iScience, Volume *27*

Supplemental information

Tumor-infiltrating mast cells confer resistance to immunotherapy in pancreatic cancer

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Supplementary Materials

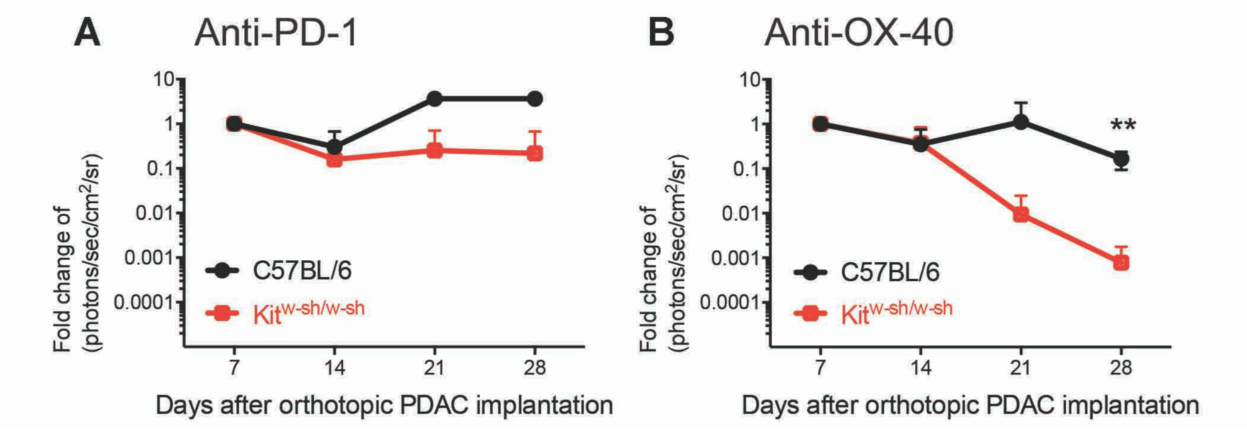


Fig. S1. Mast Cells influenced the efficacy of in vivo antibody therapy. Related to Figure 2 and 4.

Luciferase-transfected PDAC were injected on day 0, tumor growth was monitored by IVIS imaging. Tumor size in normal (C57BL/6) and mast cell-deficient ($Kit^{W\text{-}sh/W\text{-}sh}$) mice treated with: (A) anti-PD-1; (B) agonist anti-OX-40.

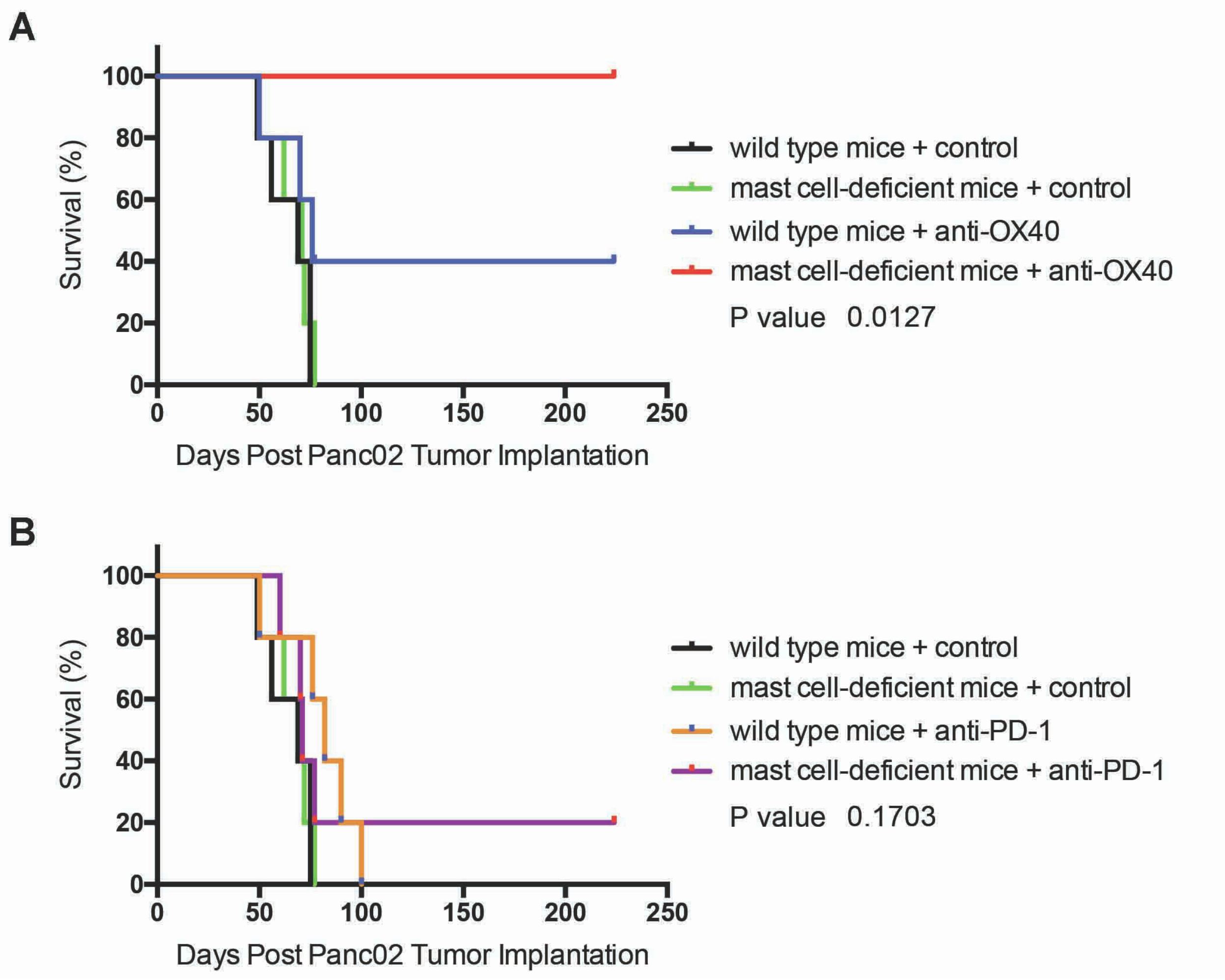


Fig. S2. Anti-OX40 treatment improves the survival of mast cell-deficient mice with orthotopic tumor implantation, compared to survival in wild-type tumor-bearing mice. Related to Figure 2 and 4.

