Supplemental figure legends

Figure S1: The DYRK1B kinase is enriched in PDAC tumor cells.

A Genomic data on *DYRK1B* from the TCGA data set (www.cbioportal.org). **B** Kaplan-Meier plot depicting *DYRK1B*-associated overall survival of PDAC patients (n=146 patients, TCGA cohort, scan split, logrank test). **C** Single cell RNA sequencing results demarcating *DYRK1B*-expressing cells in human PDAC [1]. **D** Cell type-resolved *DYRK1B* mRNA expression (summarized reads) in human PDAC. Tumor cells are represented as "Ductal cell type 2"; normal ductal cells are named "Ductal cell type 1" [1]. **E** DYRK1B IHC of allografted WT/KO5.3 tumor tissue demonstrating antibody specificity. Mouse skeletal muscle was included as positive control for strong DYRK1B expression. Scale bar: 100 µm.

Figure S2: DYRK1B blockade facilitates PDAC cell proliferation.

A Western blot of *Dyrk1b* WT/KO cells showing the levels of DYRK1A/B protein. Shown is one representative blot of n=2. **B** Growth of WT/KO mKpc4 cells in soft agar (number of counted colonies present, n=1 performed in technical triplicate, mean ±SD). **C** Representative flow cytometry plots (related to figure 2D) of BrDU and propidium iodide (PI) labelling of mKpc4 cells. Left low population (violet) – cells in G1-phase; middle population (green) – BrDU-positive cells in S-phase; right low population (pink) – cells in G2-phase. **D** Representative flow cytometry plots (related to figure 2E) of mKPC4 cells treated with DMSO as solvent or 1 µM AZ191 and stained with anti-BrDU antibody and propidium iodide. Left low population – cells in G1-phase; middle population – BrDU-positive cells in S-phase; right low population – cells in G2-phase. **E** WB showing changes in mTOR, AKT and S6 phosphorylation in *Dyrk1b* WT/KO mKpc4 cells. Shown is one representative blot of n=2.

Figure S3: Transcriptomic changes in *Dyrk1b*-KO tumors.

A Tumor size changes over time (subcutaneous allograft growth in C57BL/6 mice). Animals received either *Dyrk1b*-WT (black curve) or KO (clone 2.7; red curve) mKpc4 cells on day 0. Shown is the mean ±SEM. **B** Downregulated gene signatures in *Dyrk1b*-KO tumors (vs *Dyrk1b*-WT tumors). **C** F4/80 IHC of WT/KO2.7 allograft tumors as depicted in panel A. Scale bar: 100 µm. **D** Myeloperoxidase (MPO) staining of tumor-associated neutrophils (TANs) in WT/KO tumor tissue. Human PDAC was included as positive control (the antibody recognizes mouse and human MPO, see e.g. [2]). Scale bar: Left/middle panel 100µm; right panel 200 µm. **E** Quantification of IF imaging of WT/KO5.3 allograft tumor depicting F4/80-TNF double-positive Mphs (M1 TAMs). Mean of several viewing areas from four animals each ±SD (1-tailed t-test). **F** F4/80-CD206 IF of WT and KO5.3 tumor tissue (M2 TAMs). Scale bar: 30 µm. **G**

Quantification of IF imaging as depicted in panel F. Mean of several viewing areas from four animals each ±SD (1-tailed t-test). **H-J** Macrophage marker gene expression (bulk RNAseq, in CPM) in WT/KO5.3 allograft tumors. Each dot represents one tumor (mean ±SD). **K** F4/80-Ki67 IF of WT and KO5.3 tumor tissue to assess proliferating Mphs. Scale bar: 30 µm. **L** Quantification of IF imaging as depicted in panel K. Mean of several viewing areas from five animals each ±SD (1-tailed t-test). **M** Quantification of pan-Cytokeratin (CK)/Ki67 IF imaging (not shown) to assess proliferating cancer cells. Mean of several viewing areas from five animals each ±SD (1-tailed t-test). **N** Flow cytometric determination of CD4/IFNγ double-positive T-cells in WT/KO5.3 allograft tumors. Each dot represents one tumor (mean ±SD). **O** Flow cytometric determination of CD4/TNF double-positive T-cells in WT/KO5.3 allograft tumors. Each dot represents one tumor (mean ±SD).

