Supplementary Figures and Tables

Supplementary Figures

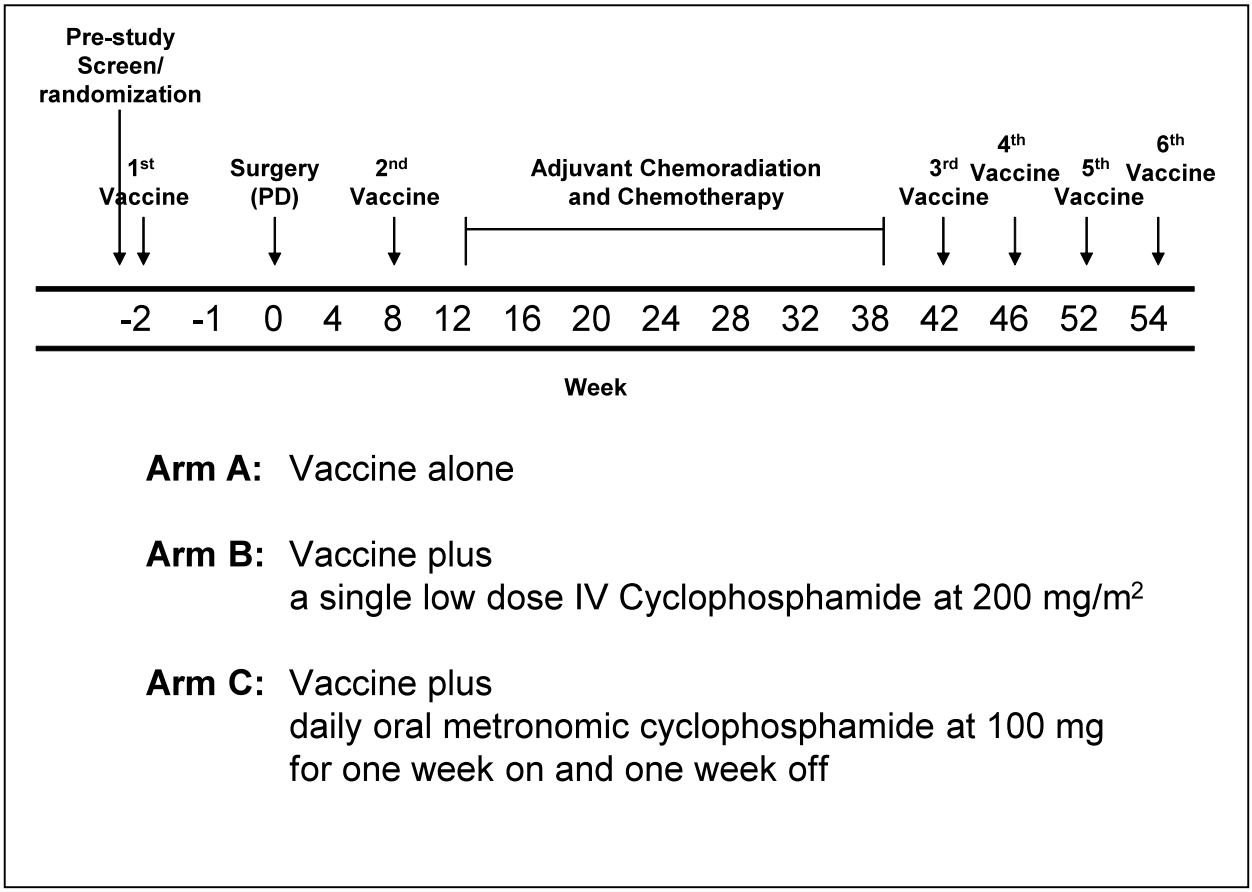
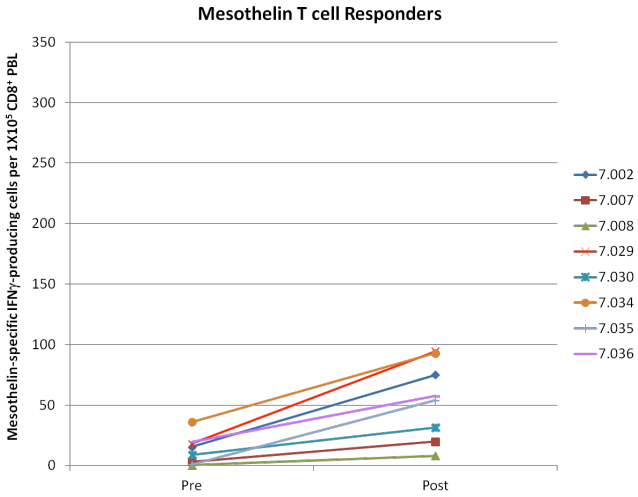


Figure S1 Schema of the Neoadjuvant and Adjuvant Study of Panc GVAX

Between July 2008 and September 2012, 59 patients were enrolled into an ongoing study of an irradiated, allogeneic GM-CSF-secreting pancreatic tumor vaccine (Panc GVAX) administered intradermally either alone or in combination with immune modulatory doses of cyclophophamide (Cy) as neoadjuvant and adjuvant treatment for patients with resectable PDAC. Patients were randomized 1:1:1 to three treatment arms. In Arm A, patients received Panc GVAX alone; in Arm B, patients received Panc GVAX plus a single intravenous dose of Cy at 200 mg/m2 one day prior to each vaccination; in Arm C, patients received Panc GVAX plus oral Cy at 100 mg once daily for one week on and one week off. Up to six Panc GVAX treatments were administered and all of the patients remained in their initial treatment arms throughout the duration of the study.

A



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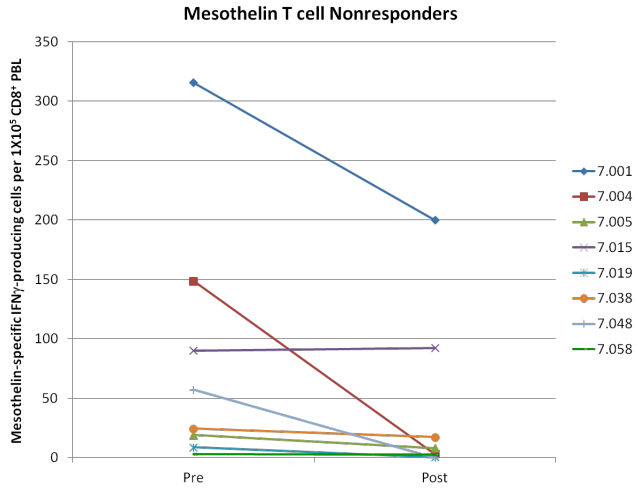


Figure S2 Pre- and post-vaccination mesothelin peptide-specific T cell levels in responders versus nonresponders. Mesothelin peptide-specific $CD8^+$ T cells were measured in peripheral blood lymphocytes collected prior to the first GVAX treatment (Pre) and 12-14 days following treatment just prior to surgery (Post) using IFNγ ELISPOT assays as previously described by Lutz et al[1]. **A.** Mesothelin-specific T-cell levels measured in eight patients who demonstrated enhanced post-vaccination mesothelin–specific responses. **B**. Mesothelin-specific T-cell levels measured in eight patients who did not demonstrate enhanced post-vaccination mesothelin –specific responses.

