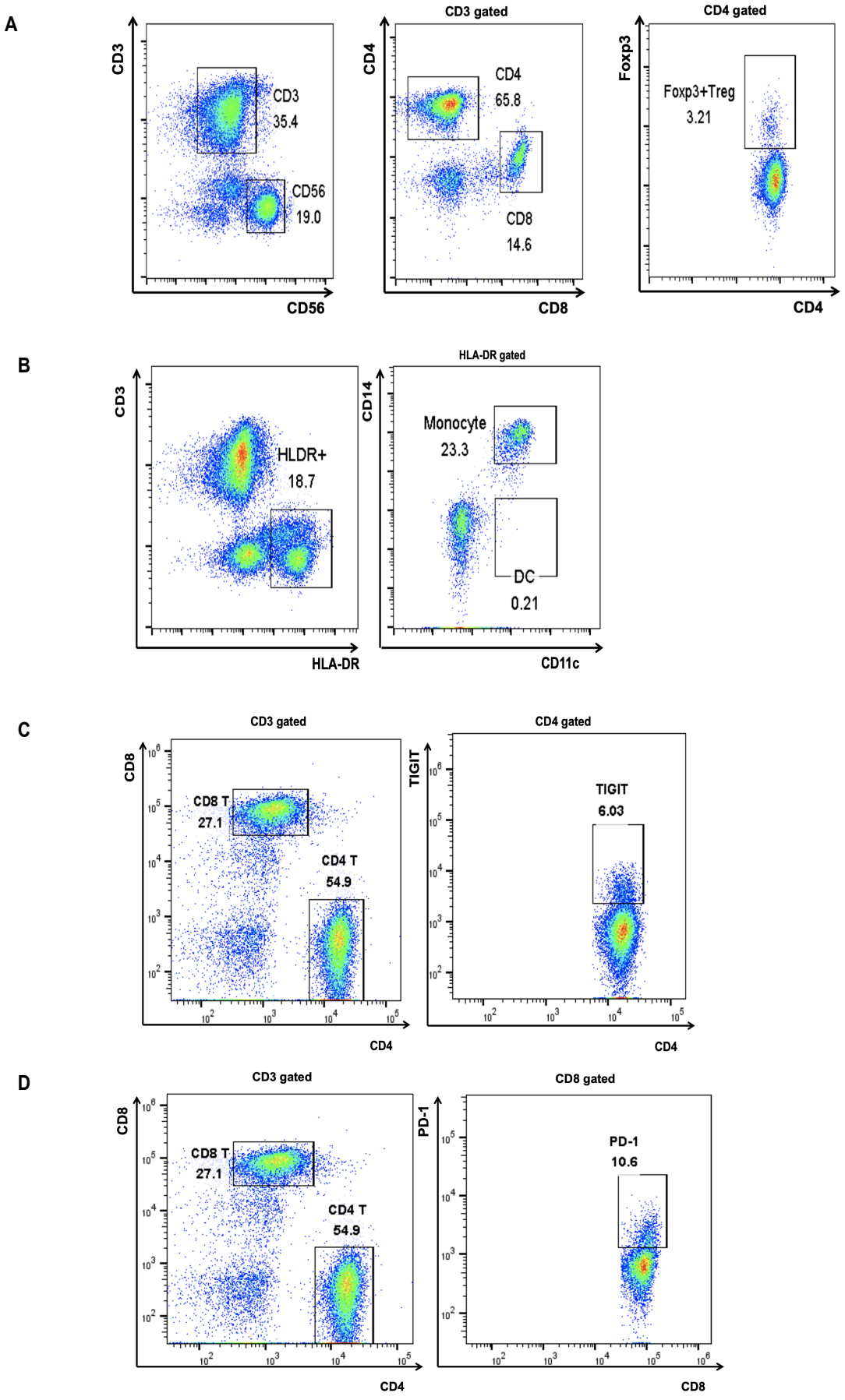
Table S7. Directed enrichment score of gene sets from nanostring data

Table S8. Clinical characteristics of pancreatic cancer patients from the TCGA data (the Human Protein Atlas).

