NFATC2 in pancreatic cancer-associated fibroblasts predicts treatment response and facilitates ERBB-targeted therapies

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SUMMARY

Pancreatic ductal adenocarcinoma (PDAC) remains a lethal malignancy, with therapeutic resistance
influenced by a dense desmoplastic stroma dominated by cancer-associated fibroblasts (CAF). Using
single-cell RNA-sequencing and gene regulatory network modeling of 42 PDAC tumors, we identified a
CAF subpopulation characterized by elevated NFATC2 expression that is enriched in patients with
improved therapeutic response and survival. NFATC2+ CAFs exhibited tumor-suppressive features,
including enhanced apoptotic signaling and suppression of ERBB pathway activity. Co-culture
experiments demonstrated that NFATC2+ CAFs restrain pancreatic cancer cell growth and enhance
chemotherapy-induced apoptosis, increasing sensitivity to standard-of-care chemotherapy regimens and
synergizing with ERBB-targeted therapies. The favorable effect of NFATC2+ CAFs on chemotherapy
response was validated in two other PDAC cohorts and in rectal cancer. Together, these findings identify
NFATC2+ CAFs as a therapy-conditioned stromal state linked to improved treatment response and
uncover a context-dependent vulnerability within the tumor microenvironment that may be exploited to
rationally optimize combination therapies.
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29 **KEYWORDS**

30 Pancreatic cancer, neoadjuvant chemotherapy, treatment response, cancer-associated fibroblasts.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most treatment-refractory human malignancies[1,2], its dismal outcome reflecting aggressive tumor biology, lack of effective early detection strategies, and profound resistance to existing therapies[3-5]. Despite advances in surgery and systemic therapies including multi-drug chemotherapy regimens such as FOLFIRINOX[3,6], median overall survival for most patients remains less than one year, underscoring a critical unmet need for strategies that overcome therapeutic resistance. A defining feature of PDAC is its exceptionally dense, desmoplastic tumor microenvironment (TME), which not only limits drug delivery but also actively shapes tumor cell adaptation under therapeutic pressure[5,7-9]. Notably, there are currently no validated molecular biomarkers to stratify patients for existing therapies or guide rational combination approaches, highlighting the need to better understand stromal determinants of treatment response in PDAC.

The desmoplastic stromal characteristic of PDAC can comprise up to 90% of the total tumor mass[10-12]. Cancer-associated fibroblasts (CAF) are the predominant cellular population in stroma that play pivotal roles in tumor progression, metastasis, and therapeutic resistance[13-15]. CAFs create a tumor-supportive microenvironment through growth factor secretion, extracellular matrix (ECM) remodelling, angiogenesis facilitation, metabolic crosstalk and immune modulation. Because CAFs dominate the PDAC tumor mass and actively constrain drug delivery and immune infiltration, therapeutic responses in PDAC are inherently shaped by stromal biology. Thus, CAF-directed approaches may be particularly impactful in complementing therapies targeting malignant cells[16], and defining treatment-responsive CAF populations and their molecular regulators may reveal new targets for combination therapies that enhance treatment efficacy and overcome resistance.

Single-cell RNA sequencing (scRNA-seq) analyses have revealed substantial CAF heterogeneity within the PDAC TME[17-23] and, importantly, mapped the stromal transcriptional responses to chemotherapy[24]. Recent evidence highlights transcriptional adaptation as a major determinant of both cancer progression[25] and treatment outcome[26]. However, which CAF states actively contribute to therapy response and the transcriptional regulators that orchestrate CAF state transitions during PDAC progression remain largely unknown.

Transcriptional adaptation and cell-state transitions are controlled by transcription factors (TF), but their functional activity in the PDAC stroma is embedded within complex, dynamic regulatory networks that are not readily captured by gene expression alone. Gene regulatory network (GRN) approaches provide a powerful framework to infer TF activity and identify regulators of cell-state transitions[27]. While recent studies have linked stromal features to PDAC patient outcome and treatment response using machine-learning analyses of bulk RNA sequencing and histopathological images[28-30], GRN-methods have not yet been applied to interrogate therapy-induced stromal reprogramming at single-cell resolution, leaving TF-level mechanisms of CAF plasticity largely unexplored.

Here, using an integrated GRN and single-cell framework, we identify NFATC2 as a transcriptional regulator defining a treatment-responsive CAF state in PDAC. Our precision oncology pipeline integrates GRN inference and scRNA-seq analysis of treatment-naïve and neoadjuvantly treated PDAC tumors, followed by functional validation in human CAF models. NFATC2, a calcium-responsive TF of the nuclear factor of activated T cells (NFAT) family, was selectively expressed in a distinct subset of CAFs associated with improved treatment response and patient survival. The NFAT family TFs are best characterized for their roles in T cells, but have also been shown to regulate for example cell proliferation, differentiation, and apoptosis in non-immune cells, exerting either tumor suppressive or oncogenic roles depending on the cancer type and cellular context [31-33]. However, the specific role of NFATC2 in CAFs, and its contribution to PDAC progression and therapeutic response, remains largely unexplored.

Our results show that inducing NFATC2 expression in human CAFs impairs the growth of PDAC cells in co-culture systems and increases their sensitivity to chemotherapy and synergizes with ERBB-targeted inhibition. Together, these findings identify NFATC2 as a treatment-inducible regulator of CAF biology in PDAC and imply a stromal mechanism linked to therapeutic benefit. By defining a TF-driven CAF state associated with improved clinical outcomes, this study provides mechanistic insight into therapy-induced stromal reprogramming and highlights a targetable vulnerability within the PDAC TME.

RESULTS

Gene regulatory network inference identifies NFATC2 as a treatment-responsive transcription factor in PDAC

To implement a precision oncology platform for identifying key TFs mediating transcriptional adaptation during PDAC treatment, we integrated GRN inference analysis with functional validation using co-culture systems (Figure 1A). We analyzed single-nuclei RNA sequencing data from primary tumor samples of 42 PDAC patients, comprising a total of 224,988 cells[34]. Cells were classified into 20 major cell types (Figure 1B) and annotated according to patient treatment response based on the original dataset definitions (Figure 1C).

As a proof of concept, we first applied GRN inference using CellOracle[35] to identify cell identity-specific TFs in pancreatic epithelial cell types. Eigenvector centrality analysis (a measure of node importance within regulatory networks that highlights putative master TFs) revealed several cell type-specific TFs, including ONECUT1 (HNF6) and ESRRG in ductal cells, MECOM in acinar cells, and NR5A2 in both populations (Supplementary Figure 1A), consistent with enriched expression of ONECUT1 and MECOM in ductal and acinar populations, respectively (Supplementary Figure 1B). These findings align with established roles of these TFs in pancreatic lineage specification: we previously identified ONECUT1 as a master TF in direct conversion of human fibroblasts into pancreatic ductal-like epithelial cells[36,37], whereas MECOM is a known regulator of acinar-to-ductal lineage transitions[38,39]. To test the accuracy of the CellOracle predictions, we performed *in-silico* perturbation of MECOM, which induced a clear transition of acinar cells towards ductal identity when compared to random perturbation controls (Supplementary Figure 1C), reinforcing the validity of the inferred GRN.

Having validated GRN performance in epithelial lineages, we next applied this framework to identify key transcriptional regulators in stromal populations and to map their enrichment during chemotherapy treatment. GRN analysis between the ductal epithelial cells and the CAFs showed ductal cells being enriched for known pancreatic cell-specific TFs such as ONECUT1, ESRRG and NR5A2[40,41], whereas the CAFs were enriched for ZEB1 and NFATC2 (Figure 1D). While ZEB1 has been reported to be associated with epithelial to mesenchymal transition (EMT) in multiple cancers and is a known mesenchymal cell marker[42], the enrichment of NFATC2, a T-cell-associated TF, was particularly interesting. UMAP analysis showed that NFATC2 was almost exclusively expressed in CAFs (Figure 1E). Importantly, NFATC2 had the highest eigenvector centrality score in CAFs among the treated patients compared to untreated ones (Figure 1F), suggesting that neoadjuvant treatment may lead to NFATC2 expression in CAFs, whereas no such difference was observed for ZEB1.

To further support our findings on the transcriptional heterogeneity across the TME, we performed TF activity analysis across all cell types using SCENIC[43]. Hierarchical clustering revealed five distinct transcriptional modules (M1-M5) characterized by differential pathway enrichments (Supplementary Figure 1D). In module 5, NFATC2 emerged as a key factor associated with metabolic remodeling, TGF-β suppression, and cellular plasticity, suggesting its role in transcriptional reprogramming across the tumor ecosystem. Collectively, these results demonstrate the strength of GRN approaches in studying the transcriptional determinants of distinct cell populations in PDAC tumors and highlight NFATC2 as a treatment-responsive transcription factor in PDAC TME.

NFATC2 defined a treatment-responsive CAF state linked to TME remodeling and improved outcome in PDAC

To further characterize the role of NFATC2 in CAFs and its association with therapeutic response in PDAC, we focused on single-cell analysis of stromal populations. Based on established markers[7,17], we identified four distinct stromal subpopulations: inflammatory CAFs (iCAFs), myofibroblastic CAFs (myCAFs), pericytes, and vascular smooth muscle cells (vSMC; Figure 2A & Supplementary Figure 2A). In addition, we identified a CAF subcluster characterized by strong upregulation of NFATC2 (Figure 2A). These NFATC2-positive CAFs (NFATC2+ CAFs) constituted a subset within the iCAF population, concomitantly showing downregulation of myCAF markers such as MYH11 and aSMA (ACTA2)[44,45] (Figure 2A, Supplementary Figure 2A & B). Stratification of stromal cells by treatment response revealed higher NFATC2 expression in tumors from patients showing better responses (moderate and minimal

response groups) compared to untreated patients and those with poor response (Figure 2B).
Consistently, density mapping demonstrated enrichment of the NFATC2+ CAF subpopulation in
responders, whereas this population was markedly reduced in poor responders and almost undetectable
in tumors of untreated patients (Supplementary Figure 2C).

In parallel with increased abundance of NFATC2+ CAFs and higher NFATC2 expression levels in
treatment responders, therapeutic response was further associated with shifts in the cellular composition
of the TME. Tumors from the treatment-responding patients exhibited higher relative proportions of
normal epithelial cells, iCAFs, CD8+ T cells, and myeloid cells compared to poor responders and
untreated patients, whereas the relative proportions of malignant cells and myCAFs were reduced (Figure
2C). Together, these findings suggest a broad therapy-associated remodeling of the tumor ecosystem,
rather than changes restricted to the CAF compartment, consistent with previous single-cell and spatial
analyses of neoadjuvant-treated PDAC[34].

NFATC2 expression in CAFs also correlated with favorable clinical features, including smaller tumor size
and reduced lymph node involvement (lower T and N stages; Supplementary Figure 2D), and was highest
in patients who had received neoadjuvant FOLFIRINOX-based chemoradiotherapy (CRT), compared to
treatment-naïve patients and those receiving other neoadjuvant therapies (Supplementary Figure 2D). At
the transcriptional level, the expression of marker genes associated with a recently described reactive
subTME linked to chemosensitivity[20] were increased in NFATC2+ CAFs compared to iCAF and myCAF
populations, whereas the markers for deserted subTME were reduced (Supplementary Figure 2E).
Importantly, stratification based on NFATC2 expression in CAFs revealed significantly prolonged
progression-free survival in patients with high NFATC2 levels (Figure 2D), with concordant improvements
in overall survival in the TCGA PDAC cohort (Supplementary Figure 2F), suggesting a protective,
treatment-associated role for NFATC2 in PDAC.

To validate the clinical relevance of our findings in another patient cohort, we performed
immunohistochemical analysis of NFATC2 expression in a tissue microarray comprising PDAC tumor
samples with documented chemotherapy response. Consistent with our scRNA-seq observations,
NFATC2+ CAFs were rare in treatment-naive tumors but became enriched in post-treatment samples
(Figure 2E & Supplementary Table 1). Importantly, NFATC2 positivity in stromal compartments correlated
strongly with treatment response, with good responders showing higher percentages of NFATC2+ CAFs
(~40%) compared to poor responders (~5%) (Figure 2F). This clinical validation supports the translational
relevance of NFATC2 as a stromal biomarker of treatment response in PDAC.

To understand the downstream signaling pathways associated with NFATC2+ CAFs, we performed Gene
Ontology enrichment analysis for differentially expressed genes across all stromal cell types (Figure 2G &
Supplementary Table 2). NFATC2+ CAFs showed enrichment for pathways related to regulation of
extrinsic apoptotic signaling and negative regulation of ERBB signaling, whereas iCAFs were associated
with complement activation. Other CAF types were enriched for pathways related to ECM organization
and muscle system processes.

Collectively, these results identify NFATC2+ CAFs as a treatment-responsive stromal state in PDAC that
is linked to improved patient survival and treatment outcome, enhanced apoptotic signaling, and
suppression of ERBB pathways, suggesting that NFATC2 marks a stromal state associated with therapy-
responsive remodeling of the PDAC TME.

