

RESEARCH ARTICLE SUMMARY

IMMUNOLOGY

ILC2s regulate a fibroblast progenitor niche in the pancreas

Thomas Yip *et al.*

Full article and list of author affiliations:
<https://doi.org/10.1126/science.aea5113>

INTRODUCTION: Fibroblasts are central regulators of organ health and disease, although it remains unclear how different fibroblast subsets are controlled within tissues. Recently, both murine and human fibroblast atlases have revealed the presence of a conserved fibroblast subset that is present in many organs and disease states, marked by *Pi16⁺Dpp4⁺Ly6c⁺* gene expression, and computationally predicted to have progenitor capacity. Notably, these fibroblasts are also reported to express high *Il33*, encoding the alarmin IL-33, which can stimulate Group 2 innate lymphoid cells (ILC2s). ILC2s are tissue-resident immune cells that can promote fibroblast activation and are also emerging as regulators of epithelial stem cell niches.

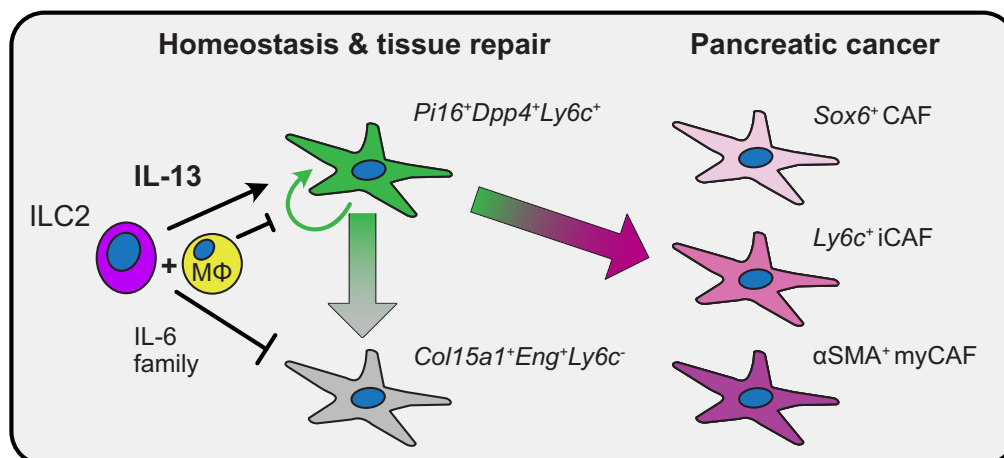
RATIONALE: The exocrine pancreas contains endogenous fibroblast populations and although fibroblasts play key roles in pancreatic inflammation or neoplasia, little is known about their spatial organization or ontogeny. Moreover, ILC2s can influence pancreatic endocrine function and pancreatic cancer. We asked where ILC2s localized in the pancreas in relation to different fibroblast subsets, and how their potential interactions influenced fibroblast biology during homeostasis, inflammation, and carcinogenesis.

RESULTS: We found that ILC2s colocalized with fibroblasts expressing the genes *Pi16⁺Dpp4⁺Ly6c⁺* in an interstitial niche of the exocrine pancreas, which encapsulates the organ parenchyma. Activation of ILC2 through alarmin IL-33 resulted in the specific activation of *Pi16⁺Dpp4⁺Ly6c⁺* fibroblasts, which had progenitor capacity and could differentiate into intraparenchymal *Col15a1⁺* fibroblasts. In healthy mice, ILC2s were important for establishing baseline number of fibroblasts in the pancreas, which represented an

inflammatory set point during acute pancreatitis. Moreover, the release of IL-33 during acute pancreatitis triggered the ILC2–*Pi16⁺Dpp4⁺Ly6c⁺* fibroblast axis to repopulate and repair the organ after injury. We found that ILC2-derived IL-13 was important for stimulating *Pi16⁺Dpp4⁺Ly6c⁺* fibroblast proliferation. However, ILC2-extrinsic negative feedback mechanisms also constrained fibroblast numbers during IL-33–driven inflammation. For instance, using fibroblast-targeted in vivo proximity labeling reagents, we found that *Tim4⁺* tissue-resident macrophages may regulate *Pi16⁺Dpp4⁺Ly6c⁺* fibroblast population dynamics through direct phagocytosis. Last, we found that the ILC2–*Pi16⁺Dpp4⁺Ly6c⁺* fibroblast niche expanded early during pancreatic carcinogenesis and locally enveloped pancreatic intraepithelial neoplastic (PanIN) lesions. Using lineage-trace mice, we found that *Pi16⁺Dpp4⁺Ly6c⁺* fibroblasts represent a potent precursor pool for specific cancer-associated fibroblast (CAF) subtypes. Moreover, ILC2 deficiency resulted in reduced CAF density in an orthotopic model of pancreatic ductal adenocarcinoma (PDAC).

CONCLUSION: We found that ILC2s are important regulators of fibroblast biology in the pancreas, and both colocalize and control *Pi16⁺Dpp4⁺Ly6c⁺* fibroblasts, which represent an important source of *Col15a1⁺* fibroblasts or specific CAF subtypes. Hence, tissue-resident ILC2s and other immune cells are important choreographers of local fibroblast topography. □

Corresponding authors: Timotheus Y. F. Halim (tim.halim@cruk.cam.ac.uk); Matthew B. Buechler (matthew.buechler@utoronto.ca) Cite this article as T. Yip *et al.*, *Science* **393**, eaea5113 (2026). DOI: 10.1126/science.aea5113.



ILC2 regulate fibroblast biology in the pancreas. ILC2s colocalize with *Pi16⁺Dpp4⁺Ly6c⁺* fibroblasts in interstitial and capsular niches of the exocrine pancreas. ILC2-derived IL-13 stimulates *Pi16⁺Dpp4⁺Ly6c⁺* fibroblasts, which can differentiate into intraparenchymal *Eng⁺Col15a1⁺* fibroblasts; this ILC2–fibroblast axis sets tissue fibroblast densities at steady-state, reinforces fibroblast repopulation after acute injury, and influences CAF development and abundance in cancer.

IMMUNOLOGY

ILC2s regulate a fibroblast progenitor niche in the pancreas

Thomas Yip¹, Julie Stockis^{1†}, Charlotte Simpson¹, Erika E. McCartney², Shwetha Raghunathan¹, Martha M. Rangel-Sosa¹, Sydney N. Hummel^{1‡}, Julia Moreno-Vicente¹, Gianmarco Raddi^{1§}, Celine Garcia¹, Rugile Linkute¹, Silvain Pinaud^{1¶}, Maye F. Cheng², Lesley A. Hill³, T. Michael Underhill³, Hans-Reimer Rodewald⁴, Christoph Schneider⁵, Claus Jørgensen⁶, Andrew N.J. McKenzie⁷, Sophie E. Acton⁸, Patrick Seale^{9,10}, Menna R. Clatworthy¹¹, Matthew B. Buechler^{2,12*}, Timotheus Y. F. Halim^{1*}

Local fibroblast development and densities influence organ health and disease, although it remains unclear how tissue fibroblast topography is controlled *in situ*. Here, we defined Group 2 innate lymphoid cells (ILC2s) as key regulators of fibroblast homeostasis in the pancreas. ILC2s colocalized with fibroblasts expressing the genes *Pi16⁺Dpp4⁺Ly6c⁺* in an interstitial niche of the exocrine pancreas, which encapsulates the organ parenchyma. ILC2s specifically regulated the expansion of *Pi16⁺Dpp4⁺Ly6c⁺* fibroblasts, which have progenitor capacity, while restraining differentiated intraparenchymal *Col15a1⁺* fibroblasts during inflammation. These circuits reinforced fibroblast numbers after injury and set an inflammatory threshold. The ILC2 and *Pi16⁺Dpp4⁺Ly6c⁺* fibroblast progenitor niche expanded around tumors and controlled cancer-associated fibroblast ontogeny and density. Hence, ILC2-fibroblast dialogue represents a regulatory node that locally orchestrates tissue homeostasis and pathology.

Fibroblasts represent a heterogeneous population of tissue-resident cells found in almost all organs (1) with roles in tissue structure, organ homeostasis, inflammation, and tissue repair (2). Conversely, their dysregulation drives fibrotic diseases and tumorigenesis (2, 3). The stromal niche is attuned to both specific organ sites and diverse physiological or pathological cues. An increased appreciation of stromal cell diversity under both homeostatic and pathological conditions has renewed questions about fibroblast ontogeny (4). A fibroblast subset marked by expression of the genes *Pi16*, *Dpp4*, and *Ly6c1* is present in multiple organs at rest or in disease states (1); however, how this population is locally maintained and its biological or pathological functions remains unknown.

Tissue-resident lymphocytes, including Group 2 innate lymphoid cells (ILC2s), are important mediators of tissue homeostasis (5–7). ILC2s inhabit epithelial stem cell niches at mucosal barrier sites, where they directly influence epithelial cell biology (8, 9). Moreover, ILC2-derived interleukin (IL)–13 promotes fibroblast activation and fibrosis; additionally, ILC2 are directly activated by epithelial or fibroblast-derived cytokines, including the damage-associated alarmin IL-33 (10–14). Hence, local fibroblast-ILC2 interactions are proposed to drive a feed-forward loop that underpins tissue fibrosis. However,

much remains unknown about the function of ILC2 during steady-state, resolution of inflammation, and cancer.

The exocrine pancreas produces digestive enzymes and consists of acinar and ductal epithelial cells that make up the bulk of this organ. Fibroblast subsets are locally maintained (1) and participate in injury-driven inflammation (15). Stressed or necrotic fibroblasts release IL-33 (16), which implies the concomitant liberation of other damage-associated molecular patterns (DAMPs). Nevertheless, how local fibroblast composition dictates the acute injury response remains uncertain. Moreover, molecularly and functionally diverse cancer-associated fibroblast (CAF) subtypes play crucial roles in pancreatic ductal adenocarcinoma (PDAC); however, many questions remain about their ontogeny (17, 18).

Results

ILC2 colocalized with IL-33⁺ fibroblasts in an interstitial niche of the pancreas

We used 2-photon microscopy of *IL13^{tdTom/+}IL33^{Cit/+}* mouse pancreata to visualize the anatomical location of ILC2s in relation to IL-33-producing cells (Fig. 1A and fig. S1A). Mice were pretreated with IL-33 to enhance fluorescent IL13-tdTomato signal, required for ILC2 visualization. Although ILC2s are reported in the islets of Langerhans and surrounding large blood vessels (13, 19), our data indicated that IL-13^{tdTom+} cells were enriched in the interstitial septae and capsule that surround the organ parenchyma. Moreover, we found that IL-33^{Cit+} cells inhabited the same collagen-rich niche and colocalized with IL-13^{tdTom+} cells (fig. S1B). Given the limitations in imaging depth of 2-photon microscopy, we also performed multiplex immunofluorescence (IF) imaging of larger tissue sections. We identified ILC2s by expression of the transcription factor Gata3 and exclusion of the T cell marker CD3 (Fig. 1B). Corroborating our 2-photon microscopy data, we observed that most pancreatic ILC2 positioned closely with IL-33⁺ cells, which also expressed podoplanin (Pdpn), a marker for fibroblasts (fig. S1C) (1, 20), in the septal/capsular space (Fig. 1C and fig. S1D). A smaller proportion of ILC2 could be detected in the adventitial cuff, parenchyma, or near the islets of Langerhans. Last, using three-dimensional (3D) imaging of clarified pancreas, we similarly observed ILC2 in the capsular regions of the exocrine pancreas in untreated mice (Fig. 1D, fig. S1E, and movie S1).

Flow cytometry profiling of untreated pancreata indicated that most IL-33^{Cit+} cells coexpressed Pdpn and the pan-fibroblast marker PDGFR α (Fig. 1, E and F, and fig. S1F), thus confirming their fibroblast identity. A smaller percentage of IL-33^{Cit+} cells were CD45⁺CD31⁺Pdpn^{high}PDGFR α mesothelial-like cells. IL-33 protein was largely sequestered to the nuclei of Pdpn⁺ cells, in line with its role as alarmin cytokine (fig. S1G). We intravenously administered mice with anti-CD45.2-PE to profile the pancreatic tissue-resident immune compartment by flow cytometry (Fig. 1G and fig. S1H). CD45⁺CD3⁺B220⁺NK1.1⁺Lineage⁺CD127⁺Gata3⁺ ILC2 were almost exclusively tissue-resident. We also found a small fraction of tissue-resident Gata3⁺ Th2 cells (fig. S1I); similarly, ILC2 were the major population of *IL13^{tdTom+}* cells in PBS-treated and IL-33-treated mice (fig. S1J). Conversely, other circulating immune cells such as B cells readily stained for CD45.2. In terms of myeloid cells, we identified tissue-resident macrophages and dendritic cells (fig. S1H); multiplex IF indicated that F4/80⁺Lyve-1⁺ macrophages (21) also inhabited the interstitium (fig. S1K). Last, ROR γ t⁺ ILC3 were present in the pancreas-draining lymph nodes but almost absent in the pancreata of both PBS-treated and IL-33-treated mice (fig. S1L). In summary,

¹University of Cambridge, CRUK Cambridge Institute, Cambridge, UK. ²University of Toronto, Department of Immunology, Toronto, ON, Canada. ³Department of Cellular and Physiological Sciences and Biomedical Research Centre, University of British Columbia, Vancouver, BC, Canada. ⁴Division of Cellular Immunology, German Cancer Research Center; Heidelberg, Germany. ⁵Department of Physiology, University of Zurich, Zurich, Switzerland. ⁶University of Manchester, Cancer Research UK Manchester Institute, Manchester, UK. ⁷MRC Laboratory of Molecular Biology, Cambridge, UK. ⁸Laboratory for Molecular Cell Biology, University College London, London, UK. ⁹Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. ¹⁰Institute for Diabetes, Obesity and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. ¹¹Cambridge Institute for Therapeutic Immunology and Infectious Diseases, University of Cambridge, Department of Medicine, Cambridge, UK. ¹²Schroeder Arthritis Institute, University Health Network, Toronto, Ontario, Canada. *Corresponding author. Email: tim.halim@cruk.cam.ac.uk (T.H.); matthew.buechler@utoronto.ca (M.B.) †Present address: UCLouvain, de Duve Institute, Brussels, Belgium. ‡Present address: Massachusetts Institute of Technology, Boston, MA, USA. §Present address: Department of Emergency Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA. ¶Present address: MIVEGEC, Université de Montpellier, IRD, CNRS, Montpellier, France.

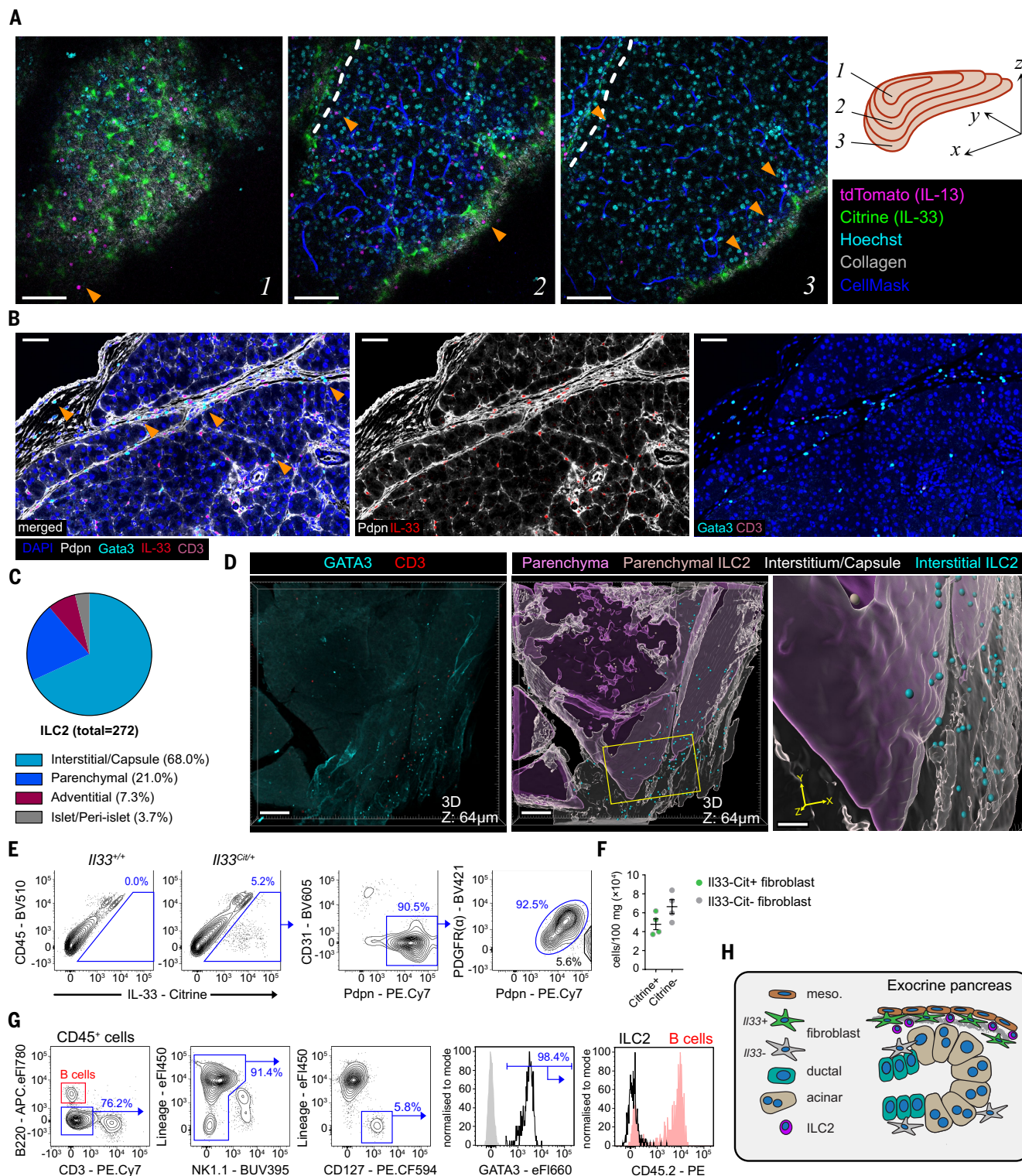


Fig. 1. ILC2s colocalize with IL-33⁺ fibroblasts in an interstitial niche of the pancreas. (A) Pancreas explants from *Il13^{tdTom/+}*/*Il13^{Cit/+}* mice pretreated with IL-33 on days 3 and 2 were imaged by 2-photon microscopy immediately after dissection at different depths (see z-stack orientation, right). Dotted line indicates interstitial septae, arrows highlight representative tdTom⁺ cells. CellMask was used to stain cell membranes of vasculature and parenchymal acinar cells. Scale bar, 100 μ m. (B and C) Formalin-fixed, paraffin-embedded (FFPE) pancreata from C57Bl/6 mice pretreated as in (A) were sectioned and analyzed using multiplex immunofluorescence (IF) imaging. A representative region is shown in (B), indicating localization of Gata3⁺CD3⁻ ILC2 (arrow highlights representative ILC2s), Podoplanin (Pdpn), and IL-33 staining. HALO image analysis on tissue sections determined the proportion of ILC2s in the indicated anatomical regions (C). Scale bar, 50 μ m. (D) Maximum intensity projection of 3D images of an optically clear thick section from an untreated C57Bl/6 mouse pancreas stained for GATA3 and CD3 (left). GATA3⁺CD3⁻ cells were reconstructed as ILC2s and stratified based on their location as parenchymal (light pink) and interstitial (cyan); parenchyma (purple) and interstitium (white) were reconstructed based on tissue autofluorescence (middle). The indicated region of interest (yellow) was magnified and rotated to provide a clearer view of interstitial ILC2s (right). Scale bar, 100 μ m (left and middle); 30 μ m (right). (E and F) Pancreata

from untreated $Il33^{Cit/+}$ and C57Bl/6 ($Il33^{+/+}$) control mice were analyzed by flow cytometry to determine the phenotype of $Il33^{Cit/+}$ cells (E), and the absolute number of fibroblasts was calculated (F). Datapoints represent individual mice. (G) Untreated C57Bl/6 mice were injected with anti-CD45.2-PE antibody before sacrifice to label circulating cells; pancreata were analyzed by flow cytometry for CD45.2 expression on the indicated immune cell types (related to fig. S1I). (H) Diagram of pancreatic ILC2-fibroblast niche. Dot plots show representative gating strategy; numbers indicate percent of gated cells. Additional gating strategy for (F) shown in fig. S1F. Imaging data are representative of two, three, and two individual mice (A), (B), and (D), respectively. Bar graphs indicate mean ($\pm$ SEM) and show data representative of two independent experiments ($n = 4$ per group) (F).