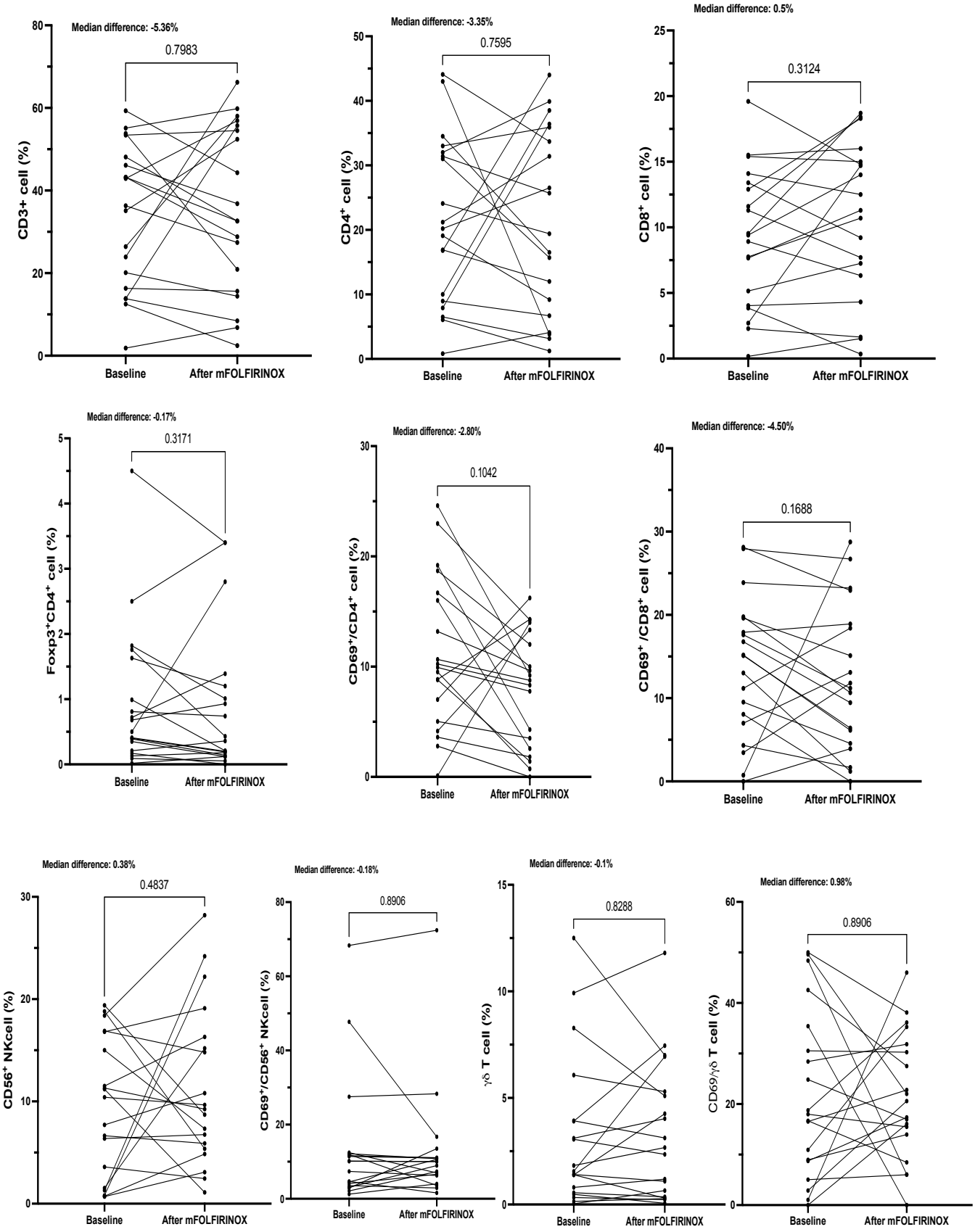
Supplementary figure 1.