NFATC2 expression in CAFs limits PDAC cell growth and enhances apoptosis

To directly test the functional role of NFATC2 in CAFs, we established an optimized in vitro co-culture
system using fluorescently labelled PANC1 pancreatic cancer cells expressing mCherry and human
pancreatic CAFs (pCAF) expressing EGFP. Because pCAFs do not endogenously express NFATC2, we
generated pCAFs with stable NFATC2 expression (NFATC2+ pCAFs; Figure 3A & Supplementary Figure
3A) and optimized CAF-to-cancer cell co-culture ratios based on previous reports[45-47]. pCAF and cancer
cells cultured in a 3:1 ratio yielded stable co-culture conditions without either cell type outcompeting the
other over time (Supplementary Figure 3B&C) and was used for all subsequent experiments.

PANC1 cells co-cultured with NFATC2+ pCAFs exhibited markedly altered growth dynamics compared to
those co-cultured with wild-type (WT) CAFs: NFATC2+ pCAF co-cultures resulted in reduced cancer cell
expansion within 48 hours (Figure 3B, top). Treatment of the co-cultures with the standard-of-care
chemotherapy regimen FOLFIRINOX further strengthened this effect, resulting in a marked reduction in
cancer cell numbers in NFATC2+ pCAF co-cultures compared to WT controls (Figure 3B, bottom).
Quantification by fluorescence-based cell counting and flow cytometry confirmed that NFATC2
expression in CAFs limits cancer cell growth, and that FOLFIRINOX treatment further enhances this
effect (Figure 3C&D). A similar response was observed in independent experiments using another
pancreatic cancer cell line MIA PaCa-2 (Supplementary Figure 3D&E), supporting the generalizability of
this effect.

Given that NFATC2+ CAFs were associated with apoptotic signaling in patient-derived single-cell
analyses, we next assessed whether NFATC2 expression in CAFs promotes cancer cell apoptosis in the
co-culture system. Flow cytometric analysis using Annexin V-FITC staining showed enhanced cancer cell
apoptosis in NFATC2+ pCAF co-cultures, particularly under FOLFIRINOX treatment (Figure 3E).
Together, these results establish that NFATC2 expression in CAFs attenuates pancreatic cancer cell
growth, enhances chemotherapy-induced apoptosis, and sensitizes cancer cells to FOLFIRINOX.

203 Transcriptional programs and paracrine effects underlying NFATC2-dependent CAF-cancer cell
204 interactions

To define transcriptional programs underlying the NFATC2-dependent phenotypes observed in co-culture
(Figure 3), we performed bulk RNA-seq under four complementary conditions: PANC1 cells co-cultured
directly with WT or NFATC2+ pCAFs, or exposed to conditioned medium from WT or NFATC2+ pCAF
cultures, each treated with either FOLFIRINOX or vehicle. Differential gene expression for each condition
was analyzed between NFATC2+ and WT samples (Supplementary Table 3-6).

Pathway-level analysis using Gene Set Variation Analysis (GSVA) revealed robust activation of
apoptosis-related programs, including Hallmark Apoptosis and p53 pathways in FOLFIRINOX-treated
PANC1 cells exposed to NFATC2+ CAF-conditioned medium (Figure 4A), consistent with enhanced
chemotherapy-induced DNA damage response. Both intrinsic (mitochondrial) and extrinsic (death
receptor) apoptotic pathways were induced by NFATC2+ CAFs, with extrinsic signaling further enhanced
by FOLFIRINOX (Figure 4B), consistent with increased measured apoptosis.

In direct CAF-PANC1 co-cultures, NFATC2 expression in CAFs was associated with prominent activation
of inflammatory pathways and immune response programs (Figure 4A). Upregulation of inflammatory
chemokines and cytokines (e.g. CXCL2/3/8, IL1A, IL1B, IL33) and immune signaling molecules (TREM1,
TNFSF9/15, PTGS2), together with downregulation of myCAF-associated genes (e.g. CXCL12,
COL11A1, MFAP5, MYLK) indicated NFATC2-dependent stromal reprogramming towards an iCAF
phenotype (Figure 4A & Supplementary Figure 4A & Supplementary Table 3&4). Interestingly, genes
linked to Ca^{2+}-signaling, such as GRIA1, were also up-regulated in NFATC2+ conditions (Supplementary
Figure 4A), suggesting a potential mechanism for calcium-dependent NFATC2 activation[48]. FOLFIRINOX
further reinforced inflammatory and stress-adaptive programs and was associated with enrichment of
immune response and T cell-related pathways, suggesting a more immune-permissive stromal
environment. Concordantly, NFATC2 expression correlated with reduced CXCL12 levels (Supplementary
Figure 4A), a stromal feature that has previously been linked to enhanced T-cell infiltration in pre-clinical
PDAC models[49]

Conditioned medium from NFATC2+ CAFs exerted a strong transcriptional effect on PANC1 cells,
particularly in the presence of FOLFIRINOX (Supplementary Table 5&6), indicating that NFATC2+ CAFs
modulate tumor cell responses, at least in part, through paracrine mechanisms. Notably, ERBB signaling
was strongly suppressed in FOLFIRINOX-treated, conditioned-medium-exposed PANC1 cells (Figure
4A), consistent with ERBB pathway downregulation observed in the patient-derived scRNA-seq data (c.f.
Figure 2F). Notably, the cytokine storm observed in direct co-cultures was absent in conditioned-medium–
treated PANC1 cells, indicating that inflammatory signals predominantly originate from the CAF
compartment.

Analysis of ECM programs revealed context-dependent NFATC2 effects. In direct CAF–PANC1 co-
cultures, NFATC2 expression was associated with attenuation of myCAF programs and induction of
dynamic, wound-healing–associated remodeling, marked by COL15A1, TNFAIP6, SERPINE2 and matrix-
modifying enzymes such as ADAMTS5 and CTSK (Supplementary Figure 4A). In contrast, conditioned-
medium-treated PANC1 cells showed a tumor-intrinsic response characterized by epithelial stabilization
and anchoring to the basement membrane, with upregulation of structural ECM scaffolding components
(HSPG2, LAMA5, COL18A1, COL8A2, COL5A3) and adhesion molecules, including factors maintaining
epithelial architecture such as SPINT2[50] (Supplementary Figure 4B). This basement-membrane-
associated epithelial containment, most pronounced under FOLFIRINOX treatment, provides a potential
mechanistic link between NFATC2 expression and improved therapeutic response.

To explore potential signaling mechanisms underlying these paracrine effects, we performed ligand–
receptor interaction analysis[51] using scRNA-seq data across CAF subtypes. NFATC2+ CAFs exhibited
distinct secretory profiles, including elevated expression of candidate factors such as MMP14 and
nicotinamide phosphoribosyltransferase (NAMPT) (Supplementary Figure 4C), suggesting potential
routes of CAF–cancer cell communication.

Having established broad functional and transcriptional effects of NFATC2+ CAFs on cancer cells, we
next investigated how this CAF state could arise within the TME. Because NFATC2+ CAFs were largely
absent in untreated condition but enriched following therapy, we asked whether treatment-associated
microenvironmental cues could induce NFATC2 expression in otherwise NFATC2-negative CAFs. Given
the well-established role of NFATC2 in T cells, we examined whether immune cells could promote
NFATC2 induction in pCAFs. While NFATC2 was undetectable in WT pCAFs cultured alone, robust
induction was observed upon co-culture with T cells (Figure 4C), with the strongest upregulation observed
in triple co-culture conditions involving WT pCAFs, T cells, and PANC1 cancer cells. Of note, these
quantitative PCR measurements were performed after removing the T cells from the co-cultures,
confirming that NFATC2 induction occurred within the CAF compartment. These findings suggest that
immune-derived signals represent one potential trigger for the emergence of NFATC2+ CAF states within
the TME, providing a potential link between treatment-associated immune activity and stromal
reprogramming.

Collectively, these results position NFATC2 as a regulator of CAF plasticity, linking immune-mediated
induction of NFATC2 to paracrine transcriptional programs that modulate cancer cell behavior and
chemotherapy response.

ERBB pathway and proliferative programs are suppressed in treatment-responsive cancer cells

The enrichment of extrinsic apoptosis pathway and negative regulation of ERBB signaling pathway in the
NFATC2+ CAFs from better-responding patients and NFATC2+ CAF-PANC1 co-cultures (c.f. Figures
2F&4A) prompted us to examine transcriptional changes within the cancer cell population of these
patients from the scRNA-seq data[34]. For this, we separated malignant cells from other epithelial cells
based on their copy number variation profiles (Supplementary Figures 5A-C) and performed
transcriptomic analyses of malignant cells stratified by neoadjuvant therapy response. This revealed
distinct transcriptional states based on neoadjuvant therapy response (Figure 5A).

Differential expression analysis identified genes associated with distinct treatment responses, with 72
genes consistently differentially expressed in moderate responders compared to each of the other
response groups (Figure 5B). Gene Ontology enrichment analysis of the shared genes revealed
significant enrichment of biological processes central for cancer cell growth and survival (Figure 5C),
including regulation of cell proliferation, ERBB signaling, and epidermal growth factor receptor signaling
pathways.

Gene Set Enrichment Analysis (GSEA) revealed significant negative enrichment of several cancer-
relevant gene sets in treatment-responsive tumors (Figure 5D). Notably, ERBB2 signaling was markedly
suppressed in moderate responders, consistent with reduced ERBB pathway activity observed in the
stromal compartment of better-responding tumors enriched for NFATC2+ CAFs. In addition, malignant
cells from moderate responders exhibited coordinated suppression of TGF-beta signaling, hypoxia
response, p53 pathway activity, and proliferation programs, all of which are well-established drivers of

tumor cell survival, plasticity, and therapy resistance[52]. These pathways are also central in CAF biology
and tumor-stroma crosstalk[16], providing an important link between transcriptional states in malignant cells
and NFATC2-associated stromal environment. In particular, TGF-β signaling is a primary driver of myCAF
differentiation and CAF activation across multiple cancer types[53], while hypoxia, a characteristic feature of
the PDAC TME, has been shown to promote iCAF phenotype through HIF-1α stabilization and cytokine-
mediated paracrine signaling[54].

Together, these results indicate that favorable therapeutic response in PDAC is associated with
coordinated suppression of proliferative and ERBB signaling programs in cancer cells, highlighting ERBB
pathway modulation as a potential vulnerability in treatment-responsive disease states.

299   ERBB-targeted combination therapies enhance chemotherapy efficacy in NFATC2+ pCAF co-
300   cultures

EGFR- and ERBB-targeted inhibitors have previously been tested in PDAC, but have shown no survival
benefit in patients[55] and only limited efficacy in pre-clinical models[56]. Our results from the PDAC patient
data demonstrated that the better responding tumors are characterized by NFATC2+ pCAFs, suppression
of ERBB signaling programs, and enhanced sensitivity to chemotherapy. This suggests that NFATC2
expression could mark treatment-responsive stromal contexts, potentially facilitating patient stratification
for adjuvant or combination therapies. This prompted us to investigate whether ERBB-targeted therapies
could potentiate standard-of-care chemotherapy in an NFATC2-positive stromal context.

For this, we performed a combinatorial drug testing using optimized PANC1-pCAF co-culture system
comparing the effects in WT and NFATC2+ pCAFs conditions. Standard chemotherapy regimens
(FOLFIRINOX or gemcitabine plus nab-paclitaxel) were administered alone or in combination with
clinically relevant ERBB-targeted inhibitors including trastuzumab (anti-ERBB2 (HER2) monoclonal
antibody), erlotinib (EGFR tyrosine kinase inhibitor), and neratinib (pan-ERBB inhibitor). Fluorescence
microscopy analysis under vehicle-treated conditions confirmed the growth-suppressive effect of NFATC2
expression in CAFs on PANC1 cells (c.f. Figure 3). While FOLFIRINOX alone reduced cancer cell
numbers, the addition of ERBB-targeting agents produced significantly greater cytotoxic effects,
particularly in NFATC2+ pCAF co-cultures (Figure 6A&B). The most pronounced effect was observed with
the FOLFIRINOX–trastuzumab combination. To exclude the possibility that these effects were driven by
baseline differences in cancer cell numbers between WT and NFATC2+ pCAF conditions, responses
were normalized to vehicle-treated controls. This analysis confirmed a synergistic interaction between
FOLFIRINOX and trastuzumab specifically in NFATC2+ co-cultures (Figure 6C). These findings were
further validated by flow cytometry–based quantification of cancer cell populations following combination
treatments (Figure 6D).

Collectively, these results demonstrate that the tumor-suppressive effects associated with NFATC2+ CAF
states can be exploited in preclinical models through rational combination strategies targeting the ERBB
signaling pathway. Overall, these findings suggest that NFATC2 expression in the tumor stroma may be
used for stratification of tumors that are more likely to benefit from ERBB-targeted therapies in
combination with standard chemotherapy.

329   Validation of NFATC2 effect on chemotherapy response in another PDAC cohort as well as in
330   rectal cancer

To validate and extend the link between NFATC2 expression and chemotherapy response, we analyzed
an independent scRNA-seq dataset from PDAC patients treated with neoadjuvant chemotherapy as an
external validation cohort[24]. UMAP analysis of CAF populations revealed distinct transcriptional states
associated with treatment status (Figure 7A) and treatment response (Figure 7B). In line with our findings
from the primary cohort, NFATC2 expression in CAFs was markedly higher in chemotherapy-treated
patients compared to treatment-naïve tumors (Figure 7C). Moreover, NFATC2 expression was enriched
in partial responders following neoadjuvant therapy compared untreated or progressive disease (Figure
7D), supporting treatment-associated activation of NFATC2-linked stromal programs.