ILC2 comprise a tissue-resident immune population that colocalizes with $Il33^{+}$ fibroblasts in a distinct anatomical niche of the exocrine pancreas at both steady-state or $Il33$ -driven inflammation (Fig. 1H).

Topographical and transcriptomic analysis of the pancreas inferred niche-specific interactions between ILC2 and fibroblast subsets

To understand more about the potential symbiotic relationship between fibroblasts and ILC2, we performed single-cell RNA sequencing (scRNA-seq) on nonimmune cells enriched from the exocrine pancreas of untreated C57Bl/6 mice (fig. S2A). Using unsupervised clustering, we found nine discrete populations (Fig. 2A). We annotated all known exocrine pancreatic nonimmune cell types, including epithelial acinar and ductal cells, endothelial cells, neuronal cells, mesothelial cells, pericytes, and fibroblast clusters (Fig. 2B). In line with our exocrine enrichment, we detected few endocrine cells. Focusing on $Pdpr^{+}Pdgfra^{+}$ fibroblasts, we observed two distinct subclusters that segregated based on the expression of $Pi16$ and $Col15a1$, among other genes (Fig. 2C and data S1). The $Pi16^{+}$ fibroblast cluster is described in a pan-tissue fibroblast atlas as a putative precursor for more differentiated $Col15a1^{+}$ fibroblasts (7). Our dataset indicated that $Pi16^{+}$ fibroblasts also selectively expressed $Il33$, $Ly6c$, and $Dpp4$ (Fig. 2D). Conversely, we found that $Col15a1^{+}$ fibroblasts coexpressed Eng , which encodes the coreceptor Endoglin (CD105), among other genes. $Pdpr^{+}Eng^{+}$ cells were observed throughout the parenchyma of the exocrine pancreas in untreated C57Bl/6 mice, whereas $Pdpr^{+}Dpp4^{+}Il33^{+}$ cells localized in the capsule and interstitium close to ILC2 (mean distance 20 to 30 μ m) (Fig. 2 E and F, and fig. S2, B to D).

$Col15a1^{+}$ and $Pi16^{+}$ fibroblast subsets were both enriched in genes encoding cell adhesion, ECM processes, and cell migration signatures. $Pi16^{+}$ fibroblasts showed specific associations with protein translation, tissue repair, and axonogenesis pathways, whereas $Col15a1^{+}$ fibroblast-specific pathways included ossification and angiogenesis (fig. S2E). Moreover, $Col15a1^{+}$ fibroblasts expressed some genes related to retinol metabolism and storage (fig. S2F), in line with recognized properties of pancreatic stellate cells (22); nevertheless, other stellate cell markers such as alpha smooth muscle actin ($Acta2$) and fatty acid binding protein 4 ($Fabp4$) were not highly expressed by fibroblasts in the pancreas at steady-state. Thus, $Col15a1^{+}$ fibroblast likely represent mature fibroblasts of the pancreas whereas $Pi16^{+}$ fibroblasts constitute a putative progenitor that colocalizes with tissue-resident ILC2 in a discrete anatomical niche.

We next performed scRNA-seq on flow cytometry-enriched $CD45^{+}CD3^{-}B220^{-}NK1.1^{-}$ Lineage $^{-}CD127^{+}$ pancreatic ILCs from untreated C57Bl/6 mice (fig. S2A). This ILC-enriched dataset also revealed ILC2 as the major ILC subset at steady-state. Using CellChat, we inferred two-way communication networks between ILC2 and different cell types of the exocrine pancreas (figs. S3 and S4, A to C). Focusing on $Pi16^{+}$ and $Col15a1^{+}$ fibroblast subsets, we found that ILC2 might engage both subsets in the healthy pancreas through numerous juxtacrine or paracrine mechanisms, including $Il6$ -family ligands (Lif , Osm), the EGFR-ligand amphiregulin ($Areg$), TGF- β superfamily ligands ($Tgfb1$, $Bmp7$), and macrophage inhibitory factor (Mif). Stereotypical ILC2-derived cytokine networks, such as $Il13$, were relatively weak in the steady-state pancreas. Conversely, $Pi16^{+}$ fibroblasts were predicted to signal to ILC2 through extracellular matrix (ECM) components including fibronectin 1 (Fni) and different collagens, as well as $Il33$, chemokines, and other factors.

Multiplex IF analysis indicated that Fni was enriched in the interstitial extracellular matrix and localized in the same region as $Dpp4^{+}$ fibroblasts, in physical association with ILC2 (fig. S2G). We utilized $Il5^{tdTom-iCre/+}R26^{uLIPSTIC}$ mice (23), where $Il5^{tdTom-iCre/+}$ cells, which are primarily ILC2 in healthy control mice, expressed a bacterial sortase (SrtA) which enabled them to covalently transfer a biotinylated peptide tag to physically interacting recipient cells (Fig. 2G and fig. S2, H and I). We found that pancreatic ILC2 did not label many $Ly6c^{+}$ or $Ly6c^{-}$ fibroblasts, or other cell types in PBS-treated control mice (Fig. 2H and fig. S2H). Even during acute $Il33$ -driven inflammation, we observed no direct ILC2-fibroblast interactions, although $CD172a^{+}CD11b^{+}cDC2$ acquired a biotin signal (Fig. 2I and fig. S2H).

ILC2 influenced fibroblast homeostasis and promoted the in situ expansion of putative $Pi16^{+}Dpp4^{+}Ly6c^{+}$ fibroblast progenitors

Based on our proximity labeling experiments, we hypothesized that tissue-resident ILC2 regulated a $Pi16^{+}Dpp4^{+}Ly6c^{+}$ fibroblast progenitor niche not by cell contacts, but primarily through secreted factors in the pancreas. We refined our flow cytometry panel to distinguish and investigate the different fibroblast subsets (Fig. 3, A to C). Staining for additional surface markers, we identified both $Ly6c^{+}Dpp4^{+}Thy1.2^{high}$ ($Pi16^{+}Dpp4^{+}Ly6c^{+}$) and $Ly6c^{-}Dpp4^{+}Thy1.2^{high}CD105^{high}$ ($Col15a1^{+}Eng^{+}Ly6c^{-}$) clusters in untreated C57Bl/6 mice; $Ly6c^{+}$ fibroblasts also highly expressed $Il33^{Cit/+}$ in reporter mice (fig. S5A), whereas $Pi16$ and $Col15a1$ were expressed by flow sorted $Ly6c^{+}Dpp4^{+}$ or $Ly6c^{-}Dpp4^{+}$ subsets, respectively (fig. S5B). Moreover, unsupervised clustering of flow cytometry data clearly distinguished the two fibroblast populations (fig. S5C). Hence, we could accurately identify and interrogate pancreatic $Pi16^{+}Dpp4^{+}Ly6c^{+}$ and $Col15a1^{+}Eng^{+}Ly6c^{-}$ fibroblasts in mice using flow cytometry.

We quantified pancreatic ILC2 and fibroblasts in C57Bl/6 [wildtype (WT)], $Il7r^{Cre/+}Rora^{fl/fl}$ (ILC2-deficient) and $Il7r^{Cre/+}$ (Cre-only control) mice (Fig. 3D). Pancreatic ILC2 were nearly absent in $Il7r^{Cre/+}Rora^{fl/fl}$ mice and reduced in $Il7r^{Cre/+}$ compared with C57Bl/6 mice, likely due to reduced surface $IL7R\alpha$ expression (fig. S5D), which is required for the maintenance of lymphocytes (24). We observed a strong positive correlation between the numbers of ILC2 and both $Pi16^{+}Dpp4^{+}Ly6c^{+}$ ($Ly6c^{+}$) and $Col15a1^{+}Eng^{+}Ly6c^{-}$ ($Ly6c^{-}$) fibroblasts in the pancreas at steady-state; conversely, there was no correlation between ILC2 and endothelial cell numbers (Fig. 3D and fig. S5E). These findings were supported using alternative ILC2-deficient $Rag2^{-/-}Il2rg^{-/-}$ mice or ILC2-hypomorphic $Il5^{tdTom-Cre/+}Rosa26^{lsL-DTA}$ mice (Fig. 3D and fig. S5E), indicating that ILC2 deficiency correlated with reduced fibroblasts densities. When considering myeloid cells in a correlation matrix, we also found effects of ILC2 numbers on dendritic cells and macrophages, as previously reported in other tissues (25, 26) (fig. S5, F and G). Overall, this suggested that ILC2 influenced steady-state fibroblast density.

We administered $Il33$ to mice to activate ILC2. Pancreatic ILC2 expressed the $Il33$ receptor ($Il1rl1$) (fig. S2A) and up-regulated $Il13$ production after activation (fig. S5H). Fibroblasts, which do not express $Il1rl1$ (fig. S5I), increased Ki67 staining, indicative of proliferation, after $Il33$ administration (Fig. 3E). Although Ki67 staining was observed in both $Ly6c^{-}$ and $Ly6c^{+}$ fibroblasts after $Il33$ administration, there was more in the $Ly6c^{+}$ fibroblast subset, especially at early time points. We did not observe Ki67 staining in endothelial cells but detected some staining in acinar and ductal cells (fig. S5J). To assess whether ILC2 were important for fibroblast cycling, we stimulated

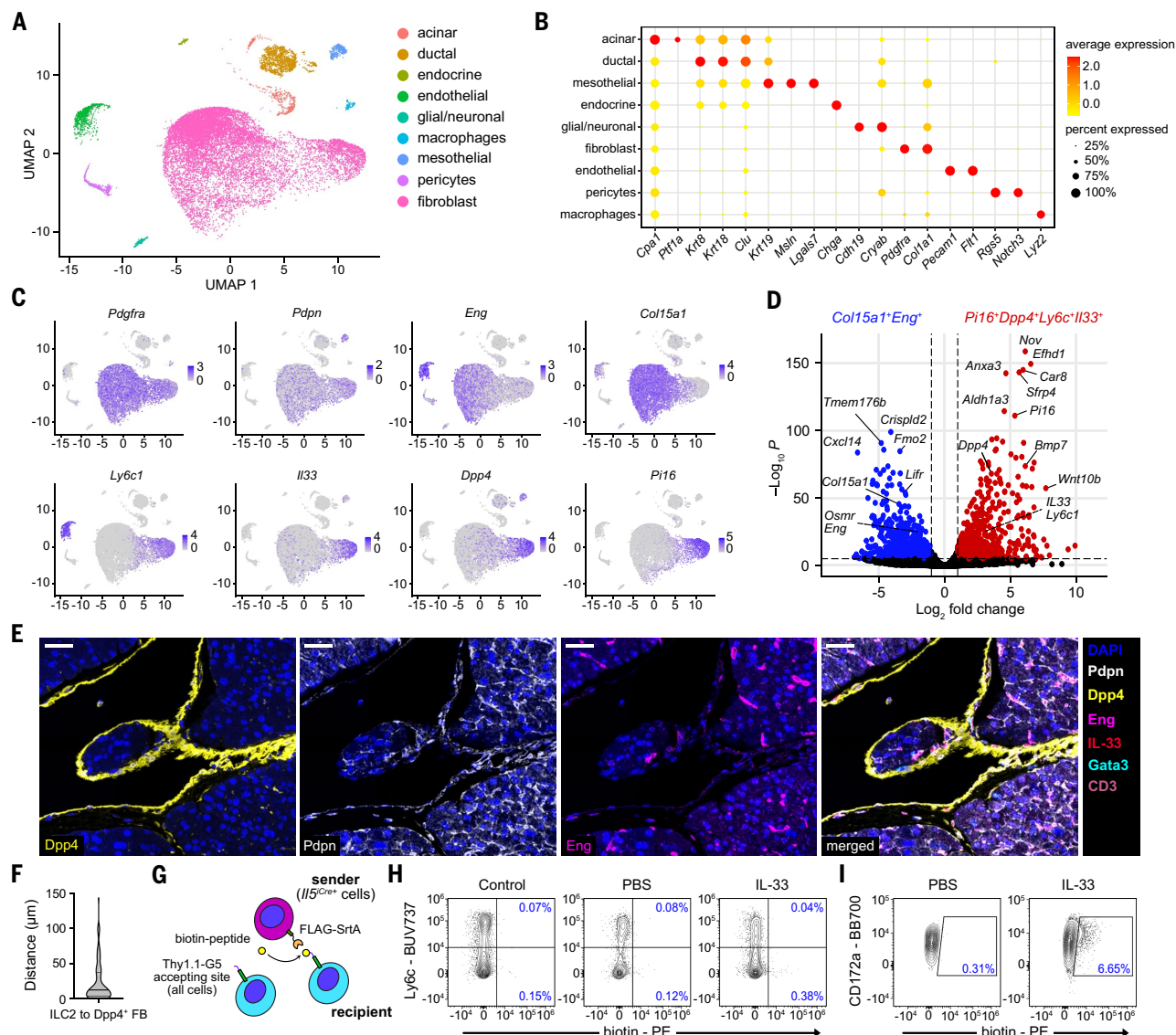


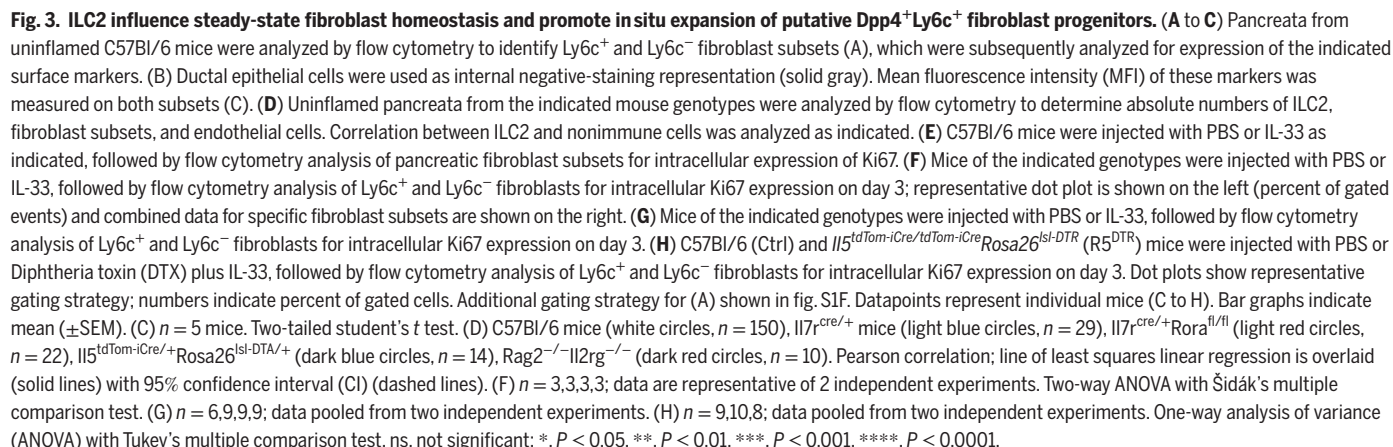
Fig. 2. Topographical, cell proximity, and transcriptomic analysis of the pancreas infer niche-specific interactions between ILC2 and fibroblast subsets. (A and B) Untreated C57Bl/6 mouse exocrine pancreata were flow-enriched for nonimmune cells followed by droplet-based scRNA-seq (17,358 cells from three mice). Uniform manifold approximation and projection (UMAP) dimensionality reduction revealed clusters of cells (A) that were annotated based on expression of lineage-specific genes (B). (C and D) Pancreatic fibroblasts universally expressed some genes (*Pdpn* and *Pdgfra*) but also revealed two subclusters that expressed *Pi16* and *Col15a1*, respectively (C), among other differentially expressed genes (D). (E and F) Antibodies toward the protein products of differentially (*Dpp4*, *Il33*, and *Eng*) and mutually (*Pdpn*) expressed genes were used to visualize both fibroblast subsets in FFPE sections of untreated pancreas using multiplex IF imaging; ILC2s (and T cells) were simultaneously detected by staining for Gata3 and CD3 (E). Distance between ILC2 and nearest *Dpp4*⁺*Pdpn*⁺ fibroblasts was quantified (F). (G to I) *Il5^{tdTom-iCre/+}R26^{uLIPSTIC}* mice enabled in vivo biotinylation of physically interacting cells in the pancreas, which can be detected by flow cytometry (G). Control mice (*R26^{uLIPSTIC}*), or *Il5^{tdTom-iCre/+}R26^{uLIPSTIC}* mice treated with PBS or IL-33 were pulsed with biotin-peptide substrate, followed by flow analysis of pancreatic *Pdpn*⁺*Pdgfra*⁺ fibroblasts (H) or MHCII⁺CD11c⁺ dendritic cells (I) for biotin signal (gating strategy in Fig. S2H). Violin plots show data pooled from two independent experiments; lines represent quartiles ($n = 4$ mice) (F). Imaging data are representative of two independent experiments (E). Data are representative of four mice from two independent experiments (H and I). Scale bar, 50 μ m (E).

Il7r^{Cre/+}Rora^{fl/fl} or *Il7r^{Cre/+}* mice with IL-33 (Fig. 3F). Although IL-33 induced *Ly6c*⁺ fibroblast cycling in control mice, this effect was ablated in the absence of ILC2. To validate these results in different mouse models we used C57Bl/6 mice, *Rag2*^{-/-} mice, which lack adaptive lymphocytes but retain IL-33-responsive ILC2 and alymphoid *Rag2*^{-/-}*Il2rg*^{-/-} mice (Fig. 3G); here, IL-33 administration rapidly induced similar cycling of *Ly6c*⁺ fibroblasts in C57Bl/6 and *Rag2*^{-/-} mice, while *Rag2*^{-/-}*Il2rg*^{-/-} mice failed to respond. Last, Diphtheria toxin-mediated depletion of ILC2 in *Il5^{tdTom-iCre/tdTom-iCre}Rosa26^{sl-DTR}* mice also impaired fibroblast cycling compared to IL-33-treated controls

(Fig. 3H and Fig. S5K). Overall, these data indicated that ILC2 control fibroblast homeostasis and mediate the acute effect of IL-33 on *Pi16*⁺*Dpp4*⁺*Ly6c*⁺ fibroblast proliferation in the exocrine pancreas.

