

Supplementary Figure 1

(A) Brightfield images of PDLOs \pm Dox treatment. Scale bar = 250 μ m.

(B) Flow cytometry quantifying mCherry reporter expression after Dox treatment.

(C) Dot plot showing expression of cell type markers in PDLO scRNA-seq data set.

(D) *KRAS* copy numbers for hESC lines, PDAC lines and patient-derived organoids (PDOs) using probes targeting either the 3' untranslated region (3'UTR) of the endogenous locus or exon 3, which detects the respective sequence in the endogenous locus and in the inserted piggyBac *KRAS*^{G12D} construct. Copy numbers are shown relative to the respective *KRAS* wild-type cell lines (parental hESCs, BxPC3 and PDO#38188). BU3 = monoallelic BU3 NGST-TetOn:*KRAS*G12D line^[92].

(E) Protein expression of phospho-ERK1/2 and ERK1/2 in PDLOs after indicated time-points of Dox treatment, in PDAC lines and PDOs. For quantification, phospho-ERK1/2 levels were normalized to ERK1/2 levels for each sample. n = 3 for *KRAS*-PDLOs, n = 2 for parental PDLOs and n = 1 for PDAC lines and PDOs.

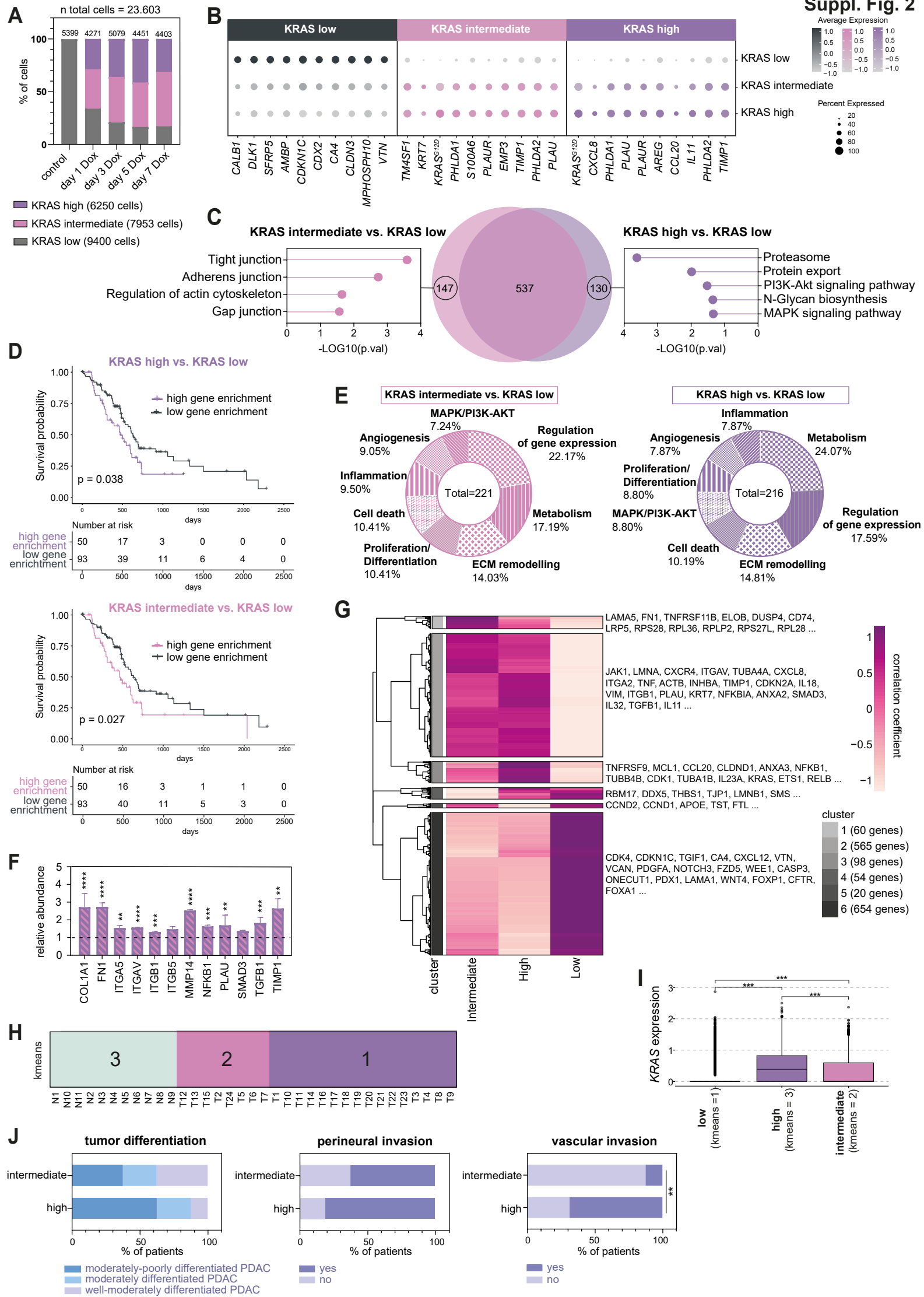
(F) Scatter plot showing correlation between *KRAS*^{G12D} expression and module scores of different pathways over the time of Dox treatment within the scRNA-seq data set. R = Pearson's correlation coefficient.

