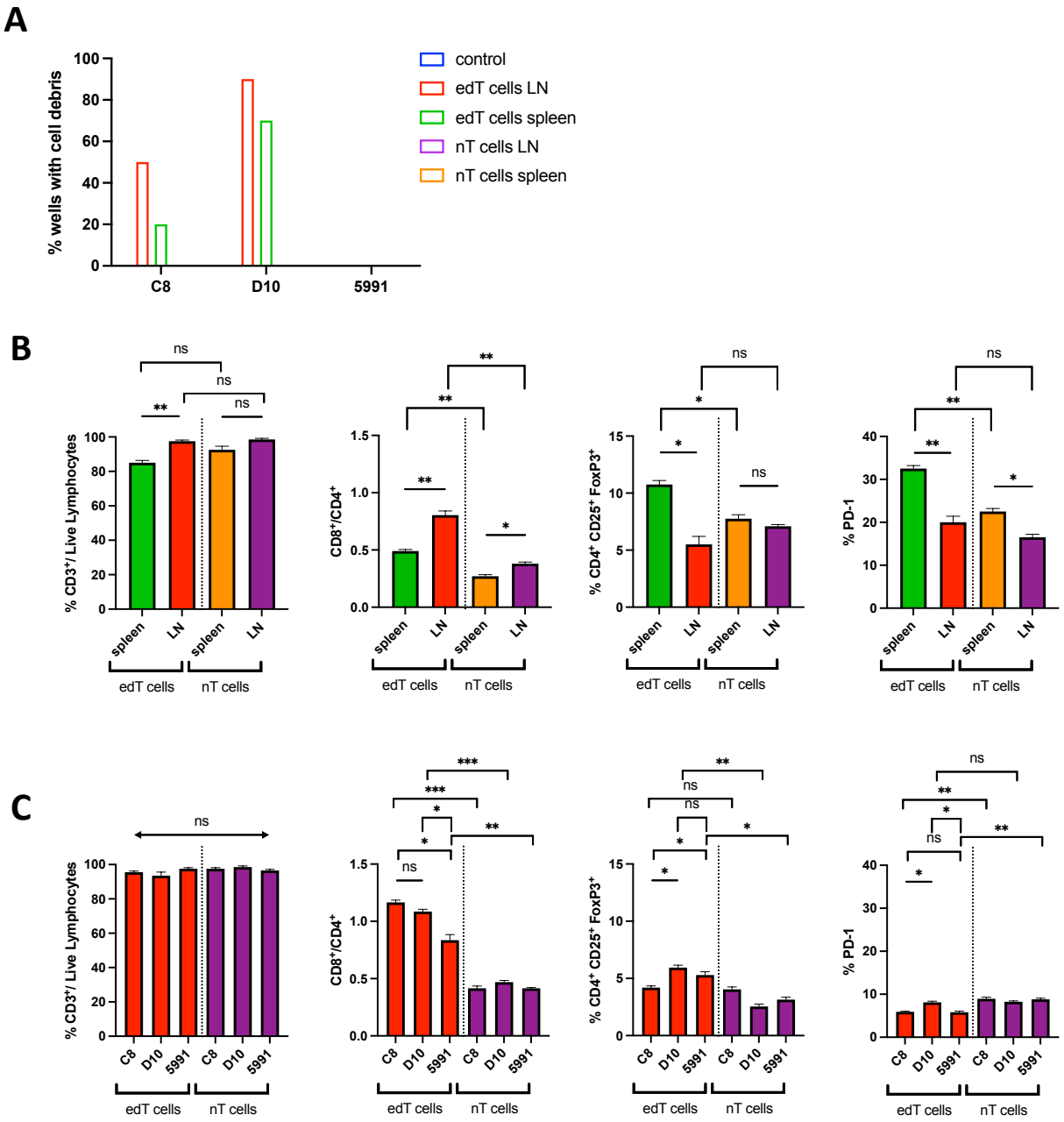
Supplementary Figure S1

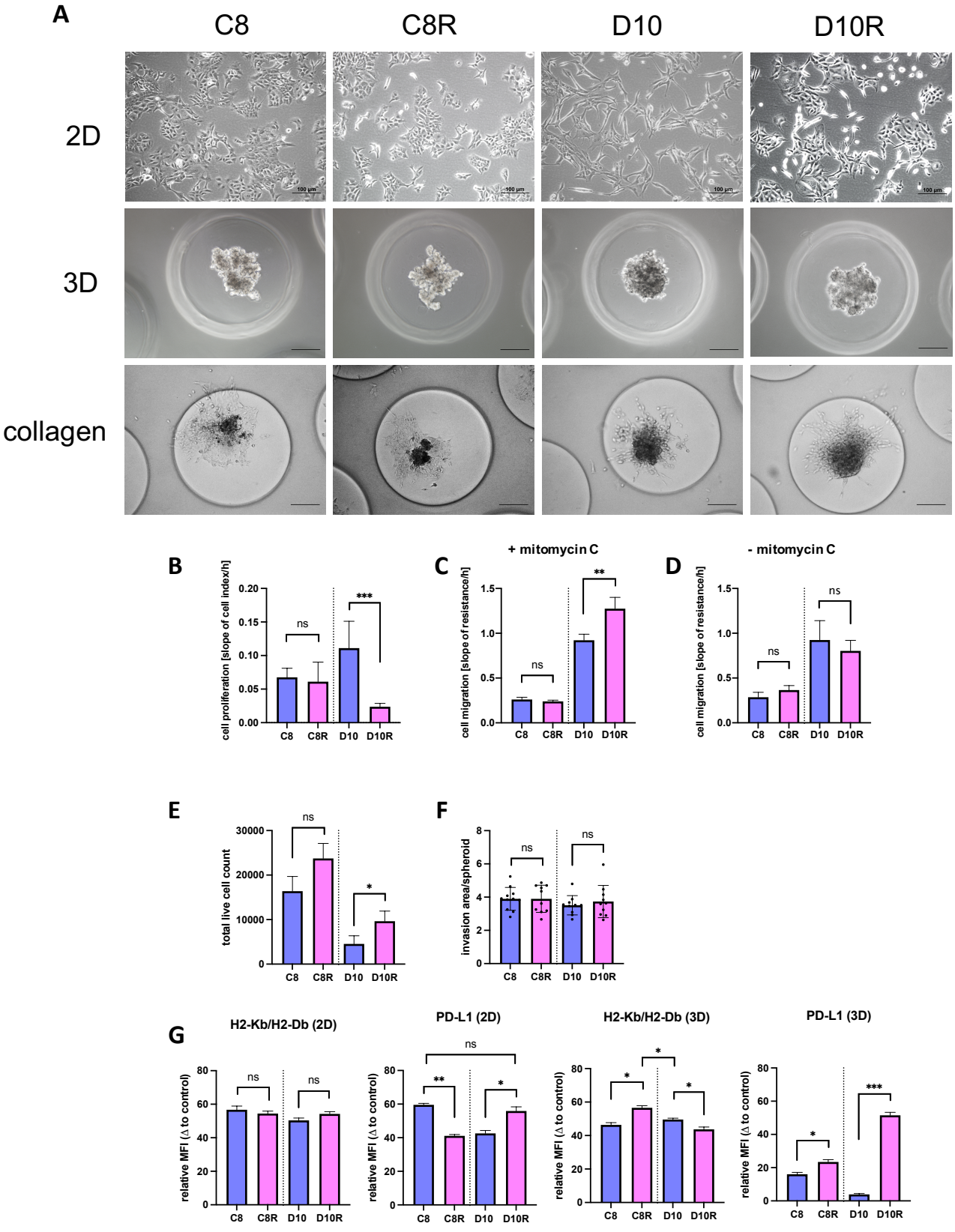


Supplementary Figure S1

Cell debris of PDAC cancer cells in co-culture and T cell characterization. (A) Cell debris in co-cultures. Characterization of T cells before **(B)** and after **(C)** co-culture with cancer spheroids via flow cytometry. edT and nT cells were from lymph nodes. Shown is the percentages of CD3+/Live Lymphocytes after isolation, CD4+CD25+FoxP3+ cells as regulatory T cells and PD-1+ T cells. CD8+/CD4+ is given as ratio of positively stained

CD8+ to CD4+ T cells, gated from live CD3+ T cells. (Student t test, *P < 0.05, **P < 0.01, ***P < 0.001, n.s., not significant).