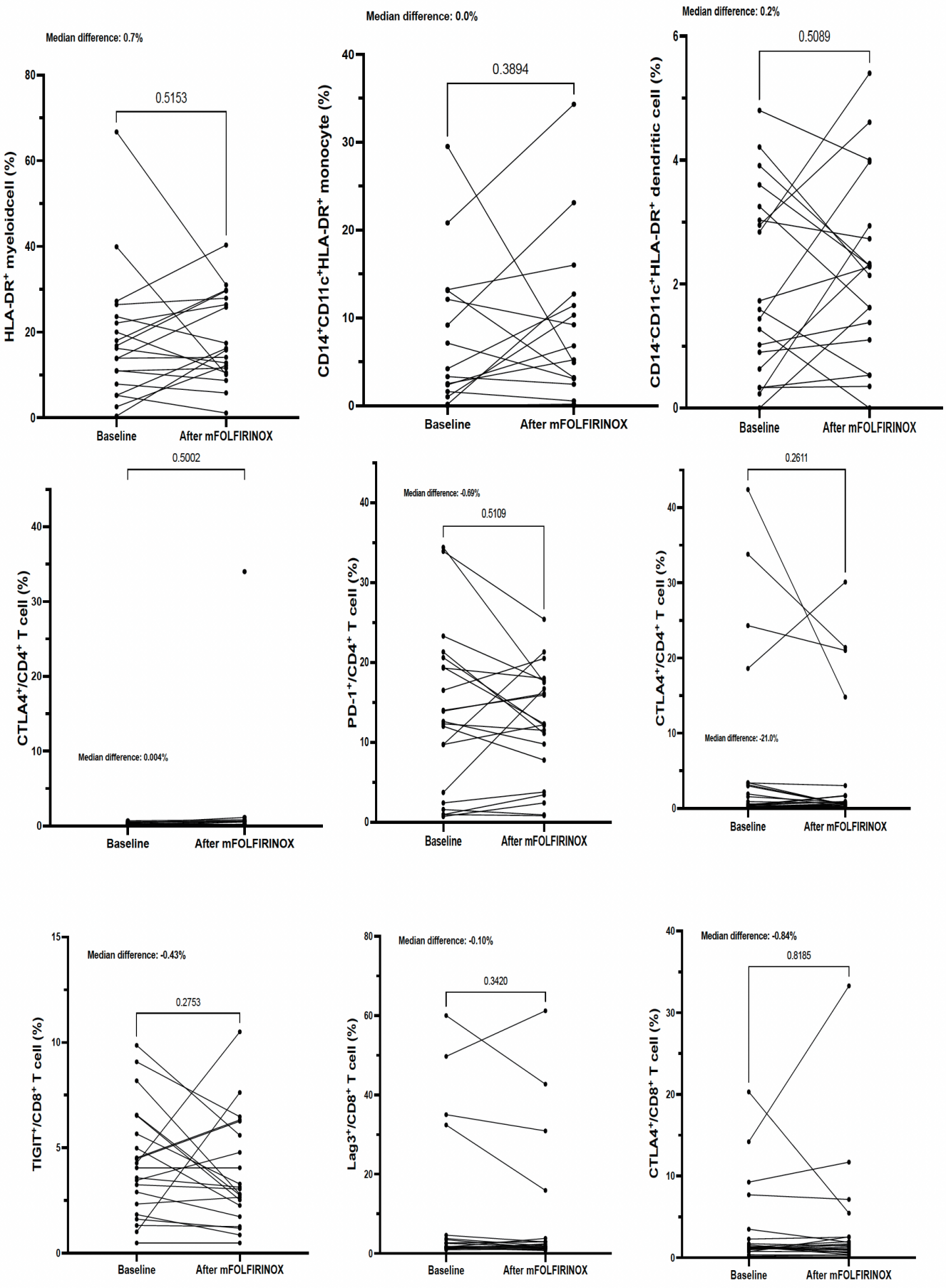
Supplementary figure 2.