Figure S4: A *Dyrk1b*-controlled cancer cell secretome affects macrophage functions.

A-C Relative mRNA expression determined by qRT-PCR of M1-like (A-B) and M2-like (C) genes in mouse BMDM either untreated or stimulated with SN collected from WT or KO mKpc4 cells. Shown is one representative of n=3-6 measured in triplicate (mean ±SD). **D** Western blot depicting DYRK1B protein levels in Panc1 cells stably expressing a non-targeting control shRNA (shCon) or *DYRK1B*-targeting shRNA. The latter included the clones #9 (generated with sh*DYRK1B*_1) and #7 (generated with sh*DYRK1B*_3). Actin was used as loading control. Shown is one representative of n=2 western blots. **E** WB of cells in panel D depicting DYRK1A protein levels. Shown is one representative of n=2 western blots. **F** WB of cells in panel D depicting phosphorylated levels of mTOR, AKT and S6. Shown is one representative of n=2 western blots. **G-I** Relative mRNA expression determined by qRT-PCR of M1-like (G-H) and M2-like (I) genes in human PBMC-derived macrophages either untreated or stimulated with SN from control (shCon) or *DYRK1B*-KD clones #7 and #9. Shown is one representative of n=3 measured in triplicate (mean ±SD). **J** Migration of human PBMC-derived macrophages towards plain 0.5%-containing medium (no CM) or medium containing 50% of SN from control (shCon) Panc1 cells or from *DYRK1B*-KD clones #7 and #9. Shown is one representative of n=3 measured in triplicate wells (±SD).

Figure S5: DYRK1B-dependent impact of cancer cells on macrophage phagocytosis.

A Representative flow cytometry plots depicting the phagocytic capability of BMDM to engulf either WT or KO mKpc4 cells. BMDM are labelled in green, tumor cells are in deep red. **B** Relative phagocytosis of control (shCon) or *DYRK1B*-KD Panc1 cells by human PBMC-derived macrophages phagocyte as percentage of double-positive macrophages. Mean of n=3 ±SD. **C** Representative flow cytometry plots of data shown in B. Macrophages are labelled in green, and tumor cells are in deep red. **D** Relative

phagocytic capability of BMDM to engulf mKpc4 cells treated with either DMSO or 1 µM AZ191 for 96 h. Results are presented as fold change in percentage of double-positive macrophages. Mean of n=5 ±SD. **E** Representative flow cytometry plots of data shown in D. Macrophages are labelled in green, and tumor cells are in deep red. **F** Relative phagocytic capability of human PBMC-derived macrophages to engulf Panc1 cells treated with either DMSO or 1 µM AZ191 and presented as fold change in percentage of double-positive macrophages. Mean of n=3 ±SD. **G** Representative flow cytometry plots of data shown in F. Macrophages are labelled in green, and tumor cells are in deep red. **H** Relative phagocytic capability of human PBMC-derived macrophages treated with conditioned media from either control (shCon) or *DYRK1B*-KD towards Panc1 cells. Shown is one representative of n=2 measured in triplicate (±SD) (Paired two-tailed t-test). **I** Representative flow cytometry plots of mean fluorescence intensity (MFI) of surface CD24 on WT and KO mKpc4 cells (relates to figure 5G). **J** Western blot depicting absolute CD24 protein levels in mKpc4 cells treated with DMSO or 1 µM AZ191 for 48 h. Actin was used as loading control. Shown is one representative blot of n=2. **K** Relative mean fluorescence intensity (MFI) of surface CD24 on mKpc4 cells treated either with DMSO or with 1 µM AZ191 for 96 h. Mean of n=3 ± SD. **L** Representative flow cytometry plots of mean fluorescence intensity (MFI) of surface CD24 on mKpc4 cells treated either with DMSO or with one of the following DYRK1B inhibitors: 1 µM AZ191, 1 µM Harmine, 1 µM Indy. Relates to Fig. S5K. **M** Relative mRNA expression of *Cd24* determined by qRT-PCR in mKpc4 cells treated either with DMSO or with 1 µM AZ191 for 48 h. Mean n=3 ±SD. **N** Representative flow cytometry plots of mean fluorescence index (MFI) of surface CD24 on control (shCon) Panc1 cells and *DYRK1B*-KD clones #7 and #9. Relates to figure 5L. **O** Relative mRNA expression of *CD24* determined by qRT-PCR in control (shCon) or *DYRK1B*-KD Panc1 cells. Mean of n=3 ±SD.