***Pi16*⁺*Dpp4*⁺*Ly6c*⁺ fibroblasts with progenitor capacity could trans-differentiate and repopulate the exocrine pancreatic fibroblast compartment**

We next integrated our pancreatic fibroblast dataset with published data from a pan-organ atlas that identified putative *Pi16*⁺ “universal” fibroblasts (I) (Fig. 4A). Our data closely overlapped, while pseudotime



We used *Dpp4^{CreERT2/+}Rosa26^{Isl-tdTom}* mice to lineage-trace *Dpp4*⁺ fibroblasts; tamoxifen administration induced expression of tdTom in fibroblasts, which were primarily *Dpp4*⁺*Ly6c*⁺ at early time points (Fig. 4, J and K, and fig. S6F). The percentage of tdTom⁺ *Dpp4*⁺*Ly6c*⁺ fibroblasts increased after 140 days and decreased the *Ly6c*⁺-to-*Ly6c*⁺ ratio (Fig. 4, K and L). Moreover, *Pi16* expression decreased as cells

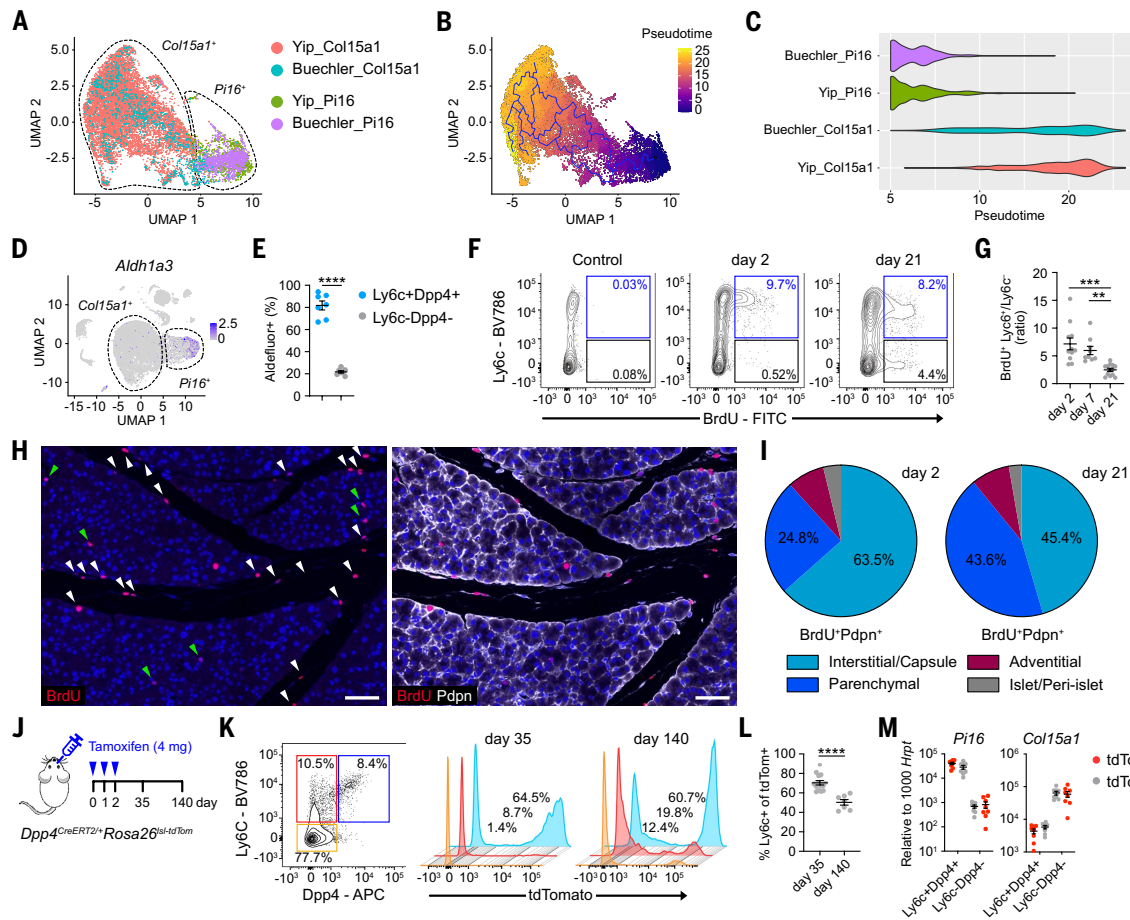


Fig. 4. ILC2-induced *Dpp4*⁺*Ly6c*⁺ fibroblast progenitors differentiate and repopulate the intraparenchymal fibroblast compartment. (A to C) Untreated pancreas fibroblast datasets from ourselves and Buechler et al. (1) were integrated and visualized using UMAP; previously identified *Pi16*⁺ and *Col15a1*⁺ subclusters in both datasets are indicated (A) 15,946 cells. Monocle pseudotime analysis was performed on the integrated dataset, showing trajectories in UMAP space (B), or in individual clusters in violin plots (C). (D and E) Aldehyde dehydrogenase 1 family member a3 (*Aldh1a3*) expression in the untreated pancreas (D); Aldehyde dehydrogenase activity was measured in different pancreatic fibroblast populations in C57Bl/6 mice using the Aldefluor assay (E). (F to I) C57Bl/6 mice were injected with IL-33 or PBS (Ctrl) and BrdU as indicated in fig. S6C, followed by analysis of BrdU incorporation into *Ly6c*⁺ and *Ly6c*⁻ fibroblast subsets at the indicated time points (representative dot plots shown) (F). The ratio of BrdU⁺ subsets (*Ly6c*⁺ versus *Ly6c*⁻) was measured at the indicated time points (G). FFPE sections of pancreas from the day 2 and day 21 timepoints were stained for the indicated markers (representative day 2 image shown): white, interstitial/capsular; green, parenchymal BrdU⁺Pdpn⁺ cells (H), followed by automated image analysis to quantify localization of BrdU⁺Pdpn⁺ cells (I). (J to M) *Dpp4*^{CreERT2/+} *Rosa26*^{Isl-tdTom} mice were treated with tamoxifen and sacrificed as indicated (J); the percent of tdTomato⁺ cells within the indicated pancreatic fibroblast subsets (K), or the percent of *Ly6c*⁺ cells among all tdTomato⁺ cells (L), was measured on days 35 and 140 (day 35 control groups were performed in the same batches as Fig. 5K). The indicated tdTomato⁺ and tdTomato⁻ fibroblast subpopulations were flow sorted from mice on day 35, followed by qRT-PCR for the indicated transcripts (M). Dot plots show representative gating strategy; numbers indicate percent of gated cells. Bar graphs indicate mean (±SEM). Data in (F and K) were pre-gated on live, CD45⁺Epcam⁺CD31⁺Pdpn⁺PDGFR(α)⁺ cells, as in Fig. 3A. Datapoints represent individual mice (E), (G), (L), and (M). (E) *n* = 7; data pooled from two independent experiments. Two-tailed Student's *t* test. (G) *n* = 11,9,13 pooled from four independent experiments. (L) *n* = 14, 7; data pooled from six independent experiments using male mice. Two-tailed Student's *t* test. (M) *n* = 8,8; data pooled from two independent experiments. *, *P* ≤ 0.05, **, *P* ≤ 0.01, ***, *P* ≤ 0.001, ****, *P* ≤ 0.0001. Scale bar, 50 μm (H).

transitioned from *Ly6c*⁺*Dpp4*⁺ into *Ly6c*⁻*Dpp4*⁻ cells, which instead expressed higher levels of *Col15a1* (Fig. 4M). Hence, we concluded that *Pi16*⁺*Dpp4*⁺*Ly6c*⁺ fibroblasts can act as bona fide fibroblast progenitors in the pancreas.

ILC2 and macrophage-dependent mechanisms regulated *Pi16*⁺*Dpp4*⁺*Ly6c*⁺ fibroblast homeostasis

Given the known and inferred role of the ILC2-derived cytokine IL-13 on fibroblast activation, we tested *Il13*^{-/-} mice for IL-33-driven cell cycling (fig. S7A). IL-13-deficiency partially reduced fibroblast proliferation for both *Ly6c*⁺ and *Ly6c*⁻ subsets, suggesting that other ILC2-derived factors could contribute. We excluded a role for IL-4 in fibroblast proliferation after IL-33 administration using a neutralizing antibody (fig. S7B). We found a similar reduction in fibroblast

proliferation in *Nmur1*^{Cre}*Il4*/*13*^{fl/fl} mice, in which ILC2s are deficient in IL-4 and IL-13 (fig. S7C), implying an important role for ILC2-derived IL-13. We assessed fibroblast subsets under homeostatic conditions in *Il13*^{-/-} and C57Bl/6 mice, which revealed a reduction in both *Ly6c*⁺ and *Ly6c*⁻ fibroblast numbers, although endothelial cell numbers were comparable in both genotypes (fig. S7, D and E).

We used cocultures of ex vivo purified pancreatic ILC2 and fibroblasts to interrogate direct cross-talk mechanisms (fig. S7, F and G). The addition of C57Bl/6-derived WT ILC2 resulted in an increase in cell density over fibroblast monoculture, whereas coculture with *Il13*^{-/-} ILC2 yielded an intermediate phenotype (fig. S7G). We performed bulk RNA-seq on purified fibroblasts to understand how ILC2 influenced fibroblast differentiation states through IL-13-dependent and -independent mechanisms and performed

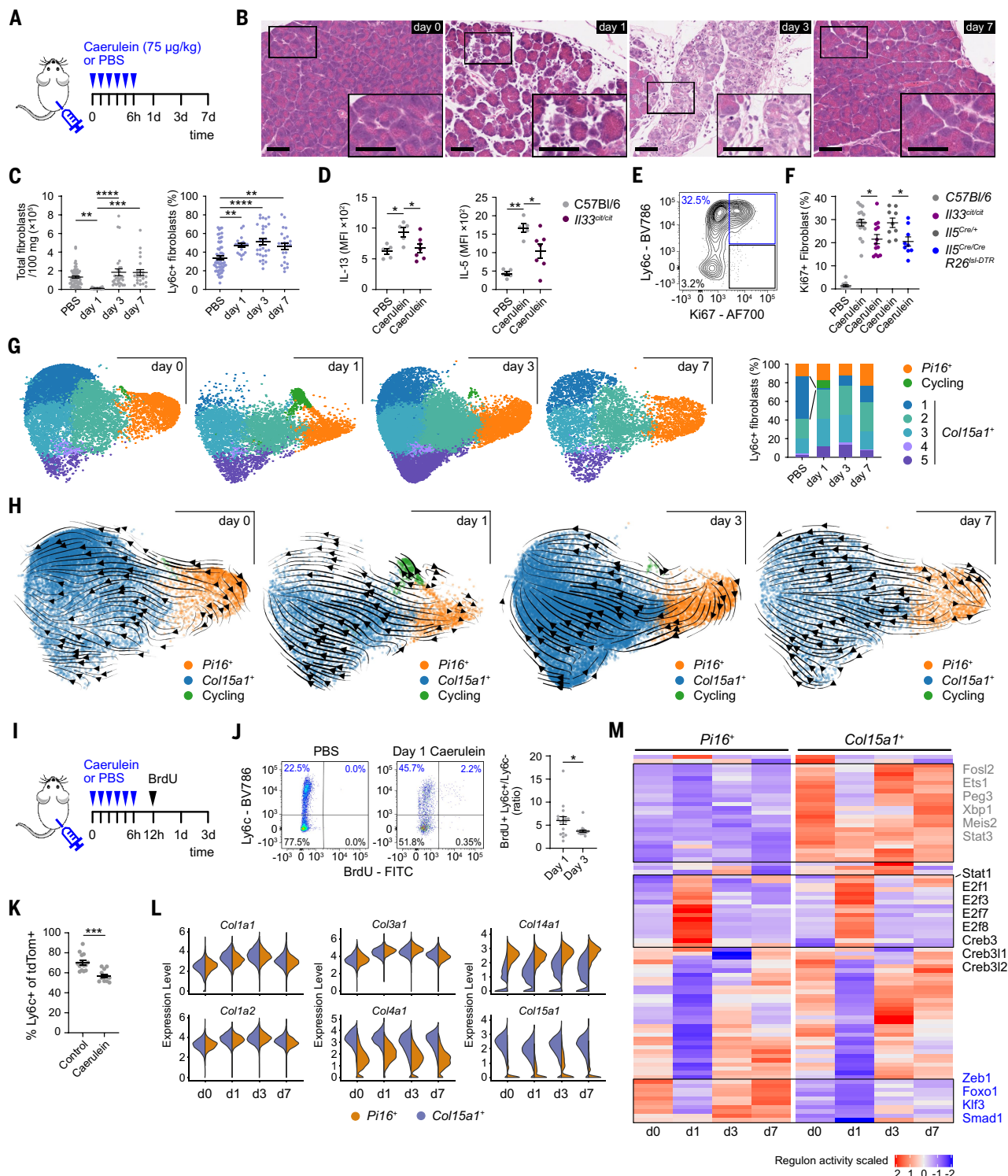


Fig. 5. ILC2 promote the expansion of fibroblast progenitors in acute pancreatitis. (A to C) Mouse pancreas analysis at different timepoints after induction of acute pancreatitis (A) Representative haematoxylin and eosin (H&E)-stained FPPE sections representing steady-state (0 hours), acinar cell necrosis and acute injury (day 1), acinar-to-ductal metaplasia (day 3), and resolution of tissue architecture (day 7) (B) Flow cytometry quantification of the absolute number of pancreatic fibroblast subsets at the indicated timepoints (C). (D to F) C57Bl/6 or *Il13*^{-/-} mice were injected with PBS or caerulein followed by detection of IL-13 and IL-5 mean fluorescence intensity (MFI) in ILC2 using intracellular flow cytometry at the 6-hour timepoint (D); similarly, at 24 hours we measured fibroblast proliferation by intracellular staining for Ki67 (E), which was quantified in the indicated genotypes and conditions (F). (G and H) Untreated or caerulein-treated C57Bl/6 mice were sacrificed at the indicated time points, followed by flow cytometry enrichment of nonimmune cells and subsequent droplet-based single cell transcriptomic analysis (13,779, 6572, 21,627, and 5424 cells, respectively). RNA velocity analysis was performed on datasets from the indicated timepoints (H); similarly, with major cluster identities color coded (G). (I and J) C57Bl/6 mice were treated with caerulein followed by administration of BrdU and sacrificed at the indicated timepoints (I). BrdU⁺ fibroblasts (pre-gated as in Fig. 6D) were identified by flow cytometry analysis (J); representative dot plots (left) indicating BrdU expression in Ly6c⁺ and Ly6c⁻ fibroblasts, which allowed quantification of ratios at days 1 and 3 (right). (K) *Dpp4*^{CreERT2/+} *Rosa26*^{tdTomato} mice were treated with tamoxifen on days 0, 1, and 2, with or without repeated caerulein administration on day 20 to induce acute pancreatitis and subsequent recovery for flow cytometry analysis of the percentage of Ly6c⁺ cells among tdTom⁺ fibroblasts on day 35 (see fig. S9G).

(L) Expression of the indicated genes by $Pi16^+$ and $Col15a1^+$ fibroblast clusters were visualized at the indicated timepoints. (M) Regulome activities within the $Pi16^+$ and $Col15a1^+$ fibroblast clusters at the indicated timepoints were assessed using SCENIC analysis. Dot plots show representative gating strategy; numbers indicate percent of gated cells. Data in (E and J) were pre-gated on live, CD45⁺Epcam⁺CD31⁺Pdpr⁺PDGFR(α) cells, as in Fig. 3A. Datapoints represent individual mice (C), (D), (F), (J), and (K). Bar graphs indicate mean ($\pm$ SEM). (C) $n = 61, 20, 29, 23$; data pooled from 15 independent experiments. One-way ANOVA with Tukey's multiple comparisons test. (D) $n = 5, 5, 7$; data pooled from two independent experiments. One-way ANOVA with Tukey's multiple comparisons test. (F) $n = 11, 17, 13, 9, 9$; data pooled from five independent experiments. One-way ANOVA with Tukey's multiple comparisons test. (J) $n = 17, 18$; pooled from three independent experiments. Two-tailed Student's t test. (K) $n = 14, 12$; data pooled from six independent experiments using male mice. Two-tailed Student's t test. *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$, ****, $P \leq 0.0001$. Scale bar, 50 μ m.

gene set enrichment analysis (GSEA) using published and our own signatures of $Pi16^+$ $Dpp4^+$ $Ly6c^+$ fibroblasts (fig. S7, G to K, and data S2). The presence of ILC2 enriched for progenitor signatures, whereas monoculture skewed gene expression toward differentiated $Col15a1^+$ Eng^+ $Ly6c^+$ fibroblast signatures. Comparison of WT and $Il13^{-/-}$ ILC2 cocultured fibroblasts indicated that IL-13 helped promote a $Pi16^+$ $Dpp4^+$ $Ly6c^+$ fibroblast cell state in ex vivo cultures (fig. S7L).

Given that IL-33-mediated ILC2 activation induced proliferation of $Pi16^+$ $Dpp4^+$ $Ly6c^+$ fibroblasts, we asked whether chronic stimulation would result in fibrosis. Long-term dosing with IL-33 did not result in fibrosis of the exocrine pancreas, whereas flow cytometry revealed a reduction in absolute $Ly6c^+$ fibroblast numbers (fig. S7, M and N). Indeed, we observed progressive reductions in absolute $Ly6c^+$ fibroblast numbers on day 3 and 5 after IL-33 administration, whereas $Ly6c^+$ fibroblast numbers were unchanged or even slightly reduced, despite the large fraction of cycling cells (fig. S7O). Hence, negative feedback mechanisms could locally counterbalance the profibrotic effects of IL-33.

We used $Pdgfra^{mGFP-CreERT2/+}$ $Rosa26^{uLIPSTIC}$ mice to identify other cells that interacted with fibroblasts. Similar to $Il5-uLIPSTIC$ mice, $Pdgfra^{Flag-SrtA+}$ fibroblasts did not biotinylate ILC2 (fig. S7P), but did biotinylate F4/80⁺MHCII^{+/low} macrophages in the pancreas after IL-33 administration (fig. S7, P and Q). Moreover, biotin-tagged F4/80⁺ macrophages increased GFP signal, which suggested the uptake of $Pdgfra$ -GFP⁺ cells (fig. S7R). More precise analysis in $Pdgfra^{mGFP-CreERT2/+}$ mice indicated that Tim4⁺MHCII^{+/low} tissue-resident macrophages acquired more GFP signal (fig. S7S). Depletion of tissue-resident Tim4⁺MHCII^{+/low} macrophages in the pancreas increased the number of proliferating and total $Ly6c^+$ fibroblasts after IL-33 administration (fig. S7, T to V). This indicated that tissue-resident macrophages functioned to limit $Pi16^+$ $Ly6c^+$ $Dpp4^+$ fibroblast numbers post inflammation.