(G) Cell cycle analysis of PDLOs after indicated time-points of Dox treatment assessed by EdU quantification via flow cytometry. n = 4.

(H) Scatter plot showing correlation between *MKI67* expression and module scores for senescence-related genes over the time of Dox treatment within the scRNA-seq data set. R = Pearson's correlation coefficient.

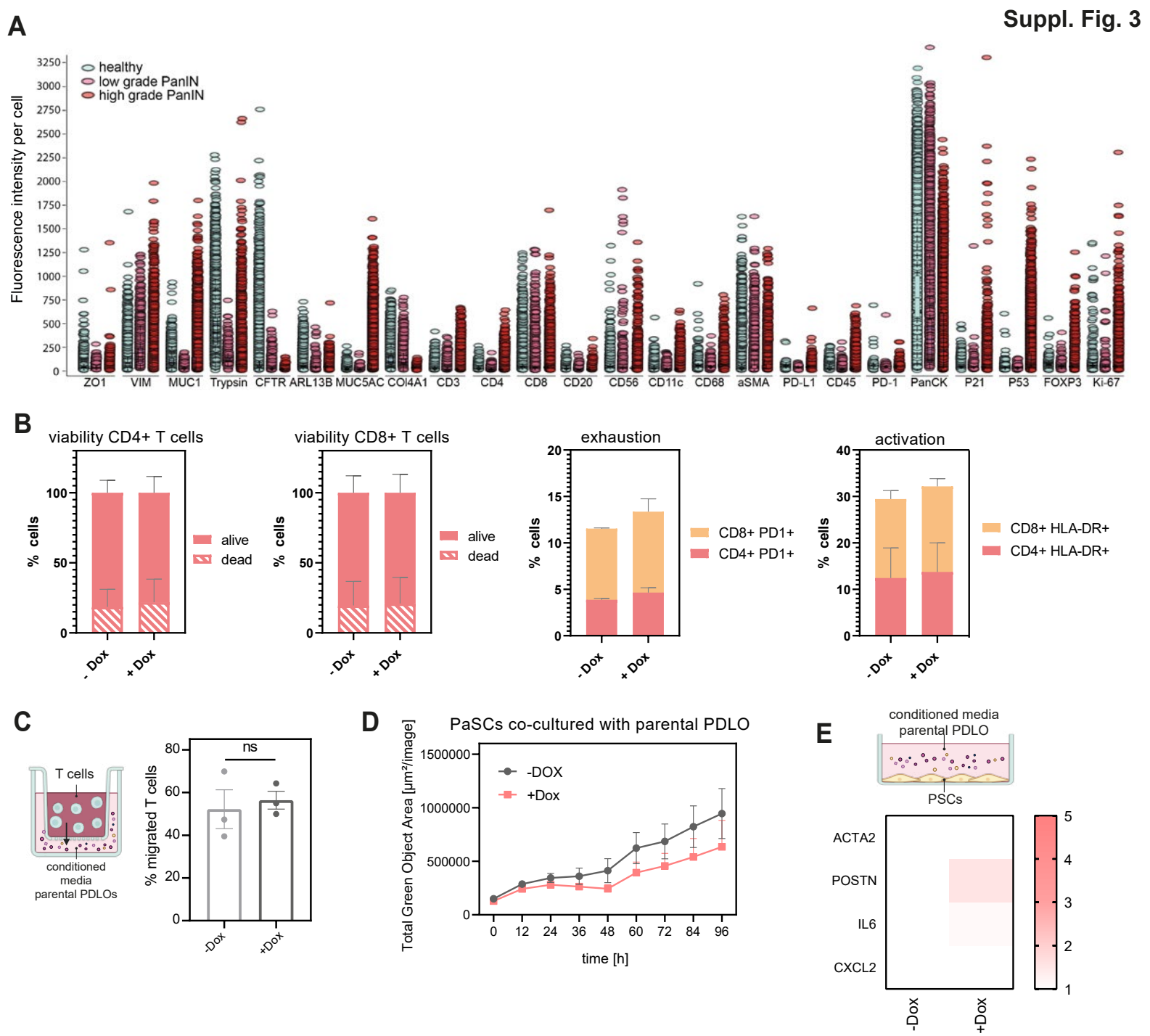
(I) PCA plot demonstrating variance of chromatin accessibility between ATAC-seq samples.