(A) Anti-OX40 treated mast cell-deficient Panc02 tumor-bearing mice achieved a long-term tumor free survival, *P* = .0127. (C) Survival curve of mice with Panc02 tumor treated with anti-PD-1. Tumors were implanted on day 0.

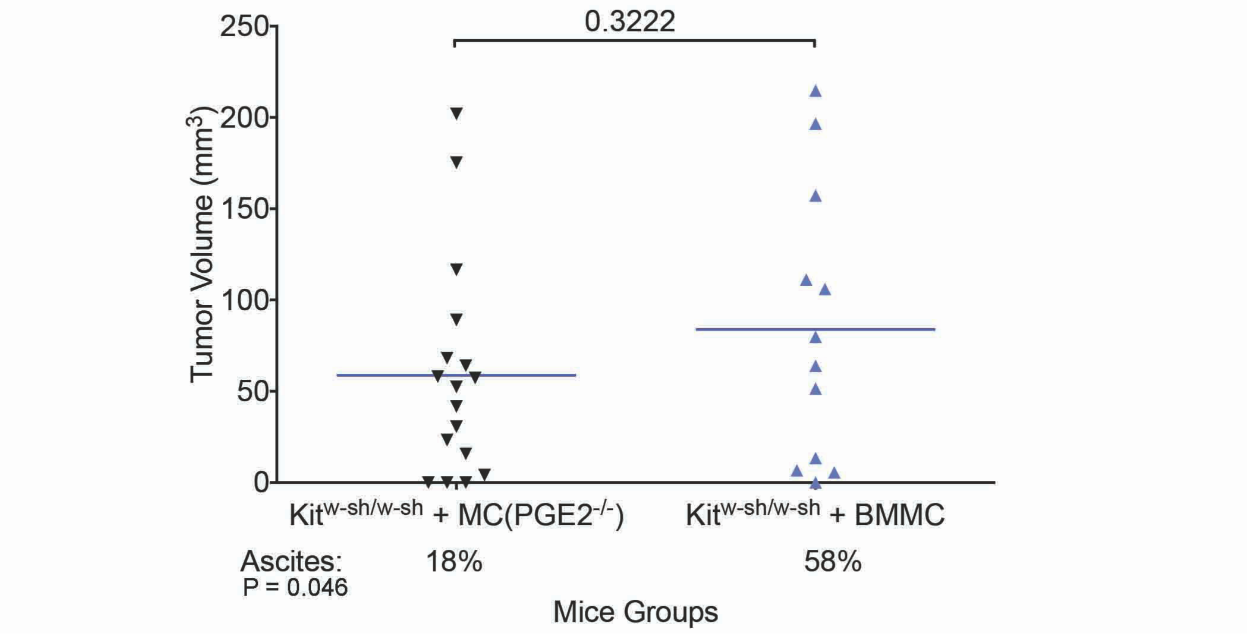


Fig. S3. Mast cell-derived PGE_2 does not influence tumor volume. Related to Figure 3.

28 days post tumor cell implantation, mast cell-deficient mice reconstituted with $PGE_2^{-/-}$ BMMC developed less ascites than those reconstituted with wild-type BMMC. P = 0.046 for ascites incidence. There was no significant effect on tumor size (*P* = .3222).

