EXTRACELLULAR

Antigen	Fluorochrome	Clone	Manufacturer	Reference
CD103	FITC	2E7	Miltenyi	130-118-681
CD11b	APC	M1/70	BD bioscience	553312
CD11c	BV711	HL3	BD bioscience	563048
CD206	BV650	C068C2	Biolegend	141723
CD3	BV650	17A12	Biolegend	100229
CD3	PE-Cy5	145-2C11	BD bioscience	553065
CD4	BV786	GK1.5	BD bioscience	563331
CD4	FITC	RM4-4	ebioscience	11-0043-85
CD45	Alexa Fluro 700	30-F11	Invitrogen	56-0451-82
CD45	APC-Cy7	30-F11	BD bioscience	557659
CD8a	BV711	53-6.7	BD bioscience	563046
CD8a	PE-Vio770	REA601	miltenyi	130-109-249
CD80	BV786	16-10A1	BD bioscience	740888
CD86	PE	GL1	BD bioscience	553692
F4/80	PE-CF594	T45-2342	BD bioscience	565613
FOXP3	eFluor 450	FJK-16S	ebioscience	48-5773-82
Ly-6C	BV605	AL-21	BD bioscience	563011
Ly6G	PE-Cy™7	1A8	BD bioscience	560601
I-A/I-E	BV421	M5/114.15.2	BD bioscience	562564
NK1.1	BV510	PK136	BD bioscience	563096
PD-1	BV605	29F.1A12	Biolegend	135219
TIGIT	PerCP eFluor 710	GIGD7	ebioscience	46-9501-82
CD120b (TNFR-II)	PE	REA228	Miltenyi	130-104-697