IL-33 activated ILC2 indirectly constrained $Col15a1^+$ Eng^+ $Ly6c^+$ fibroblast numbers

We next asked whether ILC2 were involved in the effect of IL-33 on $Ly6c^+$ fibroblasts. Three different ILC2-deficient mouse models, $Il7^{Cre/+}$ $Rora^{fl/fl}$, $Rag2^{-/-}$ $Il2rg^{-/-}$, and $Il5^{tdTom-iCre/tdTom-iCre}$ $Rosa26^{Isl-DTR}$ mice, did not show specific reduction in $Ly6c^+$ fibroblasts following IL-33 treatment compared with their respective controls (fig. S8, A to C). Hence, although ILC2-derived IL-13 promoted $Pi16^+$ $Dpp4^+$ $Ly6c^+$ fibroblasts, additional ILC2-dependent mechanisms constrained $Col15a1^+$ Eng^+ $Ly6c^+$ fibroblasts under inflammatory conditions.

We used computational approaches to predict how ILC2 communicate with fibroblasts (fig. S8D and fig. S9). In addition to IL-13, we identified IL-6-family, TGF- β superfamily, CGRP, and EGFR cytokines as candidates. Exogenous IL-13 promoted growth in ex vivo pancreatic fibroblast monocultures; however, Areg, BMP2 or CGRP had no effect in vitro or in vivo (fig. S8, E to H). Also, LIGHT ($Tnfsf14$), which controls $Pi16^+$ reticular cells (28), did not influence fibroblast growth or expression of $Pi16$ (fig. S8, F and I). Pancreatic ILC2 also secreted leukemia inhibitory factor (Lif), oncostatin M (Osm), and IL-13 when stimulated with IL-33 and IL-7; notably, we detected low IL-13 production with only IL-7 stimulation (fig. S8J). Recombinant OSM inhibited pancreatic fibroblast proliferation in monocultures and promoted apoptosis (fig. S8, E and K). Moreover, OSM receptor ($Osmr$) and LIF receptor ($Lifr$) transcripts were selectively expressed by

$Col15a1^+$ Eng^+ $Ly6c^+$ fibroblasts in the normal pancreas (fig. S8L), and OSM or LIF administration reduced pancreatic $Ly6c^+$ fibroblasts, while not affecting the $Ly6c^+$ subset (fig. S8, M and N).

ILC2-specific deletion of Lif or germline Osm deletion failed to rescue IL-33-mediated reductions in $Ly6c^+$ fibroblast numbers (fig. S8O). Fibroblast-specific deletion of gp130 (required for OSM and LIF signaling) also did not rescue IL-33-mediated fibroblast reduction (fig. S8P), although treatment with Tofacitinib (Jak-inhibitor) or SC144 (gp130 inhibitor) partially rescued $Ly6c^+$ fibroblast reduction (fig. S8Q). Moreover, unlike OSM, addition of LIF to ex vivo fibroblast cultures had no effect (fig. S8R). From these data we reasoned that the effect of IL-33 and ILC2 on $Ly6c^+$ fibroblasts is likely mediated by other cell types that rely on gp130.

ILC2 promoted the expansion of $Pi16^+$ $Dpp4^+$ $Ly6c^+$ fibroblasts with progenitor potential in acute pancreatitis

IL-33 is sequestered in nuclei and is released in bioactive form upon nonapoptotic cell death (14). We hypothesized that IL-33 released following injury promotes ILC2-driven proliferation of the $Ly6c^+$ fibroblast progenitor niche. Acute pancreatitis is a common and serious disease that can be modeled in mice by administration of the cholecystokinin analog caerulein, causing overproduction and ectopic activation of digestive enzymes by acinar cells, and profound tissue necrosis on day 1 (Fig. 5, A and B, and fig. S10A). Fibroblasts were ablated rapidly, but recovered as the exocrine pancreas underwent epithelial repair, marked by acinar-to-ductal metaplasia (day 3) and organ regeneration by day 7 (Fig. 5C and fig. S10B). The fraction of $Ly6c^+$ fibroblasts was higher during acute pancreatitis. We observed ILC2 activation 6 hours after induction of acute pancreatitis in C57Bl/6 mice, as determined by intracellular staining for IL-13 and IL-5, which was reduced in $Il33^{-/-}$ mice (Fig. 5D). Fibroblast proliferation, observed largely in the $Ly6c^+$ fraction on day 1, was also reduced in $Il33^{-/-}$ compared with C57Bl/6 mice after caerulein administration (Fig. 5, E and F). Similarly, temporal ILC2 depletion using $Il5^{tdTom-iCre/tdTom-iCre}$ $Rosa26^{Isl-DTR}$ mice also impaired fibroblast proliferation (Fig. 5F and fig. S10C).

scRNA-seq at different timepoints during acute pancreatitis damage, tissue repair, and resolution identified seven fibroblast clusters in the integrated dataset, five of which represented subsets of $Col15a1^+$ Eng^+ $Ly6c^+$ fibroblasts (Fig. 5G and fig. S10D). The major $Col15a1^+$ Eng^+ $Ly6c^+$ cluster rapidly contracted during acute pancreatitis. RNA velocity on fibroblasts at individual timepoints during acute pancreatitis revealed dividing $Pi16^+$ $Dpp4^+$ $Ly6c^+$ fibroblasts that repopulated both the progenitor niche and $Col15a1^+$ Eng^+ $Ly6c^+$ fibroblasts immediately after injury (Fig. 5H). Cell cycling diminished by day 3, and $Col15a1^+$ Eng^+ $Ly6c^+$ fibroblast cluster 2 represented a likely transitory state, as also indicated by intermediate expression of $Ly6c$ (fig. S10E). Pulsing mice with BrdU 12 hours after caerulein administration labeled primarily $Ly6c^+$ fibroblasts on day 1 (Fig. 5, I and J). Subsequent analysis of BrdU⁺ fibroblasts on day 3 indicated an increase in the proportion of BrdU⁺ $Ly6c^+$ fibroblasts (Fig. 5J); notably BrdU staining intensity decreased only among the $Ly6c^+$ fibroblast population, suggesting that their differentiation into $Ly6c^+$ fibroblasts likely accounted for the change in ratio (fig. S10F). We obtained similar results using an alternative $Dpp4^{CreERT2/+}$ $Rosa26^{Isl-RFP}$ lineage trace model (Fig. 5K and fig. S10G).

We observed dynamic induction of fibrosis-associated genes, including fibril-forming Type I/III collagens, and *Acta2* (alpha smooth muscle

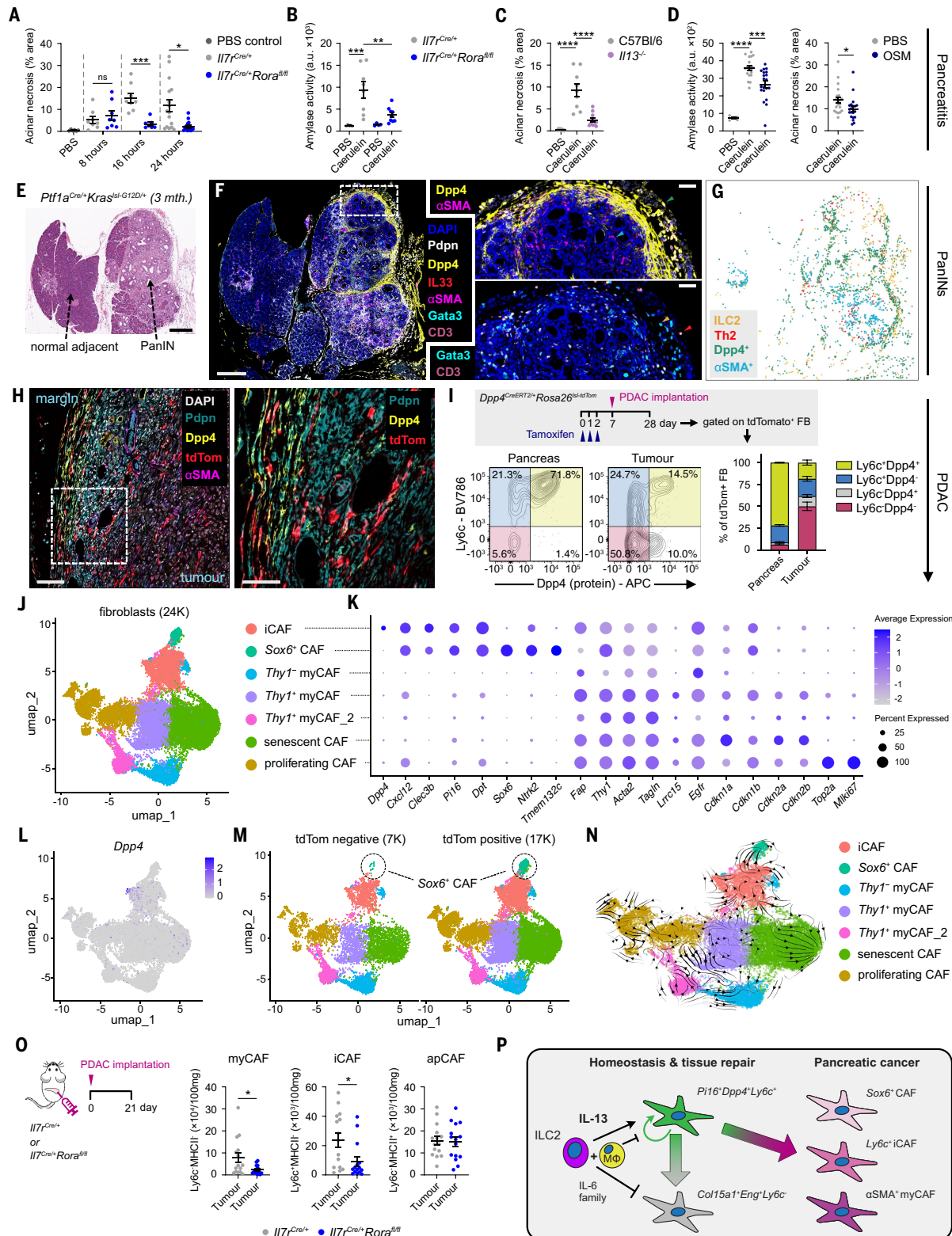


Fig. 6. Steady-state fibroblast density set an inflammatory threshold for acute injury and serve as precursors for specific CAF subsets in pancreatic cancer. (A and B) Mice of the indicated genotypes were injected with PBS or caerulein, followed by histological assessment of acinar cell necrosis at the indicated timepoints (A) or analysis of serum amylase activity at 24 hours (B). (C) Mice of the indicated genotypes were injected with PBS or caerulein, followed by histological assessment of acinar cell necrosis at 24 hours. (D) C57Bl/6 mice were pretreated with OSM or PBS at the indicated times (see fig. S110), followed by administration of caerulein and assessment of pancreatic damage by serum amylase activity (left) or acinar necrosis by histological analysis (right). (E to G) 3-month-old *Ptf1a^{Cre/+}Kras^{lsl-G12D/+}* (KC) mouse pancreas serial sections were stained for H&E (E), or for the indicated multiplex IF markers (F), which allowed identification of the indicated cell types (G). Scale bar, 200 μ m (left); 50 μ m (right). (H) *Dpp4^{CreERT2/+}Rosa26^{lsl-tdTom}* mice were orthotopically implanted with KPC cells on day 0 and pulsed with tamoxifen on days 7, 8, and 9, followed by collection of tumor-bearing pancreata on day 21; tissues were assessed by multiplex IF microscopy for the indicated markers. Scale bar, 100 μ m (left), 50 μ m (right). (I) *Dpp4^{CreERT2/+}Rosa26^{lsl-tdTom}* mice were tamoxifen-pulsed and

implanted with KPC cells as indicated, followed by dissection of tumor and surrounding pancreas; tissues were assessed by flow cytometry and pre-gated for tdTomato-positive $\text{Pdpn}^+\text{Pdgfra}^+$ fibroblasts (FB) (gating strategy in Fig. 3A). The indicated populations (left) and their relative proportions (right) are shown. (J to N) Tumours from orthotopically implanted, tamoxifen-pulsed $\text{Dpp4}^{\text{CreERT2/+}}\text{Rosa26}^{\text{Isl-tdTom}}$ mice were collected on day 21; droplet-based scRNA-seq was performed on flow-enriched tdTomato-positive (17,384 cells from four hashed tumors) and negative (7044 cells from four hashed tumors) fibroblast fractions. UMAP dimensionality reduction on the combined fractions revealed clusters of cells (J) that were annotated based on expression of lineage-specific genes (K) Dpp4 expression FeaturePlot (L). UMAP projections for tdTomato-positive and negative fractions (M). RNA velocity analysis was performed on the entire dataset (N). (O) Mice of the indicated genotypes were orthotopically implanted with KPC cells on day 0, followed by analysis of total numbers of CAF subsets by flow cytometry on day 21. (P) Graphical abstract. Bar graphs indicate mean ($\pm$ SEM). Datapoints represent individual mice (A to D and O). (A) $n = 6,8,8,8,7,12,11$; data pooled from two independent experiments. Two-tailed Student's t test. (B) $n = 5,8,4,9$; data pooled from two independent experiments. Two-way ANOVA with Šidák's multiple comparison test. (C) $n = 9,9,10$; data pooled from two independent experiments. One-way ANOVA with Tukey's multiple comparisons test. (D) $n = 5,20,20$ (left), $n = 20,20$ (right); data pooled from three independent experiments. One-way ANOVA with Tukey's multiple comparisons test (left), two-tailed Student's t test (right). (O) $n = 15,15$; data pooled from three independent experiments. Two-tailed Student's t test. ns, not significant; *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$, ****, $P \leq 0.0001$.

actin, αSMA) in the $\text{Col15a1}^+\text{Eng}^+\text{Ly6c}^-$ cluster on days 1 and 3 (Fig. 5L and fig. S10E). Other collagens were enriched in $\text{Pi16}^+\text{Dpp4}^+\text{Ly6c}^+$ fibroblasts, including Col14a1 , which is involved in fibrillogenesis (29). We also observed a substantial increase in Ccl2 and Ccl7 transcripts on day 1 only, whereas other chemokines such as Ccl11 , Cxcl1 , and Cxcl2 were constitutively expressed at all timepoints (fig. S10E). These data suggested that an inflammatory, myofibroblast phenotype was dynamically regulated during tissue repair.

We performed SCENIC (30) transcriptional network analysis on both $\text{Pi16}^+\text{Dpp4}^+\text{Ly6c}^+$ and $\text{Col15a1}^+\text{Eng}^+\text{Ly6c}^-$ clusters at each time-point (Fig. 5M and fig. S10H). At steady-state $\text{Pi16}^+\text{Dpp4}^+\text{Ly6c}^+$ fibroblasts were enriched in stem/progenitor-associated transcriptional networks (Zeb1, Foxo1, and Klf3) (31–34) and Smad1 (downstream of BMP signaling). Conversely, $\text{Col15a1}^+\text{Eng}^+\text{Ly6c}^-$ fibroblasts were enriched in networks driven by Fos12, Ets1 and Peg3 (fibroblast function) (35–37), Xbp1 (negative regulator of Foxo1) (38), Meis2 (inverse relationship with BMPs) (39) and Stat3 (downstream of EGF- and IL-6-family cytokines). Both subsets rapidly down-regulated their distinct regulatory networks early during acute pancreatitis and induced a shared transcriptional program enriched in E2F-family members (regulation of cell cycle, senescence, and apoptosis) (40), Creb3-family (ER stress, protein secretion) (41), and Stat1 (downstream of interferons, IL-6-family, and IL-13R α 1/IL-4R α) (42) signaling). Both subsets largely pivoted back to their original regulatory networks by day 3, although Stat1-driven signatures were elevated in both subsets on day 7.

ILC2-enforced fibroblast density established a set point for acute injury-induced damage

ILC2-deficient mice, which had fewer fibroblasts at baseline, showed less acinar cell necrosis and serum amylase activity (disease biomarker) compared with controls (Fig. 6, A and B, and fig. S11A). Acute ILC2-depletion in $\text{Il5}^{\text{tdTom-iCre/tdTom-iCre}}\text{Rosa26}^{\text{Isl-DTR}}$ mice (which also lack expression of Il5) did not reduce acinar cell necrosis (fig. S11B), indicating that acute ILC2 activation was disconnected from the tissue necrosis phenotype. ILC2s were important for early eosinophilia and monocyte recruitment after induction of acute pancreatitis. However, using antibody-depletion or eosinophil deficient mice, we excluded a substantial role for these immune cells on acinar cell necrosis during acute pancreatitis (fig. S11, C to F). Macrophages, which as producers of IL-6, TNF α and IL-1 β in the pancreas, could contribute to acinar cell necrosis (43). Nevertheless, in vivo neutralization of these cytokines individually, or TNF α and IL-1 β combined, had no effect on disease severity (fig. S11, G and H). Granulocyte-macrophage colony stimulating factor (GM-CSF) deficient ($\text{Csf2}^{-/-}$) mice presented with similar acinar cell necrosis compared with control animals, excluding potential ILC2-derived GM-CSF (fig. S11I). Moreover, neutrophil depletion using anti-Gr-1 or anti-Ly6G, or depletion of T or NK cells, was also ineffective at reducing acinar cell necrosis (fig. S11F). Collectively, these data suggested that ILC2 influenced AP-dependent acute pancreatitis-dependent acinar cell necrosis through a mechanism unassociated with their canonical inflammatory role.

Besides IL-33, other DAMPs such as HMGB1 (*Hmgb1*) were highly expressed by pancreatic fibroblasts at rest (fig. S11J); these or pathogen-associated molecular patterns (PAMPs) are critical amplifiers of acinar cell necrosis during acute pancreatitis (43). Co-administration of bacterial lipopolysaccharide (LPS) with caerulein increased disease severity (fig. S11K). Sparse detection of cleaved caspase 3 among necrotic acini indicated a disposition toward nonapoptotic cell death (44) (fig. S11L). Acinar cells express Toll-like receptors, including *Tlr4* (45), and SCENIC analysis of acinar scRNA-seq data revealed induction of multiple NF- κ B regulons early during acute pancreatitis (fig. S11, M and N, and fig. S12). This suggested that nonapoptotic fibroblast death can exacerbate early sterile inflammation-induced tissue necrosis in acute pancreatitis.

Hypothesizing that steady-state fibroblast densities could influence inflammation, we used $\text{Il13}^{-/-}$ mice that also have reduced fibroblast numbers at baseline (fig. S6D). Similar to ILC2-deficient mice, $\text{Il13}^{-/-}$ mice were protected against acinar cell necrosis (Fig. 6C). We used OSM to specifically induce apoptotic depletion of Ly6c^- fibroblasts before administering caerulein to WT mice, which reduced both serum biomarkers and acinar cell necrosis (Fig. 6D and fig. S11O). We used Fap^{DTR} mice, in which Diphtheria toxin-induced reductions in total pancreatic fibroblasts before caerulein administration also reduced acinar cell necrosis (fig. S11P). Conversely, $\text{Il13}^{-/-}$ and WT mice had comparable baseline fibroblast numbers, and similar levels of acinar necrosis during acute pancreatitis (fig. S11, Q and R). Notably, $\text{Il13}^{-/-}$ mice showed impaired eosinophilia after caerulein administration (fig. S11S), again discounting a substantial role for eosinophilia on acinar cell death. Thus, we concluded from complementary experimental approaches (table S1) that fibroblast density at steady-state represented a sensor for sterile tissue damage.