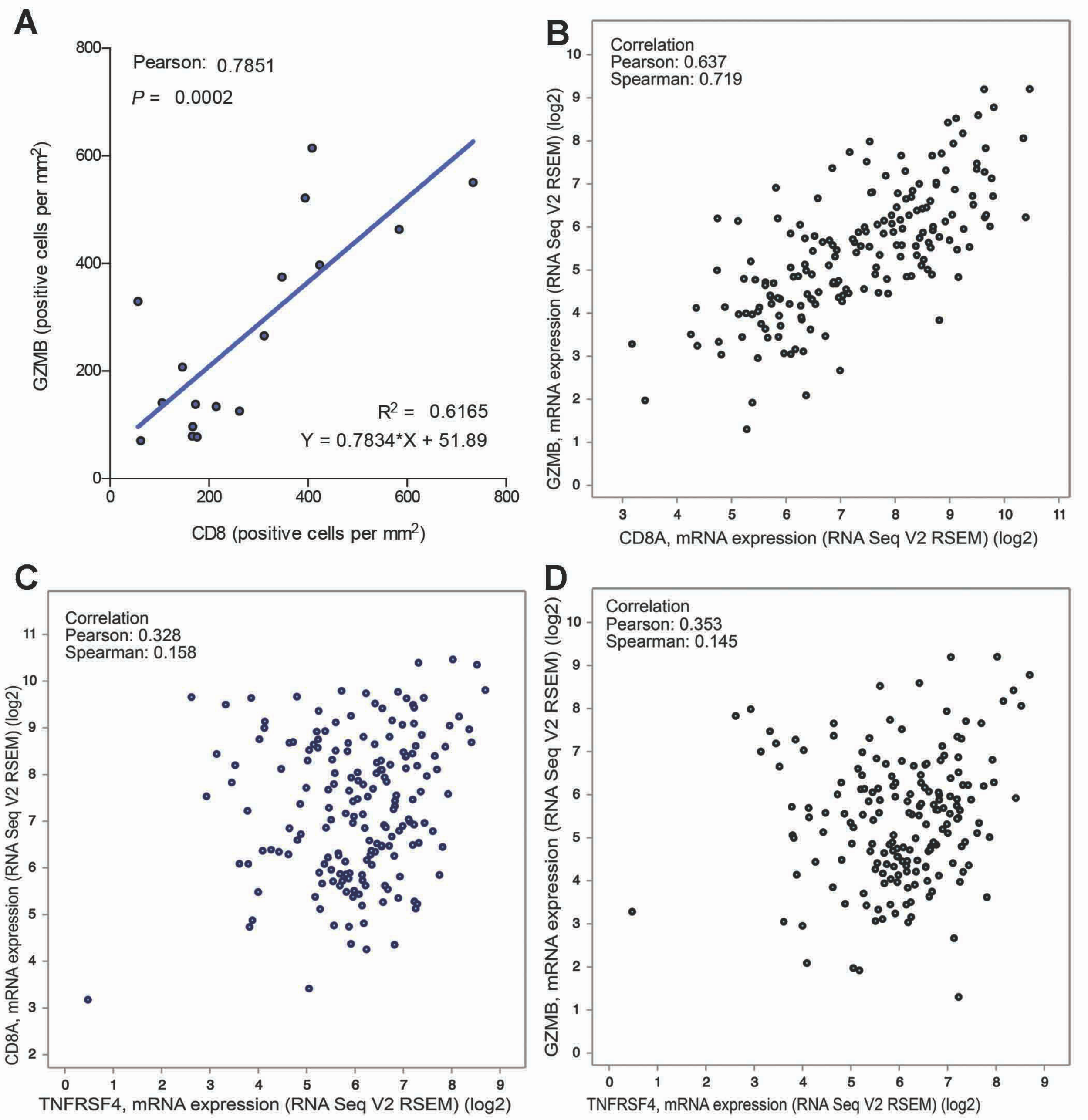
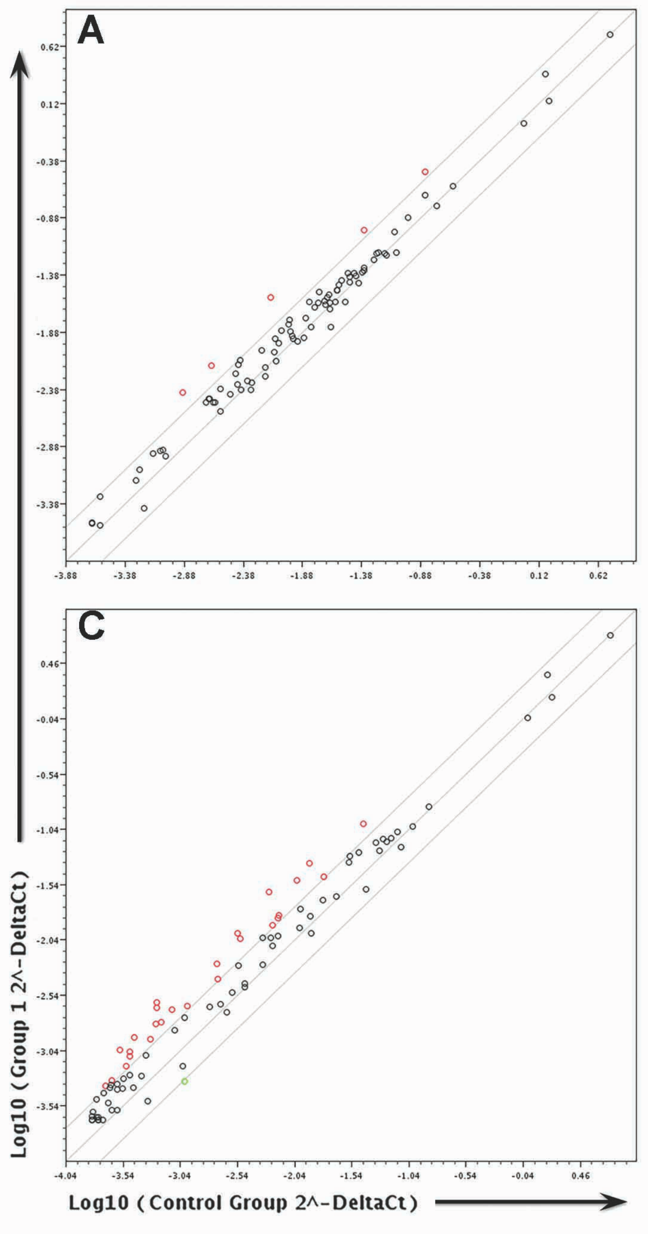


Fig. S4. Correlation of OX40 (a.k.a. TNFRSF4) and effector CD8+ T cells (CD8A) with granzyme B (GZMB) in KPC mice and PDAC patients. Related to Figure 5.

(A) KPC-GEMM mice, tumor infiltrating CD8 and GZMB positive cells (reference to Figure 5 C-F) presented strong positive linear correlation (Pearson r = 0.7851 and P =

0.0002). (B) A strong positive linear correlation between CD8A and GZMB was shown from data presented in the TCGA database. (C) Weak positive linear correlation between TNFRSF4 and CD8A or (D) GZMB analyzed from 186 PDAC patients' samples in the TCGA database.



B Toll-Like Receptor Signaling PCR array
PAMM-018Z

Position	Gene Symbol	Fold Regulation
C03	Il1a	3.7599
D03	Ly86	2.3521
D12	Muc13	3.0213
E10	Pglyrp1	2.3129
G05	Tnf	3.0702

D Allergy & Asthma PCR array
PAMM-067Z

Position	Gene Symbol	Fold Regulation
A03	Alox5	2.055
A07	Ccl11	2.1822
A09	Ccl17	4.6306
A10	Ccl22	2.8406
B02	Ccl5	3.6593
B03	Ccr3	2.2746
B04	Ccr4	2.1671
B08	Chia1	2.1706
C01	Csf2	3.3073
C04	Rnase2a	4.3895
C10	Gata3	3.0793
C12	Icos	2.1341
D10	Il17rb	2.9511
D11	Il18	2.0563
E03	Il2ra	3.3492
E05	Il33	2.8141
E07	Il4	2.7966
E09	Il5	2.6977
E10	Il5ra	3.4189
E11	Il9	2.4863
F08	Muc5ac	3.7011
F09	Pdcd1	2.3061
G01	Prg2	3.9578
G04	Satb1	2.1183
G09	Tnfrsf4	2.1813
G10	Tnfsf4	2.6267