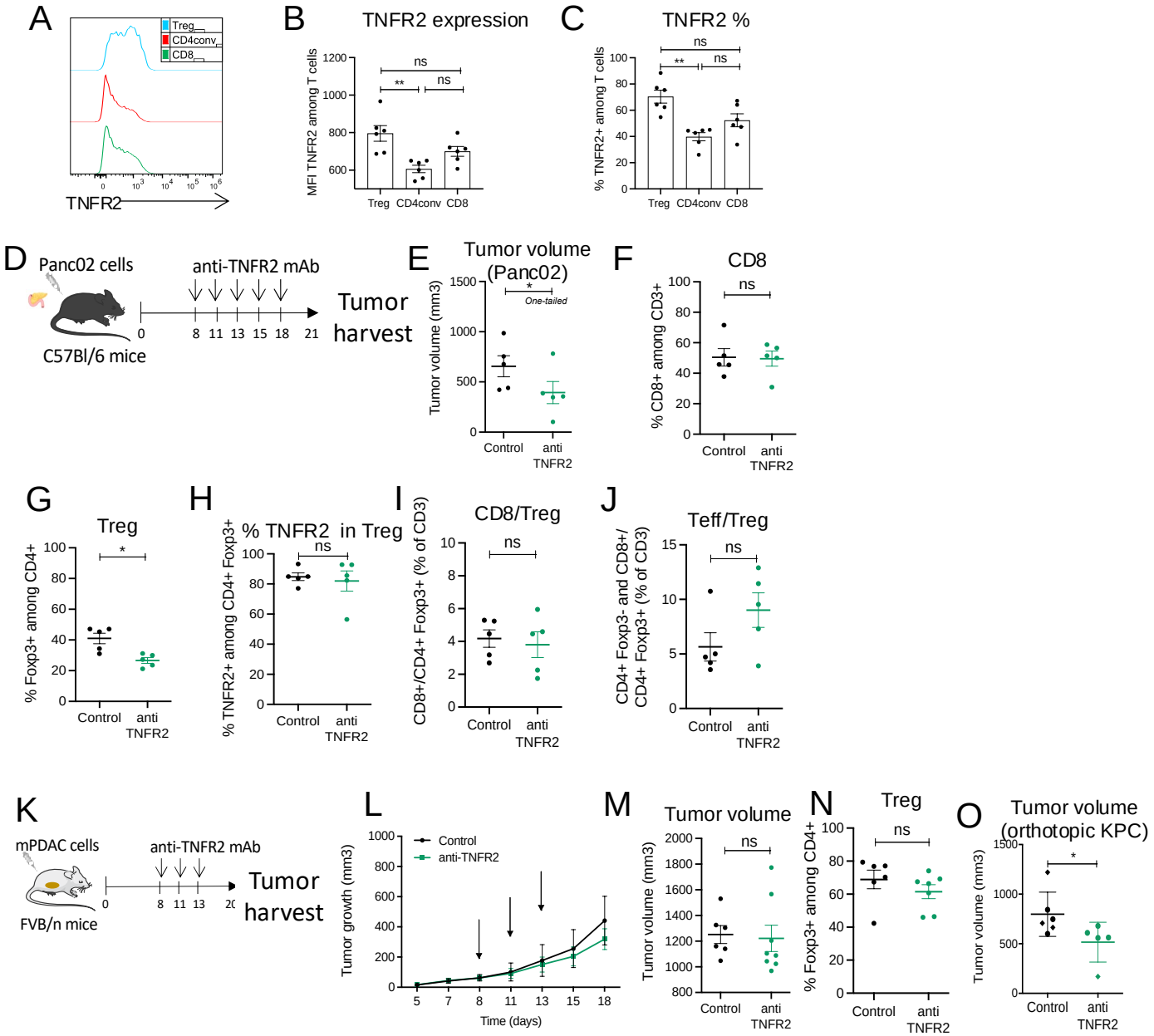
INTRACELLULAR

Antigen	Fluorochrome	Clone	Manufacturer	Reference
CTLA-4	PE/Dazzle 594	UC10-4B9	Biolegend	106318
Foxp3	eFluor 450	FJK-16S	ebioscience	48-5773-82
Foxp3	eFluro 660	FJK-16S	ebioscience	12-5773-82