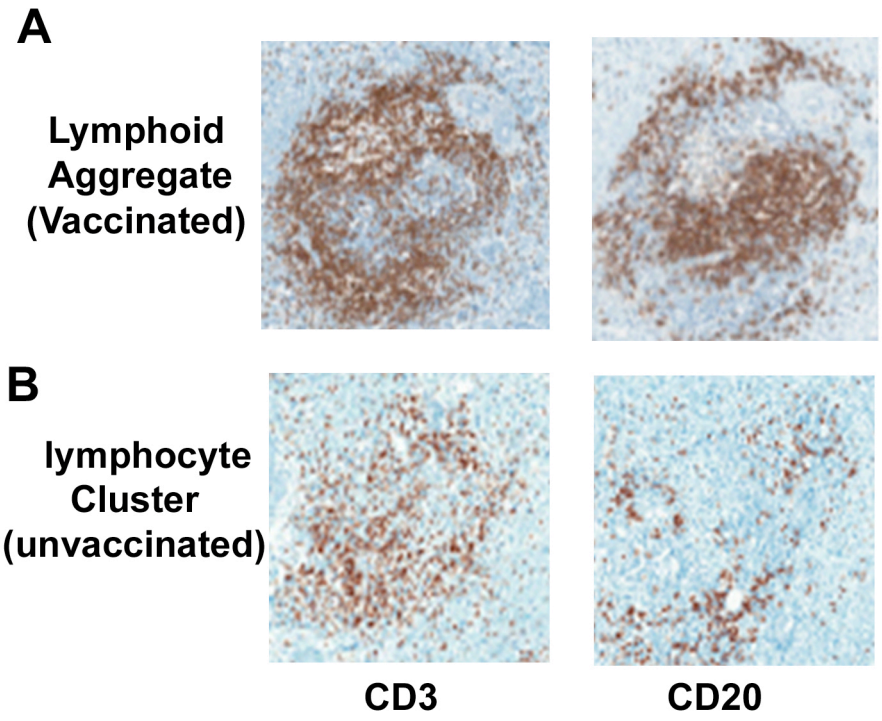


Figure S3 Intratumoral tertiary lymphoid aggregate in PDACs from patients treated with GVAX and lymphocyte cluster in PDACs from patients not treated with GVAX. A. Representative intratumoral tertiary lymphoid aggregate from patients treated with GVAX (vaccinated). Tertiary lymphoid aggregates are composed of organized T cell zone (marked by anti-CD3 staining) and B cell zone (marked by anti-CD20 staining). **B.** Occasionally, lymphocyte clusters lacking organization of T cells and B cells were seen in PDACs from patients not treated with GVAX (unvaccinated).

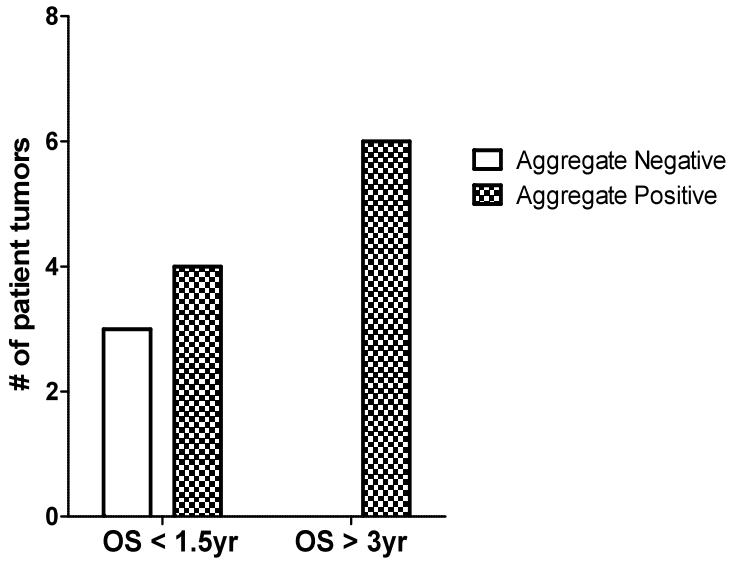


Figure S4 The development of post-vaccination intratumoral lymphoid aggregates is associated with survival.

Numbers of PDACs with or without at least one lymphoid aggregate present in the intratumoral area from patients who survived greater than 3 years (OS>3yr) (n=6) and patients who survived less than 1.5 years (OS<1.5yr) (n=7). The presence of intratumoral lymphoid aggregates is associated with but not an absolute predictor of longer survival (Chi-square p=0.069).

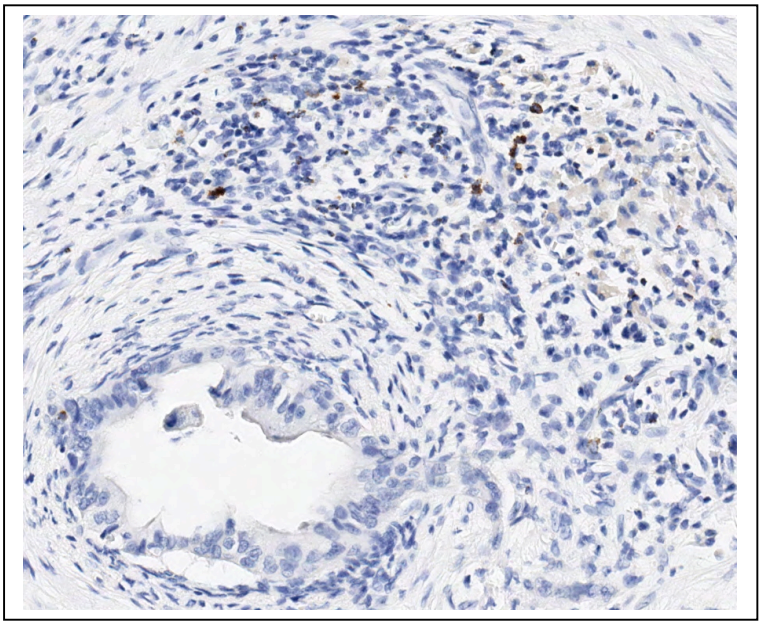


Figure S5 Relatively more Granzyme B expressing cells observed outside of the lymphoid aggregates. IHC staining of Granzyme B in a representative cluster of lymphocytes located near tumor cells but outside of lymphoid aggregates is shown.

