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YAP1 and TAZ Control Pancreatic Cancer Initiation in Mice by Direct Upregulation of JAK–STAT3 Signaling

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**Running title:** Regulation of acinar-to-duct reprogramming by KRas

**Conflict of Interest:** The authors declare that they have no conflicts of interest.

**Author contributions:** RG conceived and designed the study, performed experiments, analyzed data, and wrote the manuscript; RP assisted with in vitro ADM assay; EN and BSD performed immunohistochemistry; GS provided histopathology analysis; AB supervised the study and wrote the manuscript.
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**Abbreviations used in this paper:** ADM, acinar-to-duct metaplasia; ChIP, chromatin immunoprecipitation; DAPI, 4’,6-Diamidino-2-Phenylindole Dihydrochloride; ES, embryonic stem cell; FBS, fetal bovine serum; HBSS, Hank’s balanced salt solution; IL-6, interleukin 6; mPanIN, mouse pancreatic intraepithelia neoplasia; PanIN, pancreatic intraepithelia neoplasia; PDAC, pancreatic ductal adenocarcinoma; pfu, plaque forming units; TGFα; transforming growth factor alpha; TSS, transcription start site.
Abstract:

Background & Aims: Pancreatitis is the most important risk factor for pancreatic ductal adenocarcinoma (PDAC). Pancreatitis predisposes to PDAC because it induces a process of acinar cell reprogramming known as acinar-to-duct metaplasia (ADM)—a precursor of pancreatic intra-epithelial neoplasia lesions that can progress to PDAC. Mutations in KRAS are found at the earliest stages of pancreatic tumorigenesis, and it appears to be a gatekeeper to cancer progression. We investigated how mutations in KRAS cooperate with pancreatitis to promote pancreatic cancer progression in mice.

Methods: We generated mice carrying conditional alleles of Yap1 and Taz and disrupted Yap1 and Taz using a Cre-lox recombination strategy in adult mouse pancreatic acinar cells (Yap1fl/fl;Tazfl/fl;Ela1-CreERT2). We crossed these mice with LSL-KrasG12D mice, which express a constitutively active form of KRAS after Cre recombination. Pancreatic tumor initiation and progression were analyzed after chemically induced pancreatitis. We analyzed pancreatic tissues from patients with pancreatitis or PDAC by immunohistochemistry.

Results: Oncogenic activation of KRAS in normal, untransformed acinar cells in the pancreatic tissues of mice resulted in increased levels of pancreatitis-induced ADM. Expression of the constitutive active form of KRAS in this system led to activation of the transcriptional regulators YAP1 and TAZ; their function was required for pancreatitis-induced ADM in mice. The JAK–STAT3 pathway was a downstream effector of KRAS signaling via YAP1 and TAZ. YAP1 and TAZ directly mediated transcriptional activation of several genes in the JAK–STAT3 signaling pathway; this
could be a mechanism by which acinar cells that express activated KRAS become susceptible to inflammation.

**Conclusion:** We identified a mechanism by which oncogenic KRAS facilitates ADM and thereby generates the cells that initiate neoplastic progression. This process involves activation of YAP1 and TAZ in acinar cells, which upregulate JAK–STAT3 signaling to promote development of PDAC in mice.

**KEY WORDS:** PanINs, pancreatic cancer progression, mouse model, inflammation
Introduction

The most common and lethal tumor of the pancreas, pancreatic ductal adenocarcinoma (PDAC), exhibits the histological morphology and marker expression of pancreatic duct cells. However, many of these tumors originate from the other major exocrine cell type, acinar cells, through a reprogramming process known as acinar-to-ductal metaplasia (ADM)\textsuperscript{1}. ADM is characterized by a change in marker expression: acinar cells positive for amylase also begin to express the ductal cell marker CK19. These poorly-differentiated cells express the pancreatic progenitor cell markers Pdx1 and Nestin\textsuperscript{2,3} and are important precursors of malignancy\textsuperscript{4}. Pancreatic intraepithelial neoplasia (PanIN) lesions have a well-established histological progression towards PDAC\textsuperscript{5}, and can arise from ADM lesions\textsuperscript{1}. Thus ADM is the earliest pre-neoplastic lesion that predisposes to PDAC, making acinar-to-duct reprogramming a crucial step in pancreatic cancer initiation.

KRas mutations are found in more than 90\% of all PDAC cases\textsuperscript{6} and they occur early, in low-grade pre-neoplastic lesions\textsuperscript{7,8}. Although the importance of KRas mutation in the development of ADM, PanIN and pancreatic tumors is well established\textsuperscript{9}, the mechanisms by which oncogenic Ras leads to PDAC are not fully understood.

Pancreatitis is a well-known risk factor for PDAC development in humans. Patients with hereditary pancreatitis show a 50-fold increase in pancreatic cancer incidence\textsuperscript{10}. In mouse models, both acute and chronic inflammation of the pancreas accelerate pancreatic cancer progression\textsuperscript{1,11}. Pancreatitis can be induced experimentally by injection of caerulein, which induces acinar cell death and inflammation\textsuperscript{12}. In caerulein-induced pancreatitis, inflammation induces acinar cells to reprogram to form ADM lesions\textsuperscript{11}, which may be transient, or in the presence of a KRas\textsubscript{G12D} mutation, persistent\textsuperscript{13}. TGF\textalpha administration or overexpression also cooperates with
KRas\textsuperscript{G12D} to induce ADM in vitro\textsuperscript{3, 14}. Thus, pancreatic inflammation facilitates tumorigenesis by inducing ADM.

Expression of oncogenic KRas\textsuperscript{G12D} at endogenous levels in acinar cells triggers progression to pancreatic adenocarcinoma very inefficiently. Stronger transgenic expression of oncogenic Ras\textsuperscript{15}, or additional oncogenic stimuli, such as mutant p53\textsuperscript{16} or TGFα overexpression\textsuperscript{17} are required. Many of these additional stimuli directly or indirectly increase Ras activity, suggesting that a positive feedback loop amplifying oncogenic Ras signaling is required for PDAC progression\textsuperscript{18}.

The reliance of established PDAC tumors on KRas\textsuperscript{G12D} expression can be alleviated by upregulation of the transcriptional regulator YAP1\textsuperscript{19}. YAP1 has also been implicated in progression from PanIN to PDAC\textsuperscript{20}, but its contribution at the reprogramming stage is unclear.

Here we show that KRas mutation sensitizes acinar cells to reprogramming by activating YAP1 and TAZ signaling, which in turn upregulates components of the JAK-STAT3 pathway. This increases sensitivity to inflammatory stimuli, which induces widespread ADM among KRas-mutant acinar cells. Importantly, inhibiting KRas\textsuperscript{G12D}- and pancreatitis-induced reprogramming by inactivating YAP1/TAZ also prevents progression to PanIN, underlining the importance of this mechanism for pancreatic cancer initiation.
Materials and Methods

Mouse Lines
For the generation of conditional Yap1 and Taz/Wwtr1 knock-out mice, ES cell clones carrying the respective targeted alleles were obtained from the Knockout Mouse Project (KOMP) Repository (Yap1<tm1a(KOMP)Mbp>; ES clone E08) and the European Conditional Mouse Mutagenesis (EUCOMM) Program (Wwtr1<tm1a(EUCOMM)Wtsi>; ES clone A08). Targeted ES cell clones (agouti C57BL/6 parental cell line JM8A3.N1) were injected into blastocysts of C57BL/6 background to generate chimeric mice, which were then crossed with Flpo-expressing mice (Tg(CAG-Flpo)1Afst; C57BL/6 background) to remove the neo-selection cassette and obtain mice carrying the conditional floxed alleles Yap1<tm1c(KOMP)Mbp> and Wwtr1<tm1c(EUCOMM)Wtsi> isogenic on C57BL/6. The Flpo-allele was bred out and mice were maintained on a C57BL/6 background. For genotyping and validation of knockout alleles, see Supplementary Figure 4 and Supplementary Materials and Methods.

The Stat3fl/fl, Pdx1-Cre, R26-LSL-YFP, LSL-KRasG12D and Ela1-CreERT2 mouse lines have been described (see Supplementary Materials and Methods).

Acute pancreatitis induction
Acute pancreatitis in C57BL/6 mice was induced by eight intra-peritoneal injections of caerulein (Sigma-Aldrich) dissolved in PBS given at one-hour intervals on two consecutive days at a dose of 50 µg/kg body weight per injection (high dose). Other strains received a similar treatment schedule except with six injections at a dose of 40 µg/kg body weight per injection (low dose). Control animals received injections of PBS only.
Gene set enrichment analysis

Gene set enrichment analysis was performed on the published transcription profile of C57BL/6 Jackson mice treated with caerulein or PBS and on the transcription profile of mouse liver organoids overexpressing YAP, with the software developed by the Broad Institute of MIT and Harvard. The settings are listed in Supplementary Materials and Methods.

Primary acinar cell culture

Mice were sacrificed by cervical dislocation, the pancreas was dissected out and transferred to ice-cold Hank’s balanced salt solution (HBSS) supplemented with Penicillin/Streptomycin (Sigma-Aldrich). Pancreata were cut into small pieces and digested with 2 mg/ml Collagenase P (Roche Diagnostics) in HBSS for 15 min at 37 °C. Cells were washed three times with HBSS supplemented with 5 % FBS and then filtered through 500 µm and 105 µm nylon meshes (Spectrum Laboratories). The cell suspension was layered on top of 30 % FBS in HBSS, centrifuged at 1000 r.p.m for 2 min and the cell pellet was resuspended in acinar cell culture medium (Waymouth’s medium (Life Technologies) supplemented with 1 % FBS, Penicillin/Streptomycin (Sigma-Aldrich), 1 µg/ml Dexamethasone (Sigma-Aldrich) and 100 µg/ml Soybean trypsin inhibitor (Sigma-Aldrich)). The cell suspension was then infected with adenoviruses either obtained from the University of Iowa Carver College of Medicine (Ad5-GFP and Ad5-Cre-GFP), at a concentration of 1.25x10^7 plaque forming units (pfu)/ml, or generated by cloning (Ad-GFP, Ad-GFP-YAP1-5SA and Ad-GFP-TAZ-S89A), at a concentration of 5x10^5 pfu/ml. Acinar cells were incubated with adenoviruses for 1 h at 37 °C. 6-well tissue culture plates were coated with an 800 µl
layer of collagen solution (4 mg/ml rat tail collagen (BD Biosciences), 10 % 10x Waymouth’s medium (Sigma-Aldrich) and 0.02 mol/l NaOH (Sigma-Aldrich)). The cell suspension was mixed 1:1 with collagen solution and plated onto the collagen layer. The acinar cell/collagen mix was allowed to solidify for 1 h at 37 °C before adding medium. Medium was changed on day 1 and day 3 after the isolation. Quantifications were done on day 5 after isolation. To harvest the cells, the collagen matrix was digested with 1 mg/ml collagenase P (Roche Diagnostics) diluted in HBSS for 30 min at 37 °C.