Fig. S5. Scatter Plots (A & C) of gene expression panel and lists (B & D) of up regulated 31 genes induced by agonist anti-OX40 treatment. Related to Figure 5. Group 1 (vertical axis) represented tumor samples from 6 mice treated with anti-OX40 and Control Group (horizontal axis) represented tumor samples from 6 mice without anti-OX40 treatment. mRNA from these tissues was applied for the panel analysis of quantitative real-time PCR array. (A & B), genes from Toll-like Receptor Signaling PCR array; (C & D), genes from Allergy & Asthma PCR array. (A & C) Scatter plots were generated with Log 10 Fold change of mRNA expression determined by PCR Array assays (ΔΔCt). Red dots above the left boundary line represent the genes with more than 2 fold of up regulation in anti-OX40 treated group compared to control group; black dots between the two boundary lines represent the genes expression range from 2 to -2 fold-changes between the two groups; green dots below the right boundary line represent the genes with more than 2 fold down regulation in anti-OX40 treated group compared to control group. Genes with the red dots on the plots of A & C were listed in the tables of B & D. (B & D) Left column is the position of gene primers in the 96-well PCR Array plate; middle column is the gene symbols of the red dots on A & C; right column is the fold regulation of those red dot genes in anti-OX40 treatment group compared to control.

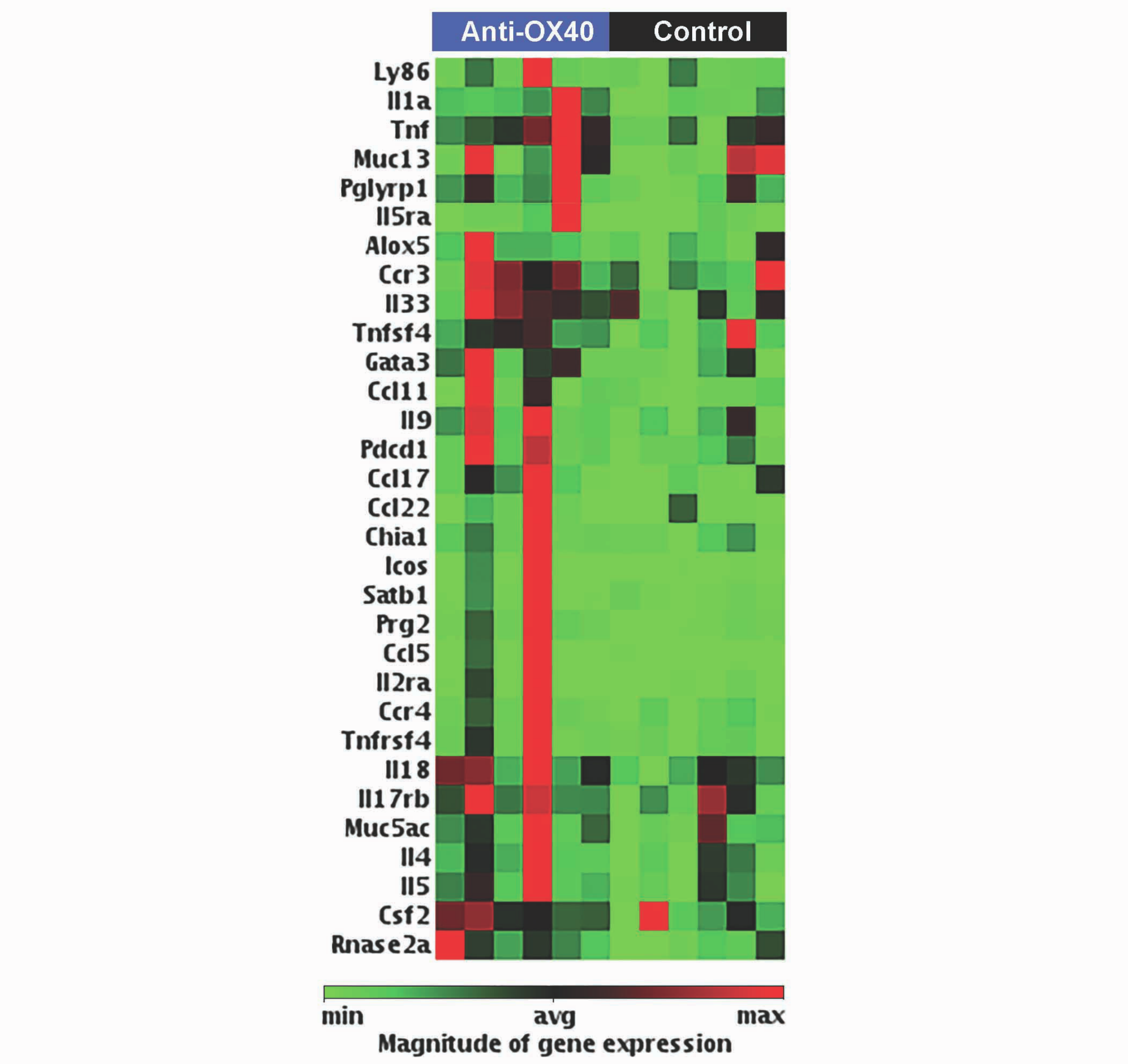
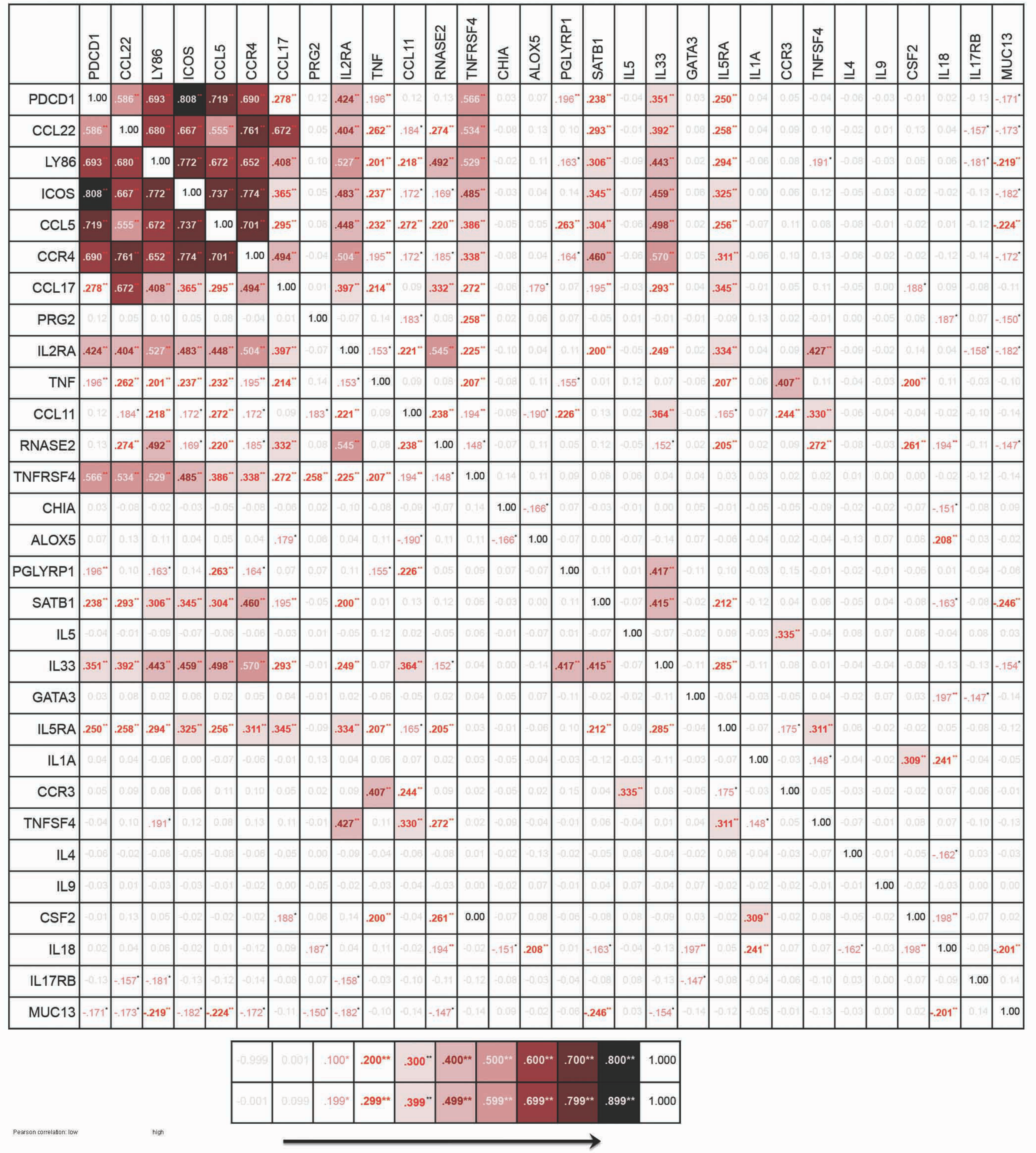