TNFa	PerCP-eFluor 710	MP6-XT22	eBioscience	46-7321-82
GzB	PE-Cy™7	QA16A02	Biolegend	372214
IFNg	APC	XMG1.2	Invitrogen	17-7311-82
Foxp3	PE	FJK-16S	ebiosience	12-5773-82

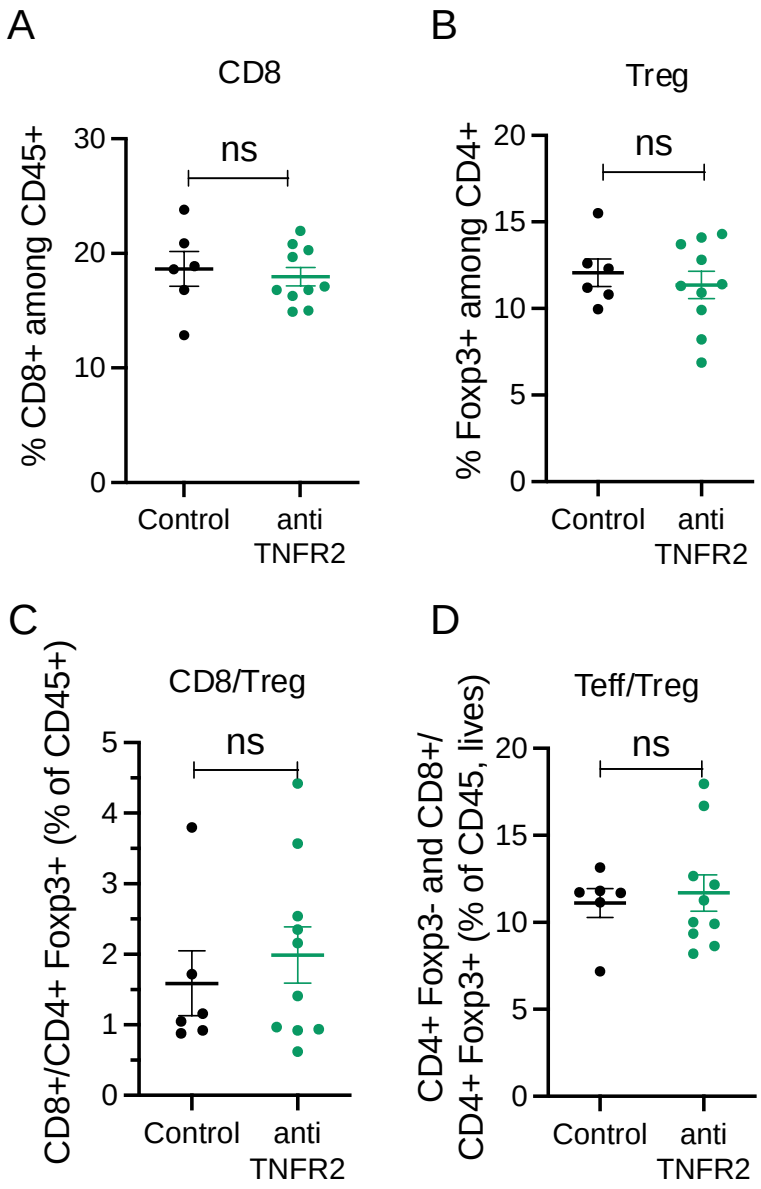
FIXABLE VIABILITY STAIN (FVS)