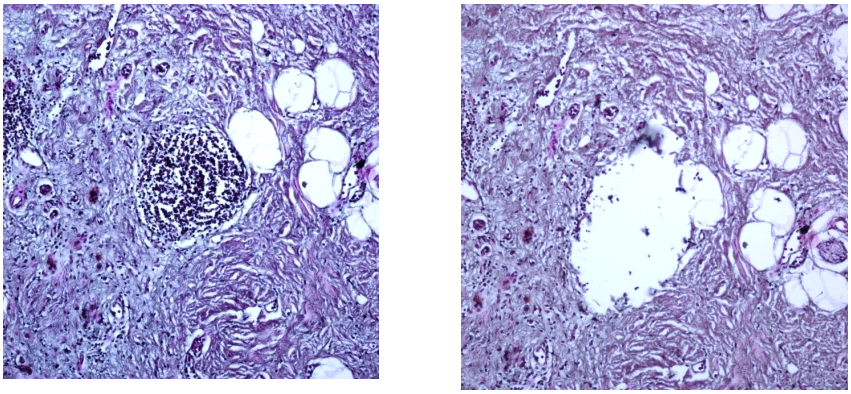


Figure S6 Microdissection of Lymphoid Aggregates for Microarray Analysis.

Lymphoid aggregates were isolated from 14 paraffin-embedded PDAC specimens by microdissection. Images of the same H&E stained PDAC tissue sample prior to and following microdissection for one representative intratumoral lymphoid aggregate are shown.

A

Integrin family genes

Gene Symbol	OS (>3yr vs <1yr)	Meso T cell Response (Enh vs Unenh)	High vs Low CD8/FOXP3 ratio
ICAM1	-15.85	1.78	-2.3
ICAM2	-4.93	1.71	-1.38
MADCAM	-1.47	-2.43	1.16
PECAM1	-14.93	-2.93	-2.68
VCAM1	-2.76	2.23	-3.51
CD44	-44.69	-3.74	-2.5
CD47	-24.45	-2.79	-8.87
LFA3 (CD58)	-4.22	-5.04	-4.20
CEACAM8 (CD66b)	4.79	1.27	1.66
ITGAL (CD11a)	-4.29	1.23	1.81
ITGAM (CD11b)	2.5	2.21	1.64
ITGAX (CD11c)	-1.05	1.47	1.14
ITGA2B	-5.1	1.24	1.42
ITGA4 (CD49D)	-12.85	-4.16	-2.75
ITGA7	3.34	1.17	1.48
ITGB1 (CD29)	-5.57	-2.41	-3.58
ITGB2 (CD18)	1.89	1.8	1.56
ITGB3	3.06	1.92	1.67
ITGB4	-2.87	2.39	1.79
ITGB6	5.37	1.35	1.50
ITGB7	1.62	1.5	1.60
ITGB8	2.92	-8.79	1.74
ITGBL1	9.88	-7.07	2.42

B

Chemokine and Chemokine receptor genes

Gene Symbol	OS (>3yr vs <1yr)	Meso T cell Response (Enh vs Unenh)	High vs Low CD8/FOXP3 ratio
CCL2 (MCP-1)	-62.22	-1.03	-3.88
CCL18	-6.09	-1.56	-4.70
CCL19	-3.14	-1.24	-1.25
CCL21	-7.31	4.77	-1.08
CXCL12 (SDF1)	-2.48	-1.81	-1.3
CCL13 (MCP-4)	-2.2	1.6	-1.67
CCL24 (Eotaxin 2)	-1.87	2.15	1.05
CCL17 (TARC)	1.43	1.65	1.43
CXCL11 (IP-9)	1.5	2.22	1.1
CX3CL1	3.01	2.16	1.85
CCL23 (MPIF-1)	7.93	-1.58	1.92
CSF1 (M-CSF)	-1.56	1.68	1.29
CSF2 (GM-CSF)	2.25	1.80	1.93
CSF3 (G-CSF)	1.84	2.39	1.52
CCR7	-7.33	1.08	-4.08
CXCR4	-7	-1.88	-1.26
CCR1	-6	-3.11	-4.19
CCR3	-2.42	1.26	1.27
CXCR2	1.78	2.58	1.16
CCR6	1.85	-3.99	2.09
CX3CR1	1.92	1.73	1.14
CCR2	4.23	1.01	1.4
CSF1R	2.49	-2.19	1.10
CSF2RA	-2.83	2.68	1.62
CSF3R	3.12	2.64	1.87

C

NF-kB pathway genes

Gene Symbol	OS (>3yr vs <1yr)	Meso T cell Response (Enh vs Unenh)	High vs Low CD8/FOXP3 ratio
NFKB1 (p105/50)	-4.48	1.14	-2.02
NFKB2 (p100/52)	3.78	1.92	1.52
RELA	-12.54	3.73	-1.98
RELB	1.41	2.01	1.39
REL (c-Rel)	-5.88	-1.86	-2.3
CHUK (IKKa)	-1.09	-2.23	-1.67
IKBKB (IKKb)	-3.73	-2.60	-1.94
IKBKG (NEMO)	-4.43	1.5	1.76
IKBKE	2.03	1.94	1.91
MAP3K14 (NIK)	-1.46	2.23	1.3
NFKBIA (IkBa)	-1.61	-2.1	-1.81
NFKBIB (IkBb)	-1.44	1.35	1.63
LTA	1.00	1.78	1.29
LTB	1.48	1.68	1.71
LTBR	1.81	2.4	1.58

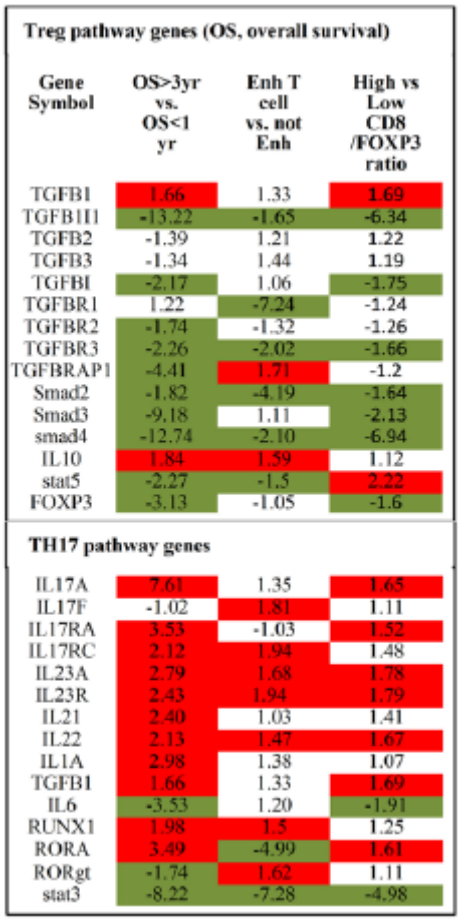
D

Ubiquitin-Proteasome System genes (fold reductions)