Fig. S6. Clustergrams of 31 genes up regulated following agonist anti-OX40 treatment. Related to Figure 5.

The left 6 columns represent tumor samples from 6 mice treated with anti-OX40 and the right columns represent tumor samples from 6 mice treated with the isotype control. These 31 genes were from both the Toll-like Receptor Signaling genes and Allergy & Asthma genes in PCR arrays. This heatmap presents the reproducibility and variation of those 31 genes with two-fold up regulation.



	PDCD1	CCL22	LY86	ICOS	CCL5	CCR4	CCL17	PRG2	IL2RA	TNF	CCL11	RNASE2	TNFRSF4	CHIA	ALOX5	PGLYRP1	SATB1	IL5	IL33	GATA3	IL5RA	IL1A	CCR3	TNFSF4	IL4	IL9	CSF2	IL18	IL17RB	MUC13
PDCD1	1.00	.586**	.693**	.808**	.719**	.690**	.278**	0.12	.424**	.196**	0.12	0.13	.566**	0.03	0.07	.196**	.238**	-0.04	.351**	0.03	.250**	0.04	0.05	-0.04	-0.06	-0.03	-0.01	0.02	-0.13	-.171*
CCL22	.586**	1.00	.680**	.667**	.555**	.761**	.672**	0.05	.404**	.262**	.184*	.274**	.534**	-0.08	0.13	0.10	.293**	-0.01	.392**	0.08	.258**	0.04	0.09	0.10	-0.02	0.01	0.13	0.04	-.157*	-.173*
LY86	.693**	.680**	1.00	.772**	.672**	.652**	.408**	0.10	.527**	.201**	.218**	.492**	.529**	-0.02	0.11	.163*	.306**	-0.09	.443**	0.02	.294**	-0.06	0.08	.191*	-0.08	-0.03	0.05	0.06	-.181*	-.219**
ICOS	.808**	.667**	.772**	1.00	.737**	.774**	.365**	0.05	.483**	.237**	.172*	.169*	.485**	-0.03	0.04	0.14	.345**	-0.07	.459**	0.06	.325**	0.00	0.06	0.12	-0.05	-0.03	-0.02	-0.02	-0.13	-.182*
CCL5	.719**	.555**	.672**	.737**	1.00	.701**	.295**	0.08	.448**	.232**	.272**	.220**	.386**	-0.05	0.05	.263**	.304**	-0.06	.498**	0.02	.256**	-0.07	0.11	0.08	-0.08	-0.01	-0.02	0.01	-0.12	-.224**
CCR4	.690**	.761**	.652**	.774**	.701**	1.00	.494**	-0.04	.504**	.195**	.172*	.185*	.338**	-0.08	0.04	.164*	.460**	-0.06	.570**	0.05	.311**	-0.06	0.10	0.13	-0.06	-0.02	-0.02	-0.12	-0.14	-.172*
CCL17	.278**	.672**	.408**	.365**	.295**	.494**	1.00	0.01	.397**	.214**	0.09	.332**	.272**	-0.06	.179*	0.07	.195**	-0.03	.293**	0.04	.345**	-0.01	0.05	0.11	-0.05	0.00	.188*	0.09	-0.08	-0.11
PRG2	0.12	0.05	0.10	0.05	0.08	-0.04	0.01	1.00	-0.07	0.14	.183*	0.08	.258**	0.02	0.06	0.07	-0.05	0.01	-0.01	-0.01	-0.09	0.13	0.02	-0.01	0.00	-0.05	0.06	.187*	0.07	-.150*
IL2RA	.424**	.404**	.527**	.483**	.448**	.504**	.397**	-0.07	1.00	.153*	.221**	.545**	.225**	-0.10	0.04	0.11	.200**	-0.05	.249**	0.02	.334**	0.04	0.09	.427**	-0.09	-0.02	0.14	0.04	-.158*	-.182*
TNF	.196**	.262**	.201**	.237**	.232**	.195**	.214**	0.14	.153*	1.00	0.09	0.08	.207**	-0.08	0.11	.155*	0.01	0.12	0.07	-0.06	.207**	0.06	.407**	0.11	-0.04	-0.03	.200**	0.11	-0.03	-0.10
CCL11	0.12	.184*	.218**	.172*	.272**	.172*	0.09	.183*	.221**	0.09	1.00	.238**	.194**	-0.09	-.190*	.226**	0.13	0.02	.364**	-0.05	.165*	0.07	.244**	.330**	-0.06	-0.04	-0.04	-0.02	-0.10	-0.14
RNASE2	0.13	.274**	.492**	.169*	.220**	.185*	.332**	0.08	.545**	0.08	.238**	1.00	.148*	-0.07	0.11	0.05	0.12	-0.05	.152*	0.02	.205**	0.02	0.09	.272**	-0.08	-0.03	.261**	.194**	-0.11	-.147*
TNFRSF4	.566**	.534**	.529**	.485**	.386**	.338**	.272**	.258**	.225**	.207**	.194**	.148*	1.00	0.14	0.11	0.09	0.06	0.06	0.04	0.04	0.03	0.03	0.02	0.02	0.01	0.00	0.00	-0.02	-0.12	-0.14