**Production of recombinant adenoviruses**

The vectors harboring the coding sequences of the phosphorylation mutants of YAP1 (5SA) and TAZ (S89A), kindly provided by S. Piccolo, were used to subclone the YAP1 and TAZ cDNAs in the pAd-Track-CMV vector (Addgene #16405). This results in the expression of YAP1/TAZ under the control of the CMV promoter and pAd-Track-CMV empty vector was used as control. The pAd-Track-CMV contains a GFP cDNA under control of a second CMV promoter. Recombinant adenoviruses expressing GFP, YAP1-5SA in combination with GFP or TAZ-S89A in combination with GFP were generated following the protocol as described. Briefly, human HEK293A cells were co-transfected with pAd-Easy-1 (Addgene #16400) together with pAd-Track-CMV, pAd-Track-CMV-YAP1-5SA or pAd-Track-CMV-TAZ-S89A. Adenoviruses were harvested 14 days after transfection. To generate higher viral titer, fresh HEK293A were infected with the adenoviruses and grown for 7 days before harvesting and this process was repeated three times. Adenoviruses were purified using the Adeno-X Maxi Purification Kit (TaKaRa Clontech) according to the manufacturer’s protocol. Virus titers were determined by infecting HEK293T cells
with a dilution series of the adenoviruses. After infection, cells were overlaid with agarose and plaques were visualized by MTT (Sigma-Aldrich) and quantified 7 days later. This protocol generated virus titer ranging from 1 to $6 \times 10^8$ pfu/ml.

**Immunoblot Analysis and Ras-GTP pulldown assay**

Protein lysates were obtained from dissected pancreata, primary acinar cells or PANC1 cells and homogenized in Cell Lysis Buffer (Cell Signaling) containing proteasome inhibitor (Sigma-Aldrich). Immunoblots were performed as described\textsuperscript{25}. PVDF membranes (GE Healthcare) were incubated with antibodies against YAP1 (Cell Signaling; #4912 and Santa Cruz; sc-15407), p-YAP1 (Ser127 in humans and Ser112 in mice; Cell Signaling; #4911); TAZ (Sigma-Aldrich; HPA007415), CK19 (DHSB; TROMA-III), STAT3 (Cell Signaling; #4904), p-STAT3 (Tyr705; Cell Signaling; #9131); LATS1 (Cell Signaling; #3477), p-LATS1 (Ser909; Cell Signaling; #9157), LIFR (Santa Cruz; sc-659), PDX1 (R&D Systems, MAB2419), SOX9 (Millipore, AB5535), p-Erk1/2 (Thr202/Tyr204; Cell Signaling; #9106), Erk1/2 (Cell Signaling, #9102), GAPDH (Abcam; ab9485) and ACTIN (Abcam; ab49900). Active Ras levels were determined by Ras-GTP pulldown assay (ThermoFisher Scientific) using the Raf Ras-binding-domain fused to GST (Raf-RBD-GST) according to the manufacturer’s instructions. Signal intensities of bands were quantified using ImageJ software.

**Statistics**

Statistical analysis was performed using GraphPad Prism 6.0a software and 2-tailed Student’s $t$-tests were used to generate $P$ values. Normalized enrichment scores and nominal $P$ values for the gene set enrichment analysis were generated using the
software developed by the Broad Institute of MIT and Harvard. \( P \) values less than 0.05 were considered significant.

**Study Approval**

Mouse experiments were carried out with the approval of the London Research Institute’s Ethical Review Committee according to the UK Animals (Scientific Procedures) Act 1986.
Results

YAP1 and TAZ are upregulated in pancreatitis

ADM caused by pancreatitis is an initiating step in pancreatic tumor development\(^1\). To find molecular regulators of this process, we performed gene set enrichment analysis\(^26\) on the published transcriptional profile of mouse pancreata treated with caerulein\(^22\) to identify pathways upregulated in pancreatitis (Supplementary Figure 1A). Many of these pathways, such as Wnt, Notch and JAK-STAT3, have previously been implicated in either acinar cell regeneration or in inducing ADM upon injury\(^13, 27-29\). Intriguingly, the highest gene set enrichment scores were for YAP1 target genes (Figure 1A and Supplementary Figure 1A).

To investigate a possible role for YAP1/TAZ signaling in pancreatitis-induced ADM, we treated C57BL/6 mice with caerulein to induce pancreatitis (Figure 1B). In untreated mice, YAP1 and TAZ proteins showed nuclear localization in duct cells of the pancreas, with low or absent expression in acinar cells and islet cells\(^20, 30\) (Supplementary Figure 1B). Consistent with the gene set enrichment analysis, the YAP1/TAZ target genes Ctgf, Cyr61, Ankrd1 and Amotl2 were overexpressed in caerulein-treated animals compared with control PBS-treated mice (Figure 1C). Caerulein treatment reduced activatory phosphorylation of the YAP1/TAZ inhibitory kinase Lats1 (Figure 1D and Supplementary Figure 2A and B). In line with this, we observed increased YAP1 and TAZ protein levels (Figure 1D and Supplementary Figure 2A and B), accompanied by strong nuclear localization, indicating more active YAP1/TAZ in caerulein-treated animals (Figure 1E). Levels of the duct cell marker CK19 were also increased, consistent with acinar-to-duct reprogramming (Figure 1D and Supplementary Figure 2A and B). To determine the identity of the YAP1/TAZ-responsive cells we performed immunofluorescence
co-staining with pancreatic cell type specific markers. Cells co-expressing CK19 and the acinar cell marker amylase showed high levels of nuclear YAP1 and TAZ, with some stromal cells also positive (Figure 1F). Thus, YAP1/TAZ activity is elevated in the injured, inflamed pancreas, particularly in the subset of cells undergoing ADM.

**YAP and TAZ are upregulated in ADM lesions induced by K\textit{Ras}^{G12D}\**

We next asked whether YAP1/TAZ upregulation is restricted to caerulein-induced ADM, or also occurs during ADM induced by oncogenic Ras. The pancreatic tumor mouse model \textit{LSL-KRas}^{G12D};\textit{Pdx1-Cre} develops PDAC with long latency and displays the whole spectrum of pre-neoplastic lesions\textsuperscript{31} (Figure 2A). We found a strong signal for both YAP1 and TAZ in ADM lesions of these mice, and an upregulation of YAP1 and TAZ in pancreatic protein lysates (Figure 2A and B and Supplementary Figure 3A and B). Analysis of YAP1 and TAZ immunostaining on human pancreatic tissue microarrays showed strong upregulation of YAP1 in human ADM and PanIN pre-neoplastic lesions compared with normal acini (Figure 2C-E). TAZ was strongly upregulated in ADM and to a lesser extent in PanIN lesions in mouse models and human tissues (Figure 2A and C-E). Surprisingly, we found a significant downregulation of YAP1 and TAZ protein levels in human PDAC samples compared to ADM lesions (Figure 2C-E), suggesting a functional role of YAP1 and TAZ particularly during tumor initiation.

**YAP1 and TAZ are necessary and sufficient for ADM induction**

To determine the functional requirement for YAP1/TAZ signaling in acinar-to-duct reprogramming, we generated conditional floxed alleles of both \textit{Yap1} and \textit{Taz} (Supplementary Figure 4A-F). YAP1 and TAZ were efficiently deleted in
Yap1\(^{fl/fl}\);Taz\(^{fl/fl}\) primary acinar cells (Supplementary Figure 5A and B). We combined the floxed Yap1\(^{fl/fl}\) and Taz\(^{fl/fl}\) alleles with the Cre-inducible oncogenic LSL-KRas\(^{G12D}\) allele, shown to induce ADM in cultured acinar cells\(^{14}\), and assayed ADM in vitro (Figure 3A). As expected, Cre-mediated activation of KRas\(^{G12D}\) transformed acinar cell clusters into tubular duct-like structures (Figure 3B). Deletion of Yap1 significantly reduced the ability of KRas\(^{G12D}\) to induce ADM, but deletion of Taz did not. However, in double mutant Yap1/Taz acinar cells, KRas\(^{G12D}\) failed to induce ADM (Figure 3B and Supplementary Figure 5C and D).

To address the requirement for YAP1 in vivo, we combined Yap1\(^{fl/fl}\) with the Pdx1-Cre mouse line. Caerulein-induced ADM lesions co-expressing CK19 and amylase were readily observed in Yap1\(^{fl/fl}\);Pdx1-Cre mice. However, these lesions were positive for YAP1 nuclear staining (Supplementary Figure 6A), suggesting that ADM occurred in cells that had escaped Yap1 deletion. Consistent with this, PCR and immunoblot analysis showed that the recombination efficiency and consequent depletion of YAP1 was incomplete in Yap1\(^{fl/fl}\);Pdx1-Cre mice (Supplementary Figure 6B and C). As an alternative model, we used a tamoxifen-inducible Cre line under the control of the acinar-specific Ela1 promoter (Ela1-Cre\(^{ERT2}\)) in combination with a GFP lineage tracer (R26-LSL-YFP) to allow identification of recombined cells (Figure 3C). In this model, GFP-positive acinar cells that had lost both Yap1 and Taz were significantly impaired in caerulein-induced ADM formation (Figure 3D), supporting a requirement for YAP1 and TAZ in ADM formation in vivo. Importantly, co-deletion of Yap1 and Taz did not affect acinar cell viability (Supplementary Figure 7A).