Antigen	Fluorochrome	Clone	Manufacturer	Reference
FVS	BV510		BD bioscience	564406
FVS	Alexa F700		BD bioscience	564997

Supplementary table 1. List of antibodies

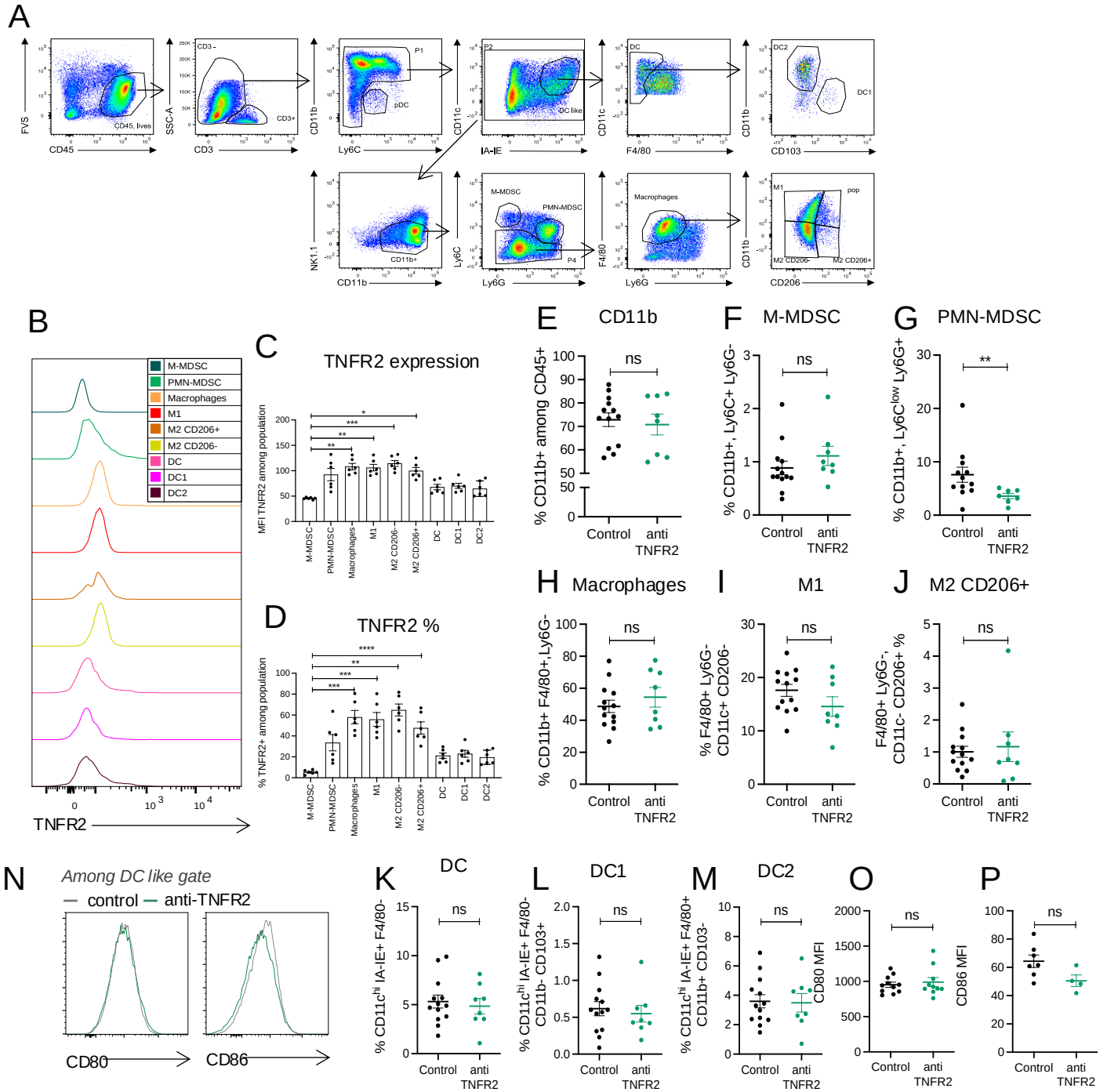


Supplementary Figure 1. TNFR2 blockade effects in Panc02 orthotopic mouse model.
Immunocompetent mice were grafted with Panc02 cells (**A-J**) or KPC (**O**) into the pancreas, or mPDAC subcutaneously (**K-N**). After 21 days, tumors were harvest for flow cytometry analysis. **(A)** TNFR2 expression in intratumoral Treg (CD4$^+$ Foxp3$^+$), CD4conv (CD4$^+$ Foxp3$^-$) and CD8$^+$ cells **(B)** Histograms show TNFR2 expression (MFI TNFR2) and **(C)** proportions among intratumoral Treg (CD4$^+$ Foxp3$^+$), CD4conv (CD4$^+$ Foxp3$^-$) and CD8$^+$ cells are shown in the graphs (n=6). **(D)** Schema of the experiment: mice were treated with anti-TNFR2 mAb or PBS at days 8,11,13 and 15 (n=6). **(E)** Scatter plot of tumor volume at day 21. **(F-G)** Scatter plots of intratumoral Treg (CD4$^+$ Foxp3$^+$), and CD8$^+$ proportions. (H) TNFR2 proportion among CD4$^+$Foxp3$^+$. **(I-J)** Scatter plots of CD8$^+$/Treg(CD4$^+$ Foxp3$^+$) ratio, and Teff (CD4$^+$Foxp3$^-$ and CD8$^+$)/Treg(CD4$^+$Foxp3$^+$) ratio. **(K)** Schema of the experiment: FvB/n immunocompetent mice were grafted with mPDAC cells subcutaneously in the right flank and were treated with anti-TNFR2 mAb or IgG control at days 8, 11 and 13 (n=10). After 20 days, tumors were harvest for flow cytometry analysis. **(L)** Tumor growth curve and **(M)** tumor volume at day 20 are shown in the graphs. **(N)** Scatter plot of intratumoral Treg (CD4$^+$ Foxp3$^+$) proportion. Data are plotted as the mean±SEM. Statistical significance between population in control was determined using Kruskal-Wallis test, *p<0,05 **p<0,01. Statistical significance from controls was determined using Mann-Whitney test (two-tailed [H-K-N-O] or one-tailed [E]. ns: non-significant p>0,05, *p<0,05, **p< 0,01.