	Ubiquitin conjugating enzyme (E2)	Ubiquitin ligage (E3)	Proteasome	Ubiquitin specific peptidase
Meso T cell Response (Enh vs Unenh)	PAXIP1 (5.57) UBE2B (2.23) UBE2K (3.13) ARIH2 (2.98) UBE2D3 (2.11) UBE2R2 (1.95) ARIH1 (1.79)	CDC26 (15.9) BARD1 (6.25) HERC4 (5.88) FBXW2 (5.32) ANAPC5 (5.01) HECTD1 (4.67) DCAF12 (4.62) FBXO3 (4.03) RNF40 (3.18) FBXO9 (3.06) UBE3C (2.95) ANAPC10 (2.41) HUWE1 (2.30) DACF13 (1.96) HERC3 (1.92) DCAF8 (1.72) FBXW2 (1.71) UBE3B (1.63)	PSME4 (11.0) PSMA4 (5.85) PSMC2 (5.14) PSMA5 (1.96) PSMD11 (1.62)	USP4 (3.08)
High vs Low CD8/ Foxp3 ratio	UBE2I (3.90) UBE2G1 (3.29) UBE2B (1.73)	CUL3 (3.90) TRIM13 (3.23) HERC4 (3.13) SIAH1 (2.75) UBE3A (2.55) STUB1 (2.24) MDM2 (2.04) HERC5 (1.82)		USP48 (6.25) USP33 (4.50) USP15 (3.71) USPL1 (3.66) USP36 (1.62)

Figure S7 Microarray analysis of lymphoid aggregates revealed gene signatures in four pathways involved in regulating immune cell trafficking and function. Microarray gene expression was compared between microdissected lymphoid aggregates from patient tumors using the same groups as described for **Figure 4**. Gene ontology analysis revealed enrichment of differentially expressed genes in the (**A**) integrin family, (**B**) chemokine and chemokine receptor

family and (**C**) the NFκB pathway and (**D**) down-regulated genes in the ubiquitin-proteasome system (UPS) The linear fold-changes in expression for each comparison are shown for individual genes. For integrin, chemokine and chemokine receptor, and NFkB pathway genes, greater than 1.5-fold increases in gene expression are highlighted in red and greater than 1.5-fold decreases in gene expression are highlighted in green. For the UPS, specific genes that were downregulated are shown with respective fold reductions in expression.



Treg pathway genes (OS, overall survival)

Gene Symbol	OS>3yr vs. OS<1 yr	Enh T cell vs. not Enh	High vs Low CD8 /FOXP3 ratio
TGFB1	1.66	1.33	1.69
TGFB1I1	-13.22	-1.65	-6.34
TGFB2	-1.39	1.21	1.22
TGFB3	-1.34	1.44	1.19
TGFBI	-2.17	1.06	-1.75
TGFBR1	1.22	-7.24	-1.24
TGFBR2	-1.74	-1.32	-1.26
TGFBR3	-2.26	-2.02	-1.66
TGFBRAP1	-4.41	1.71	-1.2
Smad2	-1.82	-4.19	-1.64
Smad3	-9.18	1.11	-2.13
smad4	-12.74	-2.10	-6.94
IL10	1.84	1.59	1.12
stat5	-2.27	-1.5	2.22
FOXP3	-3.13	-1.05	-1.6

TH17 pathway genes

IL17A	7.61	1.35	1.65
IL17F	-1.02	1.81	1.11
IL17RA	3.53	-1.03	1.52
IL17RC	2.12	1.94	1.48
IL23A	2.79	1.68	1.78
IL23R	2.43	1.94	1.79
IL21	2.40	1.03	1.41
IL22	2.13	1.47	1.67
IL1A	2.98	1.38	1.07
TGFB1	1.66	1.33	1.69
IL6	-3.53	1.20	-1.91
RUNX1	1.98	1.5	1.25
RORA	3.49	-4.99	1.61
RORgt	-1.74	1.62	1.11
stat3	-8.22	-7.28	-4.98

Figure S8 Decreased Treg and increased TH17 pathway gene expression signatures are associated with improved post-vaccination responses.

Gene expression measured by microarray was compared between microdissected lymphoid aggregates from patient tumors using the same groups as described for **Figure 4**. Gene ontology analysis revealed enrichment of downregulated genes in the Treg pathway and enrichment of upregulated genes in the TH17 pathway. The linear fold-change in expression for each comparison is shown for representative genes in each pathway. Greater than 1.5-fold increases in gene expression are highlighted in red and greater than 1.5-fold decreases in gene expression are highlighted in green.

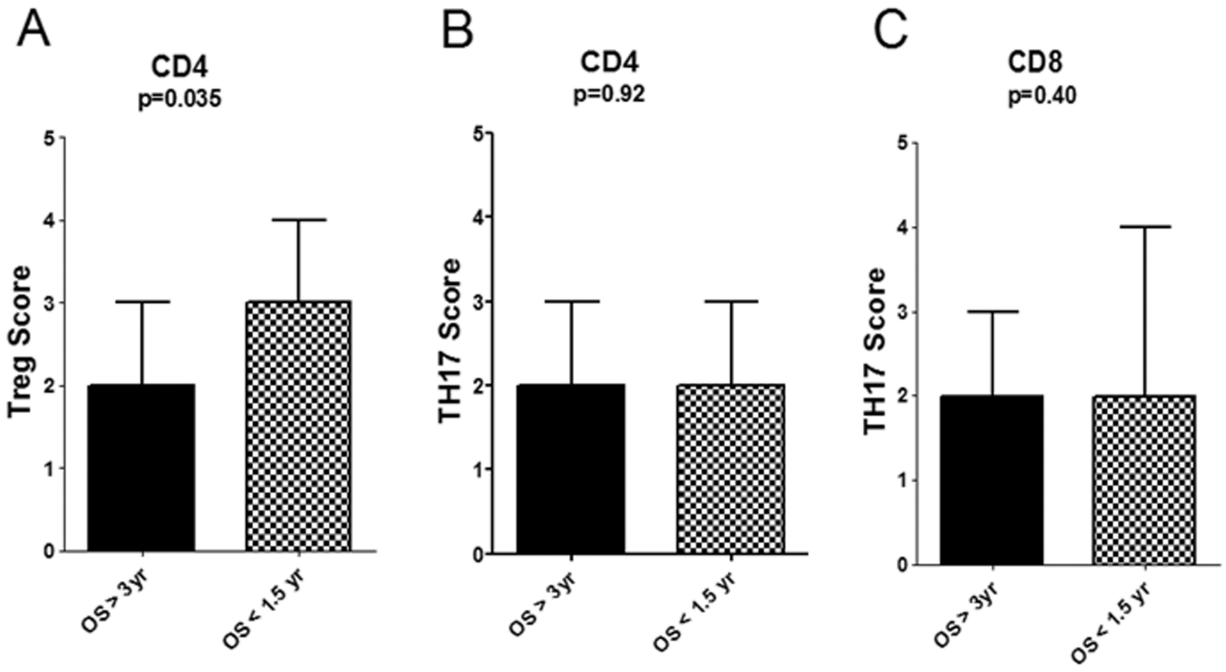


Figure S9 Downregulation of Treg pathway gene expression in TIL following treatment with PDAC GVAX is associated with longer survival.