We next examined the effects of ectopic YAP1/TAZ activation in acinar cells by generating adenoviral vectors encoding constitutively active phosphorylation mutants.
of YAP1 (Ad-GFP-YAP1-5SA) and TAZ (Ad-GFP-TAZ-S89A) (Supplementary Figure 7B-D). These mutants are predominantly nuclear and able to induce target gene expression32,33, as expected, adenoviral delivery of YAP1-5SA as well as TAZ-S89A resulted in upregulation of their target genes Ctgf and Cyr61 in primary acinar cells (Supplementary Figure 7E). Strikingly, acinar explants infected with either Ad-GFP-YAP1-5SA or Ad-GFP-TAZ-S89A, but not Ad-GFP, underwent conversion to a duct cell morphology (Figure 3E and F). These data suggest that activation of the transcriptional regulators YAP1 and TAZ in acinar cells is sufficient to induce ADM.

YAP1/TAZ activation increases expression of JAK-STAT3 pathway components

To investigate the molecular mechanisms downstream of YAP1/TAZ, we analyzed the published transcription profile of cells overexpressing YAP123. Gene set enrichment analysis of BIOCARTA-annotated pathways in this dataset34 identified the IL6-JAK-STAT3 signaling cascade as strongly regulated by YAP1 overexpression (Supplementary Figure 8A). Importantly, JAK-STAT3 signaling was also strongly upregulated in mice with pancreatitis (Supplementary Figure 1A), and STAT3 is known to be important for oncogenic KRas-induced pancreatic tumor development28,29,35. To validate the involvement of this pathway downstream of YAP1/TAZ, we analyzed the expression of JAK-STAT3 pathway components in the human PDAC cell line PANC1, which shows constitutively active YAP1/TAZ (Supplementary Figure 8B and C). The receptors LIFR, GP130, IL13RA1 and the transcription factor STAT3 were downregulated upon YAP1 and TAZ knockdown in PANC1 cells (Figure 4A). Accordingly, YAP1/TAZ knockdown reduced STAT3 and LIFR protein levels (Figure 4A and Supplementary Figure 8D). Overexpression of constitutively active
YAP1-5SA or TAZ-S89A led to increased protein levels of STAT3 and LIFR in primary mouse acinar cells (Figure 4B and Supplementary Figure 9A and B).

We next tested whether the JAK-STAT3 pathway genes are direct transcriptional targets of the YAP1/TAZ-dependent TEAD transcription factors\(^\text{36}\). The promoter regions of \textit{STAT3}, \textit{LIFR} and \textit{GP130} contain TEAD4 binding sites, as analyzed by genome-wide ENCODE chromatin immunoprecipitation (ChIP) experiments\(^\text{37}\) (Supplementary Figure 9C). We confirmed that TEAD4 binding was significantly enriched at the promoters of \textit{STAT3}, \textit{LIFR} and \textit{GP130} by ChIP (Figure 4C). In the \textit{STAT3} promoter region, one TEAD consensus binding site GT-II\(^\text{c}\) was found 312 bp to 306 bp upstream of the \textit{STAT3} transcription start site (TSS) (Figure 4D). We used the entire \textit{STAT3} promoter region including this site in a luciferase reporter construct transfected into the rat pancreatic acinar cell line AR42J. Overexpression of either YAP1-5SA or TAZ-S89A in this cell line significantly increased the luciferase activity using the \textit{STAT3} promoter construct compared with empty vector control (Figure 4D). Mutation of the TEAD binding site abolished the YAP1- and TAZ-induced luciferase activity (Figure 4D). Deletion of \textit{Stat3} did not change the expression or protein levels of YAP1 and TAZ in primary acinar cells (Supplementary Figure 10A-C). Our results suggest that, through TEAD, YAP1 and TAZ regulate the gene expression of the cytokine receptors \textit{LIFR} and \textit{GP130} and the transcription factor \textit{STAT3}.

**JAK-STAT3 pathway activation in ADM lesions**

To examine whether JAK-STAT3 signaling is involved in ADM, we analyzed STAT3 activation in response to caerulein-induced pancreatitis. Caerulein treatment led to widespread active, phosphorylated STAT3 (pTyr705-STAT3) in the injured pancreas
(Figure 4E), in accordance with previous reports\textsuperscript{29}. STAT3 phosphorylation and upregulation of STAT3 and LIFR proteins were also detected in caerulein-treated pancreas lysates (Figure 4F and Supplementary Figure 10D and E), and Stat3, Lifr, Gp130 and Jak1 were upregulated in injured pancreata at the transcriptional level (Figure 4F), in agreement with our bioinformatic analysis (Supplementary Figure 1A). Co-staining with CK19 and amylase revealed increased staining intensity of STAT3 and LIFR in \textit{KRas}^{G12D}-induced ADM cells in the \textit{Pdx1-Cre} model (Supplementary Figure 11A and B).

\textbf{YAP1/TAZ-dependent extreme ADM sensitization by oncogenic \textit{KRas}^{G12D}}

The upregulation of the JAK/STAT pathway by YAP1/TAZ predicted an increased susceptibility of \textit{KRas}^{G12D}-mutant acinar cells to inflammation, independently of oncogenic transformation. To test this, we challenged control (\textit{R26-LSL-YFP;Ela1-Cre}\textsuperscript{ERT2}) and \textit{R26-LSL-YFP;LSL-KRas}^{G12D};\textit{Ela1-Cre}\textsuperscript{ERT2} mice with caerulein to induce pancreatitis (Figure 5A). As expected, control mice treated with low doses of caerulein showed acinar cell loss, inflammation and a limited number of ADM lesions. However, \textit{R26-LSL-YFP;LSL-KRas}^{G12D};\textit{Ela1-Cre}\textsuperscript{ERT2} mice displayed a dramatic increase in ADM lesions double-positive for amylase and CK19 after caerulein treatment, although the recruitment of inflammatory F4/80-positive macrophages was similar to caerulein-treated control mice (Figure 5B, C and Supplementary Figure 12A). Lineage tracing with the Cre-inducible \textit{R26-LSL-YFP} allele confirmed the acinar cell origin of the ADM lesions (Supplementary Figure 12B). Thus oncogenic Ras mutations dramatically increase the susceptibility of acinar cells to undergo ADM, possibly by increasing acinar cell sensitivity to inflammatory cytokines through upregulation of \textit{Stat3}, \textit{Lifr} and \textit{Gp130}.
We next tested the significance of YAP1/TAZ and STAT3 in Ras-induced ADM sensitization directly. Strikingly, co-deletion of Yap1 and Taz completely blocked the enhanced formation of these pancreatitis-induced ADM lesions by KRas\textsuperscript{G12D} (Figure 5B and C). Pancreata of R26-LSL-YFP;LSL-KRas\textsuperscript{G12D};Yap1\textsuperscript{fl/fl};Taz\textsuperscript{fl/fl};Ela1-Cre\textsuperscript{ERT2} mice showed normal morphology with some signs of caerulein-induced acinar cell loss and inflammation similar to the extent observed in control mice (Figure 5B and Supplementary Figure 12A). Additionally, in agreement with previous results\textsuperscript{29}, loss of STAT3 significantly reduced caerulein-induced CK19-positive ductal structures in the mutant KRas\textsuperscript{G12D} background (Figure 5B and C). Ras-induced ADM formation resulted in increased expression of the pancreatic progenitor marker Pdx1 and the duct marker Sox9, which was dependent on YAP1/TAZ (Figure 5D and Supplementary Figure 13A). These results suggest that expression of KRas\textsuperscript{G12D} in normal, untransformed acinar cells results in extreme sensitization to pancreatitis-induced ADM, which is mediated by YAP1/TAZ and STAT3.

JAK-STAT3 pathway acts downstream of YAP1/TAZ in response to oncogenic Ras and inflammatory stimuli

We next addressed the function of YAP1/TAZ and JAK-STAT3 signaling as effectors of oncogenic Ras. KRas\textsuperscript{G12D} was activated in adult acinar cells using tamoxifen-inducible LSL-KRas\textsuperscript{G12D};Ela1-Cre\textsuperscript{ERT2} mice, and gene expression was analyzed 7 days later, when pancreas histology was unaltered and no tumor initiation had occurred. KRas\textsuperscript{G12D} activation in normal untransformed acinar cells resulted in increased expression of the YAP1/TAZ target genes Ctgf and Cyr61, accompanied by transcriptional induction of Stat3, Lifr and Gp130 (Figure 6A). Importantly, activation of STAT3 pathway genes by oncogenic KRas\textsuperscript{G12D} in this context occurred
without caerulein treatment. After deletion of $Yap1^{fl/fl}, Taz^{fl/fl}, KRas^{G12D}$ was unable to induce YAP1/TAZ target genes or Stat3, Lifr and Gp130 expression (Figure 6A). $KRas^{G12D}$ activation in primary acinar cells in vitro increased YAP1 and TAZ protein levels, STAT3 protein levels and STAT3 phosphorylation (Figure 6B and Supplementary Figure 13B) but deletion of $Yap1^{fl/fl}, Taz^{fl/fl}$ modestly reduced STAT3 protein levels and greatly impaired the $KRas^{G12D}$-induced phosphorylation of STAT3 in acinar cells in vitro (Figure 6B and Supplementary Figure 13B). In agreement with these results, caerulein treatment of tamoxifen-induced $LSL-KRas^{G12D}, Ela1-Cre^{ERT2}$ mice led to a dramatic increase in STAT3 phosphorylation at tyrosine 705 which was completely abolished in the absence of YAP1 and TAZ (Figure 6C, Figure 5D and Supplementary Figure 13A). YAP1 and TAZ deletion also diminished the caerulein-induced phosphorylation of STAT3 in pancreatic acinar cells wild type for $KRas$ (Supplementary Figure 14A and B). These data indicate that, even in the absence of an inflammatory stimulus or tumorigenesis, oncogenic Ras mutation leads to YAP1/TAZ and JAK-STAT3 pathway activation, and YAP1 and TAZ act upstream of the JAK-STAT3 signaling pathway in oncogenic KRas-induced ADM.