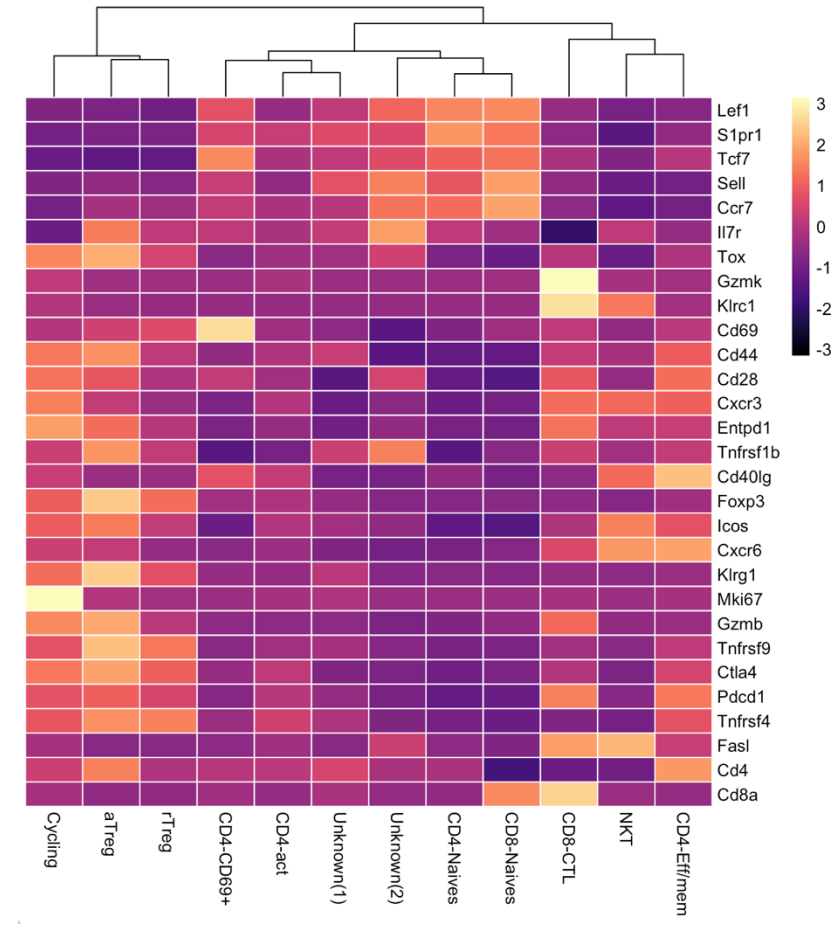
Supplementary Figure 2. TNFR2 blockade does not modify T cells proportions in tumor draining lymph nodes.

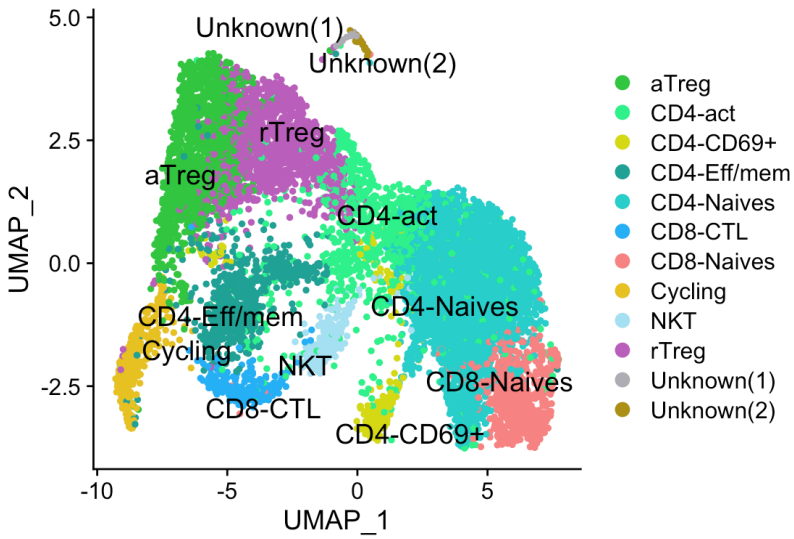
FvB/n mice injected orthotopically with mPDAC cells were treated with anti-TNFR2 mAb, or IgG control, PBS at day 11, 13 and 15 or were untreated. After 21 days, pancreatic draining lymph nodes were harvest for flow cytometry analysis. **(A-B)** Scatter plots of Treg ($CD4^+$ $Foxp3^+$) and $CD8^+$ cells proportions **(C-D)** Scatter plots of $CD8^+$/Treg($CD4^+Foxp3^+$) ratio and Teff($CD4^-Foxp3^+$ and $CD8^+$)/Treg($CD4^+Foxp3^+$) ratio (n=26 including 2 experiments, representatives of 3 experiments). Data are plotted as the mean ±SEM. Statistical significance from controls was determined using Mann-Whitney test. ns: non-significant.