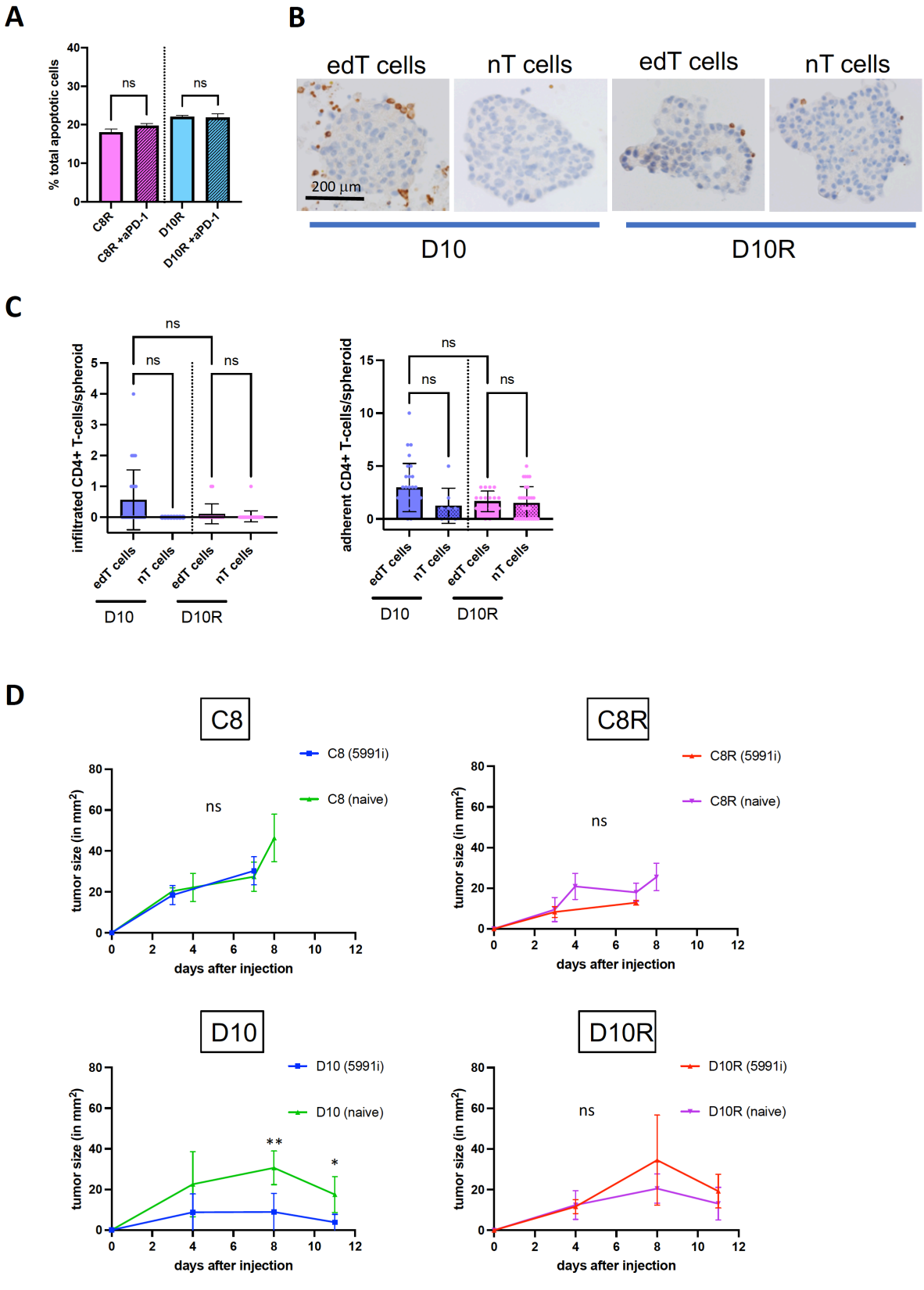
Supplementary Figure S2



Supplementary Figure S2

Parental and resistant cells show distinct phenotypic properties in 2D and 3D culture. (A) Representative images of paired parental and resistant cells grown in 2D and 3D culture conditions, or embedded in collagen. Scale bars, 100 μm. **(B)** Cancer cell proliferation in 2D is shown as slope of cell index/hours, measured by an Electric cell-substrate impedance sensing (ECIS). The impedance of cells growing on electrodes is measured as a real-time readout. Cancer cell migration in 2D, in the presence **(C)** or absence **(D)** of mitomycin C measured by ECIS in a wound-healing assay. **(E)** 3D cancer cell growth after 3 days of spheroid culture in agarose casts, is shown as total live cell counts measured via flow cytometry. **(F)** 3D cancer cell invasion into collagen is shown as relative invasion area/spheroid. **(G)** Cell surface expression of MHC-1 (H2-Kb/H2-Db) and PD-L1 of cancer cells in 2D and 3D was analyzed via flow cytometry and shown as median fluorescence intensity (MFI) relative to unstained control. All data represent the means ± SD from at least three biological replicates and three technical replicates. (Student t test, *P < 0.05, **P < 0.01, ***P < 0.001, n.s., not significant).