Deletion of YAP1 and TAZ reduces inflammation-induced Ras activation

Inflammatory insults lead to hyperstimulation of oncogenic Ras activity, and interfering with the inflammatory signaling cascade interrupts this feedback activation\textsuperscript{18}. We next tested whether YAP1/TAZ affects oncogenic Ras activity. Caerulein treatment increased Ras activity in mice expressing $KRas^{G12D}$ in acinar cells (Supplementary Figure 15A and B) as shown previously\textsuperscript{18}. Deletion of $Yap1$ and $Taz$ in the $KRas^{G12D}$ background reduced Ras activation after caerulein treatment.
(Supplementary Figure 15A and B). Overexpression of constitutively active YAP1 in primary acinar cells increased Ras activity (Supplementary Figure 15C). Hyperactivating oncogenic Ras by TGFα resulted in a more efficient differentiation of acinar to duct cells in vitro\(^39\) (Supplementary Figure 16A and B). However, YAP1 and TAZ were required for KRas\(^{G12D}\)-induced ADM even in the presence of TGFα (Supplementary Figure 16A and B).

YAP1 and TAZ are required for KRas\(^{G12D}\)-induced PanIN formation

To determine whether the block to ADM in Yap1\(^{fl/fl}\); Taz\(^{fl/fl}\); LSL-KRas\(^{G12D}\); Ela1-Cre\(^{ERT2}\) mice affects the development of pancreatic cancer, we assessed PanIN lesions 3 months after the transient induction of pancreatitis by caerulein (Figure 7A). At this stage, LSL-KRas\(^{G12D}\); Ela1-Cre\(^{ERT2}\) mice displayed CK19/amylase double-positive ADM lesions and CK19- and AB/PAS-positive PanIN lesions (Figure 7B-D). Yap1 and Taz co-deletion resulted in a striking decrease in ADM lesions and these mice were free of PanIN lesions, similar to control mice (Figure 7B-D). These results show that acinar cell-specific YAP1/TAZ signaling is essential for oncogenic KRas\(^{G12D}\)-induced PanIN formation in the context of pancreatitis, and that STAT3 signaling appears to be a critical downstream factor in this process.
Discussion

Acinar-to-duct reprogramming is now established as an important precursor to PanIN lesions and progression to pancreatic cancer, but the molecular mechanisms that drive this process have remained unclear. Our results confirm that $KRas^{G12D}$ mutation synergizes with inflammatory insults to induce widespread and persistent ADM lesions, and we elucidate how this synergy occurs (Figure 7E). We find that the transcriptional regulators YAP1 and TAZ are required for $KRas^{G12D}$ to promote acinar-to-duct reprogramming, both in vitro and in vivo. In the absence of YAP1 and TAZ, $KRas^{G12D}$ is unable to induce PanIN lesions, suggesting that acinar-to-duct reprogramming via YAP1/TAZ is an obligate step in pancreatic cancer initiation from acinar cells.

Mechanistically, active YAP1 and TAZ interact with the transcription factor TEAD, which binds and upregulates the expression of several genes in the JAK-STAT3 pathway. This pathway controls the response to inflammatory cytokines like IL-6, which is secreted during caerulein-induced pancreatitis and also present at high levels in pancreatic tumors, promoting PanIN progression in $KRas^{G12D}$ mice. A recent study found that the IL-6 coreceptor GP130, upregulated by YAP1/TAZ in our study, also activates YAP1 independently of STAT3. Additionally, LIFR has been shown to act upstream of YAP1 signaling. This would create a positive feedback loop amplifying YAP1 activity.

Our data in mouse models expressing $KRas^{G12D}$ are consistent with findings in cultured cells showing that Ras signaling both stabilizes and activates YAP1. Recent studies have implicated YAP1 as an important driver of pancreatic cancer progression at later stages in collaboration with oncogenic Ras, and as a mechanism of escape from Ras-RAF-MEK-targeted therapies. However, data from patient
samples has shown that the incidence of KRas codon 12 mutations is already over 90% even in the earliest pre-neoplastic lesions. Here we show that YAP1 acts downstream of oncogenic Ras at this early reprogramming stage. There is likely redundancy between YAP1 and TAZ, since co-deletion of Yap1 and Taz is more efficient in blocking KRasG12D-induced ADM in the in vitro assay compared to either single mutant. YAP1 seems able to compensate for TAZ deletion but not vice versa; however, overexpression of constitutively active YAP1 or TAZ alone is sufficient to induce ADM.

We noted that inactivation of YAP1 and TAZ reduced ADM formation more efficiently than Stat3 deletion (Figure 5). It has been shown that the Hippo pathway also interacts with other signaling pathways that have implications in ADM, such as Wnt and Notch pathways. YAP1/TAZ-dependent regulation of these pathways might also contribute to ADM formation.

The presence of the KRasG12D mutation in ADM cells in an inflammatory microenvironment is known to be sufficient for progression to PanIN lesions and pancreatic cancer. Additionally, inflammation in the pancreas creates a feedback loop leading to hyperactivation of oncogenic KRasG12D (Figure 7E). The KRasG12D mutation and subsequent hyperactivation promotes pancreatic cancer at two levels: it creates a susceptible “cell of origin” by inducing acinar-to-duct reprogramming; and it also acts as the transforming driver that enables progression towards malignancy. This model explains why KRas mutations in pancreatic cancer are so common, and why they frequently appear very early, as a gatekeeper mutation, in pancreatic acinar cells.
Acknowledgements

H. Gerhardt kindly provided $Yap^{fl/fl}$ mice and S. Piccolo kindly provided YAP1 (5SA) and TAZ (S89A) vectors. We thank C. Cremona and N. Tapon for comments on the manuscript and P. Chakravarty of the Francis Crick Institute Bioinformatics facility for help with gene set enrichment analysis.
Figure legends

Figure 1. YAP1 and TAZ are upregulated in pancreatitis.

A. Gene set enrichment analysis of transcription data from caerulein-treated pancreata\textsuperscript{22} identified enrichment of the conserved YAP1 signatures reported by Yimlamai\textsuperscript{23} and Cordenonsi\textsuperscript{47}. Normalized enrichment score (NES) and nominal (NOM) $P$-values are shown.

B. Scheme showing model of caerulein-induced acute pancreatitis in C57BL/6 mice.

C. Quantitative RT-PCR of YAP1/TAZ target genes in caerulein-treated versus PBS-treated pancreata. $n = 3$ mice each group; means ± SEM are shown; ** **$P<.005$, Student’s $t$-test.

D. Immunoblots of pancreas lysates from caerulein- and PBS-treated animals. $p$-Lats1, phosphorylated Lats1 (Ser909); $p$-Yap1, phosphorylated Yap1 (Ser112).

E. Immunohistochemical stains of YAP1 and TAZ in pancreata of caerulein- and PBS-treated mice. Scale bars = 50 µm. $n = 5$-7 mice analyzed.

F. Triple immunofluorescence of pancreata from caerulein- and PBS-treated animals showing the duct marker CK19, the acinar marker amylase and either YAP1 or TAZ. Arrowheads indicate ADM cells. Scale bars = 20 µm. $n = 5$-7 mice analyzed.
Figure 2. YAP1 and TAZ are upregulated in ADM lesions induced by $K_{Ras}^{G12D}$.

A. Immunohistochemical analysis of YAP1 and TAZ in normal ($LSL-K_{Ras}^{G12D}$) pancreas and pancreas from 6-month-old $LSL-K_{Ras}^{G12D};Pdx1-Cre$ mice. Scale bars = 50 µm. Dotted lines highlight mPanIN lesions. n = 4-5 mice analyzed.

B. Immunoblot analysis of pancreas lysates from control $LSL-K_{Ras}^{G12D}$ and $LSL-K_{Ras}^{G12D};Pdx1-Cre$ mice. p-Lats1, phosphorylated Lats1 (Ser909); p-Yap1, phosphorylated Yap1 (Ser112).

C. Immunohistochemical analysis of YAP1 and TAZ on human pancreatic tissue microarrays. Scale bars = 50 µm.

D and E. Quantification of immunohistochemical staining intensity, ranging from absent (0) to highest (6) for YAP1 (D) and TAZ (E) on human pancreatic tissue microarrays. n = number of tissue cores. Means ± SD are shown. **$P<.01$, ***$P<.005$, Student’s $t$-test.

Figure 3. YAPI and TAZ are necessary and sufficient for ADM induction.

A. Scheme showing in vitro assay for $K_{Ras}^{G12D}$-induced ADM.

B. Brightfield images of pancreatic acinar cell clusters on day 5 after isolation from $LSL-K_{Ras}^{G12D}$ and $LSL-K_{Ras}^{G12D};Yap1^{fl/fl};Taz^{fl/fl}$ mice. Cells were infected with adenoviruses encoding either GFP (Ad-GFP) or GFP plus Cre (Ad-Cre) on day 0. White arrows: tubular ductal structures. Scale bars = 50 µm. Quantification of tubular...
ductal structures of the indicated genotypes with n = 3-4 mice per group; means ± SEM are shown; ***P<.005, n.s. = not significant, Student’s t-test.

C. Experimental design of caerulein-induced acute pancreatitis in the acinar-specific and tamoxifen-inducible Ela1-Cre<sup>ERT2</sup> mouse model combined with the R26-LSL-YFP lineage tracer.