Supplementary Figure 3. TNFR2 blockade effects on myeloid cells.

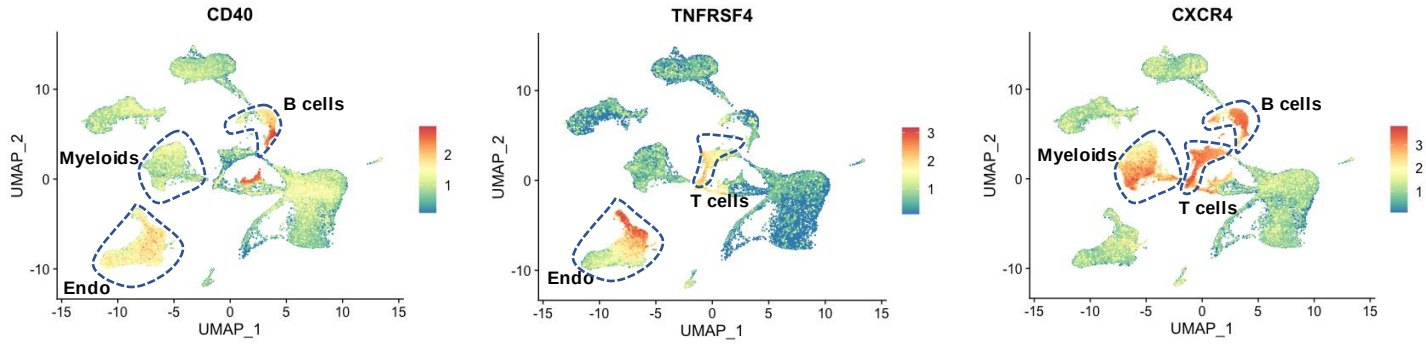
FvB/n immunocompetent mice were grafted with mPDAC cells into the pancreas. After 21 days, tumors were harvest for flow cytometry analysis. **(A)** Gating strategy used for myeloid cells identification. **(B)** Histograms of TNFR2 expression and **(C)** Representative histograms of TNFR2 expression (MFI TNFR2) and **(D)** proportions in intratumoral M-MDSC (CD11b$^+$Ly6C$^+$Ly6G$^-$), PMN-MDSC (CD11b$^+$ Ly6C^{low}Ly6G$^+$), Macrophages (CD11b$^+$Ly6G$^-$F4/80$^+$), M1 (F4/80$^+$CD11c$^+$CD206$^-$), M2 CD206$^+$ (F4/80$^+$ CD11c$^-$ CD206$^+$), M2 CD206$^-$ (F4/80$^+$ CD11c$^-$CD206$^-$) DC: dendritic cells (CD11c^{hi} IA-IE$^+$ F4/80$^-$) and DC type 1 (CD11c^{hi} IA-IE$^+$ F4/80$^-$ CD11b$^-$ CD103$^+$) and DC type 2 (CD11c^{hi} IA-IE$^+$ F4/80$^-$ CD11b$^+$ CD103$^-$) (n=6). Mice were treated with anti-TNFR2 mAb, IgG control at day 11, 13 and 15 or were untreated (n=21). After 21 days, tumors were harvest for flow cytometry analysis. **(E-M)** Scatter plots of cell proportion among CD45+ FVS- cells : (E) CD11b$^+$, (F) M-MDSC (CD11b$^+$ Ly6C$^+$ Ly6G$^-$), (G) PMN-MDSC (CD11b$^+$ Ly6C^{low} Ly6G$^+$), (H) macrophages (CD11b$^+$ Ly6G$^-$ F4/80$^+$), (I) M1 (F4/80$^+$ CD11c$^+$ CD206$^-$), (J) M2 (F4/80$^+$ CD11c$^-$ CD206$^+$), (K) DC (CD11c^{hi} IA-IE$^+$ F4/80$^-$), (L) DC1 (CD11c^{hi} IA-IE$^+$ F4/80$^-$ CD103$^+$ CD11b$^-$) and (M) DC2 (CD11c^{hi} IA-IE$^+$ F4/80$^-$ CD103$^-$ CD11b$^+$) **(N)** Representative histograms of CD80 (n=21) and CD86 (n=11) expression (MFI CD80 and MFI CD86) on CD11c$^+$ IA-IE$^+$ (DC like) cells and respective scatter plots **(O-P)** .Data are plotted as the mean±SEM. Statistical significance between population in control was determined using Kruskal-Wallis test, **p<0,01 ***p<0,001. Statistical significance from controls was determined using t-test [E-H-J-K-L-M] or Mann-Whitney two-tailed [F-G-J-O-P]. ns: non-significant p>0,05, *p<0,05, **p< 0,01, ***p<0,001.