Pancreatic carcinogenesis triggered ILC2 - $\text{Pi16}^+\text{Dpp4}^+\text{Ly6c}^+$ fibroblast niche expansion and differentiation into CAF

Given that PDAC is associated with a highly fibrotic tumor microenvironment, we asked whether ILC2 and $\text{Pi16}^+\text{Dpp4}^+\text{Ly6c}^+$ fibroblasts were engaged during pancreatic tumorigenesis. Using $\text{Ptf1a}^{\text{Cre/+}}\text{Kras}^{\text{Isl-G12D/+}}$ (KC) mice, which develop pancreatic intraepithelial neoplastic (PanIN) lesions (46), we detected low-grade PanINs among normal adjacent pancreatic parenchyma by histological analysis (Fig. 6E and fig. S13A). PanINs were encapsulated by a dense network of $\text{Dpp4}^+\text{IL33}^+\text{Pdpn}^+$ fibroblasts, whereas $\alpha\text{SMA}^+\text{Pdpn}^+$ fibroblasts, reminiscent of myCAFs, infiltrated the PanIN cores (Fig. 6, F and G). ILC2 densities increased in the expanded Dpp4^+ fibroblast niches that enveloped PanIN lesions, whereas Th2 cells were also readily detected (Fig. 6G). These data indicated that early tumorigenesis in KC mice was marked by a profound expansion of $\text{Dpp4}^+\text{IL33}^+\text{Pdpn}^+$ fibroblasts and ILC2, whereas the presence of Th2 cells suggested the establishment of an adaptive type-2 immune polarized niche as has been reported in inflamed neonatal skin (47).

Although antigen-presenting (ap)CAFs in PDAC can arise from mesothelial cells, the origin of myCAFs and inflammatory (i)CAFs remains

unclear (18). Hypothesizing that steady-state $Pi16^+Dpp4^+Ly6c^+$ fibroblasts contribute to CAF ontogeny, we orthotopically implanted $Pdx1^{Cre}Kras^{ls1-G12D/+}Trp53^{ls1-R172H/+}$ (KPC) mouse-derived pancreatic tumor cells (48) into $Dpp4^{CreERT2/+}Rosa26^{ls1-tdTom}$ mice, which were pulsed with tamoxifen early after surgery to label $Dpp4^+$ fibroblasts (fig. S13B). Histological and IHC analysis of the resultant tumors revealed that $Dpp4$ lineage-derived $tdTom^+$ cells formed dense networks that enveloped and infiltrated the tumor mass (fig. S13, C and D). Using multiplex IF staining, we found that $tdTom^+$ cells expressed Pdpn whereas $Dpp4$ protein was mainly coexpressed at the edge of the tumor margin, or in surrounding unaffected interstitial or capsular niches, but lost on $tdTom^+$ cells within the tumor core (Fig. 6H and fig. S13E). Moreover, enhanced expression of α SMA in tumors suggested the conversion into myCAFs.

We found, by flow cytometry, that although most $Dpp4$ lineage-traced $Pdpn^+CD140a$ fibroblasts retained $Dpp4$ and $Ly6c$ protein expression in the surrounding pancreas, the majority of tumor-resident cells down-regulated these markers (Fig. 6I and fig. S13F). We also traced $Pi16$ -expressing fibroblasts by subcutaneously implanting KPC-derived tumor cells into $Pi16^{iCre/+}Rosa26^{ls1-YFP}$ mice (fig. S14, A to J). Although cutaneous YFP^+ cells predominantly expressed $Dpp4$ and $Ly6c$ under steady-state conditions, these cells gave rise to the majority of $LRRC15^+$ myCAFs (49), which also exhibited transcriptional changes associated with transdifferentiation from $Pi16^+Dpp4^+Ly6c^+$ fibroblasts (fig. S14, K and L). Hence, we concluded that $Pi16^+Dpp4^+Ly6c^+$ fibroblasts served as a local progenitor pool for specific CAF subsets.

We performed scRNA-seq on $tdTom$ -positive and negative fibroblast fractions from orthotopic tumors in $Dpp4^{CreERT2/+}Rosa26^{ls1-tdTom}$ mice (fig. S13G). Unsupervised clustering of the integrated dataset revealed different CAF subsets, including previously described iCAFs, myCAFs, and a distinct mesothelium-derived apCAF cluster (17) (fig. S13G and fig. S6, J and K). We also found two CAF clusters that expressed transcripts associated with senescence or high levels of the transcription factor $Sox6$, among other genes. $tdTom$ -positive cells down-regulated $Dpp4$ transcript and could differentiate into iCAF and myCAF lineages (Fig. 6, L and M). Moreover, $Sox6$ CAFs derived selectively from $Dpp4$ lineage-traced fibroblasts (Fig. 6M and fig. S13H). We computationally inferred differentiation trajectories using RNA velocity (Fig. 6N) and CellRank (fig. S13I) (50), a method that incorporates both RNA processing and cell state dynamics, which indicated that $Dpp4^+$ fibroblasts were a prominent precursor of iCAF, myCAF, and $Sox6^+$ CAFs (fig. S13J).

Given the relationship between ILC2 and $Pi16^+Dpp4^+Ly6c^+$ fibroblasts, we investigated how ILC2 influenced CAF biology in pancreatic cancer. Orthotopic implantation of KPC cells into $Il7r^{Cre/+}Rora^{fl/fl}$ mice, lacking ILC2, showed a reduction in myCAF and iCAF numbers compared to those in $Il7r^{Cre/+}$ mice (Fig. 6O). Mesothelial-derived apCAF numbers were unchanged. These data indicate that the ILC2- $Pi16^+Dpp4^+Ly6c^+$ fibroblast niche is locally engaged early during pancreatic tumorigenesis, and both differentiates and contributes to the CAF compartment in PDAC.

Discussion

We found that $Pi16^+Dpp4^+Ly6c^+$ fibroblasts can differentiate into both $Col15a1^+Eng^+Ly6c^-$ fibroblasts, which resemble pancreatic stellate cells at steady-state, and distinct CAF lineages in pancreatic cancer. As with other “stem-like” compartments, $Pi16^+Dpp4^+Ly6c^+$ fibroblasts inhabited a defined anatomical niche that was also enriched in ILC2s. We found that ILC2s were important for maintaining steady-state fibroblast numbers in the pancreas. Although tonic production of IL-13 contributed to this function in an IL-33-independent manner, ILC2 also served as sensors of insults to the tissue fibroblast compartment. IL-33-dependent activation of ILC2 reinforced the $Pi16^+Dpp4^+Ly6c^+$ fibroblast progenitor niche to replenish tissue fibroblast numbers, representing a prototypical homeostatic circuit. The ILC2- $Pi16^+Dpp4^+Ly6c^+$

fibroblasts niche expanded profoundly around PanIN lesions, indicating a capacity to sense and respond to early pancreatic carcinogenesis. Delineation of bona fide differentiation relative to transdifferentiation and phenotypic elasticity of $Pi16^+Dpp4^+Ly6c^+$ fibroblasts remains to be fully understood. Moreover, the functions and mechanistic underpinning(s) of $Pi16^+Dpp4^+Ly6c^+$ fibroblasts in other organs and contexts is of substantive interest.

DAMPs alert surrounding cells to tissue damage, exemplified by necrotic cell death-driven sterile inflammation (51); our data now posit that necrosis of tissue fibroblasts represents a potent early source of DAMPs that are sensed by both immune and nonimmune cells. As previously shown with adipocytes (52), our data are consistent with fibroblast density setting an important tissue tone for inflammation. Indeed, obesity and fibrosis are risk factors or amplifiers of tissue damage (53).

Dialogue within tissue niches is greatly influenced by cell proximity and contact. Although ILC2 and $Pi16^+Dpp4^+Ly6c^+$ fibroblasts did not physically interact, $Tim4^+$ tissue-resident macrophages contacted fibroblasts during inflammation and constrained the number of rapidly dividing $Pi16^+Dpp4^+Ly6c^+$ fibroblasts under inflammatory conditions, likely through phagocytosis. Although ILC2-derived IL-13 promoted early $Pi16^+Dpp4^+Ly6c^+$ fibroblast proliferation, ILC2 constrained $Col15a1^+Eng^+Ly6c^-$ fibroblast numbers, possibly through indirect mechanisms involving IL-6-family cytokines and tissue-resident macrophages. This functional dualism further illustrates the central homeostatic role of ILC2 on the pancreatic fibroblast niche, with important ramifications on acute tissue injury and tissue homeostasis.

Pancreatic ILC2 are described in the anatomically distinct islets of Langerhans (19, 54) and perivascular niche (13), although these represent a fraction of total pancreatic ILC2. While pancreatic ILC2s represent a relatively minor population of immune cells in untreated mice, we find that the ILC2- $Pi16^+Dpp4^+Ly6c^+$ fibroblast niche expanded around preneoplastic lesions. Notably, ILC2s are reported to have pro- or anti-tumor roles in pancreatic cancer (55, 56), indicating our incomplete understanding of ILC2 in the pancreas. Nevertheless, pathologies of the exocrine pancreas, such as chronic pancreatitis and pancreatic ductal adenocarcinoma are marked by stromal reactions that promote disease progression (17, 18, 49). We find that $Pi16^+Dpp4^+Ly6c^+$ fibroblasts contribute to a substantial fraction and subsets of CAFs in PDAC, and determining how the ILC2-fibroblast circuit is engaged throughout disease progression is of outstanding interest.

Materials and methods

Animals

$Dpp4^{CreERT2}$ mice (57) (provided by Prof. Seale), $Pdgfra^{mGFP-CreERT2}$ mice (58) (provided by Prof. Acton), $Osm^{-/-}$ mice (59) (provided by Prof. Jorgensen), $Il6st^{fl/fl}$ mice (60) (provided by Prof. Muller), $Il5^{tdTom-iCre}$ mice (61) (JAX, 030926), $Nmur1^{iCre-eGFP}$ mice (62) (JAX, 038197), FAP^{DTR} mice (63) (provided by Prof. Fearon), $Rosa26^{ls1-DTR}$ mice (JAX, 008040), $Rosa26^{ls1-DTA}$ mice (JAX, 009669), $Rosa26^{fl-G5-Thy1.1-fl-FLAG-SrtA}$ universal LIPSTIC mice (23) (JAX, 038221), $Rosa26^{ls1-tdTom}$ mice (JAX, 007914), $Ptf1a^{Cre/+}Kras^{ls1-G12D}$ mice (provided by Prof. Brindle), $Lif^{fl/fl}$ mice (64), $Il33^{Cit/Cit}$ (65) and $Il13^{tom/tom}$ mice (66) (provided by Prof. McKenzie), $Areg^{-/-}$ mice (provided by Prof. Clatworthy), $Il7r^{Cre/+}$ mice (67) (provided by Prof. Rodewald), $Il7r^{Cre/+}Rora^{fl/fl}$ mice ($Rora^{fl/fl}$ provided by Prof. McKenzie (68)), $Rag2^{-/-}$ mice (Charles River), and $Rag2^{-/-}Il2rg^{-/-}$ mice (69) (provided by Prof. DiSanto), $Gata1^{tm6Sho/J}$ (JAX, 005653) mice, all on the C57Bl/6 background, were maintained in the Cancer Research UK – Cambridge Institute (CRUK-CI) animal facility under specific-pathogen-free conditions. $Pi16^{iCre}Rosa26^{ls1-eYFP}$ mice were maintained at the University of Toronto under specific-pathogen-free conditions (see below). C57Bl/6 background $Csf2^{-/-}$ mice were maintained at University of Zurich Laboratory Animal Sciences Center under specific-pathogen-free conditions. WT C57Bl/6J mice were

purchased from Charles River Laboratories. Animal work was conducted under project licenses PD7484FB9 or PF7993249 at the CRUK-CI (with approval from the Cancer Research UK - Cambridge Institute, Animal Welfare Ethical Review Body) all in accordance with UK Home Office regulation, or with approval from the cantonal veterinary office of Zurich (ZH054/2022 and ZH111/2022), or approval from the Animal Care Committee at the Centre for Phenogenomics (TCP) and the University of Toronto. Mice were sex and age matched whenever possible, and most mice were used at 8–14 weeks of age. All mice were humanely euthanized by rising CO₂ exposure and cervical dislocation as confirmation of death. Mice housed at the University of Toronto were kept in specific-pathogen free cages on a 13:11 hour light-dark cycle and were temperature controlled at ~22°C. Mice housed at the CRUK-CI were kept at in specific pathogen-free, individually ventilated cages on a 12h light/dark cycle, 55% ± 10% humidity, and were temperature controlled at ~21 ± 2 °C. Mice were kept on a standard diet with ad libitum water (unless indicated otherwise for specific procedures) with nesting material and cage enrichments provided. Mice were allowed to acclimate for at least 1 week in the animal facility before the beginning of any experiment.

Generation of *Pi16^{iresCre}* knock-in mice

C2 ES cells were targeted using standard protocols to generate the *Pi16*<tml(iCre/ERT2)Tep> allele (MGI:7466219) (67). The target vector included 3-kb 5' (chr17:29543925 to 29546924, mm39) and 7-kb 3' (chr17:29546925 to 29553924±, mm39) homology arms with a knock-in sequence comprised of an internal ribosomal entry site (IRES) and the iCre/ERT2 CDS with an SV40 polyadenylation signal and an FRT-flanked PGK-neo selection cassette between rox sites located between the iCre open reading frame and the ERT2 open reading frame. This was designed so that prior to Dre-mediated recombination, a constitutively active iCre is expressed under control of the *Pi16* transcriptional regulatory elements. We refer to this cassette as CreX-neo-ERT2. Quality control of the ES cells was done by short- and long-range PCR and quantitative PCR to assess copy number of the targeting cassette (Neo, ThermoFisher Mr00299300_cn) and chromosomes 1 (ThermoFisher Mm00037405_cn), 8 (ThermoFisher Mm00256539_cn), 11 (ThermoFisher Mm00004107_cn) and Y (ThermoFisher Mm00527282_cn) to assess ploidy. Sequencing of homology arm-genome junctions of long-range PCR (5lr and 3lr) and full short-range PCR (sr1 and sr2) amplicons completed QC (table S2).

Chimeras were generated by aggregation with CD-1 (70) diploid embryos and embryo transfer to in-house bred CD-1 pseudopregnant recipients (71) following published protocols. Chimeras were bred to C57BL/6NBrdCr1-Tyr<c-Brd> (Charles River 493) and black pups (evidence of ES cell genome transmission) were genotyped with short-range PCR and quality-controlled using long-range PCR and neomycin copy number. To remove the neomycin selection cassette, sperm from quality-controlled B6N-*Pi16*<tml(iCre/ERT2)Tep> was used for in vitro fertilization with C57BL/6NBrdCr1 oocytes. Zygotes with polar bodies were electroporated in 400 ng/μL Fip mRNA (Miltenyi Biotec Inc. 130-106-769), cultured overnight, and 2-cell embryos transferred into in-house bred pseudopregnant CD-1 mice. Born pups were genotyped by PCR to confirm the neo cassette was removed. The resulting allele *Pi16*<tml1(iCre)Tep> (MGI:7466221), nicknamed CreXERT2, has the IRES and iCre open reading frame followed by a rox-flanked SV40 polyA signal (STOP) after the stop codon of the *Pi16* open reading frame. This constitutively active Cre is expressed under control of the *Pi16* promoter without concern about interference from the ACTB promoter driving the neomycin resistance gene. Two founder lines were generated (E3 and D4) and crossed to *Rosa26^{isl-YFP}* reporter animals. Both founders elicited robust YFP expression in the skin. All experiments solely used the E3 founder line.

In vivo procedures

Recombinant (rm) IL-33 (0.2 μg, BioLegend), rmOSM (0.5 μg, BioLegend), rmAREG (0.5 μg, BioLegend), BrdU (2 mg, Thermo Scientific) were administered by intraperitoneal injection in 200 μl of PBS as indicated. Labeling of circulating leukocytes to evaluate tissue-residency was achieved by i.v. injection of 3 μg anti-CD45.2-PE (clone 104, BioLegend) antibody 3 min prior to sacrifice. Tamoxifen (Sigma-Aldrich) was prepared in 10% ethanol (Sigma-Aldrich) and 90% corn oil (Sigma-Aldrich) at 20mg/mL, dissolved by sonication, and administered at 4 mg by oral gavage at the indicated timepoints. Lineage tracing experiments of *Dpp4^{CreERT2}* reporter mice were performed in matched experimental batches. For acute pancreatitis experiments, mice were given subcutaneous analgesia of 50 μg/kg buprenorphine before hourly injections of 75 μg/kg Caerulein (Sigma) or endotoxin-free PBS for 6 hours. For experiments involving depletion of Tim4⁺ macrophages alongside IL-33 administration, mice were administered 250 μg anti-CSF1R antibody (clone AFS98; BioXCell #BE0213) via intraperitoneal injection on days -4, -2, and 1, and 200 ng rm-IL-33 on days 0 and 1, followed by sacrifice for flow cytometry analysis on day 3 as indicated. For experiments involving pharmacological inhibition of IL-6 family pathways, tofacitinib (Stratech) or SC144 (Selleck Chemicals) were administered at 200 μg daily from the start of IL-33 administration. For diphtheria-toxin-mediated depletion experiments, diphtheria toxin (Sigma-Aldrich) was administered at the indicated doses.

Subcutaneous tumor model

6–10-week-old *Pi16^{iresCre/+} Rosa26^{isl-YFP}* were inoculated subcutaneously in the left unilateral flank with 1x10⁵ KPC (a generous gift from T. McGaha) tumor cells in a 1:1 suspension of PBS and Geltrex Flex LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Gibco, A4000046701). Tumours were removed 21–23 days after implantation.

Orthotopic tumor model

For orthotopic allografts, mice were anaesthetized with isoflurane and an incision was made in the left abdominal area to expose the pancreas. 10 μl of 1:1 PBS and Matrigel cell suspension (2.5x10⁴ cells) was injected into the tail of the pancreas. PDAC cell-lines (6419c5 or 2838c3, (48)) were originally derived from C57BL/6 background *Pdx1^{Cre} Kras^{isl-G12D/+} Trp53^{isl-R172H/+} Rosa26^{YFP/YFP}* (KPCY) mice. The peritoneal membrane was sutured with absorbable Vicryl Rapide suture (Ethicon, W9913) and the skin was closed with tissue glue (GLUture, Zoetis) and wound clips (FST, 12022-09). Mice were given pre- and post-surgical analgesia of meloxicam (2 mg/ml) and buprenorphine (0.03 mg/ml), and wound clips were removed one-week post-surgery.