CHIA	0.03	-0.08	-0.02	-0.03	-0.05	-0.08	-0.06	0.02	-0.10	-0.08	-0.09	-0.07	0.14	1.00	-.166*	0.07	-0.03	-0.01	0.00	0.05	-0.01	-0.05	-0.05	-0.09	-0.02	-0.02	-0.07	-.151*	-0.08	0.09
ALOX5	0.07	0.13	0.11	0.04	0.05	0.04	.179*	0.06	0.04	0.11	-.190*	0.11	0.11	-.166*	1.00	-0.07	0.00	-0.07	-0.14	0.07	-0.06	-0.04	0.02	-0.04	-0.13	0.07	0.08	.208**	-0.03	-0.02
PGLYRP1	.196**	0.10	.163*	0.14	.263**	.164*	0.07	0.07	0.11	.155*	.226**	0.05	0.09	0.07	-0.07	1.00	0.11	0.01	.417**	-0.11	0.10	-0.03	0.15	-0.01	-0.02	-0.01	-0.06	0.01	-0.04	-0.06
SATB1	.238**	.293**	.306**	.345**	.304**	.460**	.195**	-0.05	.200**	0.01	0.13	0.12	0.06	-0.03	0.00	0.11	1.00	-0.07	.415**	-0.02	.212**	-0.12	0.04	0.06	-0.05	0.04	-0.08	-.163*	-0.08	-.246**
IL5	-0.04	-0.01	-0.09	-0.07	-0.06	-0.06	-0.03	0.01	-0.05	0.12	0.02	-0.05	0.06	-0.01	-0.07	0.01	-0.07	1.00	-0.07	-0.02	0.09	-0.03	.335**	-0.04	0.08	0.07	0.08	-0.04	0.08	0.03
IL33	.351**	.392**	.443**	.459**	.498**	.570**	.293**	-0.01	.249**	0.07	.364**	.152*	0.04	0.00	-0.14	.417**	.415**	-0.07	1.00	-0.11	.285**	-0.11	0.08	0.01	-0.04	-0.04	-0.09	-0.13	-0.13	-.154*
GATA3	0.03	0.08	0.02	0.06	0.02	0.05	0.04	-0.01	0.02	-0.06	-0.05	0.02	0.04	0.05	0.07	-0.11	-0.02	-0.02	-0.11	1.00	-0.04	-0.03	-0.05	0.04	-0.02	0.07	0.03	.197**	-.147*	-0.14
IL5RA	.250**	.258**	.294**	.325**	.256**	.311**	.345**	-0.09	.334**	.207**	.165*	.205**	0.03	-0.01	-0.06	0.10	.212**	0.09	.285**	-0.04	1.00	-0.07	.175*	.311**	0.08	-0.02	-0.02	0.05	-0.08	-0.12
IL1A	0.04	0.04	-0.06	0.00	-0.07	-0.06	-0.01	0.13	0.04	0.06	0.07	0.02	0.03	-0.05	-0.04	-0.03	-0.12	-0.03	-0.11	-0.03	-0.07	1.00	-0.03	.148*	-0.04	-0.02	.309**	.241**	-0.04	-0.05
CCR3	0.05	0.09	0.08	0.06	0.11	0.10	0.05	0.02	0.09	.407**	.244**	0.09	0.02	-0.05	0.02	0.15	0.04	.335**	0.08	-0.05	.175*	-0.03	1.00	0.05	-0.03	-0.02	-0.02	0.07	-0.06	-0.01
TNFSF4	-0.04	0.10	.191*	0.12	0.08	0.13	0.11	-0.01	.427**	0.11	.330**	.272**	0.02	-0.09	-0.04	-0.01	0.06	-0.04	0.01	0.04	.311**	.148*	0.05	1.00	-0.07	-0.01	0.08	0.07	-0.10	-0.13
IL4	-0.06	-0.02	-0.08	-0.05	-0.08	-0.06	-0.05	0.00	-0.09	-0.04	-0.06	-0.08	0.01	-0.02	-0.13	-0.02	-0.05	0.08	-0.04	-0.02	0.06	-0.04	-0.03	-0.07	1.00	-0.01	-0.05	-.162*	0.03	-0.03
IL9	-0.03	0.01	-0.03	-0.03	-0.01	-0.02	0.00	-0.05	-0.02	-0.03	-0.04	-0.03	0.00	-0.02	0.07	-0.01	0.04	0.07	-0.04	0.07	-0.02	-0.02	-0.02	-0.01	-0.01	1.00	-0.02	-0.03	0.00	0.00
CSF2	-0.01	0.13	0.05	-0.02	-0.02	-0.02	.188*	0.06	0.14	.200**	-0.04	.261**	0.00	-0.07	0.08	-0.06	-0.08	0.08	-0.09	0.03	-0.02	.309**	-0.02	0.08	-0.05	-0.02	1.00	.198**	-0.07	0.02
IL18	0.02	0.04	0.06	-0.02	0.01	-0.12	0.09	.187*	0.04	0.11	-0.02	.194**	-0.02	-.151*	.208**	0.01	-.163*	-0.04	-0.13	.197**	0.05	.241**	0.07	0.07	-.162*	-0.03	.198**	1.00	-0.09	-.201**
IL17RB	-0.13	-.157*	-.181*	-0.13	-0.12	-0.14	-0.08	0.07	-.158*	-0.03	-0.10	-0.11	-0.12	-0.08	-0.03	-0.04	-0.08	0.08	-0.13	-.147*	-0.08	-0.04	-0.06	-0.10	0.03	0.00	-0.07	-0.09	1.00	0.14
MUC13	-.171*	-.173*	-.219**	-.182*	-.224**	-.172*	-0.11	-.150*	-.182*	-0.10	-0.14	-.147*	-0.14	0.09	-0.02	-0.06	-.246**	0.03	-.154*	-0.14	-0.12	-0.05	-0.01	-0.13	-0.03	0.00	0.02	-.201**	0.14	1.00