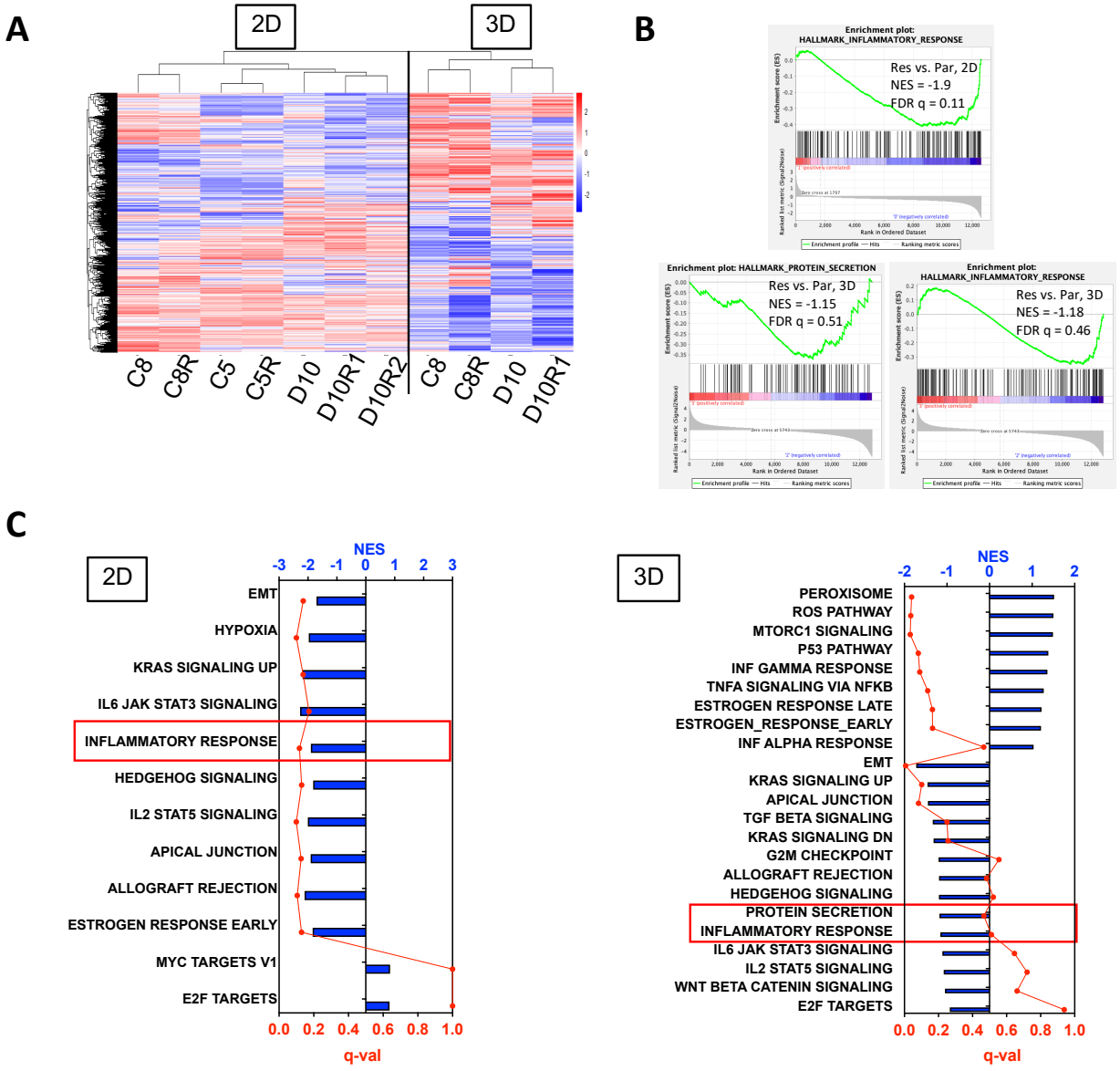
Supplementary Figure S3



Supplementary Figure S3

Anti-PD-1 effect and localization of $CD4^+$ T cells in cancer spheroids. (A) Percentage of total apoptotic resistant cancer cells ± anti-PD-1 after co-culture with edT cells. **(B)** Representative 3D co-culture sections of D10/D10R stained by immunohistochemistry for $CD4^+$. Scale bar, 200 μm. **(C)** Quantification of infiltrated and adherent $CD4^+$ T cells in cancer spheroid co-culture. **(D)** Growth rate of parental and resistant allograft tumors ± pre-immunization with KPC 5991 cells. Naïve groups are allograft tumors from mice without KPC 5991-immunization. Shown are the tumor growth curves of the individual tumors for each group. Error bars are SEM for $n \geq 3$ tumors. 5991i= 5991 cells – pre-immunized tumors. (Student t test and One-way ANOVA multiple comparison, *P < 0.05, **P < 0.01, n.s., not significant).