To determine whether CAF-associated NFATC2 enrichment was accompanied by transcriptional changes
in malignant epithelial cells, we next examined pathway activity in cancer cells from the same cohort.
Pathway-level GSVA revealed stage-dependent transcriptional programs, with apoptotic signaling
preferentially enriched in advanced stages that typically receive more intensive chemotherapy (Figure
7E). Notably, negative regulation of EGFR and ERBB2 signaling pathways was also observed in
chemotherapy-treated stages, particularly in best-responding group (partial response cases), consistent
with our primary findings linking NFATC2+ pCAFs to suppression of ERBB signaling. Together, these
data support an association between NFATC2 expression in CAFs, attenuation of ERBB-related
programs in cancer cells, and enhanced treatment response.

To assess whether this association extends beyond pancreatic cancer, we analyzed a rectal cancer
cohort[57] treated with neoadjuvant FOLFIRINOX and stratified by treatment response. CAF clustering
revealed distinct transcriptional patterns associated with treatment status and response (Figure 7F&G).
Prior to therapy, NFATC2 expression levels were comparable across different response groups (Figure
7H), indicating that baseline NFATC2 expression does not predict therapeutic response. In contrast, post-
treatment analysis revealed a strong association between NFATC2 expression and treatment efficacy,
with the highest NFATC2-levels observed in complete responders and the lowest levels in non-
responders (Figure 7I). Consistent with the PDAC cohorts, GSVA analysis further demonstrated dynamic
regulation of apoptotic and ERBB-related pathways associated with treatment response and timepoint in
cancer cells (Figure 7J), with complete responders showing enhanced apoptotic signaling and negative
regulation of ERBB pathways following treatment.

To investigate the broader clinical relevance of NFATC2 beyond pancreatic cancer, we performed
survival analysis across different tumor types using TCGA datasets. Consistent with our observations in
PDAC (c.f. Supplementary Figure 2D), elevated NFATC2 expression was associated with improved
overall survival in several malignancies, including lung adenocarcinoma, breast cancer, and head-neck
squamous carcinoma (Supplementary Figure 6). These associations are concordant with our functional
data and support a tumor-suppressive role for NFATC2 in specific TME contexts.

Collectively, these independent validation analyses demonstrate that NFATC2 expression in CAFs is
induced by chemotherapy and consistently associates with favorable treatment response and conserved
transcriptional programs across two different cancer types treated with similar neoadjuvant regimens.
Together with our functional studies, these findings support NFATC2 as a stromal biomarker associated
with chemotherapy sensitivity and suggest that tumors enriched for NFATC2+ CAFs may represent
contexts in which ERBB-targeted therapies could be rationally combined with standard chemotherapy to
improve therapeutic efficacy while potentially reducing treatment-associated toxicity.

DISCUSSION

Cancers are organ-specific and driven by distinct mutational landscapes[58,59], prompting extensive efforts
to develop tumor type-specific targeted therapies. In contrast, cells of the TME are largely non-malignant
and may rely on regulatory mechanisms shared across cancer types, potentially creating opportunities for
more generalizable therapeutic strategies. Recent single-cell studies have shown that CAFs across
multiple tumor types adopt recurrent functional states, including inflammatory and myofibroblastic
phenotypes[23,60,61]. Understanding these CAF regulatory states is particularly important in PDAC due to its
dense desmoplastic stroma with pronounced CAF heterogeneity and context-dependent effects on tumor
progression and therapy response[5,10,62]. Attempts to therapeutically target CAFs have produced mixed
outcomes[16], with either tumor restraining or promoting effects depending on context and cell state[61]. For
example, depletion of FAP+ CAFs has been associated with improved survival, whereas depletion of
αSMA+ CAFs resulted in accelerated disease progression[21], underscoring the need to distinguish tumor-
promoting from tumor-restraining stromal states.

Building on this framework, we identified NFATC2+ CAFs as a treatment-responsive fibroblast
subpopulation with tumor-suppressive properties in PDAC. Through integrated single-cell analysis, GRN
modeling, and functional validation, we demonstrate that NFATC2+ CAFs associate with enhanced
chemotherapy sensitivity, ERBB pathway suppression, activation of pro-apoptotic programs, and
improved patient survival.

Transcriptional adaptation is a central feature of chemotherapy response, and our data highlight CAF regulatory states as a critical component of this process. GRN analysis revealed NFATC2 as a key TF selectively activated in CAFs of neoadjuvantly treated tumors, in contrast to the pro-fibrotic factors predominant in untreated tumors. This pattern suggests that therapeutic pressure drives reprogramming of stromal regulatory landscapes, in agreement with recent reports linking stromal features in PDAC to treatment responses [21,28-30,62,63]. Although NFATC2+ CAFs share features with iCAFs, their strong association with treatment response, ERBB suppression, and pro-apoptotic signaling supports a functionally distinct, therapy-conditioned stromal state that emerges in response to chemotherapy, rather than a pre-existing baseline CAF subtype. This is consistent with recent perturbation-based studies showing that CAF phenotypes can be actively redirected from TGF-β–driven myofibroblastic programs toward interferon-responsive, anti-tumoral states with enhanced immune engagement[64].

The association of NFATC2+ CAFs with better treatment response is further reflected in their enrichment for reactive subTME programs previously linked to immune infiltration and treatment sensitivity[20]. Consistently, downregulation of CXCL12 observed in NFATC2+ CAFs has been implicated in improved responsiveness to immunotherapy[49] and suppression of myCAF states linked to enhanced T cell infiltration[64]. Notably, NFAT signaling has also been linked to inflammatory CAF programs driven by IL-1 pathway activation in PDAC[65], whereas our results highlight NFATC2 as a regulator of therapy-associated stromal reprogramming emerging upon neoadjuvant treatment. Together, these findings align with the established plasticity of CAF states and context-specific transcriptional adaptation in PDAC[45,52]. While the upstream signals inducing NFATC2 in CAFs remain to be fully defined, our data suggest that immune-derived signals in treated tumors, particularly from T cells, may contribute to the emergence of NFATC2+ CAF states. This is supported by a previous report demonstrating a T cell-derived chemokine switch that promotes immune-activating CAF programs[66], linking therapy-associated immune activity to stromal transcriptional reprogramming.

Experimental activation of NFATC2 in CAFs enhanced the sensitivity of cancer cells to FOLFIRINOX and other chemotherapeutic regimens, along with increased apoptotic activity. These effects were linked to coordinated modulation of downstream signaling pathways, such as suppression of ERBB signaling, a known contributor to PDAC progression and treatment resistance[67], as well as promotion of epithelial containment and matrix anchorage of cancer cells, features associated with reduced invasive potential. Together, these findings position NFATC2 not merely as a marker of CAF heterogeneity, but as a transcriptional hub capable of rewiring stromal regulatory networks in response to therapeutic pressure.

Building on these NFATC2-associated changes in ERBB signaling and apoptotic priming, we reasoned that ERBB-targeted therapies might be particularly effective in NFATC2-enriched stromal contexts. Our combinatorial drug testing revealed distinct therapeutic vulnerabilities associated with NFATC2+ pCAF-mediated stromal reprogramming. In NFATC2+ pCAF co-cultures, HER2-targeting agent trastuzumab showed greater efficacy than the EGFR inhibitor erlotinib, suggesting preferential modulation of HER2-dependent signaling rather than uniform effects across the ERBB family. This is notable given the limited clinical success of EGFR/ERBB inhibitors in PDAC when applied in unselected patient populations[53,55,67], highlighting a context-dependent sensitivity that becomes evident only when stromal states are taken into account. This preferential sensitivity may reflect several, non-mutually exclusive mechanisms, including selective interference with HER2/HER3 signaling, and lowering of the apoptotic threshold under chemotherapy-induced stress. Together, these findings suggest that NFATC2+ pCAF–associated stromal states can shape selective therapeutic sensitivities and support stratified combination strategies in PDAC.

Elevated NFATC2 expression was consistently associated with improved clinical outcomes across multiple cancer types with prominent EGFR/ERBB signaling and stromal involvement. Together with the treatment-associated NFATC2 upregulation and enhanced chemotherapy sensitivity observed in several PDAC cohorts as well as in rectal cancer, these findings support NFATC2 as a treatment-associated stromal biomarker with clinical relevance beyond pancreatic cancer. The convergence of prognostic relevance and treatment-induced NFATC2 activation suggests that NFATC2+ pCAF-mediated programs may represent a common adaptive stromal response to effective cytotoxic therapy. Accordingly, NFATC2-guided stratification could identify tumors more likely to benefit from chemotherapy and ERBB-targeted combinations, enabling the rational optimization of combination treatment strategies across selected solid tumor types.

Several considerations will be important for translating these findings toward clinical applications. While
our in vitro co-culture experiments provide strong functional evidence for the tumor-suppressive role of
NFATC2+ pCAFs, in vivo validation using complementary model systems will be required to establish
how these stromal states function within complex tumor ecosystems. Further elucidation of the upstream
cues that induce NFATC2 expression in CAFs during therapy, particularly immune-mediated signals,
represents an important direction for future investigation. Moreover, the pronounced spatial heterogeneity
of PDAC highlights the importance of spatially resolved analyses to determine how NFATC2+ CAFs are
distributed across tumor regions, disease stages, and treatment contexts. Whether NFATC2 can be
detected via minimally invasive approaches such as plasma-based assays remains to be explored but
would substantially enhance biomarker feasibility. The long-term stability and persistence of NFATC2+
pCAFs following treatment cessation also remain unknown warranting longitudinal studies, which are
challenging in pancreatic cancer given the poor patient survival. Despite these challenges, the
combination of ERBB-targeted therapies with chemotherapy in NFATC2+ stromal contexts is particularly
compelling, as it leverages clinically approved agents and could thus accelerate translational
implementation.

In conclusion, identification of NFATC2+ pCAFs elucidates stromal heterogeneity, transcriptional
adaptation, and TF-driven regulatory states in PDAC. Rather than universally targeting CAFs, our findings
identify a treatment-responsive CAF subpopulation with tumor-suppressive properties, underscoring the
need for precision therapeutic strategies that preserve beneficial stromal cells while avoiding non-
selective CAF targeting. By defining a TF-driven stromal program linked to therapeutic benefit, this study
provides mechanistic insight into therapy-induced stromal reprogramming and establishes a rational
framework for precision medicine strategies that integrate stromal state into treatment stratification.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Biswajyoti Sahu (biswajyoti.sahu@ncmbm.uio.no).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The primary single-nucleus RNA sequencing dataset analyzed in this study was previously published and is publicly available through the Gene Expression Omnibus (GEO) under accession number GSE GSE202051[34]. Validation cohort single-cell RNA sequencing data for PDAC is available under GEO accession GSE205013[24], and rectal cancer scRNA-seq data is available from China National GeneBank Database (CNGBdb) with accession number CNP0004138[57]. Bulk RNA-seq data from pCAF-PANC1 co-culture experiments and conditioned medium treatments generated in this study have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE316877 and are publicly available as of the date of publication. This study did not generate unique computational code. All analysis were performed using publicly available software and packages.

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AUTHOR CONTRIBUTIONS

B.S. conceived and supervised the study. J.G. and S.C. performed in silico analyses. J.G. performed the experiments with support from C.W C.X and L.F. E.H. and S.D. contributed with the T cell isolation. C.V and T.A. contributed to data interpretation. B.S. and J.G. wrote the manuscript with contributions from all authors.

DECLARATION OF INTERESTS

Figure 1. GRN construction reveals NFATC2 as a treatment-responsive TF in PDAC TME.

(A) Workflow of our precision oncology pipeline integrating GRN construction and functional validation.

(B) UMAP plot of 224,988 cells from 42 PDAC patients[34] showing 20 cell types based on conventional markers (see Methods for details).

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(C) UMAP plot colored by treatment response categories as defined in the original dataset[34], including tumors from untreated patients as well as patients receiving neoadjuvant therapy, classified as poor, minimal, or moderate responders, with moderate response representing the most favorable outcome.

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(D) Comparison of eigenvector centrality scores between ductal epithelial cells and CAFs, highlighting ONECUT1 enriched in ductal cell group and NFATC2 enrichment in the CAF group.

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(E) UMAP plot showing NFATC2 expression levels across different cell clusters. Color gradient represents normalized NFATC2 expression (log-transformed, purple to green: low to high expression).

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(F) Eigenvector centrality comparison between the CAFs from treated and untreated patients, highlighting NFATC2 enrichment in the treated group.

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Figure 2. PDAC therapeutic response correlates with distinct CAF subpopulation distributions and NFATC2 expression levels.

(A) Left: UMAP plots of stromal cells colored by subtype identity including inflammatory CAFs (iCAF), NFATC2-positive CAFs (NFATC2+ CAF), myofibroblastic CAFs (myCAF), pericytes, and vascular smooth muscle cells (vSMC). Right: Volcano plot for differential gene expression for NFATC2+ CAFs compared to other stromal cells.

(B) Left: UMAP plots of stromal cells colored by patient therapy response categories (untreated, poor response, minimal response, and moderate response). Right: Violin plot showing NFATC2 expression levels across different treatment response groups.

(C) Alluvial diagram quantifying the relative proportional distribution of cell types across different therapeutic response groups. Y-axis represents the percentage of cells (0-100%), with each cell type shown in distinct colors. The width of each bar reflects the relative sample size per response category.