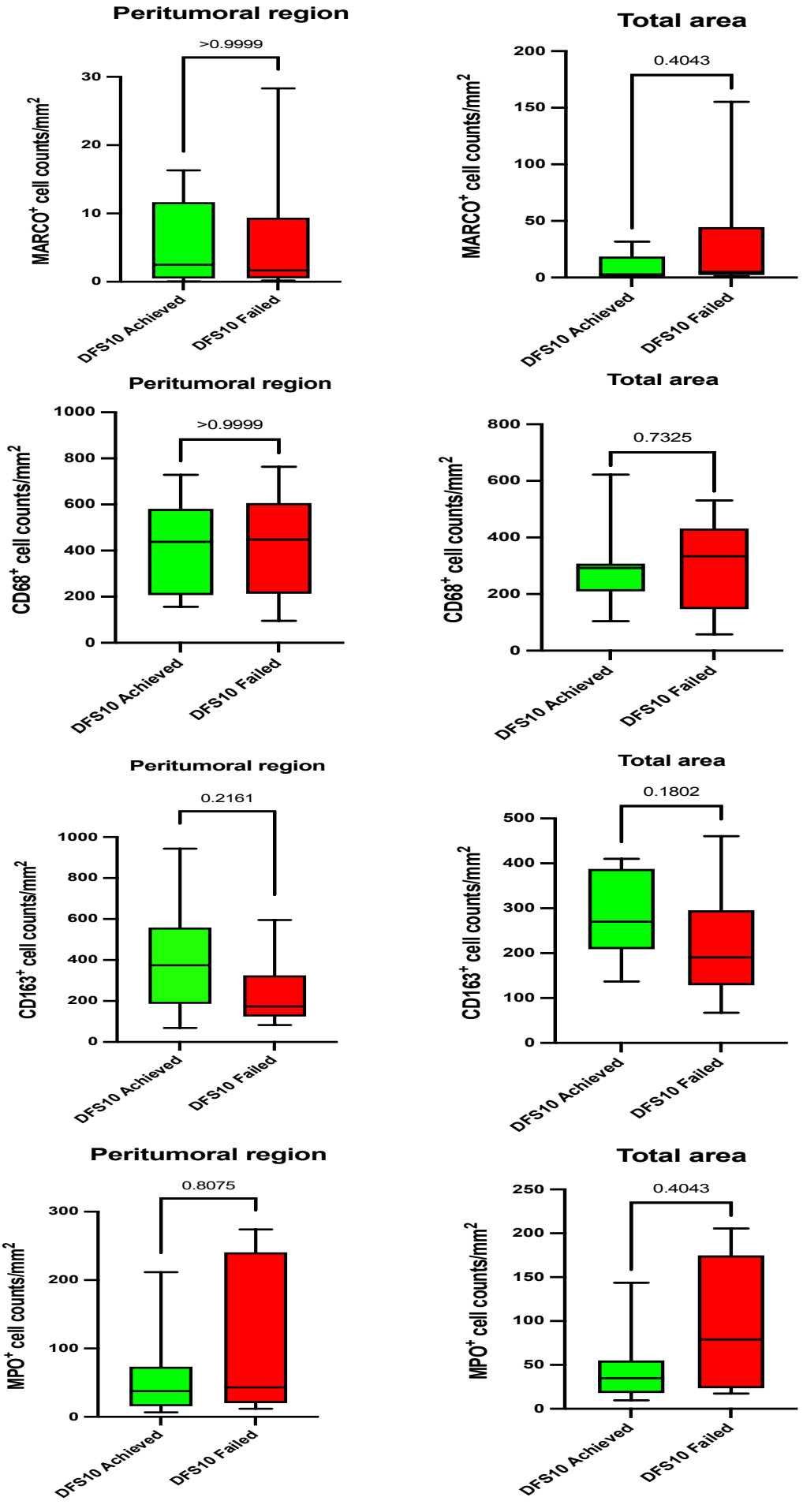
Supplementary figure 3.



Supplementary figure 3. (continued)



Supplementary figure 4.



Supplementary figure 5.