(J) Heatmap showing mean gene expression of TFs, overlapping in ATAC- and scRNA-seq.



Supplemental Figure 2

- (A) Proportion of the KRAS clusters within the individual time points of the scRNA-seq analysis.
- (B) Expression of the top 10 DEGs from each KRAS cluster compared to all other clusters.
- (C) Overlapping and unique DEGs of KRAS^{high} and KRAS^{intermediate} compared to the KRAS^{low} cluster with corresponding KEGG pathway analysis of unique DEGs.
- (D) Overall survival of TCGA-PAAD patients grouped based on KRAS cluster DEGs by gene set variance analysis (GSVA).
- (E) Pie charts showing the distribution of grouped GO terms from DEGs of KRAS^{high} and KRAS^{intermediate} compared to KRAS^{low}.
- (F) Bar plot showing the relative abundance of proteins after 7 days of Dox treatment. Mean \pm SD, n=1 in triplicates. Unpaired t-test **, p<0.01, ***, p<0.001; ****, p<0.0001.
- (G) Heatmap showing correlation coefficients of KRAS deep learning classifiers.
- (H) K-means grouping of healthy (N) and PDAC samples (T) from Peng et al.^[33] based on KRAS deep learning classifiers.
- (I) Box plot showing *KRAS* expression of PDAC samples from Peng et al.^[33] grouped by k-means based on KRAS deep learning classifiers. Wilcoxon test ***, p<0.0001.
- (J) Bar plots showing tumor differentiation (left), perineural invasion (middle), and vascular invasion (right) of PDAC samples from Peng et al. [33] grouped by k-means based on KRAS deep learning classifiers. Chi-square test **, p<0.01.