D. Triple immunofluorescence of pancreata from R26-LSL-YFP;Ela1-Cre<sup>ERT2</sup> and R26-LSL-YFP;Yap<sup>fl/fl</sup>;Taz<sup>fl/fl</sup>;Ela1-Cre<sup>ERT2</sup> mice treated with caerulein as indicated in C. Scale bars = 20 µm. Dotted white lines indicate ADM lesions. Quantification of GFP-positive ADM cells (CK19/Amylase double positive) as a percentage of total GFP-positive cells in caerulein-treated mice of the indicated genotypes. n = 3 mice per group; means ± SEM are shown; **P<.01, Student’s t-test.

E. Scheme showing in vitro assay for YAP1 and TAZ-induced ADM.

F. Brightfield images (upper panels) and GFP signal (lower panels) of acinar cells after 5 days in culture, infected on day 0 with Ad-GFP, Ad-YAP1-5SA-GFP or Ad-TAZ-S89A-GFP. Scale bars = 25 µm. Quantification of GFP-positive tubular ductal structures at day 5, in n = 3 experiments; means ± SEM are shown; ***P<.005, Student’s t-test.
Figure 4. YAP1/TAZ activation controls JAK-STAT3 signaling via transcription of STAT3.

A. Quantitative RT-PCR of the indicated genes and immunoblots of lysates from PANC1 cells expressing shRNAs against YAP1 and TAZ (shYAP/TAZ-#1 and -#2) or non-targeted shRNA control (shNT). n = 3; means ± SEM shown.

B. Immunoblots of primary acinar cell lysates 2 days after isolation and infection with Ad-GFP, Ad-YAP1-5SA-GFP or Ad-TAZ-S89A-GFP.

C. Quantitative ChIP-PCR of the LIFR, GP130 and STAT3 loci (promoter and intron regions) using either IgG or an antibody against TEAD4. ZFP37 intron = negative control region; CTGF promoter = positive control region. n = 3 experiments; means ± SEM shown.

D. Scheme showing luciferase expression construct with human STAT3 promoter region. Base pair (bp) numbers indicate position relative to STAT3 transcription start site. Red box indicates GT-IIc TEAD binding motif; genomic sequence of WT GT-IIc shown below. Mutant STAT3 promoter construct with a six-base-pair deletion of the GT-IIc site (mut GT-IIc) was used as control. Luciferase expression analysis using WT and mutant STAT3 promoter regions in AR42J acinar cells one day after transfection with empty vector, YAP1-5SA or TAZ-S89A. An artificial TEAD luciferase reporter consisting of eight GT-IIc motifs was used as positive control. n = 3-5 experiments; means ± SEM shown.

E. Immunohistochemistry showing phosphorylated STAT3 (Tyr705) in pancreata of caerulein- and PBS-treated C57BL/6 mice. Scale bars = 50 µm. n = 3 mice analyzed.

F. Immunoblots of pancreas lysates from PBS- and caerulein-treated animals on day 2 and day 7 after treatment. p-Stat3, phosphorylated Stat3 (Tyr705). Quantitative RT-
PCR of JAK-STAT3 pathway genes in caerulein-treated vs. PBS-treated pancreata 2 days after treatment. n = 3-4 mice per group; means ± SEM shown. *P<.05, **P<.01, ***P<.005, n.s. = not significant, Student’s t-test.

Figure 5. YAP1 and TAZ are required for KRas<sup>G12D</sup>-induced ADM <i>in vivo</i> in response to pancreatitis.

A. Scheme showing experimental design of caerulein-induced acute pancreatitis in cooperation with acinar-specific KRas<sup>G12D</sup> mutation.

B. H&E stain and amylase and CK19 antibody stains of pancreata from mice of the indicated genotypes. Scale bars: H&E (top row) 2 mm; (2nd row) 200 µm; (3rd row) 50 µm; Amylase 200 µm; CK19 (5th row) 200 µm; (6th row) 50 µm; double immunofluorescence 50 µm.

C. Quantification of CK19+amylase+ ADM pancreatic area in pancreatic sections of the genotypes shown in (B). n = 3 mice per genotype; means ± SEM are shown; ***P<.005, Student’s t-test.

D. Immunoblots of pancreas lysates from mice of the indicated genotypes treated as shown in (A).
Figure 6. KRas\textsuperscript{G12D} - and caerulein-mediated activation of STAT3 depends on YAP1 and TAZ.

A. Quantitative RT-PCR of the YAP1/TAZ target genes Ctgf, Cyr61 and Amotl2 and the STAT3 pathway genes Stat3, Lifr, and Gp130 in pancreas lysates from Ela1-Cre\textsuperscript{ERT2}, LSL-KRas\textsuperscript{G12D};Ela1-Cre\textsuperscript{ERT2} and LSL-KRas\textsuperscript{G12D};Yap1\textsuperscript{fl/fl};Taz\textsuperscript{fl/fl};Ela1-Cre\textsuperscript{ERT2} mice 7 days after the last tamoxifen injection. n = 3-5 mice per genotype; means ± SEM are shown; *P<.05; **P<.01; n.s. = not significant, Student’s t-test.

B. Immunoblot analysis of primary acinar cells from WT, LSL-KRas\textsuperscript{G12D} and LSL-KRas\textsuperscript{G12D};Yap1\textsuperscript{fl/fl};Taz\textsuperscript{fl/fl} mice, 2 days after isolation and infection with Ad-Cre adenovirus. pStat3, phosphorylated Stat3 (Tyr705).

C. Immunohistochemistry with antibodies against phosphorylated STAT3 (Tyr705) and STAT3 in pancreata of the indicated genotypes. Mice were treated with tamoxifen and caerulein as indicated in Figure 5A. Scale bars = 50 µm. n = 3-5 mice analyzed.
Figure 7. YAP1 and TAZ are required for KRas\textsuperscript{G12D}-induced PanIN.

A. Scheme showing experimental design of caerulein-induced PanIN formation in the KRas\textsuperscript{G12D} background.

B. H&E, amylase and CK19 antibody, and AB/PAS stains of pancreata from mice of the indicated genotypes. Scale bars: H&E 200 µm; CK19 200 µm; double immunofluorescence 50 µm; AB/PAS (4th row) 200 µm; AB/PAS (5th row) 50 µm.

C. Quantification of CK19+amylase+ ADM lesions in control, LSL-KRas\textsuperscript{G12D};Ela1-Cre\textsuperscript{ERT2} and LSL-KRas\textsuperscript{G12D};Yap1\textsuperscript{fl/fl};Taz\textsuperscript{fl/fl};Ela1-Cre\textsuperscript{ERT2} mice. n = 3-4 mice per genotype; means ± SEM are shown; *P<.05, Student’s t-test.

D. Quantification of AB/PAS-positive PanIN lesions in control, LSL-KRas\textsuperscript{G12D};Ela1-Cre\textsuperscript{ERT2} and LSL-KRas\textsuperscript{G12D};Yap1\textsuperscript{fl/fl};Taz\textsuperscript{fl/fl};Ela1-Cre\textsuperscript{ERT2} mice. n = 3-4 mice per group; means ± SEM are shown; *P<.05, Student’s t-test.

E. Scheme showing the roles of YAP1 and TAZ in pancreatic cancer initiation by inducing ADM in response to oncogenic KRas\textsuperscript{G12D} and inflammation. See text for details.
References


Author names in bold designate shared co-first authorship.
Gruber Figure 1

A. Yimlamai YAP liver

- Enrichment score (ES)
  - Caerulein: NES = 3.61, NOM_0 P-val < 0.00001
  - PBS

- Cordenonsi YAP conserved signature
  - Enrichment score (ES)
    - Caerulein: NES = 2.61, NOM_0 P-val < 0.00001
    - PBS

B. Gruber Figure 1

- C57BL/6 mouse
- Days: -1, 0, 4
- Analysis
- Caerulein or PBS

C. Relative mRNA level (normalized to Gapdh)

- Olf1
- Cyr61
- Anafrl1
- Anfrl2
- PBS
- Caerulein

D. Western blot analysis

- p-Lats1
- Lats1
- p-Yap1
- Yap1
- Taz
- CK19
- Gapdh

E. Immunohistochemistry

- PBS
- Caerulein
- Duct
- Islet
- Acinar
- PBS
- Caerulein
- Duct
- Islet
- Acinar

F. Yap1/CK19/Amylase/DAPI

- PBS
- Caerulein

- Taz/CK19/Amylase/DAPI

- PBS
- Caerulein
Gruber Figure 2

A

Mouse

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Yap1

Taz

B

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Human

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D

E

**YAP staining intensity**

**TAZ staining intensity**

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*** *** *** ***
Gruber Figure 4

A

**A**

![Graph showing relative mRNA level (normalized to Gapdh) for various targets and pathways.](image)

B

**B**

![Western blot images comparing expression of Stat3, LIFR, Yap1, and Gapdh under different conditions.](image)

C

**C**

![Bar chart showing percent of input for different genes under various conditions.](image)

D

**D**

![Diagram illustrating the STAT3 promoter and TEAD binding site with WT and mutant sequences.](image)

E

**E**

![Immunofluorescence images showing p(Tyr705)-Stat3 localization under Caerulinein treatment.](image)

F

**F**

![Western blot images comparing expression of Stat3, LIFR, Yap1, Taz, Gp130, and Jak1 under different conditions.](image)
Gruber Figure 6

A

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YAP1/TAZ targets: Ctrl, Cyr61, Amotl2, Stat3, Litr, Gp130

JAK–STAT3 pathway: Ctrl, Cyr61, Amotl2, Stat3, Litr, Gp130

B

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C

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Gruber Figure 7

A

LSL-Kras^{G12D}, Yap^{1fr}, Taz^{1fr}, Ela1-Cre^{ERT2}

Day -12 -8
Tamoxifen Caerulein
3 months
Analysis

C

CK19+Amylase+ ADM lesions per mm² pancreas area

Control Yap^{1fr}, Taz^{1fr}
LSL-Kras^{G12D}, Ela1-Cre^{ERT2}

D

Alcian Blue+ PanIN lesions per mm² pancreas area

Control Yap^{1fr}, Taz^{1fr}
LSL-Kras^{G12D}, Ela1-Cre^{ERT2}

B

Control LSL-Kras^{G12D}, Ela1-Cre^{ERT2}
Yap^{1fr}, Taz^{1fr}

H&E

CK19

Amylase/CK19/ DAPI

AB/PAS

E

Increased Ras activity
KRas^{G12D}
Increase in nuclear YAP1/TAZ
Upregulation of GP130/ LIFR/ STAT3
JAK–STAT3 pathway active
ADM
PaniN