Freshly thawed and unstimulated TIL specimens were sorted using a flow cytometer to obtain $CD4^+$ and $CD8^+$ T cells. For some TIL specimens, $CD4^-CD8^-$ non-T cells and/or $CD19^+$ B cells were also obtained. We performed quantitative real-time RT-PCR analysis for a panel of immune related genes in the TH1, TH2, Treg and TH17 pathways (**Table S2**). The expression level of each pathway gene was normalized as described in the methods section. The average score for genes in each pathway were compared between the different patient groups. Treg pathway (**A**) and TH17 pathway (**B**) scores in $CD4^+$ TIL and TH17 scores in $CD8^+$ TIL (**C**), were compared between patients who survived >3 years (n=5) and those who survived <1.5 years (n=6). Medians and ranges are shown for each group. P values calculated using Wilcoxon signed-rank tests are shown for each comparison.

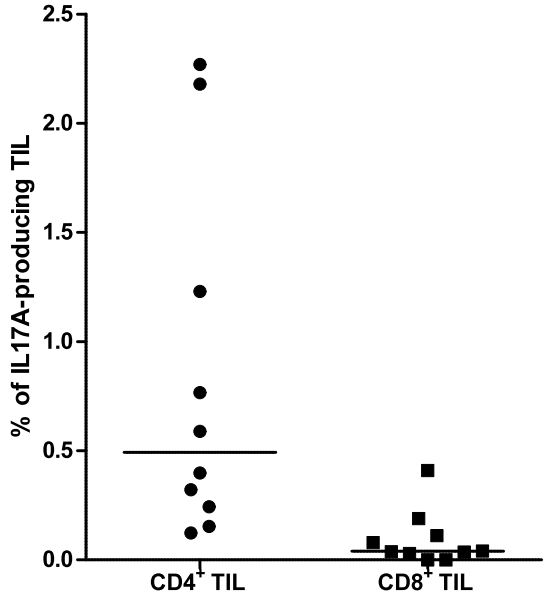


Figure S10 IL17A-producing T cells are present in low levels in TIL from PDAC GVAX treated patients.

IL17A cytokine-producing T cells were quantified in TIL isolated from ten PDAC GVAX-treated patients following overnight stimulation with anti-CD3 and anti-CD28. Percentages of $CD4^+$ IL17A-producing and $CD8^+$ IL17A-producing TIL are shown.

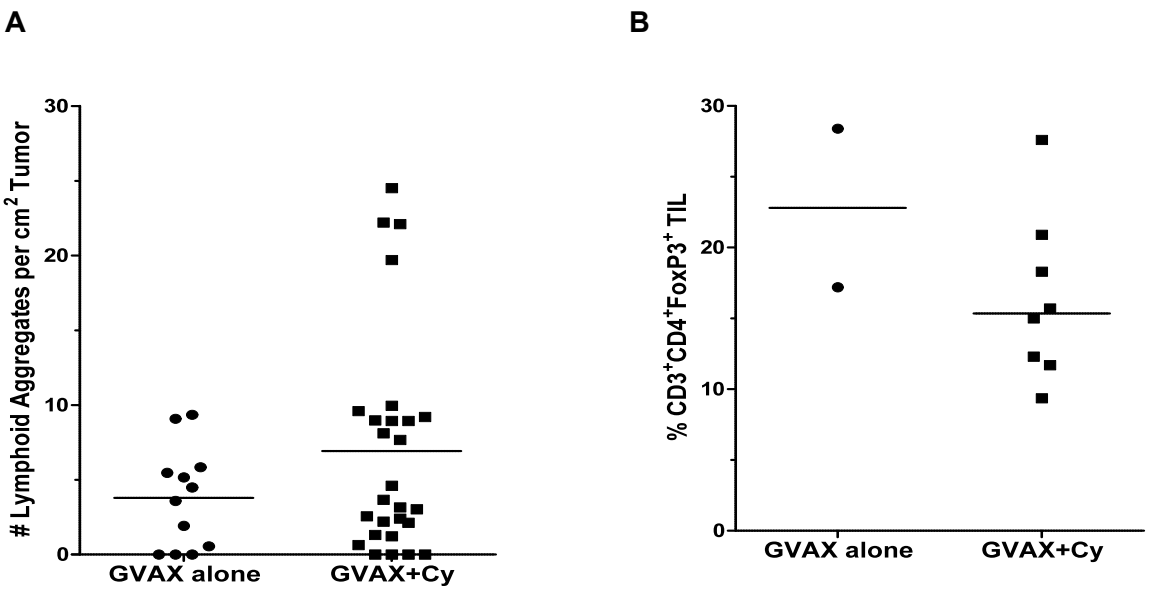


Figure S11 Treatment with low dose Cy increases post-vaccination densities of intratumoral lymphoid aggregates and reduces Treg levels in the PDAC TME.

A. Intratumoral lymphoid aggregate densities in PDACs from patients treated with GVAX alone compared to GVAX plus low dose Cy. Each symbol represents an individual patient tumor sample and means for each treatment group are shown. **B**. Levels of $CD3^+CD4^+FoxP3^+$ Tregs measured by FACS in TIL isolated from two patients who received GVAX alone and eight patients who received GVAX plus Cy. Each symbol represents an individual TIL sample and medians for each group are shown.