Supplemental Figure 3

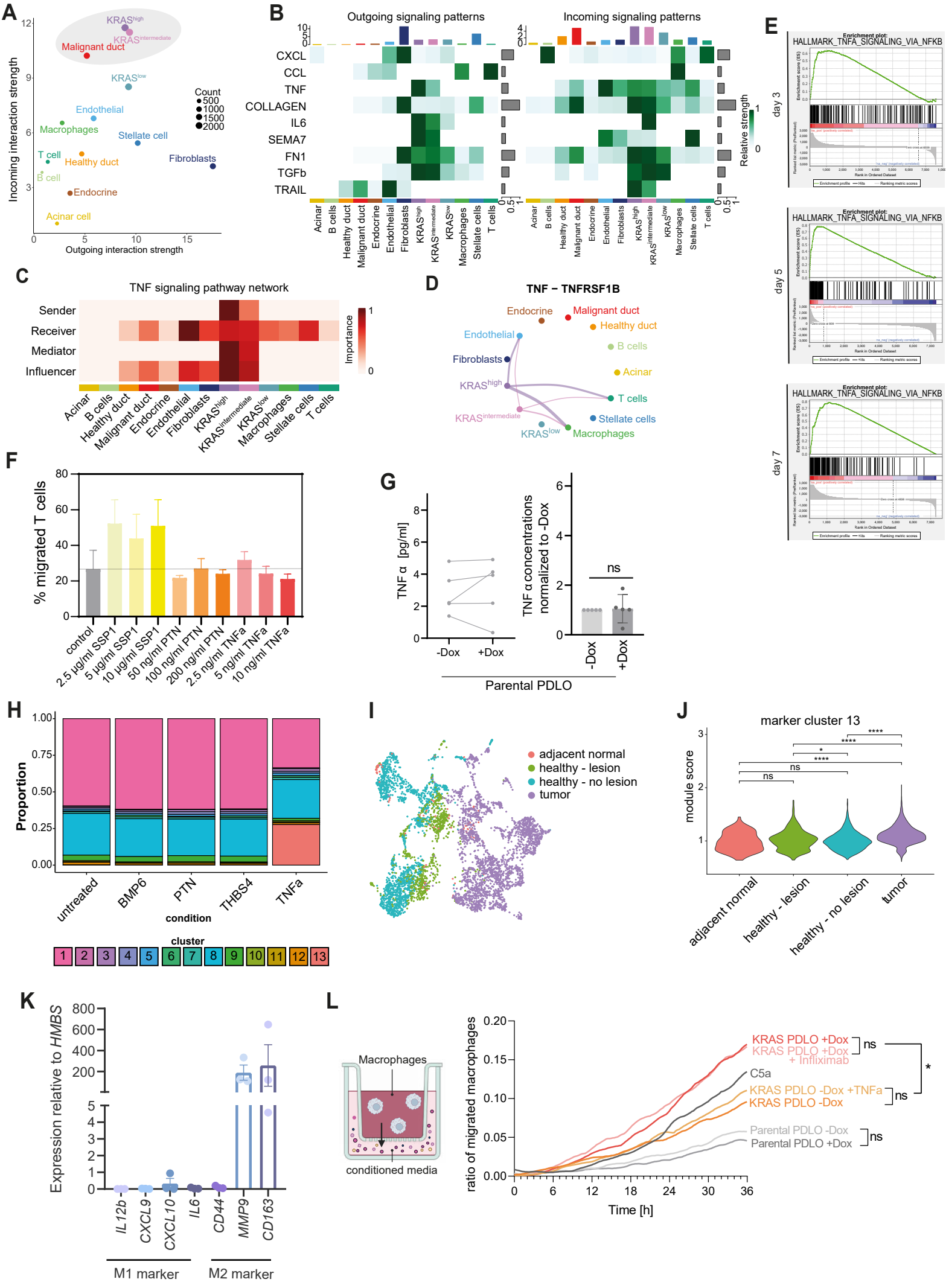
(A) Fluorescence intensities of all markers after initial background subtraction on human PanIN lesions subjected to 24-plex immunofluorescence staining.

(B) Percentage of viable, exhausted, and activated T cells after culturing in PDLO conditioned media for 72 h determined by flow cytometry. Mean \pm SD, n=2.

(C) Percentage of T cells migrating towards PDLO conditioned media derived from a control line without KRAS^{G12D} construct (parental PDLOs). Mean \pm SEM of 3 independent experiments. Unpaired t-test ns, p>0.05.

