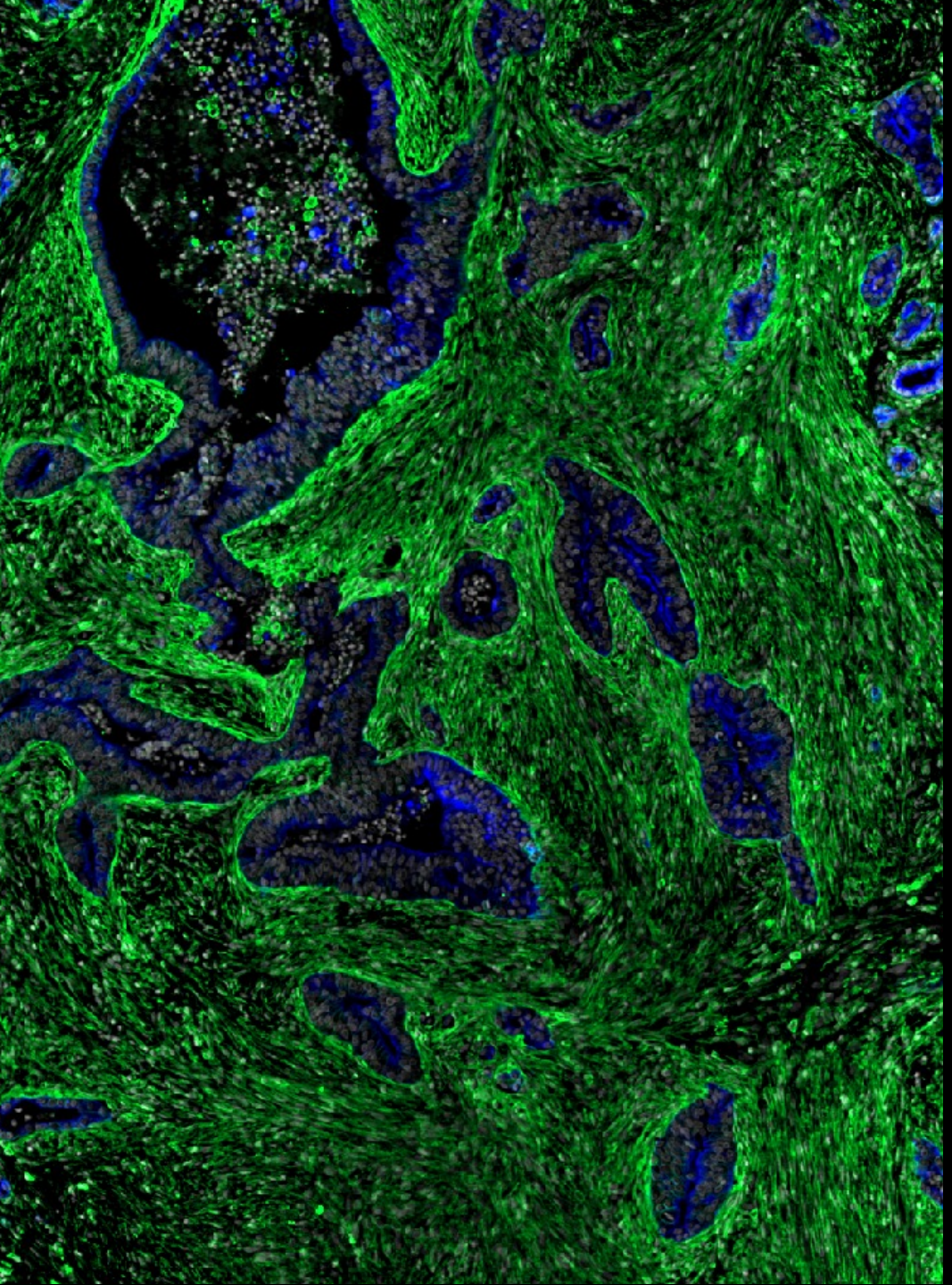


Cancer cells
Stromal cells
Nuclei (DAPI)

Crosstalk in the tumor ecosystem

Mara H. Sherman
Associate Member
Cancer Biology & Genetics
Memorial Sloan Kettering Cancer Center

Defining key terms

- Tumor microenvironment: the collective cellular and acellular components that together make up a cancer in its relevant anatomic setting