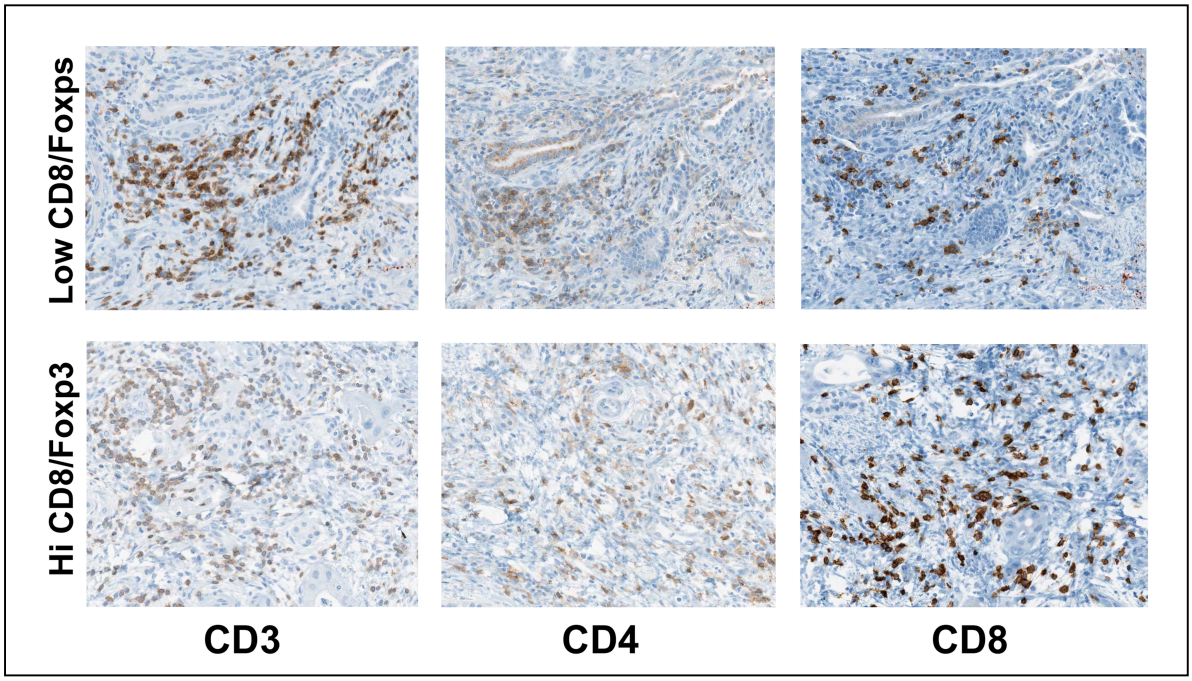


Figure S12 T cell infiltration in PDACs with low versus high CD8/Foxp3 ratio. IHC staining of CD3, CD4, and CD8 in representative PDAC tumor areas from a patient treated with the GVAX vaccine alone who had a low Teffector:Treg ratio in the TME compared to a patient treated with GVAX plus Cy who had a high Teffector:Treg ratio in the TME.

Supplementary Tables

Table S1 Immunohistochemistry protocols

Marker	Vendor	Isotype	Protocol	
			Epitope Retrieval (ER) Conditions	Dilution and incubation time of primary antibodies
CD3	Ventana	Rabbit, IgG	Ventana protocol	
CD4	Ventana	Rabbit, IgG	Ventana protocol	
CD8	Ventana	Mouse, IgG1	Ventana protocol	
CD20	Ventana	Mouse, IgG2a	Ventana protocol	
CD56	Ventana	Mouse, IgG1	Ventana protocol	
CD1a	Novocastra	Mouse, IgG1	EDTA ER, 95°C x 20 min	1:50, 15 min
CD83	Leica	Mouse, IgG1	Citrate ER, 95°C x 10 min	1:50, 15 min
DC-LAMP	Dendritics	Mouse, IgG1	EDTA ER, 95°C x 20 min	1:50, 30 min
T-Bet	Santa Cruz	Mouse, IgG1	EDTA ER, 95°C 20 min	1:50, 30 min
CD45RA	Dako	Mouse, IgG1	Ventana protocol	
CD45RO	Dako	Mouse, IgG2a	Ventana protocol	
Granzyme B	Leica	Mouse, IgG2a	EDTA ER, 95°C x 20 min	1:50, 15 min
CXCR3	BD Pharmingen	Mouse, IgG1	EDTA ER, 95°C x 20 min	1:500, 30 min
FoxP3	Abcam	Mouse, IgG1	EDTA ER, 95°Cx 20 min	1:100, 15 min
PD-1	R&D	Goat, polyclonal	Citrate ER, 95°C x 20 min	1:25, 30 min
CD163	Novocastra	Mouse, IgG1	EDTA ER, 95°C x 10 min	1:500, 15 min
CD69	Abcam	Mouse, IgG1	EDTA ER, 95°C x 20 min	1:25, 30 min
CCL21	R&D	Goat, polyclonal	Citrate ER, 95°C x 20 min	1:25, 30 min
Ki67	Novocastra	Rabbit, IgG	EDTA ER, 95°C x 20 min	1:100, 15 min
CD21	Dako	Mouse	Enzyme ER, 95°C x 10 min	1:100, 15 min
IL-17	LSBio	Rabbit, polyclonal	Diva Decloaker 125^{o}C x 30 sec	1:250, 30 min
RORgT	Millipore	Mouse, IgG2a	EDTA ER, 95°C x 20 min	1:200, 15 min
Gata3	Santa Cruz Biotechnology	Mouse, IgG1	EDTA ER, 95°C x 20 min	1:25, 45 min

Table S2 Quantitative real-time RT-PCR primers

Species	Gene Symbol	Gene Name	Accession	Product Size	Region	Forward	Reverse
Vendor: SABiosciences			(Catalogue #)				
Human	IL23R	interleukin 23 receptor	PPH18331F	149	1841-1989	Not provided by vendor	Not provided by vendor
Human	IL17RA	interleukin 17 receptor A	PPH00873A	84	807-890	Not provided by vendor	Not provided by vendor
Human	TGFB2	Transforming growth factor, beta 2	PPH00524B	83	2558-2640	Not provided by vendor	Not provided by vendor
Human	SMAD3	SMAD family member 3	PPH01921C	84	1346-1429	Not provided by vendor	Not provided by vendor
Human	STAT5B	Signal Transducer and Activator of Transcription 5B	PPH01972F	154	4676-4829	Not provided by vendor	Not provided by vendor
Vendor: Real Time Primers, LCC							
Human	IL21R	Interleukin 21 receptor	NM_021798	156	2227-2382	GGAGCTGCAAGAAGTCCATA	GGTAGCACAGCCAGAAGGTA
Human	IL22	Interleukin 22	NM_020525	187	479-665	GAGGAATGTGCAAAAGCTGA	GCTTTGGGGCATCTAATTGT
Human	IL6	Interleukin 6	NM_000600	167	549-715	ATGCAATAACCACCCCTGAC	GAGGTGCCCATGCTACATTT
Human	RORA	RAR-related orphan receptor A	NM_134261	237	6081-6317	TCATTTTGGAGTTGGGGTTA	ACACAAGACTGACGAGCACA
Human	RORA	RAR-related orphan receptor A	NM_134261	229	302-530	TGCAAGATCTGTGGAGACAA	ACATTCGGCCAAATTTTACA
Human	Runx1	Runt-related transcription factor 1	NM_001754	175	4847-5021	ACAGAGACATTGCCAACCAT	CAGGACATTTGAGTGGAACC
Human	IL10	Interleukin 10	NM_000572	285	326-611	TTACCTGGAGGAGGTGATGC	GCCACCCTGATGTCTCAGTT
Human	GAPDH	Glyceraldehyde phosphate dehydrogenase	NM_002046	238	362-2450	GAGTCAACGGATTTGGTCGT	TTGATTTTGGAGGGATCTCG
Human	TGFB2	Transforming growth factor, beta 2	NM_003238	194	754-947	AACAAGAGCAGAAGGCGAAT	TGCCATCAATACCTGCAAAT
Human	SMAD3	SMAD family member 3	NM_005902	170	5208-5377	CCAGAACCAAACCTCAACAC	TCCTCTTGCGTACGTTTTTC
Human	TGFBR2	Transforming growth factor, beta receptor II	NM_001024847	163	2826-2988	GGTTCCTGTGTGCCCTTATT	TGCAACCCATGAAGGTAAAA
Human	CTLA4	Cytotoxic T-lymphocyte-associated protein 4	NM_005214	217	392-608	AGCCAGGTGACTGAAGTCTG	CATAAATCTGGGTTCCGTTG
Human	IL17A	Interleukin 17A	NM_002190	261	1389-1649	CTCGATTTCACATGCCTTCA	GAGGGGCCTTAATCTCCAAA
Human	IL23R	interleukin 23 receptor	NM_144701	191	336-526	CAGCTCGGCTTTGGTATAAA	ATTCCAGGTGCAAGTCATGT
Human	IL23R	interleukin 23 receptor	NM_144701	172	2594-2765	TGAGGTCAGGAGTTCGAGTC	AATCTCAGCTCAGTGCAACC
Human	IFNG	Interferon, gamma	NM_000619	198	912-1109	TCCCATGGGTTGTGTGTTTA	AAGCACCAGGCATGAAATCT
Human	IL2	Interleukin 2	NM_000586	207	55-261	AATGTACAGGATGCAACTCC	TTCTTGGGCATGTAAAACTT
Human	IL5	Interleukin 5	NM_000879	237	140-376	GACCTTGGCACTGCTTTCTA	CTCCGTCTTTCTTCTCCACA
Human	IL4	Interleukin 4	NM_000589	125	200-324	CCCCAAGTGACTGACAATCT	AGGAGAAGCTAACGATGCAA
Human	IL18	Interleukin 18 (interferon-gamma-inducing factor)	NM_001562	177	61-238	GGAATTGTCTCCCAGTGCAT	ACTGGTTCAGCAGCCATCTT