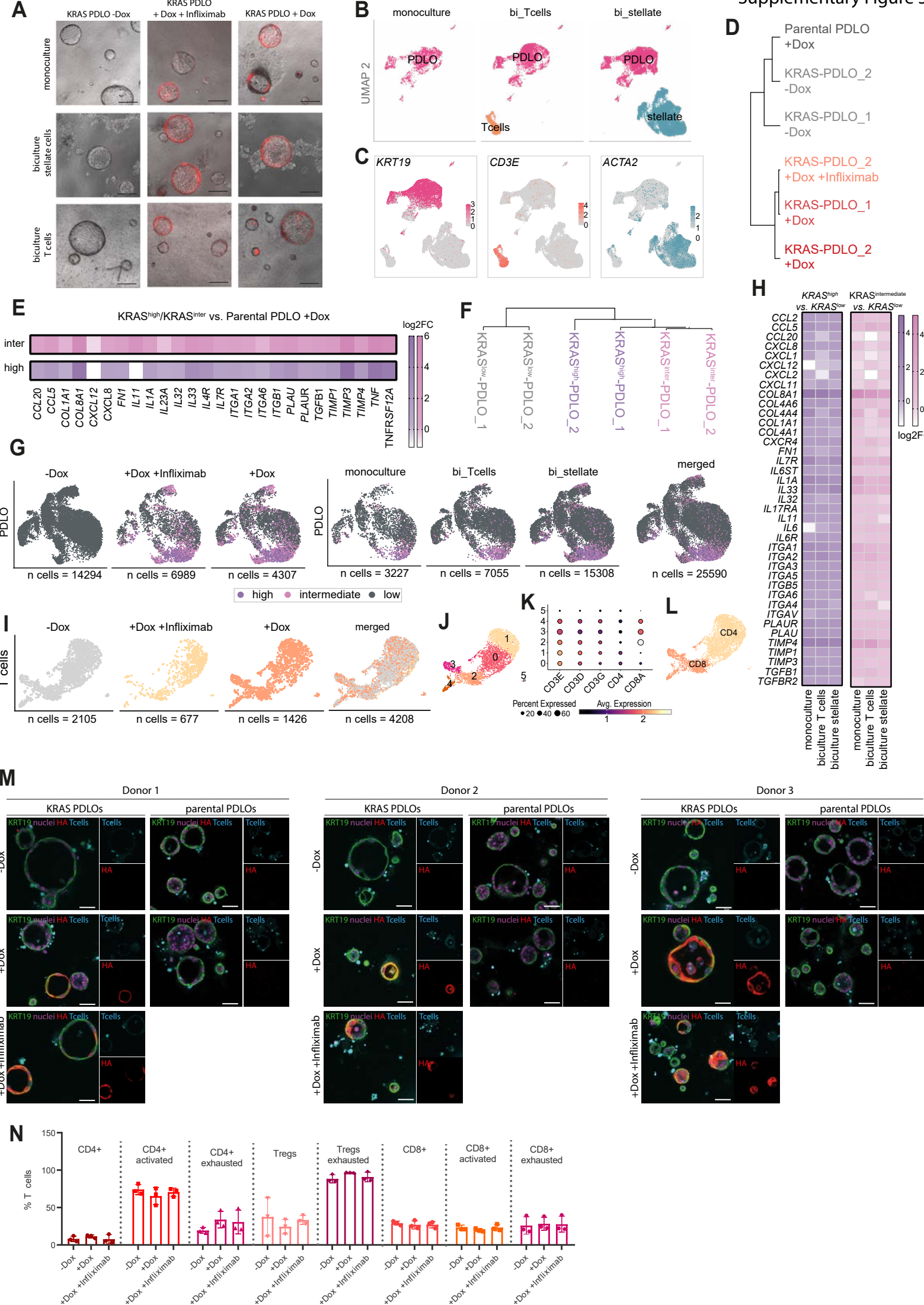
(D) Representative images of parental PDLOs co-cultured with GFP-labeled pancreatic stellate cells (PaSCs) and respective quantification of the total green area over time of live-cell imaging. Mean \pm SEM, n=3. Unpaired t-test ***, p<0.001; ****, p<0.0001. Scale bar = 400 μ m.

(E) Relative expression of CAF-related marker genes in PaSCs after culturing in parental PDLO conditioned media for 72 h. The mRNA expression was normalized to *GAPDH*, and expression is shown relative to the untreated conditions (-Dox). Mean, n=2