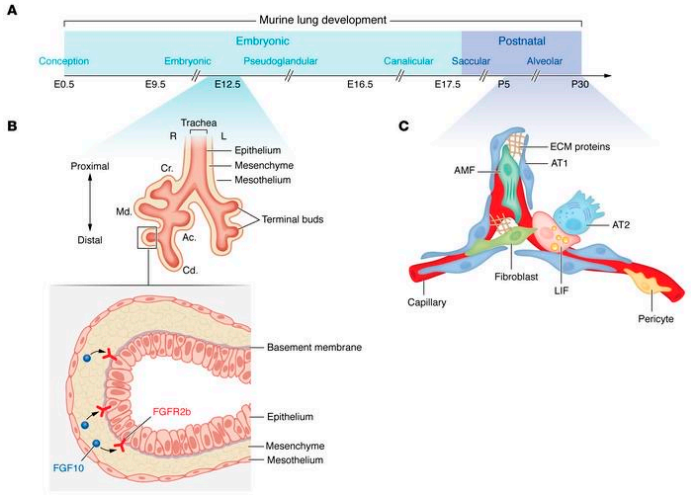
- Tumor stromal cell: an immune (i.e., T cells, macrophages) or non-immune (i.e., nerves, blood vessels) cell type within a tumor that lacks the genetic mutations that partially define cancer cells

- Fibroblast: a mesenchymal, mesoderm-derived cell that makes up connective tissue, produces extracellular matrix components (i.e., collagen), and drives wound healing reactions

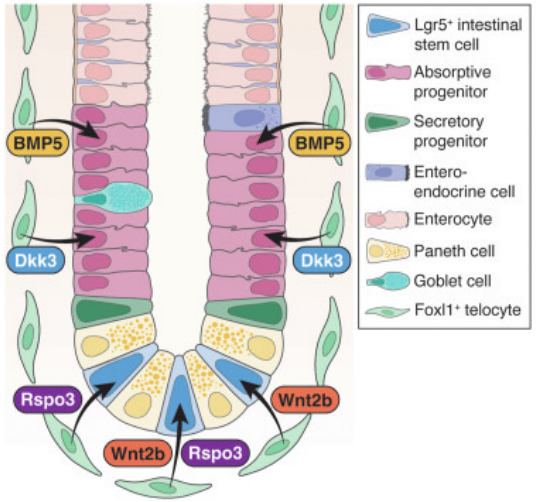
- Genetically engineered mouse model: means of altering the mouse genome that yield disease states (i.e., oncogene insertion), gene perturbations (i.e., knockout), cell perturbations (i.e., diphtheria toxin receptor expression), fluorescent tags, or other stable features that enable biological studies *in vivo*

- Orthotopic tumor model: implantable model wherein tumor cells are injected into their relevant tissue setting (i.e., breast cancer cells injected into the mammary fat pad)

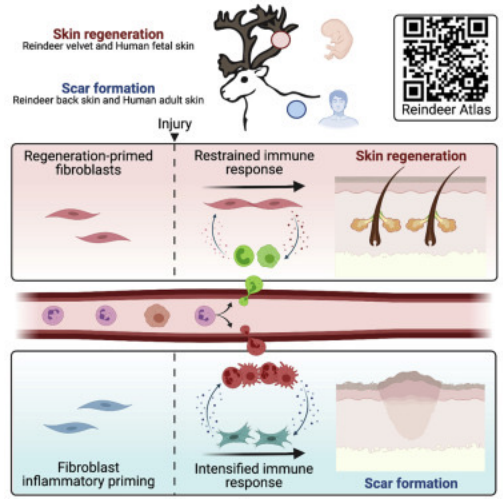
Mesenchymal elements instruct epithelial fates in development, health, and injury