Inflammation
IL-6, LIF, etc.
Increased response to inflammatory cytokines
### A

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### B

**Yap1**

**Taz**

Supplementary Figure 1. Gene set enrichment analysis of caerulein-treated mice, and YAP1/TAZ localization in untreated pancreas. (A) Table of gene sets identified by gene set enrichment analysis of data set generated by Ulmasov et al. Gene signatures significantly enriched (nominal (NOM) P-value <.05) are shown and ranked by normalized enrichment score. YAP1 gene signatures are highlighted in red and IL6-STAT3 pathway signatures are highlighted in green. (B) Immunohistochemical stains with antibodies against YAP1 and TAZ in pancreata of C57BL/6 mice. Scale bars (top row) 200 μm; (bottom row) 50 μm.
**Supplementary Figure 2.** (A) Immunoblots of phosphorylated Lats1 (Ser909), Lats1, phosphorylated YAP1 (Ser112), YAP1, TAZ, CK19 and Gapdh in pancreas lysates from caerulein- and PBS-treated animals from 3 independent experiments. Numbers under each blot represent band signal intensity normalized to Gapdh and relative to samples from PBS-treated animals. (B) Quantification of band signal intensity of immunoblots shown in (A); means ± SEM are shown.
**Supplementary Figure 3.** (A) Immunoblots of phosphorylated Lats1 (Ser909), phosphorylated YAP1 (Ser112), YAP1, TAZ and Gapdh in pancreas lysates from control LSL-KrasG12D and LSL-KrasG12D;Pdx1-Cre mice from 3-4 independent experiments. Numbers under each blot represent band signal intensity normalized to Gapdh and relative to samples from control LSL-KrasG12D mice. (B) Quantification of band signal intensity of immunoblots shown in (A); means ± SEM are shown.
Supplementary Figure 4. Generation of conditional Yap1 and Taz alleles. (A) Gene targeting strategy for conditional deletion of exon 3 in the mouse Yap1 gene. Red asterisk indicates an induced stop codon by frame shift due to exon 3 deletion. (B) PCR genotyping of mouse embryonic fibroblasts (MEFs) isolated from WT or Yap1ff embryos. Primary Yap1ff MEFs were infected with either adenovirus-GFP (GFP) or adenovirus-Cre (Cre) and harvested 3 days later. (C) Quantitative RT-PCR analysis (left) and immunoblot analysis (right) of Yap1 in primary Yap1ff MEFs infected with adenovirus-GFP (GFP) or adenovirus-Cre (Cre) 3 days after infection. Immunoblot analysis was performed with antibodies against Yap1 and Gapdh. n = 3 MEF clones isolated from 3 embryos; mean ± SEM is shown; **P<.01, Student's t-test. (D) Gene targeting strategy for conditional deletion of exon 3 in the mouse Taz/Wwtr1 gene. Red asterisk indicates an induced stop codon by frame shift due to exon 3 deletion. (E) PCR genotyping of MEFs isolated from WT or Tazff embryos 3 days after infection with adenovirus-GFP (GFP) or adenovirus-Cre (Cre). (F) Quantitative RT-PCR analysis (left) and immunoblot analysis (right) of Taz in primary Tazff MEFs infected with adenovirus-GFP (GFP) or adenovirus-Cre (Cre), 3 days after infection. Immunoblot analysis was performed with antibodies against Taz and Gapdh. n = 3 MEF clones isolated from 3 embryos; mean ± SEM is shown; **P<.01, Student's t-test.
Supplementary Figure 5. Deletion efficiency of YAP1 and TAZ in primary acinar cells. (A) Quantitative RT-PCR of Yap1 and Taz in primary acinar cells from Yap1<sup>ff</sup>, Taz<sup>ff</sup> mice 3 days after isolation and infection with either adenovirus-GFP (Ad-GFP) adenovirus-Cre (Ad-Cre). n = 3 mice per group; mean ± SEM is shown; *P<.05; **P<.01; Student’s t-test. (B) Immunoblots of YAP1, TAZ and Actin in pancreas lysates from Yap1<sup>ff</sup>, Taz<sup>ff</sup> mice 3 days after isolation and infection with either adenovirus-GFP (Ad-GFP) or adenovirus-Cre (Ad-Cre). Numbers under each blot represent band signal intensity normalized to Actin and relative to adenovirus-GFP infected cells. Graph shows quantification of band signal intensity of 3 independent experiments; means ± SEM are shown. (C and D) Quantitative RT-PCR of Yap1 (A) and Taz (B) in primary acinar cells from LSL-KRas<sup>G12D</sup>, LSL-KRas<sup>G12D</sup>;Yap1<sup>ff</sup>, LSL-KRas<sup>G12D</sup>;Taz<sup>ff</sup> and LSL-KRas<sup>G12D</sup>;Yap1<sup>ff</sup>, Taz<sup>ff</sup> mice 3 days after isolation and infection with adenovirus-Cre. n = 3 mice per group; mean ± SEM is shown; *P<.05; **P<.01; ***P<.005; n.s. = not significant, Student’s t-test.
Supplementary Figure 6. Evaluation of ADM and Yap1 recombination in the Pdx1-Cre model. (A) Triple immunofluorescence of pancreata from control and Yap1<sup>fl/fl</sup>;Pdx1-Cre mice, 4 days after PBS or caerulein treatment, with antibodies against CK19, amylase and YAP1. Scale bars = 20 μm. Arrowheads indicate ADM cells. (B) PCR genotyping of tail and pancreas DNA isolated from Yap1<sup>wt</sup> and Yap1<sup>fl/fl</sup>;Pdx1-Cre mice. (C) Immunoblot analysis of pancreas lysates from Pdx1-Cre and Yap1<sup>fl/fl</sup>;Pdx1-Cre mice with Yap1 and Actin antibodies.
Supplementary Figure 7. TUNEL staining of YAP1/TAZ-deleted pancreas and validation of YAP1/TAZ-expressing adenoviruses. (A) H&E staining, TUNEL staining and quantification showing no increase in TUNEL staining in pancreatic sections from Yap1<sup>f/f</sup>;Taz<sup>f/f</sup>;Ela1-Cre<sup>ERT2</sup> mice compared with Yap1<sup>f/f</sup>;Taz<sup>f/f</sup> mice. Inset pictures show examples of TUNEL-positive cells. Scale bars = 200 μm; insets 20 μm. n = 3 mice per group; mean ± SEM is shown; n.s. = not significant, Student’s t-test. (B) Schematic diagram of adenovirus constructs produced in the pAd-Track-CMV vector. (C) Immunoblot analysis of Gapdh and Yap1 or Taz in primary acinar cells 2 days after isolation and infection with adenovirus-GFP or adenovirus-GFP-YAP1-5SA or adenovirus-GFP-TAZ-S89A. (D) Quantitative RT-PCR analysis of Yap1 or Taz in primary acinar cells 2 days after isolation and infection with adenovirus-GFP or adenovirus-GFP-YAP1-5SA or adenovirus-GFP-TAZ-S89A. n = 3-6 experiments; means ± SEM are shown; ***P < .005, Student’s t-test. (E) Quantitative RT-PCR of the YAP1/TAZ target genes Ctgf and Cyr61 in primary acinar cells 2 days after isolation. Acinar cells were infected with adenovirus encoding either GFP (Ad-GFP), GFP with YAP1-SSA (Ad-YAP1-SSA) or GFP with TAZ-S89A (Ad-TAZ-S89A) on the day of the isolation (day 0). n = 3-6 experiments; means ± SEM are shown; *P < .05; ***P < .005, Student’s t-test.
Supplementary Figure 8. YAP1/TAZ are active in PANC1 cells and knockdown of YAP1/TAZ results in reduced STAT3 and LIFR protein levels. (A) Gene set enrichment analysis of mouse liver organoids overexpressing YAP1 identified the BIOCARTA annotated IL6 pathway. Normalized enrichment score (NES) and nominal (NOM) P-value are shown. (B and C) Immunofluorescence staining of PANC1 cells with Yap1 (B) or Taz (C) antibodies shows nuclear accumulation, indicating constitutive activation of YAP1/TAZ. Scale bars = 20 μm. (D) Immunoblots of STAT3, LIFR, YAP1, TAZ and GAPDH in lysates from PANC1 cells expressing shRNAs against YAP1 and TAZ or non-targeted shRNA control (shNT) from 3 independent experiments. Numbers under each blot represent band signal intensity normalized to GAPDH and relative to control shNT cells. Graph represents quantification of band signal intensity; means ± SEM are shown.
Supplementary Figure 9. Overexpression of active YAP1 and TAZ leads to increased STAT3 and LIFR protein levels and ENCODE dataset indicates peak for TEAD4 binding at the STAT3, LIFR and GP130 promoters. (A and B). Immunoblots of primary acinar cell lysates 2 days after isolation and infection with Ad-GFP, Ad-YAP1-5SA-GFP (A) or Ad-TAZ-S89A-GFP (B) from 3 independent experiments. Numbers under each blot represent band signal intensity normalized to Gapdh and relative to control Ad-GFP infected cells. Graphs represent quantification of band signal intensity; means ± SEM are shown. (C) UCSC Genome Browser view of TEAD4 ChIP-seq tracks from the ENCODE data set for human H1-HES cells. STAT3, GP130 and LIFR loci are shown. PCR amplicons used for quantitative ChIP assay are indicated in red with their positions relative to transcription start sites (TSS).
Supplementary Figure 10. STAT3 deletion in acinar cells has no effect on YAP1 and TAZ expression and protein levels and STAT3 is activated in response to caerulein treatment. (A) Quantitative RT-PCR of Stat3, Yap1, and Taz in primary acinar cells from Stat3££ mice 3 days after isolation and infection with either adenovirus-GFP (Ad-GFP) adenovirus-Cre (Ad-Cre). n = 3 mice per group; mean ± SEM is shown; **P < .01; Student’s t-test. (B) Immunoblots of Stat3, YAP1, TAZ and Gapdh in pancreas lysates from Stat3££ mice 3 days after isolation and infection with either adenovirus-GFP (GFP) or adenovirus-Cre (Cre). Numbers under each blot represent band signal intensity normalized to Gapdh and relative to adenovirus-GFP infected cells. (C) Quantification of band signal intensity of 3 independent experiments shown in (B); means ± SEM are shown. (D) Immunoblots of Lifr, phosphorylated Stat3 (Tyr705), Stat3, YAP1, TAZ and Gapdh in pancreas lysates from PBS- and caerulein-treated animals on day 2 (d2) and day 7 (d7) from 3 independent experiments. Numbers under each blot represent band signal intensity normalized to Gapdh and relative to samples from PBS-treated animals. (E) Quantification of band signal intensity of immunoblots shown in (D); means ± SEM are shown.
**Supplementary Figure 11.** STAT3 and LIFR are upregulated in ADM lesions. (A and B) Triple immunofluorescence of pancreata from LSL-KRasG12D and LSL-KRasG12D;Pdx1-Cre mice with antibodies against the duct marker CK19, the acinar marker amylase and either STAT3 (A) or LIFR (B). Arrowheads indicate ADM cells. Scale bars = 20 μm.
Supplementary Figure 12. Inflammation and lineage tracing in caerulein-treated pancreata. (A and B) Immunohistochemistry with antibodies against the macrophage marker F4/80 (A) or GFP (B) in pancreata from R26-LSL-YFP;Ela1-CreER<sup>T2</sup>, R26-LSL-YFP;LSL-KRas<sup>G12D</sup>;Ela1-CreER<sup>T2</sup>, and R26-LSL-YFP;LSL-KRas<sup>G12D</sup>;Yap<sup>fl/fl</sup>;Taz<sup>fl/fl</sup>;Ela1-CreER<sup>T2</sup> mice. Mice were injected 5 times with tamoxifen and treated with caerulein one week later as indicated in Figure 5A. Inset pictures show examples of F4/80-positive cells (A) and GFP-positive cells (B) at higher magnification. Scale bars = 200 μm; insets 20 μm.
Supplementary Figure 13. (A) Immunoblots and quantification of band signal intensities of phosphorylated Lats1 (Ser909), Lats1, phosphorylated Stat3 (Tyr705), Stat3, Pdx1, Sox9 and Gapdh in pancreas lysates from caerulein-treated R26-LSL-YFP;Ela1-CreERT2, R26-LSL-YFP;LSL-KRasG12D;Ela1-CreERT2, and R26-LSL-YFP;LSL-KRasG12D;Yap1f/f;Tazf/f;Ela1-CreERT2 mice. Mice were injected 5 times with tamoxifen and treated with caerulein one week later as indicated in Figure 5A. Numbers under each blot represent band signal intensity normalized to Gapdh and relative to R26-LSL-YFP;Ela1-CreERT2 mice; means ± SEM are shown. (B) Immunoblots and quantification of band signal intensity of immunoblots from WT, LSL-KRasG12D and LSL-KRasG12D;Yap1f/f;Tazf/f primary acinar lysates 2 days after isolation and adenovirus-Cre infection. Immunoblots of phosphorylated Stat3 (Tyr705), Stat3, Yap1, Taz and Actin are shown. Numbers under each blot represent band signal intensity normalized to Actin and relative to WT cells; means ± SEM are shown.
Supplementary Figure 14. Caerulein-mediated activation of STAT3 depends on YAP1 and TAZ. (A) Scheme showing experimental design of caerulein-induced acute pancreatitis in the acinar-specific and tamoxifen-inducible Ela1-Cre\textsubscript{ERT2} mouse model combined with the R26-LSL-YFP lineage tracer. (B) Double immunofluorescence of pancreata from R26-LSL-YFP;Ela1-Cre\textsuperscript{ERT2} and Yap\textsuperscript{1f/f};Taz\textsuperscript{1f/f};R26-LSL-YFP;Ela1-Cre\textsuperscript{ERT2} mice with antibodies against GFP, labeling recombined cells, and phosphorylated Stat3 (Tyr705). Mice were treated with tamoxifen and with either PBS or caerulein as indicated in (A). Scale bars = 50 μm.
Supplementary Figure 15. Deletion of YAP1 and TAZ reduced the caerulein-induced increase in Ras activity. (A) Scheme showing experimental design of caerulein-induced acute pancreatitis in cooperation with acinar-specific tamoxifen-inducible KRasG12D mutation. (B) Immunoblots (IB) of pancreas lysates from LSL-KRasG12D;Ela1-CreERT2 and Yap1ff;Tazff;LSL-KRasG12D;Ela1-CreERT2 mice treated with tamoxifen and either PBS or caerulein as indicated in (A). Antibodies against Ras, phosphorylated p-Erk1/2 (Thr202/Tyr204), Erk1/2 and Gapdh were used. Ras-GTP levels were determined with an active Ras pull-down assay using the Raf-binding-domain from Raf fused to GST (Raf-RBD-GST). Representative immunoblots are shown and graphs show quantification of band signal intensity of Ras-GTP normalized to Gapdh and relative to control PBS-treated LSL-KRasG12D;Ela1-CreERT2 mice (left graph) and band signal intensity of phosphorylated p-Erk1/2 (Thr202/Tyr204) normalized to Gapdh and relative to control PBS-treated mice (right graph) from 3-4 independent experiments; means ± SEM are shown. (C) Immunoblots (IB) of primary acinar cells from LSL-KRasG12D mice, 3 days after isolation and infection with adenovirus-Cre together with either adenovirus-GFP (GFP) or adenovirus-YAP1-5SA (YAP1-5SA), with antibodies against Ras, phosphorylated p-Erk1/2 (Thr202/Tyr204), Erk1/2 and Gapdh. Ras-GTP levels were determined with an active Ras pull-down assay using the Ras-binding-domain from Raf fused to GST (Raf-RBD-GST). Asterisks indicate non-specific bands.
Supplementary Figure 16. YAP1 and TAZ are required for ADM after TGF-α-induced increase in oncogenic Ras activity. (A) Immunoblots (IB) of primary acinar cells from WT, LSL-KrasG12D and LSL-KrasG12D;Yap1ff;Tazff mice 3 days after isolation and adenovirus-Cre infection with antibodies against Ras, phosphorylated p-Erk1/2 (Thr202/Tyr204), Erk1/2 and Gapdh. Ras-GTP levels were determined with an active Ras pull-down assay using the Ras-binding-domain from Raf fused to GST (Raf-RBD-GST). Cells were either untreated or treated with TGF-α (50 ng/ml) for 3 days. Asterisks indicate non-specific bands. Representative immunoblots are shown and graphs show quantification of band signal intensity from 3 independent experiments. Band signal intensities of Ras-GTP (left graph) or phosphorylated p-Erk1/2 (right graph) were normalized to Gapdh and are shown as relative to LSL-KrasG12D mice without TGF-α treatment; means ± SEM are shown. (B) Brightfield images of pancreatic acinar cell clusters on day 5 after isolation from WT, LSL-KrasG12D and LSL-KrasG12D;Yap1ff;TaZff mice. Cells were infected with adenovirus-Cre on day 0 and either untreated or treated with TGF-α (50 ng/ml) from day 0 for 5 days. Scale bars = 100 μm. Graph shows quantification of tubular ductal structures of the indicated genotypes with n = 3 mice per group; means ± SEM are shown; ***P<.005, *P<.05, Student’s t-test.
### Supplementary Table 1. List of primers for quantitative RT-PCR