Supplementary Figure S4

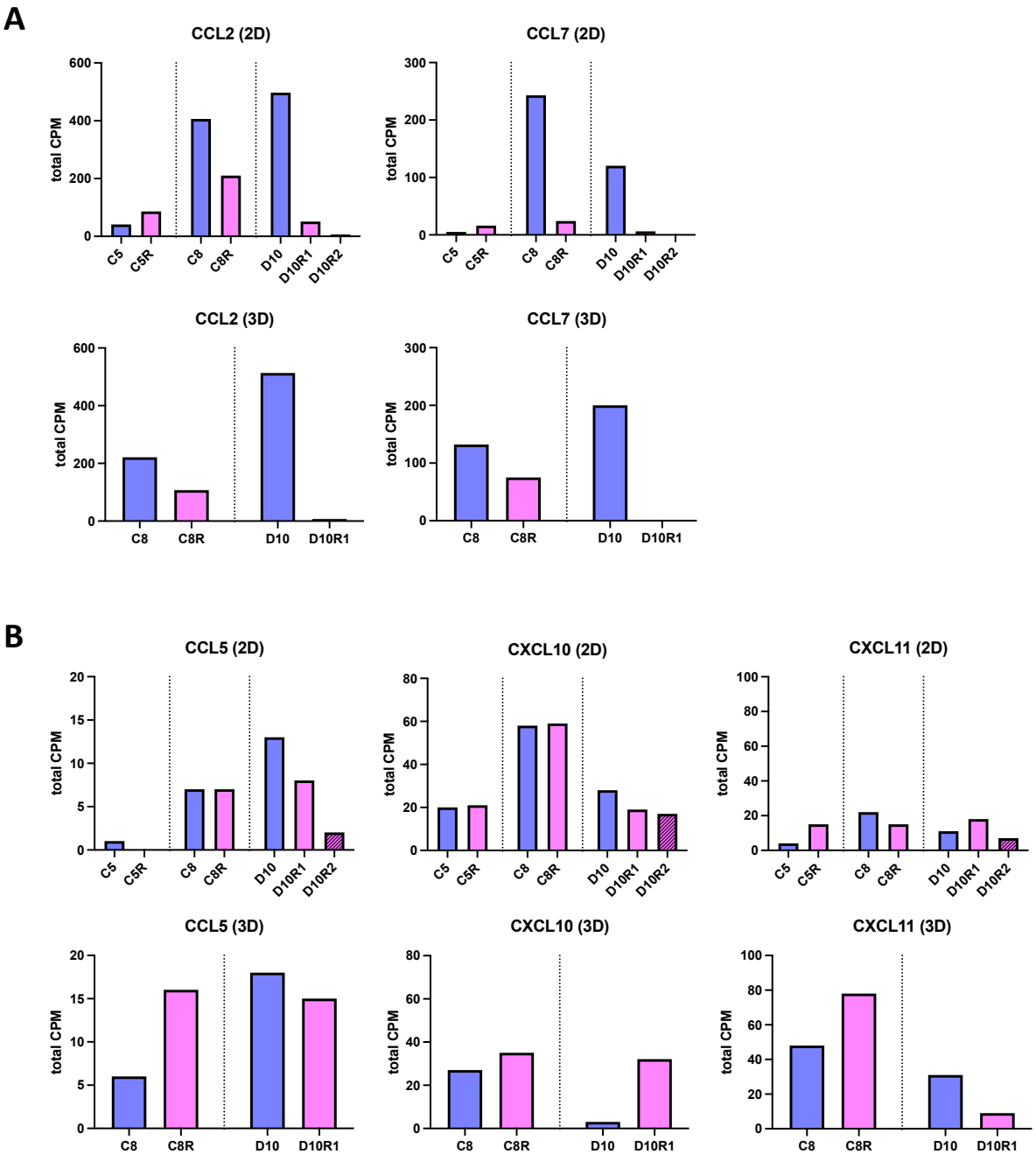


Supplementary Figure S4

Differential gene expression in resistant vs. parental cells. (A) Heatmap of gene expression from paired parental/resistant PDAC cell lines in 2D and 3D based on an RNA-sequencing analysis. C5R has been isolated from the PDAC clonal cancer cell line C5 in a separate co-culture experiment. C5/C5R were included in the transcriptomic analysis. D10R1 and D10R2 are two different resistant cell lines derived from D10 from two independent co-culture experiments. **(B)** Gene set enrichment analysis (GSEA) hallmark

pathways of resistant vs. parental cells. **(C)** Overview of GSEA hallmark pathways for resistant vs. parental cells in 2D and 3D. Normalized GSEA enrichment score and FDR q-value are shown as indicated. Highlighted are differentially regulated hallmark pathways for *Inflammatory Response* and *Protein Secretion* between resistant vs. parental cells.