(D) Kaplan-Meier survival analysis demonstrates that patients with high NFATC2 (red line) based on the median pseudobulk expression in CAFs exhibit significantly longer progression-free survival compared to those with low expression (blue line, p = 0.039, log-rank test). The risk table shows patient numbers at different time points.

(E) Representative images from tissue microarray analysis of PDAC patient samples stratified by treatment response. Upper panels show H&E staining of stromal regions from treatment-naive tumors (left), poor responder tumors (middle), and good responder tumors (right) following chemotherapy. Lower panels display corresponding NFATC2 immunohistochemistry staining of serial sections from the same regions. Black boxes indicate representative fields shown at higher magnification. NFATC2-positive CAFs (brown nuclear staining) are more abundant in good responder samples compared to poor responders and treatment-naive samples. Scale bars = 100 µm.

(F) Quantification of NFATC2-positive CAF percentage across patient response groups. Bar graph shows percentage of NFATC2-positive stromal cells in treatment-naive tumors (orange, n=5), poor responders (pink, n=2), moderate responders (green, n=6), and good responders (blue, n=3). Each dot represents an individual patient sample. NFATC2 positivity progressively increases with better treatment response, with good responders showing markedly higher NFATC2+ CAF percentage (~40%) compared to poor responders (~5%) and treatment-naive samples (~12%). Data are presented as mean ± SEM.

(G) Heatmap displaying functional enrichment analysis results for each stromal cell subtype across different therapeutic response groups and N stage. Gene Ontology Biological Process (GO BP) terms are grouped by functional categories. The color scale represents normalized enrichment scores, with red and blue indicating positive and negative enrichment, respectively.

Figure 3. NFATC2 expression in CAFs limits PDAC cell growth and enhances apoptosis.

(A) Western blot showing ectopic NFATC2 expression in pancreatic CAFs (pCAF) generated using lentiviral transduction. GAPDH served as loading control. WT, wild-type pCAFs; NFATC2+, NFATC2-expressing pCAFs. Uncropped blots are shown in Supplementary Figure 3A.

(B) Representative fluorescence microscopy images of PANC1-pCAF co-cultures. PANC1 cells are labeled with mCherry (red) and pCAF cells with EGFP (green). WT and NFATC2+ pCAF co-cultures are shown on days 1-3 under vehicle or FOLFIRINOX treatment. Scale bar, 10μm.

(C) Quantification of PANC1 cell numbers using CellProfiler from fluorescence images showing that NFATC2+ CAFs significantly reduce cancer cell proliferation, particularly under FOLFIRINOX treatment. Data are presented as mean ± SEM (n=3 independent experiments). Upper panel: p<0.0001. Lower panel: p=0.0092, two-way ANOVA.

(D) Flow cytometry analysis of PANC1-pCAF co-culture systems with WT pCAFs (left) and NFATC2+ pCAF (right). Scatter plots display mCherry fluorescence (x-axis) versus eGFP fluorescence (y-axis), PANC1 cells (mCherry+, eGFP-) seen in the lower right quadrant.

(E) Annexin V-FITC apoptosis assay by flow cytometry. Density plots showing the fraction of apoptotic cells (Q2, top-right quadrant) relative to live cancer cells (Q3, bottom-right quadrant) following co-culture with WT or NFATC2+ pCAFs ± FOLFIRINOX. In FOLFIRINOX-treated cells, the apoptotic fraction increased from 19.1% in WT pCAF co-cultures to 33.0% in NFATC2+ pCAF co-cultures, indicating enhanced chemotherapy-induced apoptosis in cancer cells co-cultured with NFATC2+ pCAFs.

Figure 4. Transcriptional programs and paracrine effects underlying NFATC2-dependent CAF-cancer cell interactions.

(A) GSVA heatmap showing pathway activity differences between WT and NFATC2+ CAF-conditioned PANC-1 cells across four experimental conditions (direct co-culture and conditioned medium, each with vehicle or FOLFIRINOX treatment). Each condition contains paired samples (WT and NFATC2+, representing merged biological triplicates). GSVA enrichment scores were calculated for each sample and averaged across biological triplicates. Rows represent selected pathways from Hallmark, KEGG and GO BP databases. Color intensity indicates GSVA enrichment Z-score (red, upregulated; blue, downregulated). Left annotation bar indicates pathway database source, top annotation bars CAF genotype (WT, blue; NFATC2+, red), culture method (direct, blue; CM, teal), and treatment (vehicle, cyan; FOLFIRINOX, orange).

(B) GSVA enrichment analysis of apoptotic pathways from RNA-seq data. Box plots comparing extrinsic (left) and intrinsic (right) apoptosis pathway scores between WT CAF and NFATC2+ pCAF co-cultures ± FOLFIRINOX. ***$p < 0.001$, *$p < 0.05$ (t-test).

(C) NFATC2 expression levels in different cell populations after 48-hour co-culture with activated T cells under vehicle (blue) and FOLFIRINOX (red) treatments. Five experimental groups were analyzed: (1) WT CAFs cultured alone; (2) WT CAFs co-cultured with T cells; (3) PANC-1 cells co-cultured with T cells; (4) PANC-1 and WT CAF co-culture system stimulated with T cells (PANC-1+ CAF+ T cell); (5) T cells cultured alone. Of note, T cells were removed from the co-cultures by washing before RNA extraction and qPCR analysis, ensuring that measured NFATC2 expression represents only CAF or PANC-1 cells. Data are presented as mean ± SEM of NFATC2 expression relative to vehicle CAF (n=3 biological replicates per group). Statistical significance was determined by unpaired Student's t-test comparing vehicle vs FOLFIRINOX within each cell type. P-values compared with CAF Vehicle group are indicated above bars. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (t-test).

Figure 5. Transcriptional signatures of tumor cells stratified by NFATC2 expression in CAFs and treatment response.

(A) UMAP visualization of single-cell transcriptomes of malignant cells from patients stratified by CAF NFATC2 expression levels and treatment response. Tumor cells are colored by patient treatment response classification: untreated (orange), poor response (purple), minimal response (green), and moderate response (blue).

(B) Venn diagram illustrating the overlap of differentially expressed genes in tumor cells between response group comparisons. Numbers indicate unique and shared genes between Moderate vs Untreated (334 unique), Moderate vs Minimal (70 unique), and Moderate vs Poor (97 unique), with 72 shared genes commonly differentially expressed across all three comparisons and varying degrees of pairwise overlap.

(C) Dot plot showing enriched biological processes and signaling pathways in the 72 overlapped genes from GO and KEGG pathway enrichment analysis. The x-axis represents the enrichment factor, the y-axis shows statistical significance as -log10(p-value), dot size indicates the number of genes in each category (3-8 genes), and dot color intensity represents the p-value.

(D) Gene Set Enrichment Analysis (GSEA) plots of six cancer-relevant pathways in tumor cells comparing moderate responders to other response groups. Each panel displays the enrichment plot (left) with running enrichment score and rank metric, accompanied by a heatmap (right) showing normalized expression of leading-edge genes across response groups. Normalized negative enrichment scores (NES) indicate pathway downregulation in moderate response group compared to other responders. Analyzed pathways include ERBB2 Signaling Pathway (NES = -1.57, P = 0.01), TGF Beta Signaling Pathway (NES = -1.59, P < 0.0001), Hypoxia (NES = -1.4, P < 0.0001), p53 Pathway (NES = -1.56, P < 0.001), Epithelial Cell Proliferation (NES = -1.22, P = 0.04), and Stem Cell Proliferation (NES = -1.5, P < 0.001). Leading-edge genes driving pathway enrichment are labeled on the enrichment curves. Heatmap displays z-score normalized expression of those genes ranging from -2 (blue, low expression) to +2 (red, high expression).

Figure 6. ERBB-targeted therapies enhance chemotherapy efficacy in NFATC2+ pCAF co-culture system.

(A) Representative fluorescence microscopy images of PANC1-pCAF co-culture system under different treatment conditions. Cancer cells (mCherry+, red) and CAFs (eGFP+, green) were treated with vehicle control, standard chemotherapy regimens (FOLFIRINOX or Gemcitabine plus Paclitaxel) alone, or chemotherapy combined with ERBB-targeted inhibitors including Trastuzumab, Erlotinib and Neratinib. Scale bar, 10μm.

(B) Quantification of PANC1 cancer cell numbers from fluorescence microscopy analysis. Data represent mean ± SEM from three independent experiments. Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001, t-test.

(C) Normalized cell numbers of PANC1 from fluorescence microscopy analysis based on vehicle control from each group. Data represent mean ± SEM from three independent experiments. Same statistical significance marks used as (B).

(D) Flow cytometry analysis of the co-culture system after three-day treatment. Density plots display mCherry fluorescence (x-axis) versus eGFP fluorescence (y-axis), revealing distinct cell populations: PANC1 cells (mCherry+, eGFP-, lower right quadrant), CAFs (mCherry-, eGFP+, upper left quadrant), and cellular debris (lower left quadrant). Quantification of PANC1 cell proportions demonstrating a marked reduction in the cancer cell population following chemotherapy combined with ERBB-targeted inhibitors, particularly Trastuzumab and Neratinib, in the presence of NFATC2+ pCAFs.

Figure 7. External data validation cohorts demonstrate that NFATC2 expression in CAFs is associated with chemotherapy response and treatment sensitivity across cancer types.

(A) UMAP visualization of CAFs from an independent pancreatic cancer validation cohort[24], colored by treatment status: untreated (light blue) and treated (dark blue) patients showing distinct transcriptional states between chemotherapy-exposed and treatment-naïve stromal populations.

(B) UMAP visualization of the same CAFs colored by treatment response categories: untreated (orange), progressive disease (purple), stable disease (green), and partial response (blue), demonstrating response-associated distribution patterns of CAF subpopulations.

(C) NFATC2 expression levels in CAFs stratified by treatment status, showing markedly elevated expression in chemotherapy-treated patients (μmean = 623.75, n=7) compared to untreated patients (μmean = 62.64, n=20) (p<0.05, t-test).

(D) NFATC2 expression in CAFs stratified by treatment response, showing higher NFATC2 expression in partial response compared untreated or progressive disease (* p<0.05, ** p<0.01, *** p<0.001, t-test).

(E) GSVA heatmap showing pathway enrichment scores in cancer cells from the same pancreatic cancer validation cohort[24], stratified by treatment response. Rows represent different Gene Ontology Biological Process (GOBP) pathways including negative regulation of EGFR, ERBB signaling pathway, extrinsic apoptotic signaling, and intrinsic apoptotic signaling. Columns represent different treatment responses (Untreated, PD, SD, PR) with color intensity indicating pathway enrichment scores.

(F) UMAP visualization of CAFs from a rectal cancer cohort[57] colored by treatment response: no responder (NR, purple), partial responder (PR, green), and complete responder (CR, blue), showing distinct clustering patterns associated with therapeutic outcomes.

(G) UMAP visualization of the same CAFs colored by timepoint: pre-treatment (orange) and post-treatment (blue), illustrating treatment-associated shifts in CAF transcriptional states.

(H) NFATC2 expression levels in CAFs from the rectal cancer cohort prior to treatment, stratified by response group.

(I) Violin plot displaying post-treatment NFATC2 expression levels in CAFs across treatment response
groups, showing higher NFATC2 expression in complete response group (** p<0.01, t-test).

(J) GSVA heatmap showing pathway enrichment scores in cancer cells from the rectal cancer cohort[57],
stratified by treatment response groups (NR, PR, CR) and timepoint (pre- and post-treatment). The same
GOBP pathways are analyzed as in panel E, showing dynamic changes in pathway activities across
response categories and treatment timepoints.