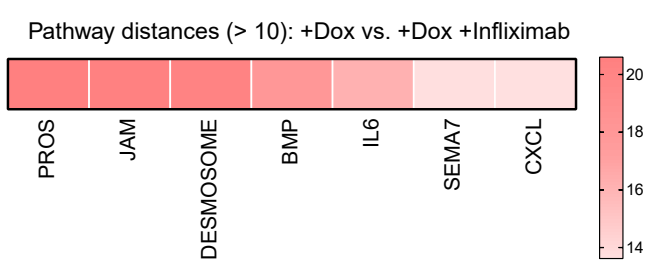
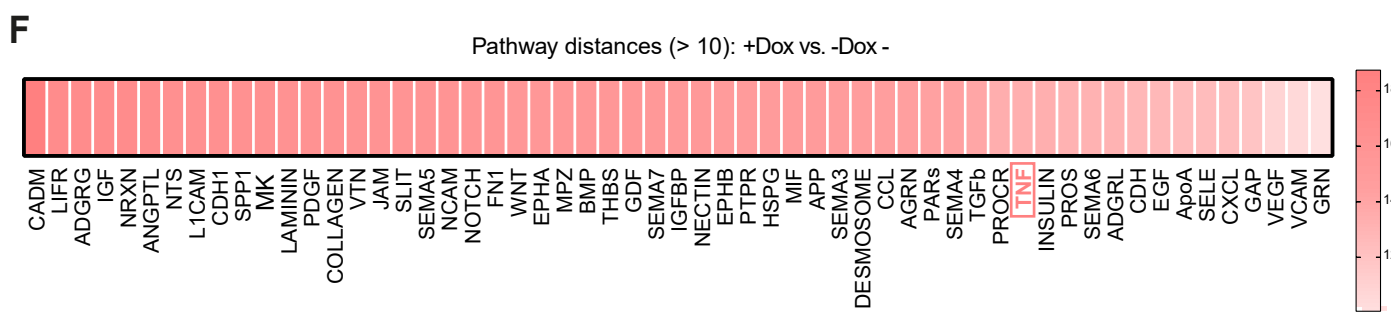
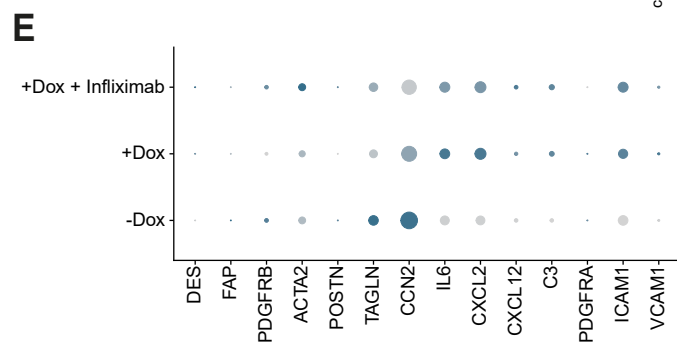
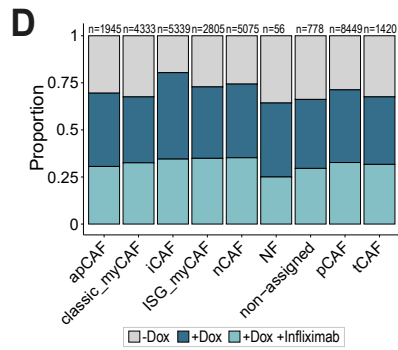
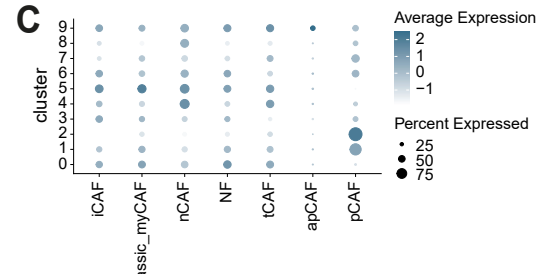
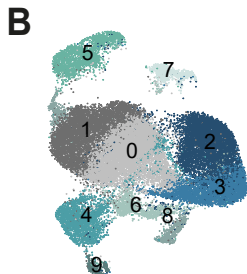
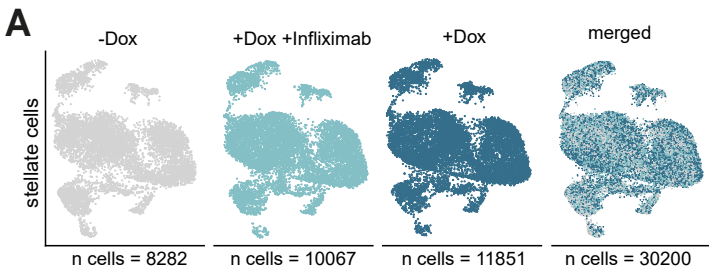
Supplemental Figure 4