Supplementary Figure S5

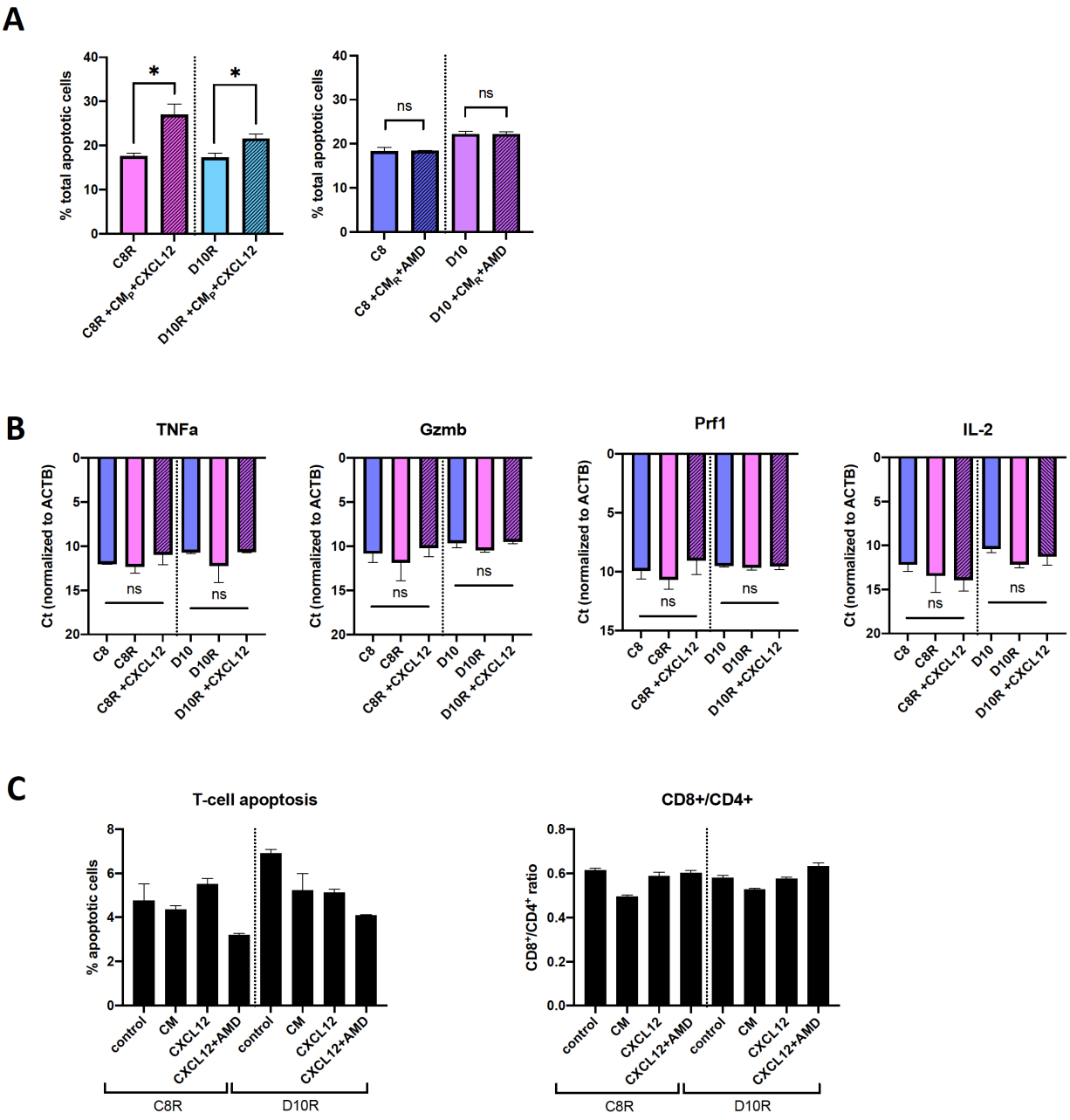


Supplementary Figure S5

T cell recruiting chemokine expression in parental and resistant cancer cells. (A) Gene expression level of T cell chemoattractant chemokines and **(B)** Th1-type chemokines in paired parental/resistant cell lines in 2D and 3D. Expression data are based

on an RNA-sequencing analysis. C5R has been isolated from the PDAC clonal cancer cell line C5 in a separate co-culture experiment. C5/C5R were included in the transcriptomic analysis. D10R1 and D10R2 are two different resistant cell lines derived from D10 from two independent co-culture experiments. D10R1 is also referred to as D10R in 3D co-culture experiments. CPM= transcripts per million.