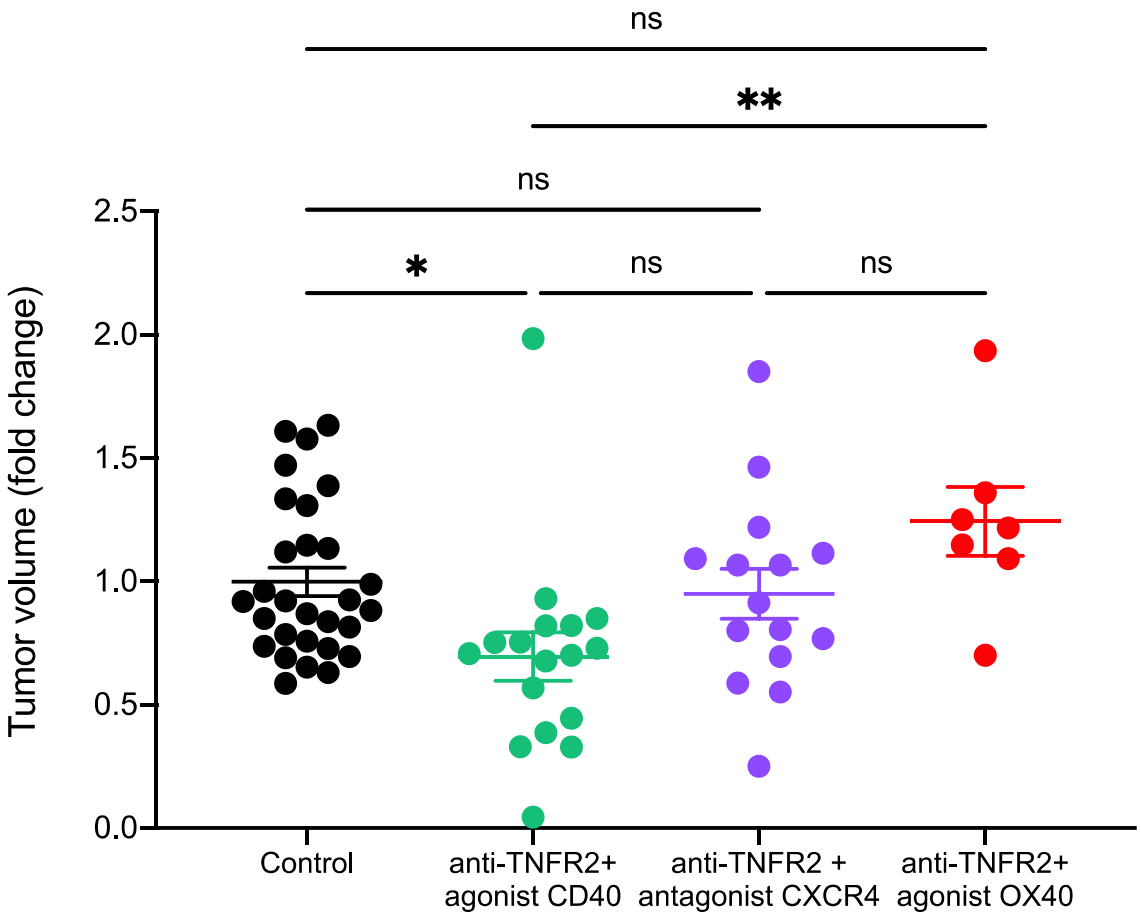


Supplementary Figure 4. Identification of the cell clusters from single cell RNAseq analysis of PDAC.