 STAR★METHODS

 KEY RESOURCES TABLE

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse NFATC2 Monoclonal Antibody	Thermo Fisher Scientific	MA1-025, RRID: AB_2152763
Mouse GAPDH Monoclonal Antibody	Thermo Fisher Scientific	437000, RRID: AB_10374327
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP	Thermo Fisher Scientific	31430, RRID: AB_228307
Horse Anti-Mouse IgG Antibody (H+L), Peroxidase	Vector Laboratories	PI-2000-1, RRID: AB_2336177
Bacterial and virus strains		
One Shot™ Stbl3™ Chemically Competent E. coli	Thermo Fisher Scientific	C737303
Chemicals, peptides, and recombinant proteins		
DMEM (Dulbecco's Modified Eagle Medium)	Gibco	41965-039
MSC-GRO™ Pancreatic Stellate CAFs Maintenance Medium	VitroBiopharma	PC00B5
Annexin V Conjugates for Apoptosis Detection	Invitrogen	A13199
Fetal Bovine Serum (FBS)	Life Technologies	A5256701
Penicillin/streptomycin (P/S)	Gibco	15140122
L-glutamine	Gibco	25030024
Sodium Pyruvate	Thermo Fisher Scientific	11360039
MEM Non-essential amino acids	Thermo Fisher Scientific	11140035
Lipofectamine 2000	Thermo Fisher Scientific	11668019
Flow Cytometry Buffer	Invitrogen	00-4222-26
Enhanced Chemiluminescence (ECL) Reagent	Bio-Rad	1705060
0.5% TrypsinEDTA	Thermo Fisher Scientific	25200056
Gemcitabine	Selleckchem	S1149
Nab-paclitaxel	Selleckchem	E1068
5-Fluorouracil	Selleckchem	S1209
Oxaliplatin	Selleckchem	S1224
Leucovorin	Selleckchem	S2588
Irinotecan	Selleckchem	S5026
Trastuzumab	Selleckchem	A2007
Erlotinib	Selleckchem	S1023
Neratinib	Selleckchem	S2150
Critical commercial assays		
KAPA mRNA HyperPrep Kit	Roche	8098123702
DAB detection system kit	DAKO	K8000
Deposited data		
PANC1 co-culture RNA-sequencing data	This paper	GEO: GSE316877
Single-nucleus RNA sequencing data of pancreatic cancer	Hwang et al.[34]	GEO: GSE202051
Single-cell RNA sequencing data of pancreatic cancer	Werba et al.[24]	GEO: GSE205013

Single-cell RNA sequencing data of rectal cancer	Qin et al.[57]	CNGBdb: CNP0004138
Experimental models: Cell lines		
PANC1	ATCC	CVCL_0480
MIA Paca-2	ATCC	CVCL_0428
Pancreatic cancer associated fibroblasts	VitroBiopharma	CAF08
FT293	Thermo Fisher Scientific	CVCL_6911
Oligonucleotides		
Primer: *GAPDH* Forward: GGCCTCCAAGGAGTAAGACC	This paper	N/A
Primer: *GAPDH* Reverse: AGGGGAGATTCAGTGTGGTG	This paper	N/A
Primer: *NFATC2* Forward: ATCCCTGAATATCGGAACAA	This paper	N/A
Primer: *NFATC2* Reverse: TGGGTGGTAGGTAAAGTGC	This paper	N/A
Recombinant DNA		
psPAX2	Didider Trono Lab	Addgene_12260
pMD2.G	Didider Trono Lab	Addgene_12259
pLV-eGFP	Pantelis Tsoulfas Lab	Addgene_36083
pLV-mCherry	Pantelis Tsoulfas Lab	Addgene_36084
TFORF1069	Feng Zhang Lab	Addgene_143463
Software and algorithms		
Scanpy (v1.10.1)	Wolf et al.[68]	SCR_018139
Seurat (v4.4.0)	Hao et al.[69]	SCR_016341
CellOracle (v0.16.0)	Kamimoto et al.[35]	https://github.com/morris-lab/CellOracle
NicheNet (v2.2.0)	Browaeys et al.[51]	https://github.com/saeyslab/nichenetr/tree/master
Fiji (v1.53)	NIH	https://imagej.net/software/fiji/
FlowJo (v10.6.1)	BD	https://www.flowjo.com/
GraphPad Prism (8.3.0)	GraphPad	https://www.graphpad.com/
CellProfiler (v4.2.5)	Stirling et al.[70]	SCR_007358
FastQC (v0.12.1)	Babraham Bioinformatics	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
STAR aligner (v2.5.2b)	Dobin et al.[71]	https://github.com/alexdobin/STAR
Trim Galore (v0.6.10)	Krueger et al.	SCR_011847
Samtools (v1.7)	Li et al.[72]	https://samtools.sourceforge.net/
HTSeq (v2.0.5)	Anders et al.[73]	https://github.com/htseq/htseq
DESeq2 (v1.40.0)	Love et al.[74]	https://github.com/thelovelab/DESeq2
KMplotter	Posta et al.[75]	https://kmplot.com/analysis/

METHOD DETAILS

Single-nucleus sequencing data analysis

We analyzed a publicly available snRNA-seq dataset from pancreatic ductal adenocarcinoma patients[34],
comprising 42 samples (18 chemotherapy-treated and 24 treatment-naive). Raw count matrices were
processed using Scanpy (v1.10.1, RRID: SCR_018139)[68] in Python (3.9.22) and Seurat (v4.4.0, RRID:
SCR_016341)[69] in R (4.4.1). Nuclei were filtered based on the following criteria: 200-6000 detected genes,
<20% mitochondrial gene content, and <1% hemoglobin gene content. Data were normalized using the
standard log-normalization method, and the top 2000 highly variable genes were identified using the
function 'FindVariableFeatures'. Principal component analysis was performed on scaled data, and the top
30 principal components were used for downstream analysis. UMAP dimensionality reduction was applied
for visualization. Leiden clustering (resolution = 0.5) was performed to identify cell clusters.

Major cell types were annotated as previously described in the original publication[34], including epithelial
cells, endothelial cells, immune cells (T cells, myeloid cells, B cells), and other stromal populations. Then,
stromal cell populations (fibroblasts, pericytes, and vascular smooth muscle cells) were subset and re-
clustered at higher resolution (resolution = 1.0) to identify distinct subtypes. CAF subtypes were classified
based on established marker gene expression patterns[17,76,77]: Inflammatory CAFs (iCAFs): characterized
by high expression of inflammatory markers including C3, C7, FBLN1 and IGF1. Myofibroblastic CAFs
(myCAFs): defined by expression of FAP, ACTA2, INHBA and POSTN. Pericytes were characterized by
RGS5, MCAM and TAGLN expression. Vascular smooth muscle cells (vSMCs) were defined by high
expression of ACTA2, TAGLN and SMTN.

To validate our findings, we analyzed a second independent scRNA-seq dataset from PDAC patients[24],
comprising samples from both chemotherapy-treated (n=7) and treatment-naive (n=20) primary PDAC
and liver metastasis tissues. This dataset was obtained through direct correspondence with the original
authors. Raw count matrices were processed using Seurat (v4) in R (4.4.1) with identical quality control
criteria as applied to the primary dataset: 200-6000 detected genes, <20% mitochondrial gene content,
and <1% hemoglobin gene content. Data were normalized using standard log-normalization, and the top
2000 highly variable genes were identified. Cell type annotations provided by the original authors were
utilized to examine gene expression patterns across treatment conditions and cellular populations.

For additional validation across cancer types, we analyzed a third independent scRNA-seq dataset from
rectal cancer patients treated with neoadjuvant FOLFIRINOX[57], containing 52 samples (25 treated and 27
treatment- Naïve) from 29 patients. The data was downloaded from China National GeneBank Database
(CNGBdb) with accession number CNP0004138 and processed using the same computational pipeline
and quality control thresholds.

Construction of gene regulatory network (GRN) and identification of hub TFs in PDAC

To identify specific relations between TFs and genes, we created a gene regulatory network (GRN). This
network allows to identify relations between TFs and target genes (TGs) at a deeper level, revealing
dependencies between gene-gene regulation. The network is built using CellOracle (v0.16.0 on Python
3.8)[35], a state-of-the-art tool specifically designed to generate GRNs for single cell datasets. As initial
configuration, the tool requires a set of pre-existing TF-TG relations. This was created by using the well-
known gimmemotifs (v5 vertebrate) human dataset[78] provided by the authors of the tool. After this step,
we split the single cell dataset into different subgroups of interest, like cell type and treatment information.
After this initial sub-classification, the set of cells is reduced to allow a faster and more accurate
computation of the network. We included 10k cells (randomly sampled) and 3k genes (highly variable
genes) in each network. One network contained the cells coming from treated samples and the other
contained cells coming from treatment-naive samples. Both networks used epithelial cells (malignant and
benign) and CAF. Then the method uses Bayesian ridge regression model to predict the contribution of
each TF to modulate a target gene expression. After this step, we have two distinct GRN that can be used
to extract information about the connections of each TF and TG. By using common networks measures

like the eigenvector centrality, we retrieved a set of TFs representing hubs (heavily connected genes). These hubs are heavily connected to genes with many connections in the network, meaning that the influence of these TF in the network is high.

Differential gene expression analysis

Differential expression analysis was performed using a pseudobulk approach. First, malignant cells from each patient were aggregated by patient ID to generate pseudobulk expression profiles, which reduces single-cell noise while preserving biological variability between patients. Gene expression counts were summed across all malignant cells within each patient, and only genes expressed in at least 10% of patients were retained for downstream analysis. Pseudobulked samples were grouped according to neoadjuvant therapy response categories (untreated, poor response, minimal response, and moderate response) and analyzed using DESeq2[74]. Differentially expressed genes (DEGs) were identified based on following threshold: absolute log2 fold change ≥ 1 and adjusted p-value < 0.05.

Functional pathway enrichment analysis

Gene Ontology biological process (GO BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted using the R package 'clusterProfiler'[79]. Threshold of p value < 0.05 was used for filtering. Gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) were implemented with the 'GSVA' package[80], and visualized using the 'GseaVis' package. Gene sets used including Hallmark, GO and KEGG were obtained from MSigDB[81].

Survival analysis

Kaplan-Meier analysis was conducted using the R packages 'survival' and 'survminer'. For single-cell data, patients were stratified into high and low NFATC2 expression groups based on the median pseudobulk expression level of NFATC2 in CAFs as the cutoff threshold. Kaplan-Meier survival curves were generated for high and low NFATC2 expression groups, and the log-rank test was used to assess statistical significance between survival curves, with p-values < 0.05 considered statistically significant.

Bulk RNA sequencing data from The Cancer Genome Atlas (TCGA) pancreatic adenocarcinoma cohort (PAAD, n=178) were obtained through UCSC Xena browser[82]. Patients were similarly stratified based on median NFATC2 expression levels in tumor samples for Kaplan-Meier analysis. The database 'kmplotter'[75] was utilized for following pan-cancer survival analysis.

Cell crosstalk analysis

Ligand-receptor interaction analysis was performed using the R package 'nichenetr' [51]. Cell types with sufficient cell numbers (>50 cells) were included in the analysis. Potential ligands were identified from sender cells (CAF subtypes) based on expression levels and cell type specificity. Ligand activity scores were calculated using NicheNet's built-in ligand-target regulatory network.

Immunohistochemistry

Human pancreatic cancer tissues were obtained from Oslo University Hospital patients undergoing neoadjuvant treatment (FOLFIRINOX, 4 cycles) followed by surgery (n=11) or upfront surgery (n=4). The collection of samples was approved by the Regional Ethics Committee (REK 2015/738). All patients had given written informed consent for the use of tumor tissue for research purposes.

Formalin-fixed paraffin-embedded 3.5-micron thick whole tissue sections were incubated with mouse anti-NFATC2 (1:25, overnight incubation, Thermo Fisher Scientific, #MA1-025, RRID: AB_2152763) following antigen-retrieval (95°C, 30 min, citrate buffer solution, pH 6.0). Subsequently, sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Vector Laboratories, #31430, RRID: AB_2336177) for 30 minutes, stained with a DAB detection system kit (EnVision Flex DAB+; DAKO North America, Santa Clara, CA, USA), and counterstained with hematoxylin. Immunostaining was assessed on digital whole slide images by manual counting of positive and negative stromal cells in 5 hot spots (each

782 measuring 0.6 mm^2) containing at least 200 stromal cells. The percentage of positive stromal cells
783 averaged over the 5 hot spots was compared between cases.

Cell lines and plasmids

Human pancreatic carcinoma cell lines PANC1 (RRID: CVCL_0480), MIA PaCa-2 (RRID: CVCL_0428)
were cultured in DMEM (Gibco, Life Technologies) supplemented with 2 mM L-glutamine (Gibco,
25030024), 10% fetal bovine serum (FBS), and 1% Penicillin-Streptomycin (Gibco, 15140122). Human
pancreatic adenocarcinoma cancer-associated fibroblasts (VitroBiopharma, #CAF08) were cultured in
MSC-GRO™ Pancreatic Cancer-Associated Fibroblast (CAF) Maintenance Medium (VitroBiopharma,
#PC00B5) with 1% Penicillin-Streptomycin. 293FT (Thermo Fisher Scientific, #R70007, RRID:
CVCL_6911) cells were cultured in DMEM supplemented with 10% FBS, 6mM L-glutamine (Gibco,
25030024), 1% Penicillin-Streptomycin, 1% Sodium Pyruvate (Thermo Fisher Scientific, #11360039) and
1% MEM Non-essential amino acids (Thermo Fisher Scientific, #11140035). All cell lines were tested for
mycoplasma contamination, cultured at low passages and maintained in a humidified incubator at 37 °C
and with 5% CO2. NFATC2 full-length ORF plasmid was a gift from Feng Zhang (Addgene, #143463,
RRID: Addgene_143463)[83].

Lentivirus production and stable transduction

For virus production, NFATC2 full-length ORF plasmid was co-transfected with the packaging plasmids
psPAX2 (Addgene #12260, RRID: Addgene_12260) and pMD2.G (Addgene, #12259, RRID:
Addgene_12259) in a ratio of 4:3:1 into 293FT cells with Lipofectamine 2000 (Thermo Fisher Scientific,
#11668019). Then, culture medium was replenished on the following day and supernatant containing the
viral particles was collected after 48 h ours, filtered with 0.45 mm filters (Merck, #SE1M003M00), and
concentrated using Lenti-X concentrator (Clontech, #631232), tittered using qPCR Lentivirus Titer Kit
(ABM, # LV900) following the manufacturer's protocol and stored as single-use aliquots in-80 °C.

For stable transduction, an empty vector was transfected as control. A total of 4 × 10^5 pCAFs in 2 mL of
medium with 2 µg/mL polybrene were infected with 30 uL of lentivirus supernatant. After 72 h, 2 µg/ml
puromycin was used for NFATC2 overexpression selection of a stable cell pool. Cell sorting was used for
eGFP and mCherry fluorescent marker selection. The efficiency of overexpression in a stable cell line was
validated by qRT-PCR and Western blot assays as described in following methods.