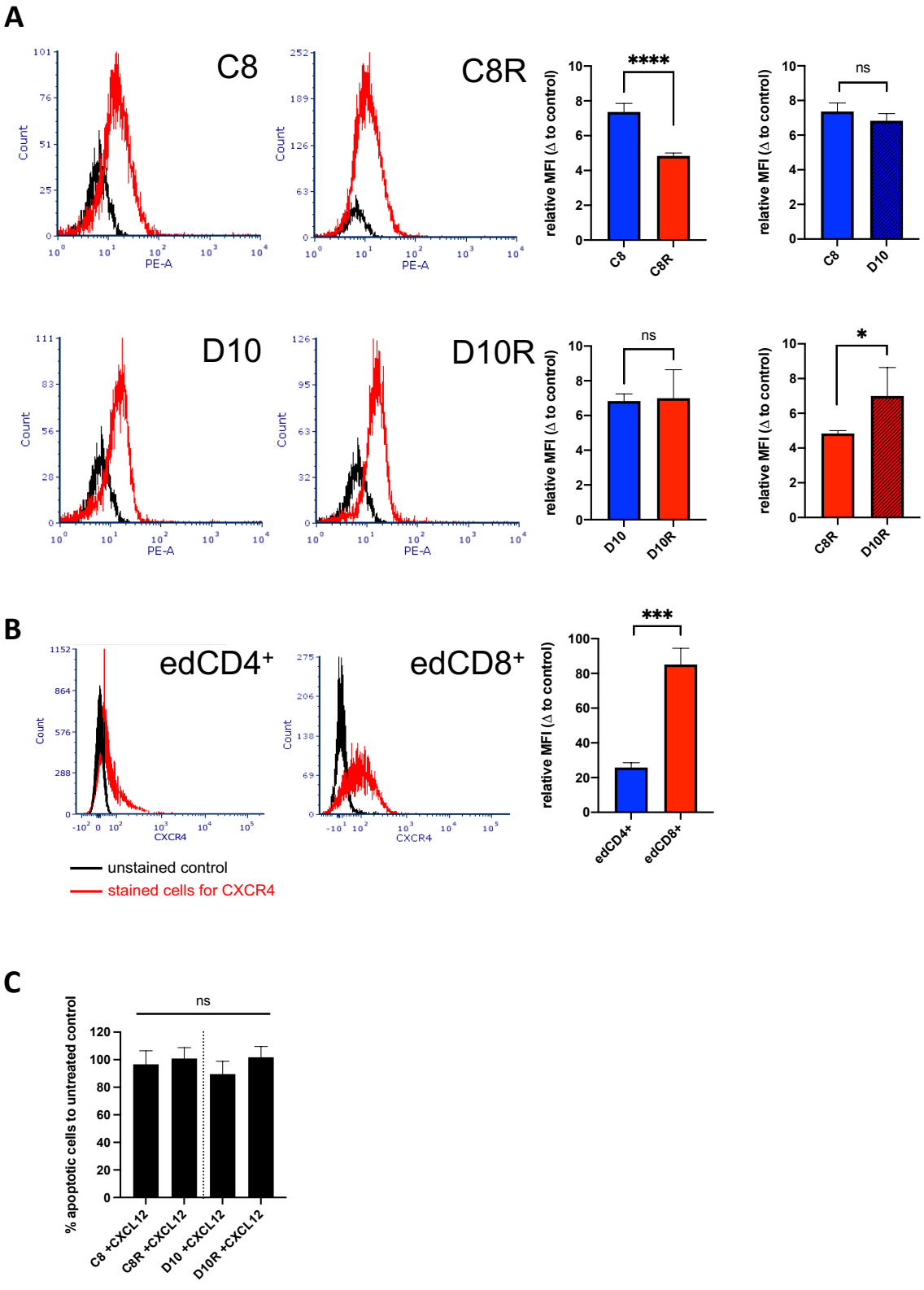
Supplementary Figure S6



Supplementary Figure S6

Conditioned media- and CXCL12-effect on edT cell killing of PDAC cells. (A) Percentage of total apoptotic resistant cancer cells +CM_P or +CXCL12 after co-culture with edT cells. Controls are cancer spheroid co-culture with edT cells without drug treatment. **(B)** Expression of T cell activation markers by RT-qPCR. Shown are mRNA transcript levels in cancer spheroid with T cells co-culture as Ct-values normalized to beta-actin. **(C)** Total percentage of apoptotic edT cell after 4 days of edT cell co-culture with resistant cell lines +/- drug treatment as indicated. $CD8^+/CD4^+$ ratio of edT cells after co-culture. All data represent the means ± SD from at least three biological replicates and two technical replicates. (Student t test, *P < 0.05, n.s., not significant). CM_R= conditioned media of resistant cells; CM_P or CM= conditioned media of parental cells.