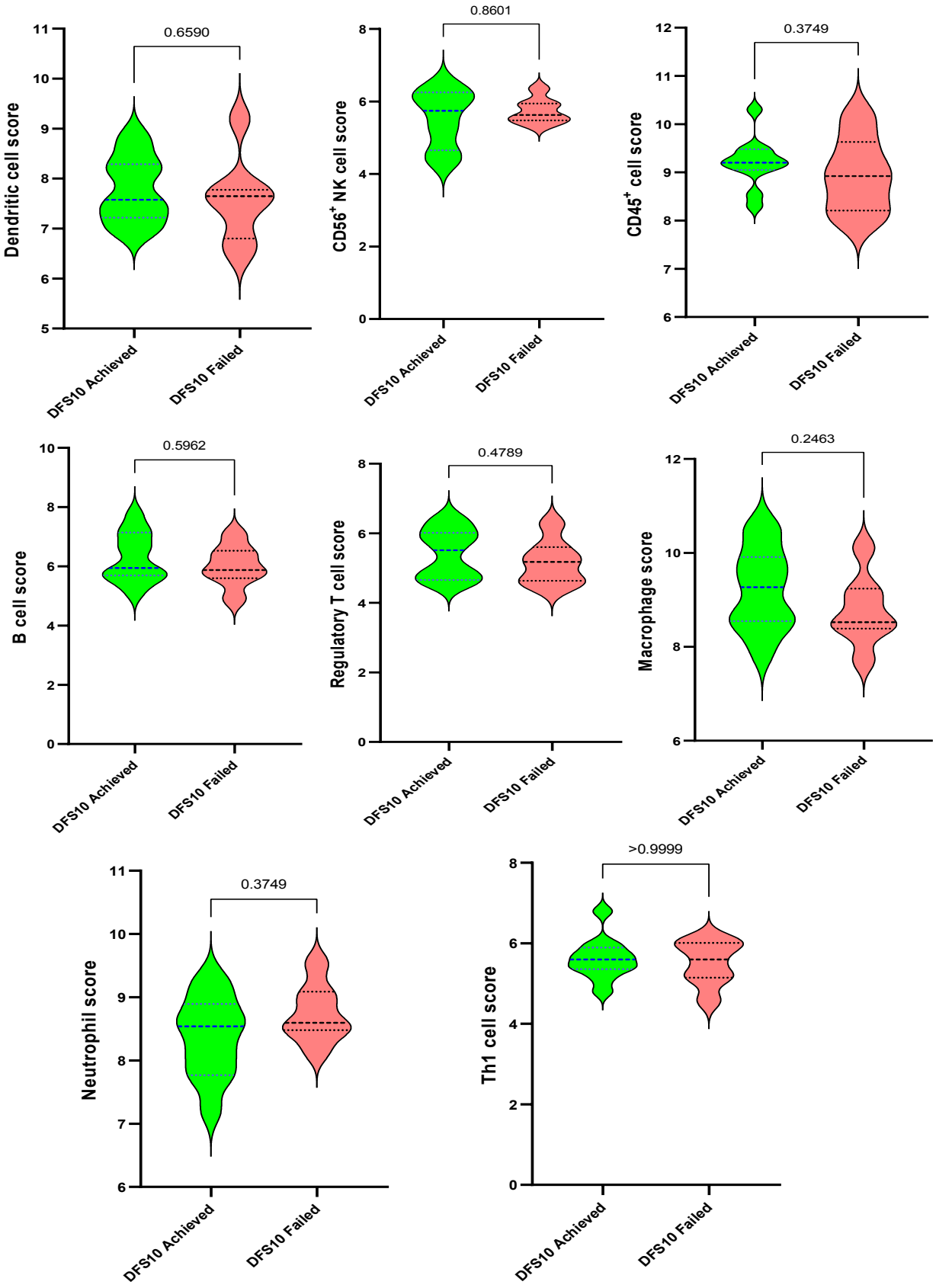


Table S1. Baseline characteristics of patients treated with neoadjuvant mFOLFIRINOX

	Total patients (N=44)
Clinical variables	
Median age	60 (range, 35-76)
Sex	
Male	26 (59.1%)
Female	18 (40.9%)
Location of primary tumor	
Head	26 (59.1%)
Body or tail or multi-centric	18 (40.9%)
Baseline serum CA 19-9	
Not elevated	11 (25.0%)
Elevated	33 (75.0%)
Median cycles of mFOLFIRINOX	8 (range, 2-10)
Response to mFOLFIRINOX	
PR	15 (34.1%)
SD	28 (63.6%)
PD	1 (2.3%)
Surgical resection after preoperative mFOLFIRINOX	
Yes	27 (61.4%)
No	17 (38.6%)

Table S2. Availability of biomarker samples of patients treated in the clinical trial (NCT02749136)