Table S3 Scores of TH1, TH2, Treg, and TH17 pathway gene expression in PDAC TIL analyzed by quantitative real-time RT-PCR.

Table S3 Part 1/6											
	OS>3 yr (n=5)					OS<1.5 yr (n=6)					
Gene ID	**7.012 CD4**	**7.011 CD4**	**7.019 CD4**	**7.024 CD4**	**7.007 CD4**	**7.005 CD4**	**7.036 CD4**	**7.006 CD4**	**7.017 CD4**	**7.029 CD4**	**7.030 CD4**
IFNG	1.5	1.5	1.5	4	1.5	4	4	1.5	1.5	1.5	1.5
IL2	2	2	2	4	4	2	2	2	2	4	2
IL18	2	2	1	2	3	3	4	3	3	1	1
TH1_average	**2**	**2**	**2**	**3**	**3**	**3**	**3**	**2**	**2**	**2**	**2**
IL4	2	2	4	2	2	2	2	2	2	2	2
IL6	1	1	4	1	1	1	1	3	1	4	4
IL10	1	1	1	1	3	3	2	4	3	2	4
STAT5b	3	2	4	1	4	4	1	1	4	3	2
TH2_average	**2**	**2**	**3**	**1**	**3**	**3**	**2**	**3**	**3**	**3**	**3**
IL17A	2	2	2	2	2	2	4	2	2	4	4
IL22	2	2	4	2	2	2	2	4	2	2	2
IL17RA	1.5	1.5	1.5	3	4	1.5	1.5	4	1.5	1.5	1.5
IL21R	1	1	4	1	4	2	2	1	4	1	3
IL23R	4	4	2	2	2	2	2	2	4	2	2
RORA	1	2	3	4	4	3	2	4	4	1	1
TH17_average	**2**	**2**	**3**	**2**	**3**	**2**	**2**	**3**	**3**	**2**	**2**
STAT5b	3	2	4	1	4	4	1	1	4	3	2
CTLA4	2	2	1	3	4	3	4	4	1	3	4
IL10	1	1	1	1	3	3	2	4	3	2	4
TGFB2	1	1	4	1	1	4	3	3	3	3	4
Treg_average	**2**	**2**	**3**	**2**	**3**	**4**	**3**	**3**	**3**	**3**	**4**

Table S3 Part 2/6											
	OS>3 yr (n=5)					**OS<1.5 yr (n=6)**					
Gene ID	**7.012 CD8**	**7.011 CD8**	**7.019 CD8**	**7.024 CD8**	**7.007 CD8**	**7.005 CD8**	**7.036 CD8**	**7.006 CD8**	**7.017 CD8**	**7.029 CD8**	**7.030 CD8**
GZMB	1	3	1	3	1	3	4	2	1	1	2
PRF1	3	2	2	1	3	4	4	3	2	4	2
IFNG	3	3	1	2	1	1	4	2	1	4	4
IL2	4	1	1	3	1	3	4	1	1	3	1
TNF	1.5	1.5	1.5	3	1.5	3	1.5	1.5	4	1.5	3
TH1_average	**3**	**2**	**1**	**2**	**2**	**3**	**4**	**2**	**2**	**3**	**2**
IL4	2	2	2	4	2	2	4	2	2	2	2
IL5	2	2	2	2	2	2	2	2	2	2	4
TH2_average	**2**	**2**	**2**	**3**	**2**	**2**	**3**	**2**	**2**	**2**	**3**
TGFBR2	4	1	1	1	2	1	1	3	2	4	4
IL17A	4	2	2	2	2	2	2	2	2	2	4
IL18	1	1	4	2	3	2	4	1	1	3	4
TH17_average	**3**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**4**
CTLA4	3	2	4	1	3	2	4	3	2	3	1
TGFBR2	4	1	1	1	2	1	1	3	2	4	4
Treg_average	**4**	**2**	**3**	**1**	**3**	**2**	**3**	**3**	**2**	**4**	**3**

Table S3 Part 3/6													
	Enhanced mesotheline-specific T cell response (n=6)						**Unenhanced mesotheline-specific T cell response (n=7)**						
Gene ID	**7.036 CD4**	**7.035 CD4**	**7.030 CD4**	**7.029 CD4**	**7.007 CD4**	**7.034 CD4**	**7.004 CD4**	**7.005 CD4**	**7.019 CD4**	**7.048 CD4**	**7.058 CD4**	**7.015 CD4**	**7.038 CD4**
IFNG	4	1.5	1.5	1.5	1.5	1.5	1.5	4	1.5	1.5	1.5	1.5	1.5
IL2	2	2	2	4	4	4	2	2	2	2	2	2	2
IL18	4	2	1	1	3	3	4	3	1	4	4	4	1
TH1_average	**3**	**2**	**2**	**2**	**3**	**3**	**3**	**3**	**2**	**3**	**3**	**3**	**2**
IL4	2	2	2	2	2	4	2	2	4	2	2	2	2
IL6	1	1	4	4	1	3	3	1	4	3	3	1	4
IL10	2	1	4	2	3	2	4	3	1	2	3	1	4
STAT5b	1	3	2	3	4	2	2	4	4	2	1	4	2
TH2_average	**2**	**2**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**2**	**2**	**2**	**3**
IL17A	4	2	4	4	2	2	2	2	2	2	2	2	2
IL22	2	2	2	2	2	2	2	2	4	2	2	2	2
IL17RA	1.5	4	1.5	1.5	4	1.5	1.5	1.5	1.5	4	1.5	1.5	1.5
IL21R	2	4	3	1	4	2	3	2	4	2	4	2	3
IL23R	2	2	2	2	2	4	2	2	2	2	4	2	2
RORA	2	4	1	1	4	2	2	3	3	1	1	3	4
TH17_ average	**2**	**3**	**2**	**2**	**3**	**2**	**2**	**2**	**3**	**2**	**2**	**2**	**2**
STAT5b	1	3	2	3	4	2	2	4	4	2	1	4	2
CTLA4	4	1	4	3	4	2	3	3	1	2	3	3	4
IL10	2	1	4	2	3	2	4	3	1	2	3	1	4
TGFB2	3	3	4	3	1	1	4	4	4	1	1	1	4
Treg_ average	3	2	4	3	3	2	3	4	3	2	2	2	4