LIPSTIC

For studying of physical cell-cell interactions by proximity labeling using *Il5^{id10m-icre/+} Rosa26^{uLIPSTIC/+}* and *Pdgfra^{eGFP-CreERT2/+} Rosa26^{uLIPSTIC/+}* mice, 7–12-week-old mice were administered tamoxifen and/or IL-33 at the indicated timepoints. Mice were intraperitoneally administered with 100 μL 20 mM Biotin-Ahx-LPETGS-NH₂ (LifeTein) every 20 min for 2 hours prior to sacrifice, and mice sacrificed 20 min after the final dose.

Histology

Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin before sectioning into 4 μm slices. Sample embedding, sectioning, and hematoxylin and eosin staining were conducted by the CRUK-CI Histology Core. Mouse CK19 (clone TROMA-III, DSHB) staining was performed on Leica's automated Bond-III platform. Acinar necrosis was quantified on H&E sections using Halo Software (Indica Labs).

Single cell preparation

Pancreas: Pancreata were weighed, then mechanically dissociated, followed by digest in 5 ml of HBSS containing collagenase I (375 U/ml),

DNase I (0.15 mg/ml) and Soybean Trypsin inhibitor (Sigma, 0.05 mg/ml) for 30 min at 37°C on a shaker (220 rpm), followed by dissociation on a syringe and needle, filtration through a 70 µm strainer and RBC lysis.

Lymph node: Cell suspensions were prepared by mechanical dissociation, followed by digest in 0.5 ml of RPMI-1640 containing collagenase I (375 U/ml) and DNase I (0.15 mg/ml) for 45 min at 37°C on a shaker (1100 rpm), followed by red blood cell (RBC) lysis.

Flow cytometry: Single cells were incubated with anti-mouse CD16/32 (Thermo Fisher) to block Fc receptors. For intracellular staining we used the Foxp3/Transcription Factor Kit (Thermo Fisher). For cytokine staining we used the BD Cytofix/Cytoperm kit (BD) after restimulation of single cell suspensions for 2h at 37°C with PMA/ionomycin in the presence of protein transport inhibitors (eBioscience Cell Stimulation cocktail). Data was acquired on a BD Fortessa, BD Symphony, or Cytex Aurora instrument. Cells were quantified using CountBright beads. Data was analyzed using FlowJo X (Tree Star).

The following antibodies were used in this study, with clones, vendors, and fluorochrome as indicated:

Thy1.2 (30-H12, eBioscience, FITC), PDGFR(α) (APA5, BV421 (BioLegend), PE (BioLegend), Super Bright 702 (Invitrogen)), CD31 (390, BV605 (BioLegend), Pacific Blue (BioLegend), BUV737 (eBioscience)), CD31 (MEC13.3, BioLegend, MEC13.3), EpCAM (G8.8, BioLegend, BV711, PE-Cy7), Ly6c (HK1.4, BV785 (BioLegend), BV421 (BioLegend), PE-Cy7 (eBioscience), BUV737 (BD)), Sca-1 (D7, BioLegend BV711), DPP4 (H194-112, APC (BioLegend), PE/Cy7 (BioLegend)), CD105 (MJ7/18, BioLegend, PE, PE/Dazzle 594), CD133 (315-2C11, BioLegend, PE/Dazzle 594), Podoplanin (8.1.1, PE/Cy7 (BioLegend), APC/Fire 750 (BioLegend), APC (BioLegend), Super Bright 600 (Invitrogen)), Ki67 (B56, BD, BUV395), Ki67 (Sola15, eBioscience, AF700), BrdU (BD, FITC), Biotin (Bio3-18E7, Miltenyi Biotec, PE), CD8 (53-6.7, PerCP-eFluor710 (eBioscience), BUV395 (BD), BB700 (BD)), CD45 (30-F11, BV510 (BioLegend), PE-Cy7 (BioLegend), BUV395 (eBioscience)), NK1.1 (PK136, BUV395 (BD), BUV661 (Invitrogen)), CD4 (RM4-5, eBioscience, AF700), B220 (A3-6B2, eBioscience, APC-eFluor780), CD19 (6D5, BioLegend, Spark Blue 550), CD127 (SB/199, BD, PE-CF594, APC), KLRG1 (2F1, BioLegend, BV605), ST2 (RMST2-2, eBioscience, PE), Gata3 (TWAJ, eBioscience, eFluor660), Foxp3 (FJK-16s, eBioscience, AF488, APC, PerCP-eFluor 710), RorgT (Q31.378, BD, BV650), CD172α (P84, AF488 (BioLegend), BB700 (BD)), Siglec-F (1RNM44N, eBioscience, SB600), Siglec-F (S17007L, BioLegend, BV421), XCR1 (ZET, BioLegend, BV650), CD64 (X54-5/7.1, BioLegend, BV711), CD11b (M1/70, BioLegend, BV785, Spark NIR 685), I-A/I-E (CI2G9, BD, BUV395, BV480, BB700), CD11c (N418, AF700 (eBioscience), Spark UV 387 (BioLegend)), F4/80 (BM8, APC-eFluor780 (eBioscience), PE/Fire 810 (BioLegend)), Ly6G (1A8-Ly6g, PE-eFluor610 (eBioscience), BUV805 (BD)), Tim4 (RMT4-54, BB700 (BD), PerCP-eFluor710 (eBioscience)), Thy1.1 (OX-7, BioLegend, PerCP), LRRC15 (M25, Genentech, unconjugated). Lineage cocktail contained CD5 (53-7.3), CD19 (1D3), CD11b (M1/70), CD11c (N418), FcεR1α (MAR-1), F4/80 (BM8), Ly-6C/G (Rb6-8C5), and Ter119 (TER-119), all on eFluor450 (eBioscience). Dead cells were excluded with the fixable viability dye UV455, LIVE/DEAD Fixable Blue, or eFluor780 (eBioscience).

ILC2s and fibroblast isolation and coculture

Primary fibroblasts were isolated by fluorescence-activated cell sorting (FACS) on Live, CD45⁺EpCAM⁺CD31⁺Pdnp⁺Pdgfra⁺ fibroblast populations with a BD FACSaria II cell sorter. For ILC2 cultures, ILC2s were first expanded in vivo by intraperitoneal injection of 200 ng rmIL-33 on day 0, 1, 6, and 7, and pancreata harvested on day 9. ILC2s were isolated by FACS, gating on Live, CD45⁺CD3⁺Lineage⁺CD127⁺KLRG1⁺ cells (Lineage = CD5, CD19, F4/80, Gr-1, CD11b, CD11c, Ter119, NK1.1, B220, FcεR1α).

For fibroblast monocultures in vitro, fibroblasts were seeded at an initial density of 20,000 cells/well in 48-well flat bottom plates in DMEM/F-12 supplemented with 10% FCS plus penicillin and streptomycin (P/S) and allowed to adhere for 48 hours. Subsequently, culture media was washed and replaced with DMEM supplemented with 10% FCS plus P/S. For cultures with cytokines, fibroblasts were cultured in cell culture media containing rmIL-13 (Peprotech), rmAREG (BioLegend), rmBMP-2 (BioLegend), recombinant rat CGRP (Cambridge Bioscience), rmLIGHT (BioLegend), rmLIF (BioLegend), and rmOSM (BioLegend), at concentrations indicated in respective figures. Growth kinetics were monitored on the Incucyte S3 System (Sartorius) using phase confluence as a readout, and growth normalised to initial phase confluence. Detection of phosphatidylserine exposure in apoptotic cell death was performed using the Incucyte Annexin V Red Dye (Sartorius). For ILC2 monocultures in vitro, ILC2s were isolated as described above and seeded at 10000 cells/well in 96-well U-bottom plates in RPMI supplemented with 10% FCS plus P/S.

For ILC2-fibroblast cocultures, fibroblasts were isolated as described above and allowed to adhere in DMEM-F12 supplemented with 10% FCS for 48 hours prior to coculture. Media was washed off, and ILC2s added to fibroblast cultures at a density of 2000 cells/well. Coculture media was supplemented with 10% FCS and 10 ng/ml rmIL-33 (BioLegend). Growth kinetics were monitored on the Incucyte S3 System (Sartorius) using phase confluence as a readout.

For culture of fibroblast obtained from *Pil6* reporter mice, tissues were pooled from 2-3 mice and digested in 15-min intervals in 5 ml of digest mix, as described above. Cells from digested adipose (subcutaneous) and skin were plated in a 6-well plate at 6e5-1e6 cells per well (Corning, CLS3526). Cells from digested adipose (epididymal), lung, lymph node, and pancreas were plated at 5e6-20e6 cells per 100 mm dish (Greiner Bio-One, 07-001-017). All cells were plated in DMEM (Gibco, 11995065) supplemented with 10% FBS (D-10). Cells were washed with PBS after 24 hours to remove debris and nonadherent cells. D-10 was replenished every 3-5 days. Cells were detached using 0.25% Trypsin-EDTA (Gibco, 25200056) for 5 min at 37°C prior to passaging at 80% confluency or for flow cytometry at indicated timepoints.

In vitro assays

ELISA was used for quantification of IL-13, LIF, and OSM concentration in cell culture supernatants (R&D Biosystems), in accordance with manufacturer's instructions. Quantification of serum amylase activity was performed using a colorimetric amylase assay kit (Abcam).

Microscopy

For imaging of whole pancreas explants, *Il13^{tdTom/+}Il133^{Citrine/+}* mice were injected with 200 ng rmIL-33 intraperitoneally for 2 days and sacrificed 3 days after the first injection. One day prior to sacrifice, mice were injected intraperitoneally with Hoechst 33342 (100 µg, Thermo Scientific). CellMask Deep Red (10µg, Invitrogen) was injected intravenously 30 min prior to sacrifice to label membranes of vasculature and acinar cell parenchyma. Pancreas explants were imaged on an inverted laser-scanning confocal microscope (Stellaris 8, Leica Microsystems) with an attached pulsed Coherent Chameleon Ultra II laser, using a 25X 0.95NA water immersion objective lens. Hoechst, citrine, and tdTomato were excited at 2-photon excitation wavelengths of 800 nm, 950 nm, and 1040 nm respectively.

Multiplex immunofluorescence imaging was performed on formalin-fixed paraffin-embedded sections using Opal multiplex IHC kits (Akoya Biosciences), following manufacturer instructions. Paraffin-embedded sections were deparaffinised through xylens and rehydrated through decreasing graded alcohol. Deparaffinised sections were then subject to antigen retrieval using 10 mM sodium citrate (pH 6) or TRIS-EDTA (pH9) buffers, depending on the primary antibody used. Before primary antibody incubation, tissue sections were blocked with blocking/antibody

diluent (ARD1001EA) for 20 min at RT. Subsequently, primary antibodies were incubated at working dilutions, durations, and temperatures described in (table S3). After washing, HRP-conjugated secondary antibody was then incubated for 15 min (anti-rabbit and anti-rat polymer-HRP) or 30 min (anti-Syrian Hamster-HRP). Sections were washed prior to addition of Opal reagents (1:100 in Opal amplification buffer).

Multiplex immunofluorescence sections were imaged on an inverted laser-scanning confocal microscope (Stellaris 8, Leica Microsystems) using a 25X 0.95NA water immersion objective lens. Large sections of tissue were imaged for downstream analysis as a maximum intensity projection (typically a z-stack of 10 μ m, 40–60 tiles). Image analysis was performed using the HALO software (Indica Labs). Cell types were identified using the HighPlex-FL algorithm and fine-tuned for cell types of interest detailed in the main text. Regions of pancreatic tissue were manually annotated into interstitial, parenchymal, islet, and vascular territories based on histological appearance using DAPI and podoplanin channels to delineate structural boundaries.

For experiments involving immunofluorescence and imaging of *Pi16* reporter mice, mouse samples were submitted to TCP Pathology Core for histology and immunohistochemistry. The samples were processed in a TissueTek VIP 6 tissue processor (Sakura Finetek), paraffin-embedded and 5 μ m-thick sections were collected onto charged slides (Assure, Epic Scientific). The sections were deparaffinized in a TissueTek Prisma instrument (Sakura Finetek), submitted to heat-induced epitope retrieval with citrate buffer pH 6.0 (10mM Sodium Citrate, 0.05% Tween-20) and incubated in a pressure cooker for 10 min, followed for 30 min at room temperature. The sections were incubated in Protein Block (Agilent, X090930-2) to minimize nonspecific antibody binding before each primary antibody incubation.

Primary antibodies goat anti-GFP (1:500, Abcam, ab6673) and rabbit anti-CD31 (1:100, Abcam, ab28364) were diluted in Antibody Diluent solution (Agilent, S302283-2) and incubated sequentially. Slides were incubated overnight in anti-GFP primary antibody at 4°C. On the following day, the tissue sections were washed in 1x TRIS-buffered saline with 0.1% Tween-20 (TBST), then incubated in anti-goat IgG conjugated with Alexa Fluor 647 (Invitrogen, A32849) diluted at 1:200 in Antibody Diluent for 1 hour at room temperature. After rinsing in TBST, the sections were incubated in Protein Block prior to overnight incubation in anti-CD31 primary antibody overnight at 4°C. On the following day, tissue sections were washed in TBST, incubated in anti-rabbit IgG conjugated with Alexa Fluor 555 (Invitrogen, A32732) diluted at 1:200 in Antibody Diluent for 1 hour at room temperature, rinsed in TBST then counterstained with DAPI (Sigma, D9542). Tissue autofluorescence was quenched in Sudan black B saturated solution in 70% ethanol (Sigma, 199664) for 25 min at room temperature, then coverslips were mounted with Vectashield Vibrance anti-fade mounting medium (Vector, H-1700). Slides were scanned at 20X using an Olympus VS120 slide scanner equipped with a Hamamatsu ORCA-R2 C10600 digital camera (Evident).

Cleared pancreatic thick slices and imaging analysis

Untreated C57BL/6 mice were intracardially perfused with cold PFA 4% in PBS. Digestive systems were collected and post-fixed in PFA 4% overnight followed by three washes in PBS; then pancreases were dissected. Organs were embedded in agarose 4% and cut as 500 μ m slices in a vibrating blade microtome. Slices were dehydrated (10 min consecutive baths of 20%, 40%, 60%, 80%, 100% methanol in PBS), bleached (5% hydrogen peroxide in methanol) for 1 hour and then hydrated (10 min consecutive baths of 100%, 80%, 60%, 40%, 20% methanol in PBS, followed by a bath of PBS). Slices were incubated for 1h in permeabilization solution (0.2% Triton X100, 23 g/L glycine, 20% DMSO in PBS), blocked overnight in blocking buffer (0.2% Triton X100, 5% donkey serum, 5% goat serum, 10% DMSO in PBS) and incubated overnight in primary antibodies (table S4) diluted in 5% DMSO, 3% donkey serum

in PtwH solution (0.2% Tween-20, 10 mg/L heparin in PBS). Tissues were washed 4 times (30 min) in PtwH solution, incubated overnight in secondary antibodies diluted in 3% donkey serum in PtwH, followed by 4 more washes in PtwH solution. Permeabilization, blocking and antibody incubations were done at 37°C in agitation. For their clarification, slices were dehydrated, incubated in 66% dichloromethane diluted in methanol for 3h, and then incubated in 100% dichloromethane for 10 min for their delipidation. Slices were finally incubated in ethyl cinnamate overnight for refractive index adjustment. Cleared slices were mounted in a glass slide covered by a coverslip with two coverslips acting as spacers on each side; ethyl cinnamate was used as mounting media and the coverslips were sealed to the glass slide with a melted mix of paraffin:vaseline (50:50).

Sections were imaged as Z-stacks with a 20X multi-immersion objective (glycerol as immersion medium) in a confocal Stellaris 8 microscope (Leica). 3D volumes were analyzed using the software Imaris (10.2.0). The parenchymal and interstitial volumes were automatically reconstructed based on tissue autofluorescence of the green and red channels by using the ‘surface’ tool. ‘Spots’ tool was used to automatically identify all GATA3⁺ cells; GATA3⁺ cells were then filtered based on CD3 expression as positive or negative by using the ‘intensity sum of square’ value for CD3 channel. GATA3⁺CD3⁺ cells were reconstructed as ILC2s, GATA3⁺CD3⁺ cells were not reconstructed. ILC2s were automatically stratified as parenchymal (light pink) or interstitial (cyan) based on whether their 3D location was within the reconstructed parenchymal or interstitial volumes.

Bulk-RNA-seq

For bulk RNA-sequencing of fibroblasts recovered following coculture, fibroblasts, and ILC2s were detached by incubation at 37°C with 0.25% trypsin. Fibroblasts were then sort-enriched, gating on Live, CD45-Pdpn+Pdgfra+ cells. RNA was extracted using RNeasy Plus Micro kit (Qiagen) and 10 ng RNA used as input material for library preparation. Libraries were generated with the NEBNext® Single Cell/Low Input RNA Library Prep Kit for Illumina (NEB) according to manufacturer's instructions. The pooled libraries were quantified with KAPA Library Quantification Kit for Illumina (Kapa Biosystems) and sequenced (paired end 50nt) on an Illumina NovaSeq 6000 system (Illumina).

For bulk RNA-sequencing of pancreatic ILC2s isolated from untreated and IL-33-treated mice, pancreatic ILC2s were sorted as live, CD45+, CD3-, B220-, Lineage (CD5, CD11b, CD11c, Gr-1, Ter119, CD19, F4/80, FcεR1α-), NK1.1-, CD127+, KLRG1+ cells. RNA was extracted using the RNeasy mini kit (Qiagen, 74104) and DNase was eliminated using the Turbo DNase kit according to manufacturer's instructions (ThermoFisher, AM1907). Libraries were generated with the NEBNext® Single Cell/Low Input RNA Library Prep Kit for Illumina (NEB) according to manufacturer's instructions. Library quantification and quality check of the libraries were done using the Bioanalyser DNA 1000 kit (Agilent Technologies, 5067-1505) and the KAPA library quantification kit for Illumina (KAPA Biosystems, KK4828) respectively. The libraries were then pooled and sequenced on 2 lanes of the HiSeq 4000 system (single end 50nt).

The quality of raw sequence data was assessed using FastQC (v 0.11.9). Raw sequence was trimmed for adapter content using Trimmomatic (v 0.39) and aligned to the GRCh38.p6 genome using STAR (v 2.7.7a). Quantification of gene expression was performed using Salmon. Differential gene expression analysis was carried out using DESeq2 (v 1.34.0) in R (v 4.1.2). 1 sample each from the WT and IL-13 coculture groups were excluded based on high expression of ILC2 transcripts, including *Il13*, indicating ILC2 contamination among sorted fibroblasts retrieved from coculture.

Subsequently, a heatmap of differentially expressed genes was created using the ComplexHeatmap package (v2.15.2), filtering on differentially expressed genes (padj < 0.05) with a minimum expression threshold of 200 DESeq2 normalised counts. Gene set enrichment

analysis was performed using the fgsea package¹⁷⁷ (v1.24.0). Gene signatures of Pi16+ and Col15a1+ fibroblasts were obtained from Buechler *et al.* (1) (as reported in the original publication in supplementary table 4; Markers_Col15a1vsPi16 and Markers_Pi16vsCol15a1).