Figure S6: A DYRK1B-directed therapy extends survival in an autochthonous mouse model of PDAC. **A.** Kaplan-Meier overall survival curve of KPC mice treated with vehicle (black line, n=13) or with 70 mg/kg once weekly Gemcitabine (red line, n=6) (logrank test). **B** Kaplan-Meier overall survival curve of KPC mice treated with vehicle (black line, n=13) or with twice weekly 5 mg/kg AZ191 (red line, n=5). **C** F4/80 IHC of KPC animals treated with solvent or AZ191 (treated until humane endpoint). Scale bar: 100 µm. **D** Quantification of IHC as depicted in panel C. Each dot represents one tumor (mean ±SD). **E** Kaplan-Meier overall survival curve of KPC mice treated with solvent (black line, n=13) or with a combination of KU0063794 (KU; 5 mg/kg; twice weekly) and AZ191 (AZ; 5 mg/kg; twice weekly) (red line, n=5). **F** Kaplan-Meier overall survival curve of KPC mice treated with solvent (black line, n=13) or with a combination of Gemcitabine (70 mg/kg once weekly) plus AZ191 (5 mg/kg; twice weekly) (red line, n=3).

Figure S7: Intra-tumoral macrophage abundance correlates with DYRK1B levels in human PDAC patients.

A Correlation between *CD86* and *DYRK1B* bulk mRNA expression in PDAC patients of the TCGA cohort. **B** Correlation between *HLA-DRA* and *DYRK1B* bulk mRNA expression in PDAC patients of the TCGA cohort. **C** Correlation between *MSR1* and *DYRK1B* bulk mRNA expression in PDAC patients of the Yang cohort [3]. **D** Correlation between *CD80* and *DYRK1B* bulk mRNA expression in PDAC patients of the Yang cohort. **E** Correlation between *CD86* and *DYRK1B* bulk mRNA expression in PDAC patients of the Yang cohort.

References

1. Peng, J.; Sun, B.-F.; Chen, C.-Y.; Zhou, J.-Y.; Chen, Y.-S.; Chen, H.; Liu, L.; Huang, D.; Jiang, J.; Cui, G.-S.; et al. Single-cell RNA-seq highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal adenocarcinoma. *Cell Res.* **2019**, *29*, 725–738, doi:10.1038/s41422-019-0195-y.
2. Awasthi, D.; Chopra, S.; Cho, B.A.; Emmanuelli, A.; Sandoval, T.A.; Hwang, S.-M.; Chae, C.-S.; Salvagno, C.; Tan, C.; Vasquez-Urbina, L.; et al. Inflammatory ER stress responses dictate the immunopathogenic progression of systemic candidiasis. *J. Clin. Invest.* **2023**, *133*.
3. Yang, S.; Tang, W.; Azizian, A.; Gaedcke, J.; Ströbel, P.; Wang, L.; Cawley, H.; Ohara, Y.; Valenzuela, P.; Zhang, L.; et al. Dysregulation of HNF1B/Clusterin axis enhances disease progression in a highly aggressive subset of pancreatic cancer patients. *Carcinogenesis* **2022**, *43*, 1198–1210, doi:10.1093/carcin/bgac092.