Western blot analysis

Pancreatic CAFs were lysed in RIPA Buffer (Thermo Fisher Scientific, #89900) supplemented with
protease inhibitor cocktail (Thermo Fisher Scientific, #78441). Protein concentration was determined
using the Bradford Protein Assay Kit (Bio-Rad, #5000202). Equal amounts of protein (20 µg) were
separated by SDS-PAGE and transferred onto PVDF membranes (Bio-Rad, #1620177). Membranes were
blocked with 5% non-fat milk in TBST (Tris-buffered saline with 0.1% Tween-20) The membranes were
then incubated overnight at 4°C with primary antibodies diluted in 1% BSA in TBST: anti-NFATC2 (1:1000,
Thermo Fisher Scientific, #MA1-025, RRID: AB_2152763) and anti-GAPDH (1:5000, Thermo Fisher
Scientific, #437000, RRID: AB_10374327) as a loading control. Following three washes with TBST,
membranes were incubated with Goat anti-Mouse Secondary antibody (1:10000, Thermo Fisher Scientific,
#31430, RRID: AB_228307) for 1 hour at room temperature. Protein bands were visualized using
enhanced chemiluminescence (ECL) reagent (Bio-Rad, #1705060) and detected with a ChemiDoc
imaging system (Bio-Rad, #12003153).

Direct co-culture of cancer cells and pCAFs and drug treatment

Prior to establishing the co-culture system, we performed ratio optimization experiments to determine the
optimal CAF: cancer cell ratio according to previous reports[45-47]. PANC1 cells were co-cultured with
pancreatic CAFs at four different ratios: 3:1, 2:1, 1:1, and 1:3 (CAF: cancer cell). After 72 hours of co-
culture, we found that the 3:1 ratio maintained the most stable co-culture conditions without overgrowth of
either cell type. Therefore, a ratio of 3:1 was selected for all subsequent co-culture experiments. Drug

treatment was initiated on the second day following cell seeding, and subsequent quantitative and
functional assays were performed on the fourth day.

Drug treatments were administered at the following concentrations[84-87]. All drugs were dissolved in DMSO
and diluted to ensure a final DMSO concentration of ≤1% in each well. FOLFIRINOX: 5-Fluorouracil
(Selleckchem, #S1209) at 10 µM, oxaliplatin (Selleckchem, #S1224) at 100 nM, leucovorin (Selleckchem,
#S2588) at 132 nM and irinotecan (Selleckchem, #S5026) at 123 nM. Other combination therapy:
Gemcitabine (Selleckchem, #S1149) at 100 nM, nab-paclitaxel (Selleckchem, #E1068) at 10 nM,
trastuzumab (Selleckchem, #A2007) at 10 µg/mL, erlotinib (Selleckchem, #S1023) at 1 µM, and neratinib
(Selleckchem, #S2150) at 100 nM.

Image acquisition and quantitative analysis

Fluorescence images were acquired using Zeiss AxioVert fluorescence microscope. For each
experimental condition, at least 3-4 random fields of view were captured per well, multiple with 3
biological replicates. Images were acquired with consistent exposure times and laser power settings
across all samples to ensure comparability.

Fluorescence microscopy images from co-culture experiments were quantitatively analyzed using
CellProfiler[70] (version 4.2.5, RRID: SCR_007358). Images were acquired in CZI format containing dual
fluorescence channels: mCherry-labeled cancer cells (red) and eGFP-labeled pCAFs (green). Raw
images were imported into CellProfiler and underwent the following preprocessing steps and parameters:
Multi-channel fluorescence images were separated into individual channels using the "ColorToGray".
Then, "CorrectIlluminationApply" module was used to correct for uneven background illumination. Noise
reduction with Gaussian filtering (sigma = 2-3 pixels). Total cell numbers for each fluorescence channel
(mCherry+ cancer cells and eGFP+ CAFs) were quantified per field of view.

RNA isolation, quantitative real-time PCR and RNA-sequencing

Total RNA was isolated from two experimental designs: (1) direct co-culture of PANC1 with WT CAF and
NFATC2+ CAF for 3 days with or without FOLFIRINOX treatment, (2) PANC1 cultured with both CAF-
conditioned medium for 3 days with or without FOLFIRINOX treatment. RNA extraction was performed
using the RNeasy Mini Kit (Qiagen, #74136) according to the manufacturer's protocol. RNA concentration
and purity were assessed using a NanoDrop spectrophotometer, with A260/A280 ratios between 1.8-2.0
considered acceptable. NFATC2 expression was quantified using the following primer sequences (forward:
5'-ATCCCTGAATATCGGAACAA-3', reverse: 5'-TGGGTGGTAGGTAAAGTGC-3'). GAPDH served as the
internal   reference   gene   (forward: 5'-GGCCTCCAAGGAGTAAGACC-3',   reverse: 5'-
AGGGGAGATTCAGTGTGGTG-3'). Relative gene expression was calculated using the $2^{\wedge}(-\Delta\Delta Ct)$ method,
with each sample analysed in triplicate.

RNA-seq libraries were constructed using 800-1000 ng of total RNA with the KAPA mRNA HyperPrep Kit
(Roche, #8098123702) following the manufacturer's protocol. Libraries were quantified using Qubit
dsDNA HS Assay Kit. Libraries were sequenced on Illumina NovaSeq X platform generating 150 bp
paired-end reads.

Raw sequencing reads were assessed for quality using FastQC (v0.12.1). Adapter sequences and low-
quality bases were trimmed using Trim Galore (v0.6.10) with a quality threshold of Phred score ≥20 and
minimum read length of 20 bp. Quality-filtered reads were aligned to the human reference genome
(GRCh38) using STAR aligner (v2.5.2b)[71] in two-pass mode with default parameters. Uniquely mapped
reads were retained for downstream analysis. The resulting BAM files were sorted and indexed using
Samtools (v1.7)[72]. Gene-level read counts were quantified using HTSeq (v2.0.5)[73] in union mode with
GENCODE v38 gene annotations. Differential gene expression analysis was performed using DESeq2
(v1.40.0)[74] in R (v4.3.0). Raw count matrices were normalized using the DESeq2 median-of-ratios method
to account for library size and composition biases. Genes with fewer than 10 reads across all samples

were excluded from analysis. Statistical significance was determined using the Wald test with Benjamini-
Hochberg correction for multiple testing.

Flow cytometry analysis

Following treatments for 3 days, co-cultured cells were harvested for flow cytometric analysis. Culture
medium was aspirated from the co-culture systems, and cells were washed once with PBS. Cells were
dissociated using 0.25% trypsin-EDTA solution and incubated at 37°C for 5 minutes. Trypsin activity was
neutralized by adding an equal volume of complete medium containing FBS. Single-cell suspensions
were generated by gentle pipetting, followed by centrifugation at 400g for 4 minutes at room temperature.
Cell pellets were resuspended in 1 mL flow cytometry buffer (Invitrogen, #00-4222-26) and passed
through 40 μm cell strainers (Corning, #CLS431750) to remove cell aggregates and debris. Flow
cytometric analysis was performed using a Sony SH800S Cell Sorter (Sony Biotechnology) operating in
analysis mode. The instrument was cleaned and calibrated according to manufacturer protocols before
each experimental session. Standard fluorescent beads were used for instrument calibration and quality
control. Single-stained controls (eGFP-positive CAFs only and mCherry-positive PANC1 cells only) and
unstained cells were used as negative controls for compensation adjustment and gating strategy
optimization. Cell suspensions were loaded into the flow cytometer sequentially, with data acquisition
initiated immediately upon sample loading. Data acquisition was performed until the entire sample from
every condition was processed to ensure comprehensive quantitative assessment of cell population ratios
and amounts.

SUPPLEMENTAL INFORMATION

Document S1: Figures S1–S6.
Document S2: Table S1. NFATC2 immunohistochemistry quantification in PDAC tissue
microarray. Related to Figure 2.
Table S2. Differential expressed gene list from stromal cell subtypes. Related to Figure 2.
Table S3-S6. Differential expressed genes of PANC1 in different co-culture conditions. Related to
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REFERENCES

1.   Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I., and Jemal, A. (2024).
Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36
cancers in 185 countries. CA: A Cancer Journal for Clinicians *74*, 229–263.
https://doi.org/10.3322/caac.21834.
2.   Park, W., Chawla, A., and O'Reilly, E.M. (2021). Pancreatic Cancer: A Review. JAMA *326*, 851–
862. https://doi.org/10.1001/jama.2021.13027.
3.   Conroy, T., Desseigne, F., Ychou, M., Bouché, O., Guimbaud, R., Bécouarn, Y., Adenis, A., Raoul,
J.-L., Gourgou-Bourgade, S., Fouchardière, C.d.l., et al. (2011). FOLFIRINOX versus Gemcitabine
for Metastatic Pancreatic Cancer. New England Journal of Medicine *364*, 1817–1825.
https://doi.org/10.1056/nejmoa1809775.
4.   Springfeld, C., Ferrone, C.R., Katz, M.H.G., Philip, P.A., Hong, T.S., Hackert, T., Büchler, M.W., and
Neoptolemos, J. (2023). Neoadjuvant therapy for pancreatic cancer. Nature Reviews Clinical
Oncology *20*, 318–337. https://doi.org/10.1038/s41571-023-00746-1.
5.   Halbrook, C.J., Lyssiotis, C.A., Pasca di Magliano, M., and Maitra, A. (2023). Pancreatic cancer:
Advances and challenges. Cell *186*, 1729–1754. https://doi.org/10.1016/j.cell.2023.02.014.
6.   Grossberg, A.J., Chu, L.C., Deig, C.R., Fishman, E.K., Hwang, W.L., Maitra, A., Marks, D.L., Mehta,
A., Nabavizadeh, N., Simeone, D.M., et al. (2020). Multidisciplinary standards of care and recent
progress in pancreatic ductal adenocarcinoma. CA Cancer J Clin *70*, 375–403.
https://doi.org/10.3322/caac.21626.

7. Chen, Y., McAndrews, K.M., and Kalluri, R. (2021). Clinical and therapeutic relevance of cancer-
associated fibroblasts. Nature Reviews Clinical Oncology 18, 792–804.
https://doi.org/10.1038/s41571-021-00546-5.
8. LeBleu, V.S., and Kalluri, R. (2018). A peek into cancer-associated fibroblasts: origins, functions
and translational impact. Disease Models & Mechanisms 11.
https://doi.org/10.1242/dmm.029447.
9. Kalluri, R. (2016). The biology and function of fibroblasts in cancer. Nature Reviews Cancer 16,
582–598. https://doi.org/10.1038/nrc.2016.73.
10. Neesse, A., Michl, P., Frese, K.K., Feig, C., Cook, N., Jacobetz, M.A., Lolkema, M.P., Buchholz, M.,
Olive, K.P., Gress, T.M., and Tuveson, D.A. (2011). Stromal biology and therapy in pancreatic
cancer. Gut 60, 861–868. https://doi.org/10.1136/gut.2010.226092.
11. Sherman, M.H., and di Magliano, M.P. (2023). Cancer-Associated Fibroblasts: Lessons from
Pancreatic Cancer. Annual Review of Cancer Biology 7, 43–55. https://doi.org/10.1146/annurev-
cancerbio-061421-035400.
12. Hingorani, S.R. (2023). Epithelial and stromal co-evolution and complicity in pancreatic cancer.
Nature Reviews Cancer 23, 57–77. https://doi.org/10.1038/s41568-022-00530-w.
13. Feldmann, K., Maurer, C., Peschke, K., Teller, S., Schuck, K., Steiger, K., Engleitner, T., Öllinger, R.,
Nomura, A., Wirges, N., et al. (2021). Mesenchymal Plasticity Regulated by Prrx1 Drives
Aggressive Pancreatic Cancer Biology. Gastroenterology 160, 346–361.e324.
https://doi.org/10.1053/j.gastro.2020.09.010.
14. Awaji, M., Saxena, S., Wu, L., Prajapati, D.R., Purohit, A., Varney, M.L., Kumar, S., Rachagani, S.,
Ly, Q.P., Jain, M., et al. (2020). CXCR2 signaling promotes secretory cancer-associated fibroblasts
in pancreatic ductal adenocarcinoma. Faseb j 34, 9405–9418. https://doi.org/10.1158/1541-
7786.MCR-15-0348.
15. Niu, N., Shen, X., Wang, Z., Chen, Y., Weng, Y., Yu, F., Tang, Y., Lu, P., Liu, M., Wang, L., et al.
(2024). Tumor cell-intrinsic epigenetic dysregulation shapes cancer-associated fibroblasts
heterogeneity to metabolically support pancreatic cancer. Cancer Cell 42, 869–884.e869.
https://doi.org/10.1016/j.ccell.2024.03.005.
16. Boeker, V., and Kalluri, R. (2025). Insights into the relevance of targeting fibroblasts to control
cancer. Cell Reports Medicine 6. 10.1016/j.xcrm.2025.102395.
17. Gao, Y., Li, J., Cheng, W., Diao, T., Liu, H., Bo, Y., Liu, C., Zhou, W., Chen, M., Zhang, Y., et al.
(2024). Cross-tissue human fibroblast atlas reveals myofibroblast subtypes with distinct roles in
immune modulation. Cancer Cell 42, 1764–1783.e1710.
https://doi.org/10.1016/j.ccell.2024.08.020.
18. Elyada, E., Bolisetty, M., Laise, P., Flynn, W.F., Courtois, E.T., Burkhart, R.A., Teinor, J.A., Belleau,
P., Biffi, G., Lucito, M.S., et al. (2019). Cross-Species Single-Cell Analysis of Pancreatic Ductal
Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts. Cancer Discov 9,
1102–1123. https://doi.org/10.1158/2159-8290.CD-19-0094.
19. Biffi, G., Oni, T.E., Spielman, B., Hao, Y., Elyada, E., Park, Y., Preall, J., and Tuveson, D.A. (2019).
IL1-Induced JAK/STAT Signaling Is Antagonized by TGFβ to Shape CAF Heterogeneity in
Pancreatic Ductal Adenocarcinoma. Cancer Discov 9, 282–301. https://doi.org/10.1158/2159-
8290.CD-18-0710.
20. Grünwald, B.T., Devisme, A., Andrieux, G., Vyas, F., Aliar, K., McCloskey, C.W., Macklin, A., Jang,
G.H., Denroche, R., Romero, J.M., et al. (2021). Spatially confined sub-tumor microenvironments
in pancreatic cancer. Cell 184, 5577–5592.e5518. https://doi.org/10.1016/j.cell.2021.09.022.
21. McAndrews, K.M., Chen, Y., Darpolor, J.K., Zheng, X., Yang, S., Carstens, J.L., Li, B., Wang, H.,
Miyake, T., Correa de Sampaio, P., et al. (2022). Identification of Functional Heterogeneity of

969   Carcinoma-Associated Fibroblasts with Distinct IL6-Mediated Therapy Resistance in Pancreatic
970   Cancer. Cancer Discovery *12*, 1580–1597. https://doi.org/10.1158/2159-8290.CD-20-1484.