Predictions of receptor-ligand interactions from bulk RNAseq data were performed using the CellChat (72) package (v1.6.1) using default parameters except where otherwise described. As CellChat was developed for use in single-cell applications, overexpressed genes were defined as genes for which at least one sample has a normalised expression count exceeding 500. Computation of communication probability was also performed using the 'truncatedMean' method, with trim=0 and rawuse = TRUE, and filterCommunication set at min.cells=1.

For bulk RNA-sequencing of fibroblasts of subcutaneously implanted KPC tumor cells, tumors were isolated and digested using 0.1 mg/ml DNase I (Roche, 10104159001), 0.8 mg/ml Dispase II (Gibco, 17105041), and 0.2 mg/ml Collagenase P (Roche, 11249002001) in RPMI with 2% FBS. Tissues were digested in 3 ml of digestion mix for 8 min at 37°C after which cell suspension was removed and filtered through a 70 µm cell strainer (Miltenyi Biotec, 130-110-916) into cold PBS with 2% FBS and 2 mM EDTA. An additional 3ml of digestion mix was added to remaining tissue and incubated for 12 min before cell suspension was removed as before. A final 3 ml of digestion mix was added to tissue for a final 15-min incubation prior to the removal and filtration of remaining cell suspension. ACK lysis buffer (Gibco, A1049201) was used to lyse red blood cells. Cells were counted using a ViCell XR (Beckman Coulter), prior to staining for flow cytometry or Fluorescent Activated Cell Sorting (FACS). Cells were gated as live, CD45-CD24-CD31-EpCAM-PDPN+ and then YFP+LRRC15+, YFP+LRRC15- or YFP-LRRC15+ and sorted by FACS. RNA was isolated using RNeasy Plus Mini Kit (Qiagen, 74136). Quantity and quality of RNA was assessed using an RNA nano chip on an Agilent Bioanalyzer (Agilent). Sequencing libraries were prepared using truseq stranded total RNA library preparation kits with RiboZero as per the manufacturer's instructions (Illumina). Samples were sequenced on an Illumina NextSeq2000 sequencer for 40 million paired end reads with a sequencing depth at read lengths 59bp × 59bp. Protein-coding genes were retained for downstream analysis using a custom list of mouse protein-coding genes generated from NCBI. Differential gene expression analysis was performed using the DESeq2 package (v1.49.3). A regularized log (rlog) transformation was applied to normalized counts, and sample-level variability was assessed by principal component analysis (PCA). Gene set enrichment analysis (GSEA) was conducted using the clusterProfiler (v4.17.0) and fgsea (v1.35.6) R packages. Normalized enrichment scores from GSEA were visualized in custom plots using ggplot2 (v3.5.2).

Single-cell RNA-seq analysis

For single-cell RNA sequencing data of nonimmune cells over the course of acute pancreatitis, acute pancreatitis was first induced in mice via administration of caerulein as described above. Mice were sacrificed and pancreata harvested at 24 hours, 3 days, and 7 days following the induction of acute pancreatitis. Cell suspensions were sort-enriched on live (Live/Dead Fixable eF780-), nucleated (DRAQ5+), nonhaematopoietic (CD45-) cells. For single-cell RNA sequencing of ILCs from untreated mice, cell suspensions were sort-enriched on live, CD45+, CD3-, B220-, Lineage (CD5, CD11b, CD11c, Gr-1, Ter119, CD19, F4/80, FcεR1α-), CD127+, NK1.1- cells. Single-cell encapsulation was performed using the Chromium Single Cell B Chip Kit (10x Genomics, PN-1000073), followed by library preparation using the Chromium Single Cell 3' GEM Library & Gel Bead Kit v3 (10x Genomics, PN-1000075). Pooled libraries were then sequenced (paired end 50nt) on a NovaSeq 6000 system.

Initial mapping and counting of 10x Genomics scRNA-seq reads was performed using Cell Ranger v3.1.0. Data was then imported into Seurat (73) for downstream analysis. For scRNA-seq of the nonimmune

compartment, quality control was performed on individual datasets, filtering for single cells with between 1000 and 6000 expressed genes, and with less than 10% expressed mitochondrial transcripts. Doublets were identified and excluded using the scDblFinder package (74). Subsequently, datasets were integrated in Seurat, and principal components 1 to 15 were provided as input for dimensionality reduction via UMAP with default parameters in Seurat. Cells were clustered using the Louvain method on a shared nearest neighbor graph using default parameters (resolution = 0.3).

Differentially expressed genes for Col15a1+ and Pi16+ fibroblast clusters were identified by first obtaining pseudobulk gene expression for each cluster on a per-sample level (AggregateExpression), then performing differential gene expression testing using the DESeq2 package. Data was visualized as a volcano plot using the EnhancedVolcano package. The top 500 up-regulated or down-regulated genes ranked by fold-change in gene expression were then used for functional gene ontology enrichment analysis with the clusterProfiler (v4.6.2) package, using the Molecular Signatures Database (MSigDB) C2:Biological Processes gene sets.

For scRNA-seq of ILCs in the untreated pancreas, initial quality control was performed by filtering for single cells with less than 5% expressed mitochondrial transcripts. Data were then log-normalised, and principal components 1 to 25 were provided as input for dimensionality reduction via UMAP with default parameters in Seurat. Cells were clustered using the Louvain method on a shared nearest neighbor graph using default parameters (resolution = 0.2).

For scRNA-seq of lineage-traced fibroblasts of *Dpp4^{CreERT2/+} Rosa26^{lsl-tdTom/lsl-tdTom}* mice, mice were orthotopically implanted with 2838c3 PDAC tumor cells. Mice were administered 4mg of tamoxifen by oral gavage on days 7-9 and sacrificed on day 21. Tumour single-cell suspensions were sort-enriched on live (Live/Dead Fixable eF780-), CD45-, EpCAM-, CD31-, Pdpn+, Pdgfra+ cells, and separately sorted into tdTomato+ and tdTomato- sort fractions per tumor. Individual sort fractions were loaded for on-chip multiplexing (GEM-X OCM 3' Chip Kit v4 4-plex), single-cell encapsulation, and library preparation following manufacturer's instructions (GEM-X Universal 3' Gene Expression v4 4-plex Reagent Kits). Pooled libraries were then sequenced (paired end 50nt) on a NovaSeqX system. A custom reference was constructed with the GRCm39-2024-A reference genome containing an appended entry for tdTomato and the WRPE of the Ai9 mouse using cellranger mkref, and reads aligned, demultiplexed, counted using cellranger multi (Cell Ranger v9.1.0). Data was subsequently imported into Seurat, subsetting for cells with fewer than 10% mitochondrial transcripts, at least 1000 UMIs, and that express at least 200 genes. Gene expression was log-normalised, and principal component analysis was performed on the top 2000 most variable genes. Individual Seurat objects were then integrated using Harmony (75) through IntegrateLayers, and the first 30 Harmony dimensions used as input for dimensionality reduction via UMAP. Subsequently, cells within the tdTomato- sort fraction that expressed tdTomato transcript and therefore represent likely sort contaminants were reannotated within the tdTomato+ group for downstream analysis. This clustering resulted in the identification of distinct populations mesothelial-derived (originating from *Msln*⁺ cells) and fibroblast-derived (originating from *Pdgfra*⁺*Pi16*⁺*Ly6c1*⁺*Dpp4*⁺) cells, of which fibroblast-derived cells were further subclustered for downstream analysis.

Analysis of publicly available scRNA-seq datasets

Single cell transcriptomes from healthy pancreas were obtained from processed data (1) (fibroXplorer.com) and integrated with fibroblast transcriptomes of untreated or PBS-injected mice, preserving original cluster identities. Pseudotime analysis was subsequently performed using the Monocle 3 package (76), defining Pi16+ fibroblasts as the starting node.

SCENIC analysis

Potential gene regulatory networks active in fibroblast subsets over the course of caerulein-induced acute pancreatitis were predicted using the SCENIC package (30). For each time point, 500 P16+ fibroblasts and 1500 Col15a1+ fibroblasts were downsampled from the original integrated object. The R implementation of SCENIC was run with default parameters, except for gene regulatory network inference, where GRNBoost was performed in Python due to its superior runtime. Due to the stochastic nature of the gene regulatory network inference step, slightly varying results are produced each run. In line with the developers' suggestions, the SCENIC pipeline was run several times, and potential regulons were considered 'high confidence' if they occurred in >80% of runs. All regulons presented in figures were inferred in >80% of runs.

RNA velocity

Counting of spliced and unspliced RNA abundances in single cell transcriptomes was performed using velocity (77). Subsequently, trajectory inference was performed with scVelo (78), using the generalised dynamical model for transcriptional dynamics. UMAP embeddings obtained from Seurat were imported for visualization of cell trajectories.

Milo

Differential abundance analysis between tdTomato+ and tdTomato- groups within scRNAseq datasets was performed using the miloR (79) package, using parameters of $k=20$ and $d=30$ for buildGraph, $\text{prop}=0.1$, $k=20$, $d=30$, $\text{refined}=T$ for makeNhoods, and $d=30$ for calcNhhoodDistance. As tdTomato+ and tdTomato- cells can be matched by tumor of origin, testNhoods was performed with the argument $\text{design} = \sim \text{original_tumor} + \text{tdTom_grouping}$.

CellRank

Modelling of CAF population fates was performed using CellRank 2 (80). scVelo was used to set up the VelocityKernel, and a combined kernel created from velocity and connectivity kernels ($0.8 * vk + 0.2 * ck$). 12 macrostates were fitted using the GPCCA estimator, and a macrostate corresponding to the Dpp4^+ fibroblast population set as the initial macrostate in accordance with the experimental ground truth. Subsequently, terminal macrostates were predicted, and per-cell fate probabilities computed.

Statistics

Analysis for two groups were calculated using an unpaired two-tailed Student's t test; comparisons of more than two groups were calculated using a one-way analysis of variance (ANOVA) with Tukey post-analysis. All data were analyzed using GraphPad Prism 9 (GraphPad Software). Results with $P \leq 0.05$ were considered statistically significant (*), $P \leq 0.01 = **$, $P \leq 0.001 = ***$, $P \leq 0.0001 = ****$.

REFERENCES AND NOTES

- M. B. Buechler *et al.*, Cross-tissue organization of the fibroblast lineage. *Nature* **593**, 575–579 (2021). doi: [10.1038/s41586-021-03549-5](https://doi.org/10.1038/s41586-021-03549-5)
- S. Davidson *et al.*, Fibroblasts as immune regulators in infection, inflammation and cancer. *Nat. Rev. Immunol.* **21**, 704–717 (2021). doi: [10.1038/s41577-021-00540-z](https://doi.org/10.1038/s41577-021-00540-z)
- L. Monteran, N. Erez, The Dark Side of Fibroblasts: Cancer-Associated Fibroblasts as Mediators of Immunosuppression in the Tumor Microenvironment. *Front. Immunol.* **10**, 1835 (2019). doi: [10.3389/fimmu.2019.01835](https://doi.org/10.3389/fimmu.2019.01835)
- H. Soliman *et al.*, Multipotent stromal cells: One name, multiple identities. *Cell Stem Cell* **28**, 1690–1707 (2021). doi: [10.1016/j.stem.2021.09.001](https://doi.org/10.1016/j.stem.2021.09.001)
- M. L. Meizlish, R. A. Franklin, X. Zhou, R. Medzhitov, Tissue Homeostasis and Inflammation. *Annu. Rev. Immunol.* **39**, 557–581 (2021). doi: [10.1146/annurev-immunol-061020-053734](https://doi.org/10.1146/annurev-immunol-061020-053734)
- D. Wu *et al.*, Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* **332**, 243–247 (2011). doi: [10.1126/science.1201475](https://doi.org/10.1126/science.1201475)
- M. Feuerer *et al.*, Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* **15**, 930–939 (2009). doi: [10.1038/nm.2002](https://doi.org/10.1038/nm.2002)
- F. Gerbe *et al.*, Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature* **529**, 226–230 (2016). doi: [10.1038/nature16527](https://doi.org/10.1038/nature16527)
- J. von Moltke, M. Ji, H.-E. Liang, R. M. Locksley, Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* **529**, 221–225 (2016). doi: [10.1038/nature16161](https://doi.org/10.1038/nature16161)
- S. Sanada *et al.*, IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J. Clin. Invest.* **117**, 1538–1549 (2007). doi: [10.1172/JCI30634](https://doi.org/10.1172/JCI30634)
- T. Mahlaköv *et al.*, Stromal cells maintain immune cell homeostasis in adipose tissue through production of interleukin-33. *Sci. Immunol.* **4**, eaax0416 (2019). doi: [10.1126/sciimmunol.aax0416](https://doi.org/10.1126/sciimmunol.aax0416)
- B. M. J. Rana *et al.*, A stromal cell niche sustains ILC2-mediated type-2 conditioning in adipose tissue. *J. Exp. Med.* **216**, 1999–2009 (2019). doi: [10.1084/jem.20190689](https://doi.org/10.1084/jem.20190689)
- M. W. Dahlgren *et al.*, Adventitial Stromal Cells Define Group 2 Innate Lymphoid Cell Tissue Niches. *Immunity* **50**, 707–722.e6 (2019). doi: [10.1016/j.immuni.2019.02.002](https://doi.org/10.1016/j.immuni.2019.02.002)
- A. B. Molofsky, A. K. Savage, R. M. Locksley, Interleukin-33 in Tissue Homeostasis, Injury, and Inflammation. *Immunity* **42**, 1005–1019 (2015). doi: [10.1016/j.immuni.2015.06.006](https://doi.org/10.1016/j.immuni.2015.06.006)
- M. V. Plikus *et al.*, Fibroblasts: Origins, definitions, and functions in health and disease. *Cell* **184**, 3852–3872 (2021). doi: [10.1016/j.cell.2021.06.024](https://doi.org/10.1016/j.cell.2021.06.024)
- C. Cayrol, J.-P. Girard, Interleukin-33 (IL-33): A critical review of its biology and the mechanisms involved in its release as a potent extracellular cytokine. *Cytokine* **156**, 155891 (2022). doi: [10.1016/j.cyto.2022.155891](https://doi.org/10.1016/j.cyto.2022.155891)
- D. Ohlund *et al.*, Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J. Exp. Med.* **214**, 579–596 (2017). doi: [10.1084/jem.20160204](https://doi.org/10.1084/jem.20160204)
- M. Shakiba, D. A. Tuveson, Macrophages and fibroblasts as regulators of the immune response in pancreatic cancer. *Nat. Immunol.* **26**, 678–691 (2025). doi: [10.1038/s41590-025-02134-6](https://doi.org/10.1038/s41590-025-02134-6)
- E. Dalmas *et al.*, Interleukin-33-Activated Islet-Resident Innate Lymphoid Cells Promote Insulin Secretion through Myeloid Cell Retinoic Acid Production. *Immunity* **47**, 928–942.e7 (2017). doi: [10.1016/j.immuni.2017.10.015](https://doi.org/10.1016/j.immuni.2017.10.015)
- C. X. Dominguez *et al.*, Single-cell RNA sequencing reveals stromal evolution into LRRC15+ myofibroblasts as a determinant of patient response to cancer immunotherapy. *Cancer Discov.* **10**, 232–253 (2020). doi: [10.1158/2159-8290.CD-19-0644](https://doi.org/10.1158/2159-8290.CD-19-0644)
- S. Chakarov *et al.*, Two distinct interstitial macrophage populations coexist across tissues in specific subcellular niches. *Science* **363**, eaau0964 (2019). doi: [10.1126/science.aau0964](https://doi.org/10.1126/science.aau0964)
- M. V. Apte, R. C. Pirola, J. S. Wilson, Pancreatic stellate cells: A starring role in normal and diseased pancreas. *Front. Physiol.* **3**, 344 (2012). doi: [10.3389/fphys.2012.00344](https://doi.org/10.3389/fphys.2012.00344)
- S. Nakandakari-Higa *et al.*, Universal recording of immune cell interactions in vivo. *Nature* **627**, 399–406 (2024). doi: [10.1038/s41586-024-07134-4](https://doi.org/10.1038/s41586-024-07134-4)
- K. S. Schluns, W. C. Kieper, S. C. Jameson, L. Lefrançois, Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells in vivo. *Nat. Immunol.* **1**, 426–432 (2000). doi: [10.1038/80868](https://doi.org/10.1038/80868)
- T. Y. F. Halim *et al.*, Group 2 innate lymphoid cells license dendritic cells to potentiate memory TH2 cell responses. *Nat. Immunol.* **17**, 57–64 (2016). doi: [10.1038/ni.3294](https://doi.org/10.1038/ni.3294)
- A. B. Molofsky *et al.*, Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J. Exp. Med.* **210**, 535–549 (2013). doi: [10.1084/jem.20121964](https://doi.org/10.1084/jem.20121964)
- G. Vassalli, Aldehyde Dehydrogenases: Not Just Markers, but Functional Regulators of Stem Cells. *Stem Cells Int.* **2019**, 1–15 (2019). doi: [10.1155/2019/3904645](https://doi.org/10.1155/2019/3904645)
- A. De Martin *et al.*, P16+ reticular cells in human palatine tonsils govern T cell activity in distinct subepithelial niches. *Nat. Immunol.* **24**, 1138–1148 (2023). doi: [10.1038/s41590-023-01502-4](https://doi.org/10.1038/s41590-023-01502-4)
- H. L. Ansoorge *et al.*, Type XIV Collagen Regulates Fibrillogenesis*. *J. Biol. Chem.* **284**, 8427–8438 (2009). doi: [10.1074/jbc.M805582200](https://doi.org/10.1074/jbc.M805582200)
- S. Aibar *et al.*, SCENIC: Single-cell regulatory network inference and clustering. *Nat. Methods* **14**, 1083–1086 (2017). doi: [10.1038/nmeth.4463](https://doi.org/10.1038/nmeth.4463)
- X. Zhang *et al.*, FOXO1 is an essential regulator of pluripotency in human embryonic stem cells. *Nat. Cell Biol.* **13**, 1092–1099 (2011). doi: [10.1038/ncb2293](https://doi.org/10.1038/ncb2293)
- N. Sue *et al.*, Targeted disruption of the basic Krüppel-like factor gene (Klf3) reveals a role in adipogenesis. *Mol. Cell. Biol.* **28**, 3967–3978 (2023). doi: [10.1128/MCB.01942-07](https://doi.org/10.1128/MCB.01942-07)
- B. Gupta *et al.*, The transcription factor ZEB1 regulates stem cell self-renewal and cell fate in the adult hippocampus. *Cell Rep.* **36**, 109588 (2021). doi: [10.1016/j.celrep.2021.109588](https://doi.org/10.1016/j.celrep.2021.109588)
- K. M. Southard *et al.*, Comprehensive transcription factor perturbations recapitulate fibroblast transcriptional states. *Nat. Genet.* **57**, 2323–2334 (2025). doi: [10.1038/s41588-025-02284-1](https://doi.org/10.1038/s41588-025-02284-1)
- M. Yan *et al.*, ETS1 governs pathological tissue-remodeling programs in disease-associated fibroblasts. *Nat. Immunol.* **23**, 1330–1341 (2022). doi: [10.1038/s41590-022-01285-0](https://doi.org/10.1038/s41590-022-01285-0)
- T. Guan, F. Fang, X. Su, K. Lin, Q. Gao, Silencing PEG3 inhibits renal fibrosis in a rat model of diabetic nephropathy by suppressing the NF- κ B pathway. *Mol. Cell. Endocrinol.* **513**, 110823 (2020). doi: [10.1016/j.mce.2020.110823](https://doi.org/10.1016/j.mce.2020.110823)
- M. Stellato *et al.*, The AP-1 transcription factor Fosl-2 drives cardiac fibrosis and arrhythmias under immunofibrotic conditions. *Commun. Biol.* **6**, 1–15 (2023).
- A. Kishino *et al.*, XBP1-FoxO1 interaction regulates ER stress-induced autophagy in auditory cells. *Sci. Rep.* **7**, 4442 (2017). doi: [10.1038/s41598-017-02960-1](https://doi.org/10.1038/s41598-017-02960-1)
- J. Capdevila, T. Tsukui, C. Rodríguez Esteban, V. Zappavigna, J. C. Izpisua Belmonte, Control of vertebrate limb outgrowth by the proximal factor Meis2 and distal antagonism of BMPs by Gremlin. *Mol. Cell* **4**, 839–849 (1999). doi: [10.1016/S1097-2765\(00\)80393-7](https://doi.org/10.1016/S1097-2765(00)80393-7)