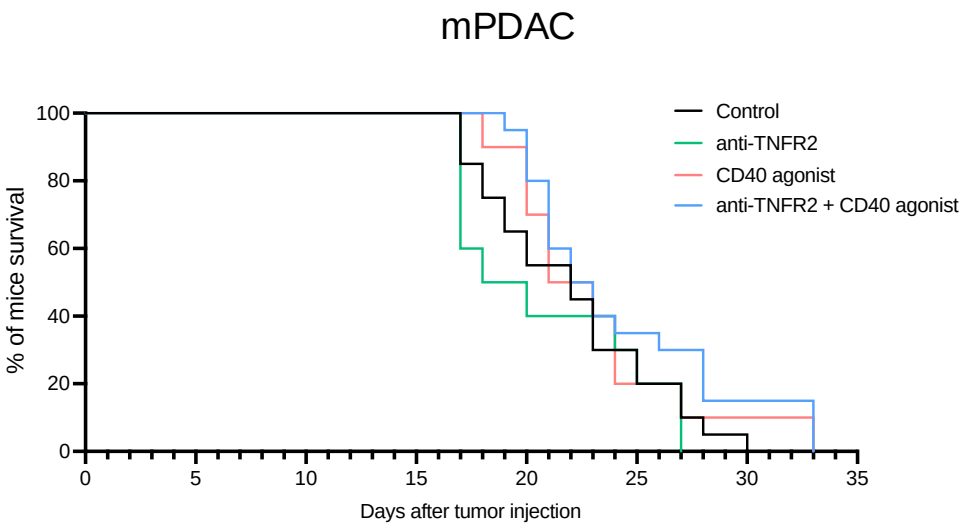
Intratumoral CD4⁺ and CD8⁺ were isolated for single–cell RNA sequencing from mPDAC control and anti-TNFR2 treated mice. (A) Heatmap showing mean gene expression of different genes used to describe CD4 and CD8 T cells cluster (clustering done using FindNeighbors on the fifty first PCs and FindClusters, resolution 0.7). (B) UMAP showing the transcriptional profile of single cells in 2D space.



Supplementary Figure 5. CD40, TNFRSF4(OX40) and CXCR4 expression in human PDAC. Expression of human *CD40*, *TNFRSF4* (OX40) and *CXCR4* among tumor samples. Adaptive thresholded low rank approximation-imputed data are depicted.



Supplementary Figure 6. Combination of blocking anti-TNFR2 with agonist anti-CD40, or agonist anti-OX40 or antagonist anti-CXCR4 mAb.
mPDAC carrying mice were treated with a blocking anti-TNFR2 mAb together with an agonist anti-CD40, or agonist anti-OX40 or antagonist anti-CXCR4 mAbs mice administered at day 11, 13, 15. Twenty one days after mPDAC cell injection, mice were euthanized and the tumor volumes were measured and data were plotted as tumor fold change to the group of control mice. Statistical analysis has been done by the Kruskal-Wallis test with a Dunn post-test for multiple comparison between all groups. ns: non-significant p>0,05, *p<0,05, **p< 0,01



Supplementary Figure 7. Combination of blocking anti-TNFR2 and agonist anti-CD40 mAbs effects in survival of mPDAC orthotopic mouse model.
FvB/n immunocompetent mice were grafted with mPDAC cells into the pancreas. Mice were treated with either anti-TNFR2 mAb or CD40 agonist or both or received PBS at day 11, 13, 15, 20 and 27. Clinical score of the mice is established by a grid of symptoms. Mice are euthanized when the limit of the clinical score is reached. **(A)** Kaplan-Meier survival curve of the following groups: Control (n = 20), anti-TNFR2 (n = 10), CD40 agonist (n = 10) and anti-TNFR2+CD40 agonist (n = 20). (Kaplan-Meier test, ns p>0,05).