Table S3 Part 4/6													
	Enhanced mesotheline-specific T cell response (n=7)						**Unenhanced mesotheline-specific T cell response (n=7)**						
Gene ID	**7.036 CD8**	**7.035 CD8**	**7.030 CD8**	**7.029 CD8**	**7.007 CD8**	**7.034 CD8**	**7.004 CD8**	**7.005 CD8**	**7.019 CD8**	**7.048 CD8**	**7.058 CD8**	**7.015 CD8**	**7.038 CD8**
GZMB	4	4	2	1	1	4	2	3	1	3	4	1	1
PRF1	4	1	2	4	3	3	1	4	2	1	3	2	1
IFNG	4	4	4	4	1	2	1	1	1	2	4	4	1
IL2	4	4	1	3	1	3	1	3	1	4	4	4	3
TNF	1.5	3	3	1.5	1.5	3	4	3	1.5	4	4	1.5	1.5
TH1_ average	**4**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**1**	**3**	**4**	**3**	**2**
IL4	4	4	2	2	2	2	2	2	2	2	2	2	2
IL5	2	2	4	2	2	2	2	2	2	2	2	2	2
TH2_ average	**3**	**3**	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
TGFBR2	1	3	4	4	2	1	1	1	1	4	4	1	1
IL17A	2	2	4	2	2	2	4	2	2	2	2	2	2
IL18	4	2	4	3	3	4	3	2	4	3	4		3
TH17_ average	**2**	**2**	**4**	**3**	**2**	**2**	**3**	**2**	**2**	**3**	**3**	**2**	**2**
CTLA4	4	1	1	3	3	4	4	2	4	1	4	3	2
TGFBR2	1	3	4	4	2	1	1	1	1	4	4	1	1
Treg_ average	**3**	**2**	**3**	**4**	**3**	**3**	**3**	**2**	**3**	**3**	**4**	**2**	**2**

Table S3 Part 5/6								
	High CD8/Foxp3 ratio (> 5) (n=5)					**Low CD8/Foxp3 ratio (<5) (n=3)**		
Gene ID	**7.012 CD4**	**7.006 CD4**	**7.035 CD4**	**7.005 CD4**	**7.004 CD4**	**7.011 CD4**	**7.017 CD4**	**7.015 CD4**
IFNG	1.5	1.5	1.5	4	1.5	1.5	1.5	1.5
IL2	2	2	2	2	2	2	2	2
IL18	2	3	2	3	4	2	3	4
TH1_average	**2**	**2**	**2**	**3**	**3**	**2**	**2**	**3**
IL4	2	2	2	2	2	2	2	2
IL6	1	3	1	1	3	1	1	1
IL10	1	4	1	3	4	1	3	1
STAT5b	3	1	3	4	2	2	4	4
TH2_average	**2**	**3**	**2**	**3**	**3**	**2**	**3**	**2**
IL17A	2	2	2	2	2	2	2	2
IL22	2	4	2	2	2	2	2	2
IL17RA	1.5	4	4	1.5	1.5	1.5	1.5	1.5
IL21R	1	1	4	2	3	1	4	2
IL23R	4	2	2	2	2	4	4	2
RORA	1	4	4	3	2	2	4	3
TH17_average	**2**	**3**	**3**	**2**	**2**	**2**	**3**	**2**
STAT5b	3	1	3	4	2	2	4	4
CTLA4	2	4	1	3	3	2	1	3
IL10	1	4	1	3	4	1	3	1
TGFB2	1	3	3	4	4	1	3	1
Treg_average	**2**	**3**	**2**	**4**	**3**	**2**	**3**	**2**

Table S3 Part 6/6								
	High CD8/Foxp3 ratio (> 5) (n=5)					**Low CD8/Foxp3 ratio (<5) (n=3)**		
Gene ID	**7.012 CD8**	**7.006 CD8**	**7.035 CD8**	**7.005 CD8**	**7.004 CD8**	**7.011 CD8**	**7.017 CD8**	**7.015 CD8**
GZMB	1	2	4	3	2	3	1	1
PRF1	3	3	1	4	1	2	2	2
IFNG	3	2	4	1	1	3	1	4
IL2	4	1	4	3	1	1	1	4
TNF	1.5	1.5	3	3	4	1.5	4	1.5
TH1_average	**3**	**2**	**3**	**3**	**2**	**2**	**2**	**3**
IL4	2	2	4	2	2	2	2	2
IL5	2	2	2	2	2	2	2	2
TH2_average	**2**	**2**	**3**	**2**	**2**	**2**	**2**	**2**
TGFBR2	4	3	3	1	1	1	2	1
IL17A	4	2	2	2	4	2	2	2
IL18	1	1	2	2	3	1	1	
TH17_average	**3**	**2**	**2**	**2**	**3**	**1**	**2**	**2**
CTLA4	3	3	1	2	4	2	2	3
TGFBR2	4	3	3	1	1	1	2	1
Treg_average	**4**	**3**	**2**	**2**	**3**	**2**	**2**	**2**

Table S4 Numbers of TIL specimens with detectable expression of immune related genes

Gene Name	CD4+ (n=20)	CD8+ (n=20)	non-T (n=7)	CD19+ (n=5)
IFNg	3	14	3	1
IL2	5	11	NT	NT
IL18	17	16	NT	NT
IL5	0	1	NT	NT
IL4	1	5	NT	NT
IL6	11	NT	7	3
IL10	14	NT	7	5
STAT5b	20	NT	NT	NT
SMAD3	20	NT	NT	NT
TGFB2	10	NT	6	2
TGFBR2	14	11	NT	NT
CTLA4	20	20	NT	NT
IL22	7	NT	0	0
IL17A	3	3	1	0
IL17RA	6	NT	2	2
IL21R	11	NT	NT	NT
IL23R	5	NT	7	5
IL23A	0	0	7	5
RUNX1	20	NT	NT	NT
RORA	20	NT	NT	NT
LAG3	NT	19	NT	NT
PDCD1	NT	20	NT	NT
CD69	NT	20	NT	NT
TNF	NT	9	NT	NT
FASLG	NT	20	NT	NT
GZMB	NT	13	NT	NT
PRF1	NT	20	NT	NT
IL1A	NT	NT	7	5

References for Supplemental data

1. Lutz E, Yeo CJ, Lillemoe KD, et al. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation. Annals of surgery 2011;253:328-35.