	Baseline sample		Post treatment sample		Nanostring and IHC data	
Study ID	Immune cell panel	T cell checkpoint panel	Immune cell panel	T cell checkpoint panel	Availability	DFS10
AMC01					Yes	Failed
AMC02						
AMC03						
AMC04						
AMC05	Yes	Yes	Yes	Yes	Yes	Achieved
AMC06						
AMC07	Yes	Yes	Yes	Yes		
AMC08						
AMC09	Yes	Yes	Yes	Yes	Yes	Failed
AMC10	Yes	Yes	Yes	Yes	Yes	Failed
AMC11	Yes	Yes	Yes	Yes	Yes	Failed
AMC12						
AMC13						
AMC14						
AMC15	Yes	Yes	Yes	Yes	Yes	Achieved
AMC16	Yes				Yes	Achieved
AMC17	Yes		Yes		Yes	Achieved
AMC18	Yes		Yes		Yes	Achieved
AMC19	Yes	Yes	Yes	Yes		
AMC20	Yes	Yes		Yes	Yes	Achieved
AMC21	Yes	Yes		Yes		
AMC22	Yes	Yes	Yes	Yes	Yes	Achieved
AMC23	Yes	Yes				
AMC24	Yes	Yes			Yes	Achieved
AMC25					Yes	Achieved
AMC26	Yes				Yes	Failed
AMC27	Yes					
AMC28						
AMC29						
AMC30	Yes					
AMC32	Yes	Yes		Yes		
AMC33	Yes	Yes			Yes	Failed
AMC34	Yes	Yes	Yes	Yes		
AMC35	Yes	Yes				
AMC36					Yes	Failed
AMC37	Yes	Yes	Yes	Yes	Yes	Achieved
AMC38	Yes	Yes	Yes	Yes		
AMC39	Yes	Yes			Yes	Achieved
AMC40	Yes	Yes	Yes	Yes		
AMC41	Yes	Yes	Yes	Yes		
AMC42	Yes	Yes	Yes	Yes		
AMC43	Yes	Yes	Yes	Yes		
AMC44	Yes	Yes	Yes	Yes		
AMC45	Yes	Yes	Yes	Yes		

Table S3. Univariate analysis of survival outcomes according to baseline peripheral immune cell level (Cox proportional hazards model, continuous variable)

Baseline peripheral level (%)	Overall survival		Progression-free survival	
	Hazards ratio (95% CI)	*P*	Hazards ratio (95% CI)	*P*
CD3+ T cell	0.99 (0.96-1.01)	0.322	0.99 (0.97-1.01)	0.559
CD4+ T cell	0.98 (0.94-1.02)	0.27	0.99 (0.96-1.02)	0.378
CD69+CD4+ T cell	0.97 (0.87-1.07)	0.524	0.99 (0.94-1.05)	0.112
Foxp3+CD4+ T cell	0.78 (0.59-1.03)	0.077	0.84 (0.72-0.97)	0.02*
CD8+	0.96 (0.88-1.05)	0.393	9.99 (0.96-1.02)	0.776
CD69+CD8+	0.93 (0.82-1.06)	0.3	0.94 (0.86-1.02)	0.143
CD56+ NK	1.07 (0.98-1.17)	0.126	1.03 (0.97-1.11)	0.32
CD69+CD56+	0.97 (0.92-1.01)	0.16	0.97 (0.95-1.10)	0.066
Gamma delta T cell	1.10 (0.94-1.29)	0.245	1.04 (0.91-1.20)	0.546
CD69+Gamma delta T cell	0.94 (0.86-1.03)	0.183	0.96 (0.93-1.00)	0.067
HLA-DR+ Myeloid cell	1.00 (0.96-1.04)	0.944	0.99 (0.96-1.02)	0.445
CD11c+CD14- Dendritic cell	0.95 (0.82-1.11)	0.544	0.96 (0.87-1.06)	0.448
CD14+CD11c+ Monocyte	1.07 (1.01-1.13)	0.026*	1.06 (1.00-1.12)	0.044*
CTLA4+CD4+	0.83 (0.57-1.21)	0.33	0.94 (0.86-1.02)	0.155
Lag-3+CD4+	0.87 (0.19-4.10)	0.865	0.98 (0.95-1.01)	0.134
PD-1+CD4+	0.98 (0.92-1.05)	0.594	0.99 (0.94-1.05)	0.699
TIGIT+CD4+	0.96 (0.84-1.10)	0.577	0.99 (0.89-1.09)	0.783
CTLA4+CD8+	0.73 (0.40-1.31)	0.288	0.89 (0.76-1.05)	0.163
Lag-3+CD8+	0.93 (0.83-1.05)	0.268	0.97 (0.94-1.01)	0.173
PD-1+CD8+	1.03 (0.96-1.09)	0.421	1.01 (0.96-1.06)	0.693
TIGIT+CD8+	1.10 (0.87-1.40)	0.427	1.05 (0.88-1.27)	0.574

(CI; confidence inverval, *; $P<0.05$)

Table S4. Non-parametric bootstrapping of paired comparison of immune cell level pre- and post-chemotherapy.