Agha & Thannickal, *JCI*, 2023



Kolev & Kaestner, *CMGH*, 2023



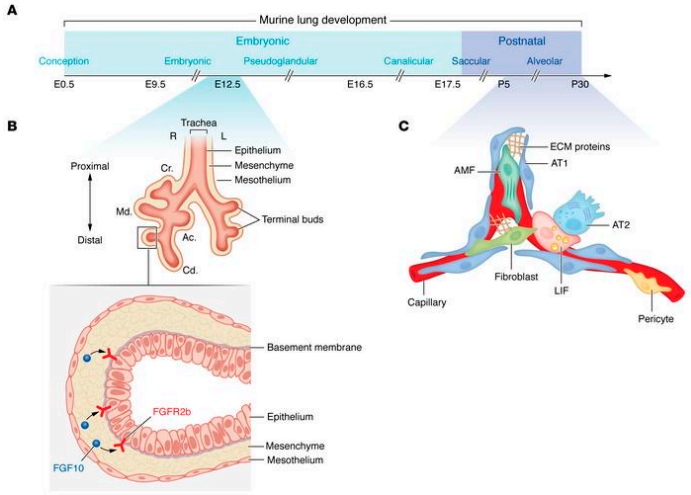
Sinha, Sparks et al., *Cell*, 2022

Fibroblasts defined by position and/or cell of origin provide instructive cues essential for tissue patterning (*i.e.*, intestinal crypts)

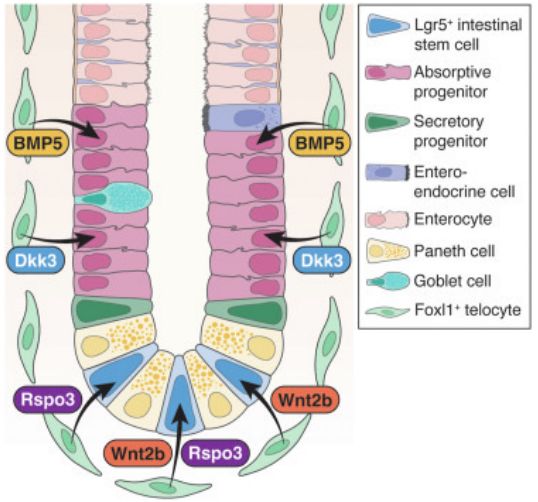
Intrinsic differences underlie functional differences in roles of fibroblasts during injury and wound repair (*i.e.*, differentially primed skin fibroblasts during wounding, different recruitment of + interactions with immune cells, different outcomes)

What about this epithelial-mesenchymal crosstalk and fibroblast heterogeneity in cancer?

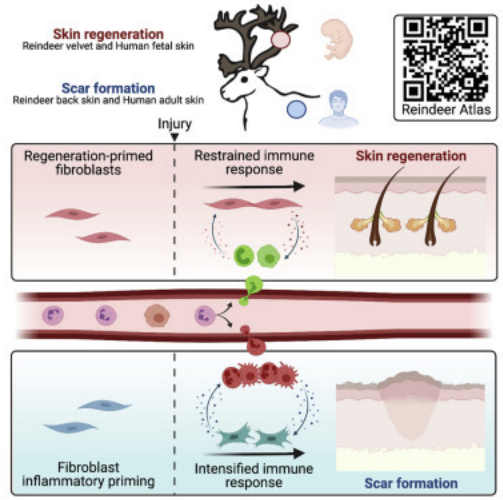
Mesenchymal elements instruct epithelial fates in development, health, and injury