-0.999	0.001	.100*	.200**	.300**	.400**	.500**	.600**	.700**	.800**	1.000
-0.001	0.099	.199*	.299**	.399**	.499**	.599**	.699**	.799**	.899**	1.000

Pearson correlation: low high

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Fig. S7. TCGA correlation of 30 inflammatory genes up-regulated by OX40. Related to Figure 6. (MUC5AC data was not available in TCGA database). Genes were sorted according to their correlation index (Pearson r value) with TNFRSF4 (both row and column) from the largest to the smallest.

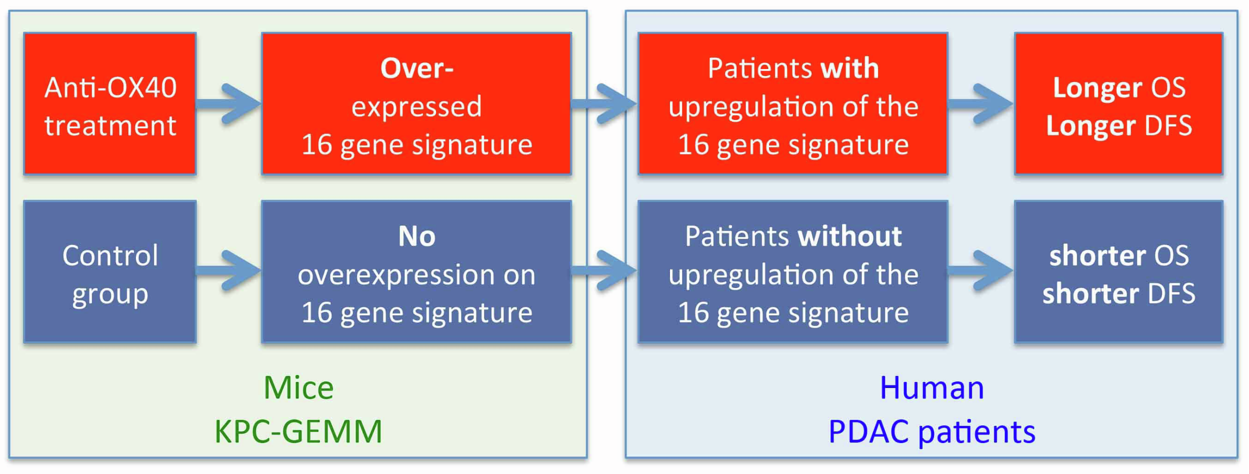
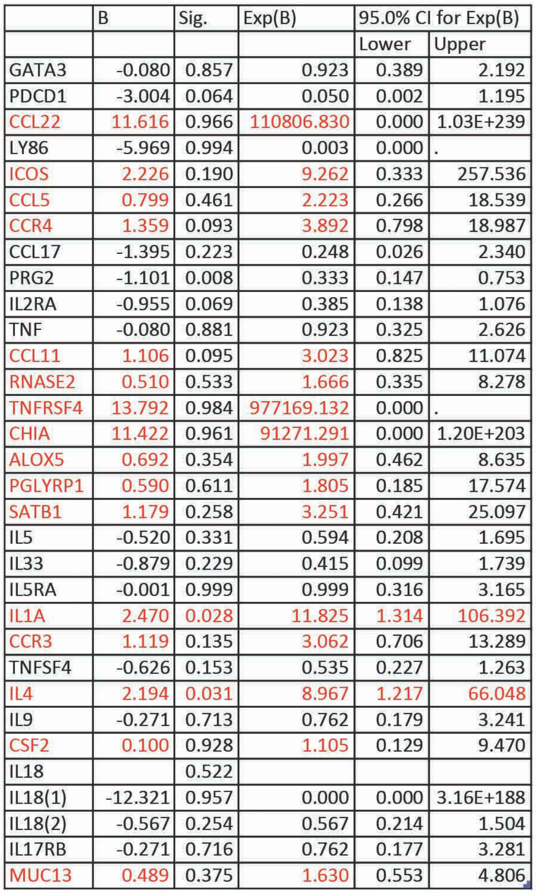


Fig. S8. OX40 signature genes derived from treated mice can be used to stratify patients for predicting survival benefit. Related to Figure 7.