971 22. Buechler, M.B., Pradhan, R.N., Krishnamurty, A.T., Cox, C., Calviello, A.K., Wang, A.W., Yang, Y.A.,
972   Tam, L., Caothien, R., Roose-Girma, M., et al. (2021). Cross-tissue organization of the fibroblast
973   lineage. Nature *593*, 575–579. https://doi.org/10.1038/s41586-021-03549-5.

974 23. Foster, D.S., Januszyk, M., Delitto, D., Yost, K.E., Griffin, M., Guo, J., Guardino, N., Delitto, A.E.,
975   Chinta, M., Burcham, A.R., et al. (2022). Multiomic analysis reveals conservation of cancer-
976   associated fibroblast phenotypes across species and tissue of origin. Cancer Cell *40*, 1392–
977   1406.e1397. https://doi.org/10.1016/j.ccell.2022.09.015.

978 24. Werba, G., Weissinger, D., Kawaler, E.A., Zhao, E., Kalfakakou, D., Dhara, S., Wang, L., Lim, H.B.,
979   Oh, G., Jing, X., et al. (2023). Single-cell RNA sequencing reveals the effects of chemotherapy on
980   human pancreatic adenocarcinoma and its tumor microenvironment. Nature Communications
981   *14*, 797. https://doi.org/10.1038/s41467-023-36296-4.

982 25. Chaligne, R., Gaiti, F., Silverbush, D., Schiffman, J.S., Weisman, H.R., Kluegel, L., Gritsch, S.,
983   Deochand, S.D., Gonzalez Castro, L.N., Richman, A.R., et al. (2021). Epigenetic encoding,
984   heritability and plasticity of glioma transcriptional cell states. Nature Genetics *53*, 1469–1479.
985   https://doi.org/10.1038/s41588-021-00927-7.

986 26. França, G.S., Baron, M., King, B.R., Bossowski, J.P., Bjornberg, A., Pour, M., Rao, A., Patel, A.S.,
987   Misirlioglu, S., Barkley, D., et al. (2024). Cellular adaptation to cancer therapy along a resistance
988   continuum. Nature *631*, 876–883. https://doi.org/10.1038/s41586-024-07690-9.

989 27. Badia-i-Mompel, P., Wessels, L., Müller-Dott, S., Trimbour, R., Ramirez Flores, R.O., Argelaguet,
990   R., and Saez-Rodriguez, J. (2023). Gene regulatory network inference in the era of single-cell
991   multi-omics. Nature Reviews Genetics *24*, 739–754. https://doi.org/10.1038/s41576-023-00618-
992   5.

993 28. Mascharak, S., Guo, J.L., Foster, D.S., Khan, A., Davitt, M.F., Nguyen, A.T., Burcham, A.R., Chinta,
994   M.S., Guardino, N.J., Griffin, M., et al. (2023). Desmoplastic stromal signatures predict patient
995   outcomes in pancreatic ductal adenocarcinoma. Cell Reports Medicine *4*, 101248.
996   https://doi.org/10.1016/j.xcrm.2023.101248.

997 29. Heger, U., Martens, A., Schillings, L., Walter, B., Hartmann, D., Hinz, U., Pausch, T., Giese, N.,
998   Michalski, C.W., and Hackert, T. (2022). Myofibroblastic CAF Density, Not Activated Stroma
999   Index, Indicates Prognosis after Neoadjuvant Therapy of Pancreatic Carcinoma. Cancers (Basel)
1000   *14*. https://doi.org/10.3390/cancers14163881.

1001 30. Dias Costa, A., Väyrynen, S.A., Chawla, A., Zhang, J., Väyrynen, J.P., Lau, M.C., Williams, H.L.,
1002   Yuan, C., Morales-Oyarvide, V., Elganainy, D., et al. (2022). Neoadjuvant Chemotherapy Is
1003   Associated with Altered Immune Cell Infiltration and an Anti-Tumorigenic Microenvironment in
1004   Resected Pancreatic Cancer. Clin Cancer Res *28*, 5167–5179. https://doi.org/10.1158/1078-
1005   0432.ccr-22-1125.

1006 31. Daniel, C., Gerlach, K., Väth, M., Neurath, M.F., and Weigmann, B. (2014). Nuclear factor of
1007   activated T cells—A transcription factor family as critical regulator in lung and colon cancer.
1008   International Journal of Cancer *134*, 1767–1775. https://doi.org/10.1002/ijc.28329.

1009 32. De Boer, M.L., Mordvinov, V.A., Thomas, M.A., and Sanderson, C.J. (1999). Role of nuclear factor
1010   of activated T cells (NFAT) in the expression of interleukin-5 and other cytokines involved in the
1011   regulation of hemopoetic cells. The International Journal of Biochemistry & Cell Biology *31*,
1012   1221–1236. https://doi.org/10.1016/S1357-2725(99)00069-2.

1013 33. Chai, W.R., Chen, Y., Wang, Q., and Gao, H.B. (2008). Mechanism of nuclear factor of activated
1014   T-cells mediated FasL expression in corticosterone -treated mouse Leydig tumor cells. BMC Cell
1015   Biol *9*, 31. https://doi.org/10.1186/1471-2121-9-31.

34. Hwang, W.L., Jagadeesh, K.A., Guo, J.A., Hoffman, H.I., Yadollahpour, P., Reeves, J.W., Mohan,
R., Drokhlyansky, E., Van Wittenberghe, N., Ashenberg, O., et al. (2022). Single-nucleus and
spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated
with neoadjuvant treatment. Nature Genetics *54*, 1178–1191. https://doi.org/10.1038/s41588-
022-01134-8.

35. Kamimoto, K., Stringa, B., Hoffmann, C.M., Jindal, K., Solnica-Krezel, L., and Morris, S.A. (2023).
Dissecting cell identity via network inference and in silico gene perturbation. Nature *614*, 742–
751. https://doi.org/10.1038/s41586-022-05688-9.

36. Fei, L., Zhang, K., Poddar, N., Hautaniemi, S., and Sahu, B. (2023). Single-cell epigenome analysis
identifies molecular events controlling direct conversion of human fibroblasts to pancreatic
ductal-like cells. Dev Cell *58*, 1701–1715.e1708. https://doi.org/10.1016/j.devcel.2023.08.023.

37. Kropp, P.A., Zhu, X., and Gannon, M. (2019). Regulation of the Pancreatic Exocrine
Differentiation Program and Morphogenesis by Onecut 1/Hnf6. Cell Mol Gastroenterol Hepatol
7, 841–856. https://doi.org/10.1016/j.jcmgh.2019.02.004.

38. Marstrand-Daucé, L., Lorenzo, D., Chassac, A., Nicole, P., Couvelard, A., and Haumaitre, C.
(2023). Acinar-to-Ductal Metaplasia (ADM): On the Road to Pancreatic Intraepithelial Neoplasia
(PanIN) and Pancreatic Cancer. Int J Mol Sci *24*. https://doi.org/10.3390/ijms24129946.

39. Backx, E., Wauters, E., Baldan, J., Van Bulck, M., Michiels, E., Heremans, Y., De Paep, D.L.,
Kurokawa, M., Goyama, S., Bouwens, L., et al. (2021). MECOM permits pancreatic acinar cell
dedifferentiation avoiding cell death under stress conditions. Cell Death & Differentiation *28*,
2601–2615. https://doi.org/10.1038/s41418-021-00771-6

1037

40. Cobo, I., Martinelli, P., Flández, M., Bakiri, L., Zhang, M., Carrillo-de-Santa-Pau, E., Jia, J.,
Sánchez-Arévalo Lobo, V.J., Megías, D., Felipe, I., et al. (2018). Transcriptional regulation by
NR5A2 links differentiation and inflammation in the pancreas. Nature *554*, 533–537.
https://doi.org/10.1038/nature25751.

41. Choi, J., Oh, T.G., Jung, H.W., Park, K.Y., Shin, H., Jo, T., Kang, D.S., Chanda, D., Hong, S., Kim, J.,
et al. (2022). Estrogen-Related Receptor γ Maintains Pancreatic Acinar Cell Function and Identity
by Regulating Cellular Metabolism. Gastroenterology *163*, 239–256.
https://doi.org/10.1053/j.gastro.2022.04.013.

42. Zhang, P., Sun, Y., and Ma, L. (2015). ZEB1: at the crossroads of epithelial-mesenchymal
transition, metastasis and therapy resistance. Cell Cycle *14*, 481–487.
https://doi.org/10.1080/15384101.2015.1006048.

43. Aibar, S., González-Blas, C.B., Moerman, T., Huynh-Thu, V.A., Imrichova, H., Hulselmans, G.,
Rambow, F., Marine, J.-C., Geurts, P., Aerts, J., et al. (2017). SCENIC: single-cell regulatory
network inference and clustering. Nature Methods *14*, 1083–1086.
https://doi.org/10.1038/nmeth.4463.

44. Grout, J.A., Sirven, P., Leader, A.M., Maskey, S., Hector, E., Puisieux, I., Steffan, F., Cheng, E.,
Tung, N., Maurin, M., et al. (2022). Spatial Positioning and Matrix Programs of Cancer-Associated
Fibroblasts Promote T-cell Exclusion in Human Lung Tumors. Cancer Discovery *12*, 2606–2625.
https://doi.org/10.1158/2159-8290.CD-21-1714.

45. Öhlund, D., Handly-Santana, A., Biffi, G., Elyada, E., Almeida, A.S., Ponz-Sarvise, M., Corbo, V.,
Oni, T.E., Hearn, S.A., Lee, E.J., et al. (2017). Distinct populations of inflammatory fibroblasts and
myofibroblasts in pancreatic cancer. Journal of Experimental Medicine *214*, 579–596.
https://doi.org/10.1084/jem.20162024.

46.   You, E., Danaher, P., Lu, C., Sun, S., Zou, L., Phillips, I.E., Rojas, A.S., Ho, N.I., Song, Y., Raabe,
M.J., et al. (2024). Disruption of cellular plasticity by repeat RNAs in human pancreatic cancer.
Cell *187*, 7232–7247.e7223. https://doi.org/10.1016/j.cell.2024.09.024.

47.   Wu, Y.-H., Huang, Y.-F., Chang, T.-H., Chen, C.-C., Wu, P.-Y., Huang, S.-C., and Chou, C.-Y. (2021).
COL11A1 activates cancer-associated fibroblasts by modulating TGF-β3 through the NF-
κB/IGFBP2 axis in ovarian cancer cells. Oncogene *40*, 4503–4519.
https://doi.org/10.1038/s41388-021-01865-8.

48.   Park, Y.J., Yoo, S.A., Kim, M., and Kim, W.U. (2020). The Role of Calcium-Calcineurin-NFAT
Signaling Pathway in Health and Autoimmune Diseases. Front Immunol *11*, 195.
https://doi.org/10.3389/fimmu.2020.00195.

49.   Feig, C., Jones, J.O., Kraman, M., Wells, R.J.B., Deonarine, A., Chan, D.S., Connell, C.M., Roberts,
E.W., Zhao, Q., Caballero, O.L., et al. (2013). Targeting CXCL12 from FAP-expressing carcinoma-
associated fibroblasts synergizes with anti–PD-L1 immunotherapy in pancreatic cancer.
Proceedings of the National Academy of Sciences *110*, 20212–20217.
https://www.pnas.org/doi/abs/10.1073/pnas.1320318110.