	Original estimates		Bootstrapping estimates (Sampling=1,000)		
T cell	Median difference (%)	*P*	Mean of bootstrapped median difference estimates (95% CI)	Standard error	P
$TIGIT^{+}CD4^{+}$ T cell	-0.84	0.0701	-0.500 (- 1.494, -0.851)	0.573	0.349
$PD\text{-}1^{+}CD8^{+}$ T cell	-3.22	0.0136	-3.273 (-6.132, -0.144)	1.537	0.047

Table S5. Comparison of clinical variables between the two recurrence groups

	DFS10		
	Achieved (N=11)	**Failed (N=7)**	**P**
Resection			0.2451
R0	10 (90.9%)	4 (57.1%)	
R1	1 (9.1%)	3 (42.9%)	
Surgery			0.326
Pancreatico-duodenectomy	9 (81.8%)	4 (57.1%)	
Distal pancreatectomy or total pancreatectomy	2 (18.2%)	3 (42.9%)	
Major vessel resection			0.3348
Yes	4 (36.4%)	5 (71.4%)	
No	7 (63.6%)	2 (28.6%)	
Histology			0.6405
W/D, M/D	10 (90.9%)	6 (85.7%)	
Others	1 (9.1%)	0	
P/D	0	1 (14.3%)	
Pathologic stage			0.8029
IA-IB	2 (18.2%)	0	
IIA	5 (45.5%)	3 (42.9%)	
IIB	4 (36.3%)	4 (57.1%)	
CAP tumor regression grade			0.4608
1	2 (18.2%)	0	
2	8 (72.7%)	5 (71.4%)	
3	1 (9.1%)	2 (28.6%)	
Adjuvant gemcitabine			0.3889
Yes	11 (100%)	6 (85.7%)	
No	0	1 (28.6%)	
Median gemcitabine cycle	6 (range, 3-6)	3 (range, 1-6)	

W/D, well-differentiated; M/D, moderately differentiated, P/D, poorly differentiated; CAP, College of American Pathologists.

Table S6. Gene list of signfiicant differential expression between the recurrence groups.

Gene	Fold Change	Log2 Fold Change	*P*	Adjusted *P*
LTF	6.53121	2.70735	0.001565	0.078862
LILRB5	2.10472	1.07363	0.000778	0.06988
FN1	-2.21427	-1.14683	0.004943	0.131122
SPP1	-2.37522	-1.24806	0.006475	0.135977
DUSP4	-2.48821	-1.31511	0.000916	0.06988
CEACAM6	-3.4277	-1.77724	0.004453	0.12933
SLC2A1	-5.11296	-2.35416	0.000101	0.048545
MARCO	-5.40041	-2.43307	0.000193	0.048545

Table S7. Directed enrichment score of gene sets from nanostring data

Gene set	Directed Enrichment Score
Th1 Differentiation	1.0996
Type II Interferon Signaling	0.6631
Th2 Differentiation	0.621
Type I Interferon Signaling	0.3501
Adaptive Immune System	0.2985
Oxidative Stress	-0.3005
Transcriptional Regulation	-0.3245
Chemokine Signaling	-0.3477
Inflammasomes	-0.3856
TNF Family Signaling	-0.4696
Cell Adhesion	-0.5037
MHC Class II Antigen Presentation	-0.5203
Complement System	-0.6036
Th17 Differentiation	-0.6241
Cytokine Signaling	-0.6252
Lymphocyte Activation	-0.6423
Hemostasis	-0.7545
Autophagy	-0.755
Lymphocyte Trafficking	-0.7895
Host-pathogen Interaction	-0.7897
MHC Class I Antigen Presentation	-0.8254
B cell Receptor Signaling	-0.8469
Phagocytosis and Degradation	-0.8566
T Cell Receptor Signaling	-0.8977
Treg Differentiation	-0.9506
Innate Immune System	-0.9625
NLR signaling	-0.9939
TLR Signaling	-1.0526
NF-kB Signaling	-1.1232
Apoptosis	-1.1364
TGF-b Signaling	-1.2129
Immunometabolism	-1.4371

Table S8. Clinical characteristics of pancreatic cancer patients from TCGA data (the Human Protein Atlas).

Variables	N=176
Age (Median)	65 years (range, 35-88)
Sex	
Male	96
Female	80
Race	**N=172**
Asian	11
African	6
White	155
TNM Stage	**N=173**
Stage I	21
Stage II	145
Stage III	3
Stage IV	4
Median FPKM	5.36