KPC GEMM models were treated with agonist OX40 antibodies, and a consistent 16-gene signature was identified upon transcriptional profiling. This signature was then interrogated in the TCGA PDAC subset (Right). PDAC with presence of the OX40 induced transcriptional signature has significantly higher disease-free and overall survival compared to tumors without the signature.



	B	Sig.	Exp(B)	95.0% CI for Exp(B)	
				Lower	Upper
GATA3	-0.080	0.857	0.923	0.389	2.192
PDCD1	-3.004	0.064	0.050	0.002	1.195
CCL22	11.616	0.966	110806.830	0.000	1.03E+239
LY86	-5.969	0.994	0.003	0.000	.
ICOS	2.226	0.190	9.262	0.333	257.536
CCL5	0.799	0.461	2.223	0.266	18.539
CCR4	1.359	0.093	3.892	0.798	18.987
CCL17	-1.395	0.223	0.248	0.026	2.340
PRG2	-1.101	0.008	0.333	0.147	0.753
IL2RA	-0.955	0.069	0.385	0.138	1.076
TNF	-0.080	0.881	0.923	0.325	2.626
CCL11	1.106	0.095	3.023	0.825	11.074
RNASE2	0.510	0.533	1.666	0.335	8.278
TNFRSF4	13.792	0.984	977169.132	0.000	.
CHIA	11.422	0.961	91271.291	0.000	1.20E+203
ALOX5	0.692	0.354	1.997	0.462	8.635
PGLYRP1	0.590	0.611	1.805	0.185	17.574
SATB1	1.179	0.258	3.251	0.421	25.097
IL5	-0.520	0.331	0.594	0.208	1.695
IL33	-0.879	0.229	0.415	0.099	1.739
IL5RA	-0.001	0.999	0.999	0.316	3.165
IL1A	2.470	0.028	11.825	1.314	106.392
CCR3	1.119	0.135	3.062	0.706	13.289
TNFSF4	-0.626	0.153	0.535	0.227	1.263
IL4	2.194	0.031	8.967	1.217	66.048
IL9	-0.271	0.713	0.762	0.179	3.241
CSF2	0.100	0.928	1.105	0.129	9.470
IL18		0.522			
IL18(1)	-12.321	0.957	0.000	0.000	3.16E+188
IL18(2)	-0.567	0.254	0.567	0.214	1.504
IL17RB	-0.271	0.716	0.762	0.177	3.281
MUC13	0.489	0.375	1.630	0.553	4.806

Fig. S9. Validation of OX40 up-regulated 16-gene signature in a publicly available single cell RNA sequencing dataset. Related to Figure 7.

(A) Heatmap of expression levels (TPM values) of 16 genes across various cell types.

(B) Correlation analysis results of the average expression levels of the 16 genes with T cell proportions in various samples.

Table S1. Multivariate Cox regression analyses of OX40 up-regulated 30 inflammatory genes for OS. Related to Figure 7.

	B	Sig.	Exp(B)	95.0% CI for Exp(B)	
				Lower	Upper
GATA3	-0.080	0.857	0.923	0.389	2.192
PDCD1	-3.004	0.064	0.050	0.002	1.195
CCL22	11.616	0.966	110806.830	0.000	1.03E+239
LY86	-5.969	0.994	0.003	0.000	.
ICOS	2.226	0.190	9.262	0.333	257.536
CCL5	0.799	0.461	2.223	0.266	18.539
CCR4	1.359	0.093	3.892	0.798	18.987
CCL17	-1.395	0.223	0.248	0.026	2.340
PRG2	-1.101	0.008	0.333	0.147	0.753
IL2RA	-0.955	0.069	0.385	0.138	1.076
TNF	-0.080	0.881	0.923	0.325	2.626
CCL11	1.106	0.095	3.023	0.825	11.074
RNASE2	0.510	0.533	1.666	0.335	8.278
TNFRSF4	13.792	0.984	977169.132	0.000	.
CHIA	11.422	0.961	91271.291	0.000	1.20E+203
ALOX5	0.692	0.354	1.997	0.462	8.635
PGLYRP1	0.590	0.611	1.805	0.185	17.574
SATB1	1.179	0.258	3.251	0.421	25.097
IL5	-0.520	0.331	0.594	0.208	1.695
IL33	-0.879	0.229	0.415	0.099	1.739
IL5RA	-0.001	0.999	0.999	0.316	3.165
IL1A	2.470	0.028	11.825	1.314	106.392

CCR3	1.119	0.135	3.062	0.706	13.289
TNFSF4	-0.626	0.153	0.535	0.227	1.263
IL4	2.194	0.031	8.967	1.217	66.048
IL9	-0.271	0.713	0.762	0.179	3.241
CSF2	0.100	0.928	1.105	0.129	9.470
IL18		0.522			
IL18(1)	-12.321	0.957	0.000	0.000	3.16E+188
IL18(2)	-0.567	0.254	0.567	0.214	1.504
IL17RB	-0.271	0.716	0.762	0.177	3.281
MUC13	0.489	0.375	1.630	0.553	4.806

Sig: P value; Exp(B): OR (odd ratio); CI: confidence interval. The 30 inflammatory genes up regulated with anti-OX40 treatment were narrowed down to a 16 genes signature (red: OR > 1), which will contribute to the survival benefit.

Abbreviations:

PDAC, pancreatic ductal adenocarcinoma
NP, normal pancreas
CP, chronic pancreatitis
PanIN, pancreatic intraepithelial neoplasia
KPC-GEMM, Kras$^{G12D/+}$;Trp53$^{R172H/+}$;Pdx-1-Cre genetically engineered mouse model
KC, Pdx-1-Cre x LSL-Kras^{G12D}; mice
KCLW, Kit $^{W\text{-}sh/W\text{-}sh}$ x Pdx-1-Cre x LSL-Kras^{G12D} x LSL-Luciferase mice
KCLB, wild-type Kit with C57BL/6 background, Pdx-1-Cre x LSL-Kras^{G12D} x LSL-Luciferase mice
BLI, bioluminescence imaging
TCGA, The Cancer Genome Atlas
BMMC, bone marrow-derived mast cells
GZMB, granzyme B
OR, odd ratio
CI, confidence interval