- (A) Scatter plot showing predicted interaction strengths of different cell types of PDAC samples from Peng et al.^[33] and PDLOs.
- (B) Signaling patterns of selected pathways in different cell types of PDAC samples from Peng et al.^[33] and PDLOs.
- (C) TNF signaling patterns in different cell types of PDAC samples from Peng et al.^[33] and PDLOs.
- (D) Circle plot showing predicted interaction strengths of the ligand-receptor pair TNF-TNFRSF1B between different cell types of PDAC samples from Peng et al.^[33] and PDLOs.
- (E) GSEA plots showing enrichment of DEGs of PDLOs from different time points of the scRNAseq analysis compared to the -Dox control against the HALLMARK_TNF_SIGNALING_VIA_NFKB gene set.
- (F) Percentage of T cells migrated towards reduced T cell media with the addition of SSP1, PTN or, TNF α at indicated concentrations. Mean \pm SD, n=2.
- (G) TNF α concentrations in parental PDLO-derived conditioned media from 5 independent pancreatic differentiation experiments (left) and relative levels normalized to the untreated -Dox control (right). Mean \pm SD. Paired t-test ns, p>0.05
- (H) Bar graph showing distribution of t-SNE clusters of murine fibroblasts, untreated or treated with respective ligands for 72 h, before being subjected to CyTOF analysis.
- (I) UMAP showing fibroblasts of a reanalyzed scRNA-seq data set from Carpenter et al.²⁰.
- (J) Violin plot showing module score of CyTOF cluster 13 markers in fibroblasts of healthy and PDAC samples from from Carpenter et al.²⁰ Unpaired t-test ****, p<0.0001, *, p<0.05, ns, p>0.05
- (K) Expression of M1- and M2-polarisation marker genes relative to *HMBS* in macrophages.
- (L) Chemotactic migration of human macrophages towards PDLO-derived conditioned medium under the indicated conditions. C5a served as positive control. The cell count on the bottom side of the membrane (migrated cells), normalized to the initial top-side value (all cells after seeding), is plotted over time. Statistical significance was determined by ratio-paired t-test on the areas under the respective curves.* p < 0.05.



Supplemental Figure 5

- (A) Brightfield images of PDLO co-cultures used as input for scRNA-sequencing. Scale bar = 250 μm .
- (B) UMAP of cell types within the different co-culture conditions.
- (C) UMAP of cell-type-specific marker expression.
- (D) Cluster tree plot illustrating transcriptional similarity between PDLOs of time-series scRNA-seq data set (KRAS-PDLO_1), PDLOs of co-culture scRNA-seq data set (KRAS-PDLO_2) and Dox-treated parental PDLOs based on treatment conditions.
- (E) Heatmap showing log₂FC values of selectively chosen ECM and inflammation-related genes from DEGs between KRAS^{high} and KRAS^{intermediate} clusters from both time-series and co-culture scRNA-seq data sets compared to parental PDLOs + Dox.
- (F) Cluster tree plot illustrating transcriptional similarity between PDLOs of time-series scRNA-seq data set (KRAS-PDLO_1) and PDLOs of co-culture scRNA-seq data set (KRAS-PDLO_2) based on KRAS clusters.
- (G) UMAP of the PDLO subset colored by KRAS groups and split by treatment condition (left) and co-culture condition (right). The samples from the monocultures were only included in one of two sequencing runs and therefore show overall lower cell numbers (see the Methods section for details).
- (H) Heatmap showing log₂FC values of selectively chosen ECM and inflammation-related genes from DEGs between KRAS^{high} and KRAS^{intermediate} clusters compared to KRAS^{low} for the different co-culture conditions.
- (I) UMAP of the T cell subset split by the treatment condition.
- (J) UMAP of the T cell subset colored by Louvain clusters.
- (K) Dot plot of the T cell subset showing the expression of T cell-specific marker genes within the respective Louvain clusters.
- (L) UMAP of the T cell subset colored by the annotated CD4 and CD8 subpopulations.
- (M) Immunofluorescence images of PDLOs co-cultured with T cells for 72h. Scale bar = 50 μm .
- (N) Quantification of T cell subpopulations after direct co-culture with PDLOs assessed by flow cytometry. n = 3 T cells donors.



Supplemental Figure 6

(A) UMAP of stellate cell subset split by the different treatment conditions.

(B) UMAP of the stellate cell subset colored by the Louvain clusters.

(C) Dot plot of the stellate cell subset grouped by the Louvain clusters showing the module scores for different CAF subpopulations using the top 100 DEGs from Toa et al[39]

(D) Proportions of different CAF-subtypes within the stellate cells annotated by module scores using the top 100 DEGs from Toa et al.^[39]

(E) Dot plot representing the average expression of canonical CAF marker in the different conditions.

(F) Pathways from cell-cell communication analysis with a Euclidean distance greater than 10 based on their functional similarities.