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Supplementary Table 2. List of primers for quantitative ChIP analysis

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Supplementary Materials and Methods

Mouse Lines

For genotyping of the *Yap1* and *Taz* conditional alleles, genomic DNA from ear snips, cells or tissues was used with PCR primers for *Yap1*: Yap1-f 5’-GCTTCCTGTAGTCATGTGGTTGT-3’, Yap1-r 5’-TGTTGTCATATGCCATTGTGTGAA-3’ and Yap1-delta-f 5’-CACAGAGATCCTCCTGTCTCAG-3’ producing PCR bands of 138 bp (wildtype), 154 bp (floxed) and 457 bp (deleted). PCR primers used for *Taz*/Wwtr1 genotyping were Taz-f 5’-AGCAAAGTAAGGCACGTATG-3’, Taz-r 5’-GCTCCAAACCACATCACAG-3’ and Taz-delta-r 5’-TCTACTCTTGCGTCTTAGCTGG-3’ producing PCR bands of 161 bp (wildtype), 295 bp (floxed) and 249 bp (deleted). See diagrams in Supplementary Fig 4A and D for allele details and Supplementary Fig 4B,C and 4E,F for validation of the knock-out alleles in *Yap1*^f/f^ and *Taz*^f/f^ primary MEFs isolated from E13.5 embryos.

Previously described mouse lines used were *Stat3*^fl/fl^, *Pdx1-Cre*^2^, *R26-LSL-YFP*^3^, *LSL-KRas*^G12D^ and *Elal1-Cre*^ERT2^.

These lines were intercrossed to obtain the different genotypes. In order to maintain a similar genetic background in the knock-out and control groups, mice carrying the *R26-LSL-YFP*, *LSL-KRas*^G12D^ and *Elal1-Cre*^ERT2^ alleles were first crossed into the *Yap1*^f/f^;*Taz*^f/f^ or the *Stat3*^f/f^ background. The resulting mice carrying the *Yap1*^f/f^ and *Taz*^f/f^ or the *Stat3*^f/f^ alleles together with the *R26-LSL-YFP*, *LSL-KRas*^G12D^ and *Elal1-Cre*^ERT2^ alleles were then intercrossed to breed out the floxed alleles (control groups) or to obtain the homozygous floxed alleles (knock-out groups). Controls for the *LSL-
**KRas\textsuperscript{G12D};Pdx1-Cre** mice were litter mates harbouring the **LSL-KRas\textsuperscript{G12D}** allele without the **Pdx1-Cre** transgene.