50.   Kawaguchi, M., Yamamoto, K., Takeda, N., Fukushima, T., Yamashita, F., Sato, K., Kitamura, K.,
Hippo, Y., Janetka, J.W., and Kataoka, H. (2019). Hepatocyte growth factor activator inhibitor-2
stabilizes Epcam and maintains epithelial organization in the mouse intestine. Commun Biol *2*,
11. https://doi.org/10.1038/s42003-018-0255-8.

51.   Browaeys, R., Saelens, W., and Saeys, Y. (2020). NicheNet: modeling intercellular communication
by linking ligands to target genes. Nature Methods *17*, 159–162.
https://doi.org/10.1038/s41592-019-0667-5.

52.   Zhang, X., Du, Y., Behrens, A., and Lan, L. (2025). Emerging insights into lineage plasticity in
pancreatic cancer initiation, progression, and therapy resistance. Developmental Cell *60*, 2391–
2406. https://doi.org/10.1016/j.devcel.2025.07.002.

53.   Mucciolo, G., Araos Henríquez, J., Jihad, M., Pinto Teles, S., Manansala, J.S., Li, W., Ashworth, S.,
Lloyd, E.G., Cheng, P.S.W., Luo, W., et al. (2024). EGFR-activated myofibroblasts promote
metastasis of pancreatic cancer. Cancer Cell *42*, 101–118.e111.
https://doi.org/10.1016/j.ccell.2023.12.002.

54.   Schwörer, S., Cimino, F.V., Ros, M., Tsanov, K.M., Ng, C., Lowe, S.W., Carmona-Fontaine, C., and
Thompson, C.B. (2023). Hypoxia Potentiates the Inflammatory Fibroblast Phenotype Promoted
by Pancreatic Cancer Cell–Derived Cytokines. Cancer Research *83*, 1596–1610.
https://doi.org/10.1158/0008-5472.CAN-22-2316.

55.   Harder, J., Ihorst, G., Heinemann, V., Hofheinz, R., Moehler, M., Buechler, P., Kloeppel, G.,
Röcken, C., Bitzer, M., Boeck, S., et al. (2012). Multicentre phase II trial of trastuzumab and
capecitabine in patients with HER2 overexpressing metastatic pancreatic cancer. Br J Cancer
106, 1033–1038. https://doi.org/10.1038/bjc.2012.18.

56.   Kimura, K., Sawada, T., Komatsu, M., Inoue, M., Muguruma, K., Nishihara, T., Yamashita, Y.,
Yamada, N., Ohira, M., and Hirakawa, K. (2006). Antitumor Effect of Trastuzumab for Pancreatic
Cancer with High HER-2 Expression and Enhancement of Effect by Combined Therapy with
Gemcitabine. Clinical Cancer Research *12*, 4925–4932. https://doi.org/10.1158/1078-0432.CCR-
06-0544.

57.   Qin, P., Chen, H., Wang, Y., Huang, L., Huang, K., Xiao, G., Han, C., Hu, J., Lin, D., Wan, X., et al.
(2023). Cancer-associated fibroblasts undergoing neoadjuvant chemotherapy suppress rectal
cancer revealed by single-cell and spatial transcriptomics. Cell Reports Medicine *4*, 101231.
https://doi.org/10.1016/j.xcrm.2023.101231.

58.   Sahu, B., Pihlajamaa, P., Zhang, K., Palin, K., Ahonen, S., Cervera, A., Ristimäki, A., Aaltonen, L.A.,
Hautaniemi, S., and Taipale, J. (2021). Human cell transformation by combined lineage

conversion and oncogene expression. Oncogene *40*, 5533–5547.
https://doi.org/10.1038/s41388-021-01940-0.

59.   Bianchi, J.J., Zhao, X., Mays, J.C., and Davoli, T. (2020). Not all cancers are created equal: Tissue
specificity in cancer genes and pathways. Curr Opin Cell Biol *63*, 135–143.
https://doi.org/10.1016/j.ceb.2020.01.005.

60.   Chen, B., Chan, W.N., Xie, F., Mui, C.W., Liu, X., Cheung, A.H.K., Lung, R.W.M., Chow, C., Zhang,
Z., Fang, C., et al. (2023). The molecular classification of cancer-associated fibroblasts on a pan-
cancer single-cell transcriptional atlas. Clinical and Translational Medicine *13*, e1516.
https://doi.org/10.1002/ctm2.1516.

61.   Flynn, J.M., Thadani, N., Gallagher, E.E., Azzaro, I., Bodnar, C.M., McCarty, C.P., Romano, G.,
Webster, M.R., and Capparelli, C. (2025). Plasticity and Functional Heterogeneity of Cancer-
Associated Fibroblasts. Cancer Research *85*, 3378–3398. https://doi.org/10.1158/0008-
5472.CAN-24-3037.

62.   Raghavan, S., Winter, P.S., Navia, A.W., Williams, H.L., DenAdel, A., Lowder, K.E., Galvez-Reyes,
J., Kalekar, R.L., Mulugeta, N., Kapner, K.S., et al. (2021). Microenvironment drives cell state,
plasticity, and drug response in pancreatic cancer. Cell *184*, 6119–6137.e6126.
https://doi.org/10.1016/j.cell.2021.11.017.

63.   Shiau, C., Cao, J., Gong, D., Gregory, M.T., Caldwell, N.J., Yin, X., Cho, J.-W., Wang, P.L., Su, J.,
Wang, S., et al. (2024). Spatially resolved analysis of pancreatic cancer identifies therapy-
associated remodeling of the tumor microenvironment. Nature Genetics *56*, 2466–2478.
https://doi.org/10.1038/s41588-024-01890-9.

64.   Li, J., Liu, H., Guo, Q., Zhang, Y., Li, J., Diao, T., Zheng, L., Deng, Z., Yang, Y., Chen, X., et al. Single-
cell screens identify ADAM12 as a fibroblast checkpoint impeding anti-tumor immunity. Cancer
Cell. 10.1016/j.ccell.2025.12.018.

65.   Guo, C., Griffin, M.F., Morgan, A.G., Foster, D.S., Parker, J.B.L., Januszyk, M., Lindsay, H.G.,
Guardino, N.J., Reveron-Thornton, R., Xie, P.Y., et al. (2026). NFAT mediates pro-tumorigenic
inflammation in cancer-associated fibroblasts in pancreatic ductal adenocarcinoma. Cell Reports
45, 116849. https://doi.org/10.1016/j.celrep.2025.116849.

66.   Yan, R., Moresco, P., Gegenhuber, B., and Fearon, D.T. (2023). T cell–Mediated Development of
Stromal Fibroblasts with an Immune-Enhancing Chemokine Profile. Cancer Immunology
Research *11*, 1044–1054. https://doi.org/10.1158/2326-6066.CIR-22-0593.

67.   Ardito, Christine M., Grüner, Barbara M., Takeuchi, Kenneth K., Lubeseder-Martellato, C.,
Teichmann, N., Mazur, Pawel K., DelGiorno, Kathleen E., Carpenter, Eileen S., Halbrook,
Christopher J., Hall, Jason C., et al. (2012). EGF Receptor Is Required for KRAS-Induced Pancreatic
Tumorigenesis. Cancer Cell *22*, 304–317. https://doi.org/10.1016/j.ccr.2012.07.024.

68.   Wolf, F.A., Angerer, P., and Theis, F.J. (2018). SCANPY: large-scale single-cell gene expression
data analysis. Genome Biology *19*, 15. https://doi.org/10.1186/s13059-017-1382-0.

69.   Hao, Y., Hao, S., Andersen-Nissen, E., Mauck, W.M., 3rd, Zheng, S., Butler, A., Lee, M.J., Wilk,
A.J., Darby, C., Zager, M., et al. (2021). Integrated analysis of multimodal single-cell data. Cell
184, 3573–3587.e3529. https://doi.org/10.1016/j.cell.2021.04.048.

70.   Stirling, D.R., Swain-Bowden, M.J., Lucas, A.M., Carpenter, A.E., Cimini, B.A., and Goodman, A.
(2021). CellProfiler 4: improvements in speed, utility and usability. BMC Bioinformatics *22*, 433.
https://doi.org/10.1186/s12859-021-04344-9.

71.   Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and
Gingeras, T.R. (2012). STAR: ultrafast universal RNA-seq aligner. Bioinformatics *29*, 15–21.
https://doi.org/10.1093/bioinformatics/bts635.

72. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G.,
Durbin, R., and Subgroup, G.P.D.P. (2009). The Sequence Alignment/Map format and SAMtools.
Bioinformatics *25*, 2078–2079. https://doi.org/10.1093/bioinformatics/btp352.

73. Anders, S., Pyl, P.T., and Huber, W. (2014). HTSeq—a Python framework to work with high-
throughput sequencing data. Bioinformatics *31*, 166–169.
https://doi.org/10.1093/bioinformatics/btu638.

74. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and
dispersion for RNA-seq data with DESeq2. Genome Biology *15*, 550.
https://doi.org/10.1186/s13059-014-0550-8

1164

75. Posta, M., and Győrffy, B. (2025). Pathway-level mutational signatures predict breast cancer
outcomes and reveal therapeutic targets. Br J Pharmacol *182*, 5734–5747.
https://doi.org/10.1111/bph.70215.

76. Brichkina, A., Polo, P., Sharma, S.D., Visestamkul, N., and Lauth, M. (2023). A Quick Guide to CAF
Subtypes in Pancreatic Cancer. Cancers (Basel) *15*. https://doi.org/10.3390/cancers15092614.

77. Zayou, F., Pearce, H., Croft, W., Nicol, S., Margielewska-Davies, S., Powell-Brett, S., Brown, R.,
Zuo, J., Roberts, K., McGettrick, H.M., and Moss, P. (2024). Abstract A030: Modulation of
lymphocyte adhesion and migration by iCAF and myCAF fibroblasts in PDAC. Cancer Research
84, A030–A030. https://doi.org/10.1158/1538-7445.PANCREATIC24-A030.

78. van Heeringen, S.J., and Veenstra, G.J.C. (2010). GimmeMotifs: a de novo motif prediction
pipeline for ChIP-sequencing experiments. Bioinformatics *27*, 270–271.
https://doi.org/10.1093/bioinformatics/btq636.

79. Yu, G., Wang, L.-G., Han, Y., and He, Q.-Y. (2012). clusterProfiler: an R Package for Comparing
Biological Themes Among Gene Clusters. OMICS: A Journal of Integrative Biology *16*, 284–287.
https://doi.org/10.1089/omi.2011.0118.

80. Hänzelmann, S., Castelo, R., and Guinney, J. (2013). GSVA: gene set variation analysis for
microarray and RNA-Seq data. BMC Bioinformatics *14*, 7. https://doi.org/10.1186/1471-2105-
14-7.

81. Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich,
A., Pomeroy, S.L., Golub, T.R., Lander, E.S., and Mesirov, J.P. (2005). Gene set enrichment
analysis: A knowledge-based approach for interpreting genome-wide expression profiles.
Proceedings of the National Academy of Sciences *102*, 15545–15550.
https://doi.org/10.1073/pnas.0506580102.

82. Goldman, M.J., Craft, B., Hastie, M., Repečka, K., McDade, F., Kamath, A., Banerjee, A., Luo, Y.,
Rogers, D., Brooks, A.N., et al. (2020). Visualizing and interpreting cancer genomics data via the
Xena platform. Nature Biotechnology *38*, 675–678. https://doi.org/10.1038/s41587-020-0546-8.

83. Joung, J., Ma, S., Tay, T., Geiger-Schuller, K.R., Kirchgatterer, P.C., Verdine, V.K., Guo, B., Arias-
Garcia, M.A., Allen, W.E., Singh, A., et al. (2023). A transcription factor atlas of directed
differentiation. Cell *186*, 209–229.e226. https://doi.org/10.1016/j.cell.2022.11.026.

84. Esfahani, S.A., Ma, H., Krishna, S., Shuvaev, S., Sabbagh, M., Deffler, C., Rotile, N., Weigand-
Whittier, J., Zhou, I.Y., Catana, C., et al. (2024). Collagen type I PET/MRI enables evaluation of
treatment response in pancreatic cancer in pre-clinical and first-in-human translational studies.
Theranostics *14*, 5745–5761. https://doi.org/10.7150/thno.100116.

85. Allen-Coyle, T.J., Niu, J., Welsch, E., Conlon, N.T., Garner, W., Clynes, M., O'Sullivan, F.,
Straubinger, R.M., Mager, D.E., and Roche, S. (2022). FOLFIRINOX Pharmacodynamic
Interactions in 2D and 3D Pancreatic Cancer Cell Cultures. The AAPS Journal *24*, 108.
https://doi.org/10.1208/s12248-022-00752-8.

86.   Awasthi, N., Zhang, C., Schwarz, A.M., Hinz, S., Wang, C., Williams, N.S., Schwarz, M.A., and
1203   Schwarz, R.E. (2013). Comparative benefits of Nab-paclitaxel over gemcitabine or polysorbate-
1204   based docetaxel in experimental pancreatic cancer. Carcinogenesis *34*, 2361–2369.
1205   https://doi.org/10.1093/carcin/bgt227.
1206   87.   Koh, E.K., Lee, H.R., Son, W.C., Park, G.Y., Kim, J., Bae, J.H., and Park, Y.S. (2023). Combinatorial
1207   immunotherapy with gemcitabine and ex vivo-expanded NK cells induces anti-tumor effects in
1208   pancreatic cancer. Sci Rep *13*, 7656. https://doi.org/10.1038/s41598-023-34827-z.

1209