40. J. DeGregori, D. G. Johnson, Distinct and Overlapping Roles for E2F Family Members in Transcription, Proliferation and Apoptosis. *Curr. Mol. Med.* **6**, 739–748 (2006).
41. L. Sampieri, P. Di Giusto, C. Alvarez, CREB3 Transcription Factors: ER-Golgi Stress Transducers as Hubs for Cellular Homeostasis. *Front. Cell Dev. Biol.* **7**, 123 (2019). doi: [10.3389/fcell.2019.00123](https://doi.org/10.3389/fcell.2019.00123)
42. I.-M. Wang, H. Lin, S. J. Goldman, M. Kobayashi, STAT-1 is activated by IL-4 and IL-13 in multiple cell types. *Mol. Immunol.* **41**, 873–884 (2004). doi: [10.1016/j.molimm.2004.04.027](https://doi.org/10.1016/j.molimm.2004.04.027)
43. R. Kang, M. T. Lotze, H. J. Zeh, T. R. Billiar, D. Tang, Cell death and DAMPs in acute pancreatitis. *Mol. Med.* **20**, 466–477 (2014). doi: [10.2119/molmed.2014.00117](https://doi.org/10.2119/molmed.2014.00117)
44. O. A. Mareninova *et al.*, Cell death in pancreatitis: Caspases protect from necrotizing pancreatitis. *J. Biol. Chem.* **281**, 3370–3381 (2006). doi: [10.1074/jbc.M511276200](https://doi.org/10.1074/jbc.M511276200)
45. H. Y. Guo, Z. J. Cui, Extracellular Histones Activate Plasma Membrane Toll-Like Receptor 9 to Trigger Calcium Oscillations in Rat Pancreatic Acinar Tumor Cell AR4-2J. *Cells* **8**, cells8010003 (2018). doi: [10.3390/cells8010003](https://doi.org/10.3390/cells8010003)
46. S. R. Hingorani *et al.*, Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* **7**, 469–483 (2005). doi: [10.1016/j.ccr.2005.04.023](https://doi.org/10.1016/j.ccr.2005.04.023)
47. I. C. Boothby *et al.*, Early-life inflammation primes a T helper 2 cell-fibroblast niche in skin. *Nature* **599**, 667–672 (2021). doi: [10.1038/s41586-021-04044-7](https://doi.org/10.1038/s41586-021-04044-7)
48. J. Li *et al.*, Tumor Cell-Intrinsic Factors Underlie Heterogeneity of Immune Cell Infiltration and Response to Immunotherapy. *Immunity* **49**, 178–193.e7 (2018). doi: [10.1016/j.immuni.2018.06.006](https://doi.org/10.1016/j.immuni.2018.06.006)
49. A. T. Krishnamurthy *et al.*, LRRCL15+ myofibroblasts dictate the stromal setpoint to suppress tumour immunity. *Nature* **611**, 148–154 (2022). doi: [10.1038/s41586-022-05272-1](https://doi.org/10.1038/s41586-022-05272-1)
50. M. Lange *et al.*, CellRank for directed single-cell fate mapping. *Nat. Methods* **19**, 159–170 (2022). doi: [10.1038/s41592-021-01346-6](https://doi.org/10.1038/s41592-021-01346-6)
51. T. Gong, L. Liu, W. Jiang, R. Zhou, DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat. Rev. Immunol.* **20**, 95–112 (2019). doi: [10.1038/s41577-019-0215-7](https://doi.org/10.1038/s41577-019-0215-7)
52. S. Navina *et al.*, Lipotoxicity causes multisystem organ failure and exacerbates acute pancreatitis in obesity. *Sci. Transl. Med.* **3**, 107ra110 (2011). doi: [10.1126/scitranslmed.3002573](https://doi.org/10.1126/scitranslmed.3002573)
53. G. I. Papachristou, D. J. Papachristou, H. Avula, A. Slivka, D. C. Whitcomb, Obesity increases the severity of acute pancreatitis: Performance of APACHE-O score and correlation with the inflammatory response. *Pancreatology* **6**, 279–285 (2006). doi: [10.1159/000092689](https://doi.org/10.1159/000092689)
54. M. Šestan *et al.*, Neuronal-ILC2 interactions regulate pancreatic glucagon and glucose homeostasis. *Science* **387**, eadi3624 (2025). doi: [10.1126/science.adi3624](https://doi.org/10.1126/science.adi3624)
55. J. A. Moral *et al.*, ILC2s amplify PD-1 blockade by activating tissue-specific cancer immunity. *Nature* **579**, 130–135 (2020). doi: [10.1038/s41586-020-015-4](https://doi.org/10.1038/s41586-020-015-4)
56. A. Alam *et al.*, Fungal microbiome drives IL-33 secretion and type 2 immunity in pancreatic cancer. *Cancer Cell* **40**, 153–167.e11 (2022). doi: [10.1016/j.ccell.2022.01.003](https://doi.org/10.1016/j.ccell.2022.01.003)
57. M. Stefkovich, S. Traynor, L. Cheng, D. Merrick, P. Seale, Dpp4+ interstitial progenitor cells contribute to basal and high fat diet-induced adipogenesis. *Mol. Metab.* **54**, 101357 (2021). doi: [10.1016/j.molmet.2021.101357](https://doi.org/10.1016/j.molmet.2021.101357)
58. H. L. Horsnell *et al.*, Lymph node homeostasis and adaptation to immune challenge resolved by fibroblast network mechanics. *Nat. Immunol.* **23**, 1169–1182 (2022). doi: [10.1038/s41590-022-01272-5](https://doi.org/10.1038/s41590-022-01272-5)
59. B. Y. Lee *et al.*, Heterocellular OSM-OSMR signalling reprograms fibroblasts to promote pancreatic cancer growth and metastasis. *Nat. Commun.* **12**, 7336 (2021). doi: [10.1038/s41467-021-27607-8](https://doi.org/10.1038/s41467-021-27607-8)
60. U. A. Betz *et al.*, Postnatally induced inactivation of gp130 in mice results in neurological, cardiac, hematopoietic, immunological, hepatic, and pulmonary defects. *J. Exp. Med.* **188**, 1955–1965 (1998). doi: [10.1084/jem.188.10.1955](https://doi.org/10.1084/jem.188.10.1955)
61. J. C. Nussbaum *et al.*, Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* **502**, 245–248 (2013). doi: [10.1038/nature12526](https://doi.org/10.1038/nature12526)
62. A. M. Tsou *et al.*, I. C. Lorenz, D. Andrew, P. Balderes, C. S. N. Klose, S. A. Lira, D. Artis, Neuropeptide regulation of nonredundant ILC2 responses at barrier surfaces. *Nature* **611**, 787–793 (2022). doi: [10.1038/s41586-022-05297-6](https://doi.org/10.1038/s41586-022-05297-6)
63. M. Kraman *et al.*, Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein- α . *Science* **330**, 827–830 (2010). doi: [10.1126/science.1195300](https://doi.org/10.1126/science.1195300)
64. M. Gogoi *et al.*, ILC2-derived LIF licences progress from tissue to systemic immunity. *Nature* **632**, 885–892 (2024). doi: [10.1038/s41586-024-07746-w](https://doi.org/10.1038/s41586-024-07746-w)
65. C. S. Hardman, V. Panova, A. N. J. McKenzie, IL-33 citrine reporter mice reveal the temporal and spatial expression of IL-33 during allergic lung inflammation: Innate immunity. *Eur. J. Immunol.* **43**, 488–498 (2013). doi: [10.1002/eji.201242863](https://doi.org/10.1002/eji.201242863)
66. J. L. Barlow, A. Bellosi, C. S. Hardman, L. F. Drynan, S. H. Wong, J. P. Cruickshank, A. N. J. McKenzie, Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *J. Allergy Clin. Immunol.* **129**, 191–8.e1–4 (2012).
67. S. M. Schlenner *et al.*, Fate mapping reveals separate origins of T cells and myeloid lineages in the thymus. *Immunity* **32**, 426–436 (2010). doi: [10.1016/j.immuni.2010.03.005](https://doi.org/10.1016/j.immuni.2010.03.005)
68. C. J. Olliphant *et al.*, MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity* **41**, 283–295 (2014). doi: [10.1016/j.immuni.2014.06.016](https://doi.org/10.1016/j.immuni.2014.06.016)
69. F. Colucci *et al.*, Dissecting NK cell development using a novel alymphoid mouse model: Investigating the role of the c-abl proto-oncogene in murine NK cell differentiation. *J. Immunol.* **162**, 2761–2765 (1999). doi: [10.4049/jimmunol.162.5.2761](https://doi.org/10.4049/jimmunol.162.5.2761)
70. M. Gertsenstein *et al.*, Efficient generation of germ line transmitting chimeras from C57BL/6N ES cells by aggregation with outbred host embryos. *PLOS ONE* **5**, e11260 (2010). doi: [10.1371/journal.pone.0011260](https://doi.org/10.1371/journal.pone.0011260)
71. A. Nagy, M. Gertsenstein, K. Vintersten, R. R. Behringer, *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory Press, ed. 3, 2002).
72. S. Jin *et al.*, Inference and analysis of cell-cell communication using CellChat. *Nat. Commun.* **12**, 1088 (2021). doi: [10.1038/s41467-021-21246-9](https://doi.org/10.1038/s41467-021-21246-9)
73. T. Stuart *et al.*, Comprehensive integration of single-cell data. *Cell* **177**, 1888–1902.e21 (2019). doi: [10.1016/j.cell.2019.05.031](https://doi.org/10.1016/j.cell.2019.05.031)
74. P.-L. Germain, A. Lun, C. Garcia Meixide, W. Macnair, M. D. Robinson, Doublet identification in single-cell sequencing data using scDblFinder. *F1000 Res.* **10**, 979 (2021). doi: [10.12688/f1000research.73600.1](https://doi.org/10.12688/f1000research.73600.1)
75. I. Korsunsky *et al.*, Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat. Methods* **16**, 1289–1296 (2019). doi: [10.1038/s41592-019-0619-0](https://doi.org/10.1038/s41592-019-0619-0)
76. J. Cao *et al.*, The single-cell transcriptional landscape of mammalian organogenesis. *Nature* **566**, 496–502 (2019). doi: [10.1038/s41586-019-0969-x](https://doi.org/10.1038/s41586-019-0969-x)
77. G. La Manno *et al.*, RNA velocity of single cells. *Nature* **560**, 494–498 (2018). doi: [10.1038/s41586-018-0414-6](https://doi.org/10.1038/s41586-018-0414-6)
78. V. Bergen, M. Lange, S. Peidli, F. A. Wolf, F. J. Theis, Generalizing RNA velocity to transient cell states through dynamical modeling. *Nat. Biotechnol.* **38**, 1408–1414 (2020). doi: [10.1038/s41587-020-0591-3](https://doi.org/10.1038/s41587-020-0591-3)
79. E. Dann, N. C. Henderson, S. A. Teichmann, M. D. Morgan, J. C. Marioni, Differential abundance testing on single-cell data using k-nearest neighbor graphs. *Nat. Biotechnol.* **40**, 245–253 (2022). doi: [10.1038/s41587-021-01033-z](https://doi.org/10.1038/s41587-021-01033-z)
80. P. Weiler, M. Lange, M. Klein, D. Pe'er, F. Theis, CellRank 2: Unified fate mapping in multiview single-cell data. *Nat. Methods* **21**, 1196–1205 (2024). doi: [10.1038/s41592-024-02303-9](https://doi.org/10.1038/s41592-024-02303-9)

ACKNOWLEDGMENTS

We thank the CRUK-CI research instrumentation, flow cytometry, genomics, bioinformatics, histopathology, microscopy, transgenics, and BRU cores for their expertise and help, and the Centre for Immune Analytics (RRID: SCR_027612) in the Department of Immunology at the University of Toronto for assistance with the FACS Analysis and Cell Sorting. We are grateful to the staff at the Centre for Phenogenomics for animal husbandry and colony maintenance assistance. We gratefully acknowledge the sequencing facility in the school of biomedical engineering at the University of British Columbia. We thank L. Nutter at The Centre for Phenogenomics for designing and the Model Production team for ES cell targeting and generation of C57BL/6N-*Pt16^{tm1(cre)Tep}* mice. We are grateful to W. Müller and D. Konrad for sharing *gp130^{fl}* mice. **Funding:** This work was supported by the following: EU Horizon 2020 - Marie Skłodowska-Curie grant (PanLC no. 840501, to J.S.); The Royal Society and Wellcome Trust (204622/Z/16/Z, to T.Y.F.H.); Wellcome Trust (220268/Z/20/Z, to M.R.C.; 220223/Z/20/Z, to A.N.J.M.; 301533/Z/23/Z, to T.Y.F.H.); Cancer Research UK (CRUK) core award (A24995, to T.Y.F.H.); Cancer Research UK (CRUK) Career Development Fellowship (CRUK-A19763; to S.E.A.); UK Medical Research Council (U105178805, to A.N.J.M.); Swiss National Science Foundation (10001794, to C.Sch); Pancreatic Cancer UK (CFF2024_Yip, to T.Y.); G102883, to C.S.); Canadian Institute of Health Research Project grant (471606, to M.B.B.); Belgian Fonds de la Recherche Scientifique - FNRS (to J.S.); Natural Sciences Research Council of Canada (RGPIN-2025-0429, NGECR-2025-00122314, to M.B.B.); University of Toronto's Medicine by Design initiative, which receives funding from the Canada First Research Excellence Fund, John Evans Leadership Funds and Ontario Research Funds (40536, to M.B.B.); Department of Emergency Medicine, Icahn School of Medicine at Mount Sinai, NY, USA (to G.R.); Churchill Scholarship, Churchill College, University of Cambridge (to S.N.H.). **Author contributions:** Conceptualization: T.Y., J.S., and T.Y.F.H. Methodology: T.Y., J.S., S.R., C.G., R.L., M.M.R.S., C.J., M.R.C., M.B.B., and T.Y.F.H. Resources: T.Y.F.H. Investigation: T.Y., J.S., S.R., C.S., E.E.M., M.M.R.S., S.N.H., J.M.V., G.R., C.G., R.L., S.P., M.F.C., L.A.H., and T.Y.F.H. Provided reagents: H.R.R., C.Sch, C.J., A.N.J.M., S.E.A., P.S., and M.B.B. Visualization: T.Y., J.S., S.P., E.E.M., L.A.H., M.B.B., and T.Y.F.H. Funding acquisition: J.S., G.R., M.B.B., and T.Y.F.H. Project administration: T.Y.F.H. Supervision: J.S., T.M.U., M.B.B., and T.Y.F.H. Writing – original draft: T.Y. and T.Y.F.H. Writing – review & editing: T.Y., J.S., M.B.B., and T.Y.F.H. **Competing interests:** The authors declare that they have no competing interests. **Data, code, and materials availability:** Transcriptomic data are available at G.E.O. under accession numbers GSE 248387 and GSE 309073. Raw and processed transcriptomic data from (1) are available at ArrayExpress repository under the accession number E-MTAB-8483, and integrated scRNA-seq objects are available at <http://www.fibroxplorer.com>. All other data needed to evaluate the conclusions in the paper are present in the main text or the supplementary materials. No new code or materials, other than those listed below, were generated. *Pdgfra^{mGFP-CreERT2}* mice are available from S. Acton via CancerTools.org under a material transfer agreement with University College London. *Dpp4^{CreERT2}* mice are available from P. Seale under a material agreement with the University of Pittsburgh. *Il33^{Cre}*, *Il13^{Cre}*, *Lil^{fl}*, and *Rora^{fl}* mice are available from A.N.J. McKenzie under a material transfer agreement with the MRC Laboratory of Molecular Biology. *Pt16^{tm1(cre)Tep}* mice are available from M.B. Buechler at the Canadian Mouse Mutant Repository (cmmr.ca; strain AFGG) under a material transfer agreement with the University of Toronto. License information: Copyright © 2026 the authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original US government works. <https://www.science.org/about/science-licenses-journal-article-reuse>. This research was funded in whole or in part by Wellcome Trust (220268/Z/20/Z, 220223/Z/20/Z, 01533/Z/23/Z), a cOAlition S organization. The author will make the Author Accepted Manuscript (AAM) version available under a CC BY public copyright license.

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.aea5113
 Figs. S1 to S14; Tables S1 to S4; Data S1 and S2; Movie S1; MDAR Reproducibility Checklist
 Submitted 3 October 2025; accepted 21 April 2026

10.1126/science.aea5113



ILC2s regulate a fibroblast progenitor niche in the pancreas

Thomas Yip, Julie Stockis, Charlotte Simpson, Erika E. McCartney, Shwetha Raghunathan, Martha M. Rangel-Sosa, Sydney N. Hummel, Julia Moreno-Vicente, Gianmarco Raddi, Celine Garcia, Rugile Linkute, Silvain Pinaud, Maye F. Cheng, Lesley A. Hill, T. Michael Underhill, Hans-Reimer Rodewald, Christoph Schneider, Claus Jørgensen, Andrew N.J. McKenzie, Sophie E. Acton, Patrick Seale, Menna R. Clatworthy, Matthew B. Buechler, and Timotheus Y. F. Halim

Science **393** (6806), eaea5113. DOI: 10.1126/science.aea5113

Editor's summary

Type 2 innate immune cells (ILC2s) function to couple host defense with tissue homeostasis. Yip *et al.* investigated cross-talk between ILC2s and fibroblasts within the mouse pancreas. ILC2s localized alongside a distinctive population of fibroblasts with the capability to differentiate into other fibroblast subtypes. They also helped these progenitor fibroblasts to proliferate and contributed to determining their total numbers within tissues. This ILC2-fibroblast axis was important for homeostasis and tissue repair after injury, and was also able to contribute to the differentiation of cancer-associated fibroblasts. —Sarah H. Ross

View the article online

<https://www.science.org/doi/10.1126/science.aea5113>

Permissions

<https://www.science.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of service](#)

Science (ISSN 1095-9203) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 2026 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works