All mouse experiments were performed on sex- and age-matched adult mice at the age of 8-14 weeks and analysed at the indicated time points. The **LSL-KRas\textsuperscript{G12D};Pdx1-Cre** and respective control mice were analysed at 6 months.

For the induction of recombination in pancreatic acinar cells using the **Ela1-Cre\textsuperscript{ERT2}** mouse line, adult mice (8-14 weeks) were injected intra-peritoneally with 100 µg/g body weight tamoxifen (Sigma-Aldrich) dissolved in peanut oil (Sigma-Aldrich) once daily for five consecutive days.

**Cell lines**

The pancreatic adenocarcinoma cell line PANC-1 and the rat acinar cell line AR42J were purchased from HPA Cultures and authenticated by Short Tandem Repeat (STR) profiling (Promega). PANC-1 cells were cultured in DMEM medium (Life technologies) containing 10 % fetal bovine serum (FBS) and Penicillin/Streptomycin (Sigma-Aldrich) and AR42J cells were cultured in RPMI medium (Life technologies) supplemented with 10 % FBS and Penicillin/Streptomycin (Sigma-Aldrich). All cell lines were passaged for a maximum of 6 months after authentication.

**Immunofluorescence and immunohistochemistry staining**

Mice were euthanized by cervical dislocation and the pancreas was dissected out and fixed overnight in 10 % neutral buffered formalin, then washed with 70 % ethanol, processed and embedded into paraffin. Sections were cut at a thickness of 4 µm and deparaffinized in Histo-Clear (National Diagnostics), rinsed in ethanol and washed in water and PBS. Sections were stained with Haematoxylin & Eosin (H&E) or with
antibodies for immunofluorescence or immunohistochemistry. Antigen retrieval was performed by incubating sections in 10 mM sodium citrate (pH 6) for 10 minutes at sub-boiling temperature and keeping them in the same buffer for 30 min before washing with PBS.

For immunohistochemistry, endogenous peroxidase was blocked with 3 % hydrogen peroxide for 5 min followed by washes with PBS. Epitope blocking was performed for 1 h at room temperature (RT) with blocking solution: 1 % bovine serum albumin (Sigma-Aldrich), 5 % normal donkey serum (Millipore) and 0.4 % Triton X-100 (Sigma-Aldrich) diluted in PBS. Sections were then incubated overnight at 4 ºC with primary antibodies diluted in blocking solution. Next day, sections were washed with PBS and incubated for 2 h at RT with secondary antibodies (Life Technologies) diluted in blocking solution, then washed again and staining was visualized with 3.3'-diaminobenzidine (DAB) followed by counterstaining with Mayer’s hematoxylin. The sections were then dehydrated and mounted.

For immunofluorescence, epitope blocking, antibody and washing steps were performed as above except the secondary antibody solution contained DAPI at a concentration of 0.75 nM, and sections were stained with 0.1 % Sudan Black B (Sigma-Aldrich) diluted in 70 % ethanol for 30 min before washing and mounting. Antibodies used were YAP1 (Cell Signaling; #4912 and #14074), TAZ (Sigma-Aldrich; HPA007415), CK19 (DHSB; TROMA-III), Amylase (Santa Cruz; sc-12821 and Sigma-Aldrich; A8273), GFP (Abcam; ab6673), STAT3 (Cell Signaling; #4904), p-STAT3 (Tyr705; Cell Signaling; #9131), LIFR (Santa Cruz; sc-659) and F4/80 (eBiosciences; #14-4801).

Confocal images were acquired on Zeiss 710, 780 or 880 confocal microscopes and processed using Zeiss AxioVision or Zeiss ZEN software. For quantifications, images
of the whole pancreatic area of three different levels for each mouse were acquired on the Zeiss Axio Scan.Z1 slide scanner, processed and quantified using the Zeiss ZEN software.

TUNEL assay was performed on paraffin-embedded sections using the DeadEnd Colorimetric TUNEL system (Promega) according to manufacturer’s protocol. Immunofluorescence staining in PANC1 cells was performed on glass coverslips. Cells were grown on glass coverslips and fixed for 10 min on ice with 4 % paraformaldehyde. After washing, cells were blocked in blocking solution for 1 h at RT and then incubated overnight at 4 °C with YAP1 (Cell Signaling; #4912) or TAZ (Sigma-Aldrich; HPA007415) antibodies diluted in blocking solution. Cells were then washed and incubated for 2 h at RT with secondary antibodies (Life Technologies) diluted in blocking solution containing 0.75 nM DAPI. After three washes with PBS, cells were mounted and confocal images were obtained with a Zeiss 710 confocal microscope.

**Gene set enrichment analysis**

Gene set enrichment analysis was performed on the published transcription profile of C57BL/6 Jackson mice treated with caerulein or PBS \(^6\) and on the transcription profile of mouse liver organoids overexpressing YAP1 \(^7\). The settings in all analyses were: number of permutations = 1000, permutation type = gene set, enrichment statistics = weighted, metric for ranking genes = t-test. The identified gene sets (nominal p-value \(P < .05\)) were rank-ordered by normalized enrichment score (NES). Published gene signatures used were: YIMLAMAI_YAP_LIVER \(^7\), CORDENONSI_YAP_CONSERVED_SIGNATURE \(^8\), LABBE_WNT3A_TARGETS_UP \(^9\), YAP_ZHAO_MCF10AND3T3 \(^10\),
shRNA knockdown

Human PANC1 cells stably expressing non-targeting shRNA (shNT) or two independent shRNAs against YAP1 and TAZ (shYAP1/TAZ-#1 and -#2) were generated using lentiviral-based vectors pLL3.7-GFP (Addgene #11795) for shRNA against YAP1 and tetracycline-inducible pLKO-Tet-On-PURO (Addgene #21915) for shRNA against TAZ and selected for GFP signal and puromycin resistance. shRNA sequences were:

- shTAZ-#1: 5’-GCCCTTTCTAACCTGGCTGTACTCGAGTACAGCCAGGTTA GAAAGGGCTTTTTG-3’;
- shTAZ-#2: 5’-CAGCCAAATCTCGTGATGAATCTCGAGATTCATCACGAGATT TTGGCTTTTTT-3’;
- shYAP1-#1: 5’-CCCAGTTAAATGTTCACCAATCTCGAGATTGGTGAACA TTTAACTGGGTTTTTG-3’;
- shYAP1-#2: 5’-CAGGTTAATGTTCACCAATCTCGAGATTGGTGTTGA TAGTATCAGCTTGGTTTGT-3’.

REACTOME_SIGNALING_BY_WNT 11, 12, DANG_MYC_TARGETS_UP 13, ALFANO_MYC_TARGETS 14, HAN_JNK_SIGNALING_UP 15, ABBUD_LIF_SIGNALING_1_UP 16, PID_IL6_PATHWAY 17, MTOR_UP.V1_UP 18, HINATA_NFKB_TARGETS_FIBROBLASTS_UP 19, DASU_IL6_SIGNALING_UP 20, AZARE_STAT3_TARGETS 21, PARENT_MTOR_SIGNALING_UP 22, DAUER_STAT3_TARGETS_UP 23, NGUYEN_NOTCH1_TARGETS_UP 24, VILIMAS_NOTCH1_TARGETS_UP 25, TGF_B_UP.V1_UP 26 and BIOCARTA_IL6_PATHWAY 27, 28.
Quantitative RT-PCR

RNA was isolated from dissected pancreata using Qiagen Micro-RNA kit (Qiagen) or from primary acinar cells using the Qiagen Mini-RNA kit (Qiagen) or the MagMAX Total RNA isolation kit (Life Technologies). The cDNA was generated using the Transcriptor First Strand cDNA synthesis kit (Roche Diagnostics) and was used for qPCR SYBR-green (Life Technologies) detection using the primers listed in Supplementary Table 1.

Human Pancreatic Tissue Array

Human YAP1 and TAZ protein levels were examined on the human pancreatic tissue arrays BIC14011 and PA207, consisting of pancreatitis, PanIN and PDAC, purchased from US Biomax, Inc. Cores were stained with YAP1 (Cell Signaling; #4912) and TAZ (Sigma-Aldrich; HPA007415) antibodies as described in Immunofluorescence and immunohistochemistry staining. The staining intensity was then scored on a scale ranging from 0 (absent) to 6 (strong positive signal).

Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) using PANC1 cells with a TEAD4 antibody (Abcam; ab58310) was performed as described. The recovery of DNA was determined by quantitative RT-PCR with SYBR-green (Life Technologies). Primers for quantitative ChIP analysis are listed in Supplementary Table 2.

Luciferase expression assay

Luciferase assays were performed in rat acinar AR42J cells with the LightSwitch Luciferase Assay System (Active Motif). The luciferase reporters used were
pLightSwitch-STAT3-PROM-722049 (Active Motif) containing 1393 bp of the human STAT3 promoter (STAT3 WT), and the same vector where the TEAD binding motif (GT-IIc) 238 bp to 233 bp upstream of the TSS was deleted by site directed mutagenesis (STAT3 mut GT-IIc). For the positive control, 8xGT-IIc motifs from the YAP/TAZ/TEAD-responsive reporter pGL3b-8xGT-IIc (Addgene #34615) were subcloned into the pLightSwitch empty promoter vector (Active Motif). Cells were co-transfected with pcDNA3 empty vector or the vectors encoding the YAP1 and TAZ phosphorylation mutants pcDNA3-HA-YAP1-5SA and pCS2-FLAG-TAZ-S89A (kind gifts from S. Piccolo). Cells were co-transfected with the pGL3-Control Vector (Promega) to normalize for transfection efficiency.

Supplementary References


Author names in bold designate shared co-first authorship.