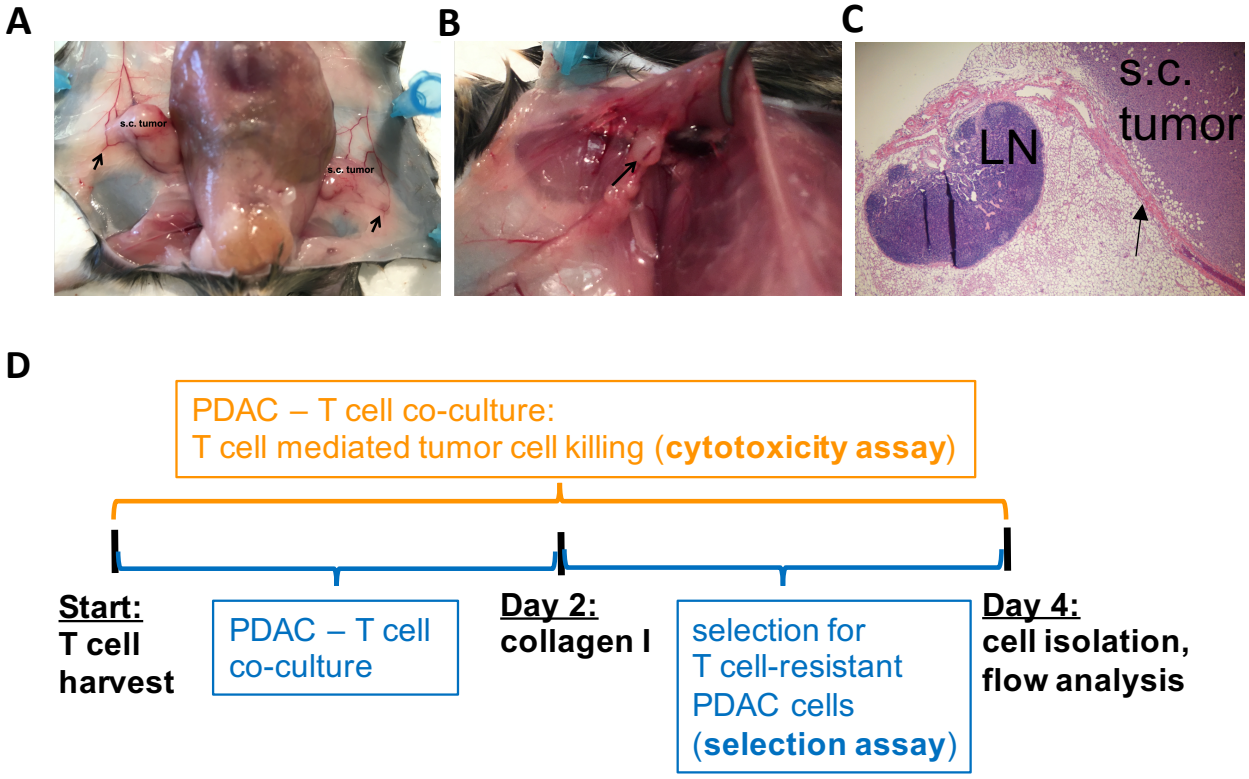
Supplementary Figure S7



Supplementary Figure S7

Cell surface expression of CXCR4 on cancer cells and edT cells. (A) Representative histograms of paired parental/resistant cancer cells, and **(B)** PDAC tumor-educated (ed) $CD4^+$ and $CD8^+$ T cells, measured for CXCR4 expression via flow cytometry. Shown is also the quantification of CXCR4 expression level as delta median fluorescence intensity (MFI) to unstained control. **(C)** Percentage of CXCL12-treated total apoptotic cancer cells to control (untreated) after 3 days of culture in agarose molds. All data represent the means ± SD from at least three biological replicates and two technical replicates. (Student t test, *P < 0.05, ***P < 0.001, ****P < 0.0001, n.s., not significant).

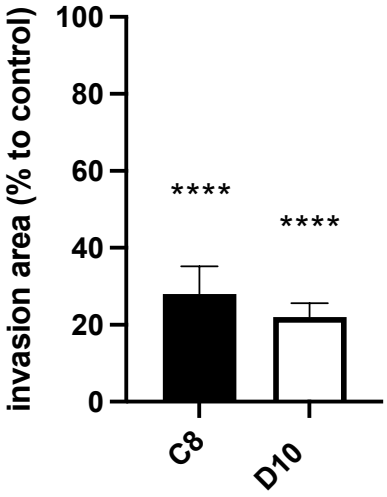
Supplementary Figure S8



Supplementary Figure S8

Anatomical location of tumor-draining lymph nodes and timeline of co-culture studies. (A-C) Anatomical exposures of bilateral inguinal **(A)** and right lateral axillary **(B)** draining lymph nodes of a PDAC tumor-immunized mouse. Arrows point to the lymph node. **(C)** Tumor section including adjacent inguinal lymph node, stained with hematoxylin and eosin. Arrow points to a vessel connecting lymph node with the tumor. LN= lymph node, s.c.= subcutaneous. **(D)** Shown is the timeline of the 3D co-culture experiments.

Supplementary Figure S9



Supplementary Figure S9

Effect of splenic edT cells from KPC mice with spontaneous PDAC on cancer cells in vitro. Invasion area of PDAC cell spheroids in 3D co-culture with splenic edT cells shown as percentage to control. Control is cancer spheroid only. At least 20 cancer spheroids were analyzed per group. Data represent the means ± SD from three biological replicates. (Student t test, ****P < 0.0001).

Supplementary Table S1

Gene	Primer
mIFNg_Fwd	GCCACGGCACAGTCATTGAA
mIFNg_Rev	GTCACCATCCTTTTGCCAGTTCC
mGzmb_Fwd	CCACTCTCGACCCTACATGG
mGzmb_Rev	GGCCCCCAAAGTGACATTTATT
mTNFa_Fwd	ACAAGCCTGTAGCCCACGTC
mTNFa_Rev	GTGAGGAGCACGTAGTCGGG
mPrf1_Fwd	AGCACAAGTTCGTGCCAGG
mPrf1_Rev	GCGTCTCTCATTAGGGAGTTTTT
mIl-2_Fwd	ATGAACTTGGACCTCTGCGG
mIl-2_Rev	GTCCACCACAGTTGCTGACT
mActb_Fwd	GTGACGTTGACATCCGTAAAGA
mActb_Rev	GCCGGACTCATCGTACTCC

Supplementary Table S1
Primer sequences of murine T cell activation markers.

Supplementary Table S2

T cell abundance markers (CD4, CD8):

	- fold cancer / normal	p-value
Breast:	1.31	2.47 x 10e-31
Kidney (RCC):	5.45	6.14 x 10e-45
Melanoma:	2.18	1.44 x 10e-72
PDAC:	6.36	4.37 x 10e-54

T cell exhausting markers [LAG3, PD1 (PDCD1), TIM3 (HAVCR2)]

	- fold cancer / normal	p-value
Breast:	1.93	5.84 x 10e-55
Kidney (RCC):	4.18	1.47 x 10e-27
Melanoma:	2.77	2.67 x 10e-83
PDAC:	8.74	9.57 x 10e-58

Supplementary Table S2. Relative expression of T cell abundance markers (CD4 and CD8) and T cell exhausting markers LAG3, PD1 (PDCD1), TIM3 (HAVCR2) comparing paired cancer and normal tissues. Gene expression is from RNAseq of cancer and normal tissues. Data from the TNMplot data base (https://tnmplot.com/analysis/) [1]. RCC = Renal clear cell carcinoma.

1. Bartha Á, Győrffy B. TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. Int J Mol Sci. 2021;22(5):2622. PMID: 33807717