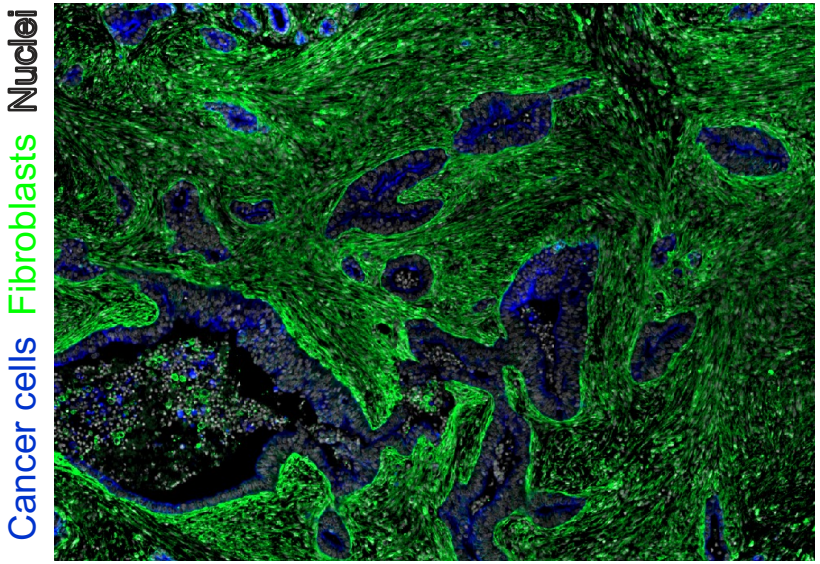
Agha & Thannickal, *JCI*, 2023



Kolev & Kaestner, *CMGH*, 2023



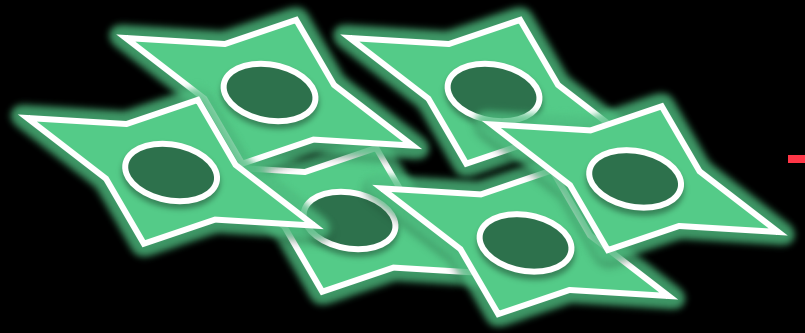
Sinha, Sparks et al., *Cell*, 2022



The pancreatic tumor ecosystem is dominated by mesenchymal elements

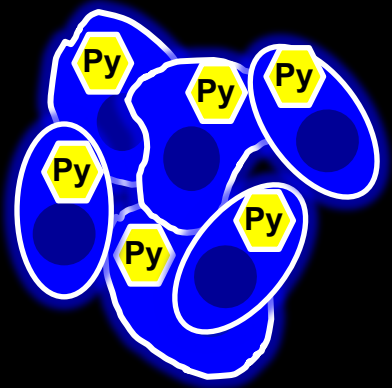
Microenvironmental cues can suppress or promote tumorigenesis

Normal, static fibroblasts

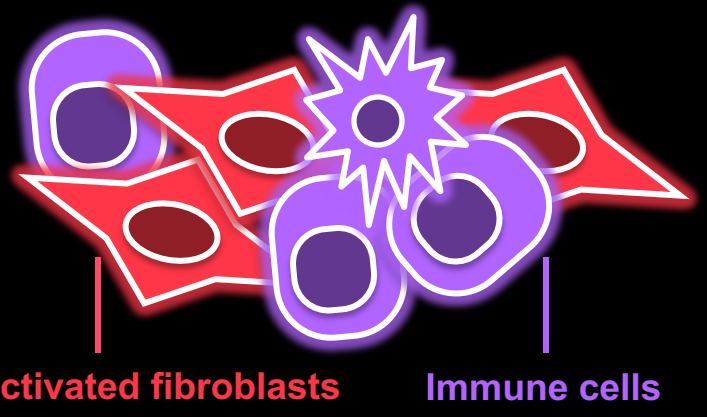


Stoker MG et al., *J. Cell Sci.*, 1966

Polyoma-transformed cell growth

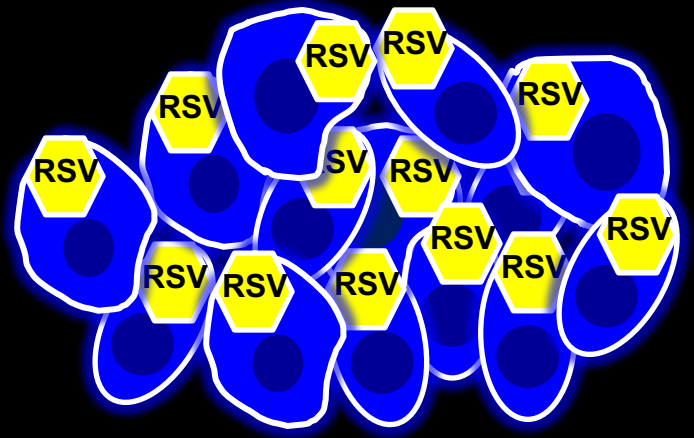


Wounding, inflammation



Dolberg DS et al., *Science*, 1985

RSV-mediated tumor formation

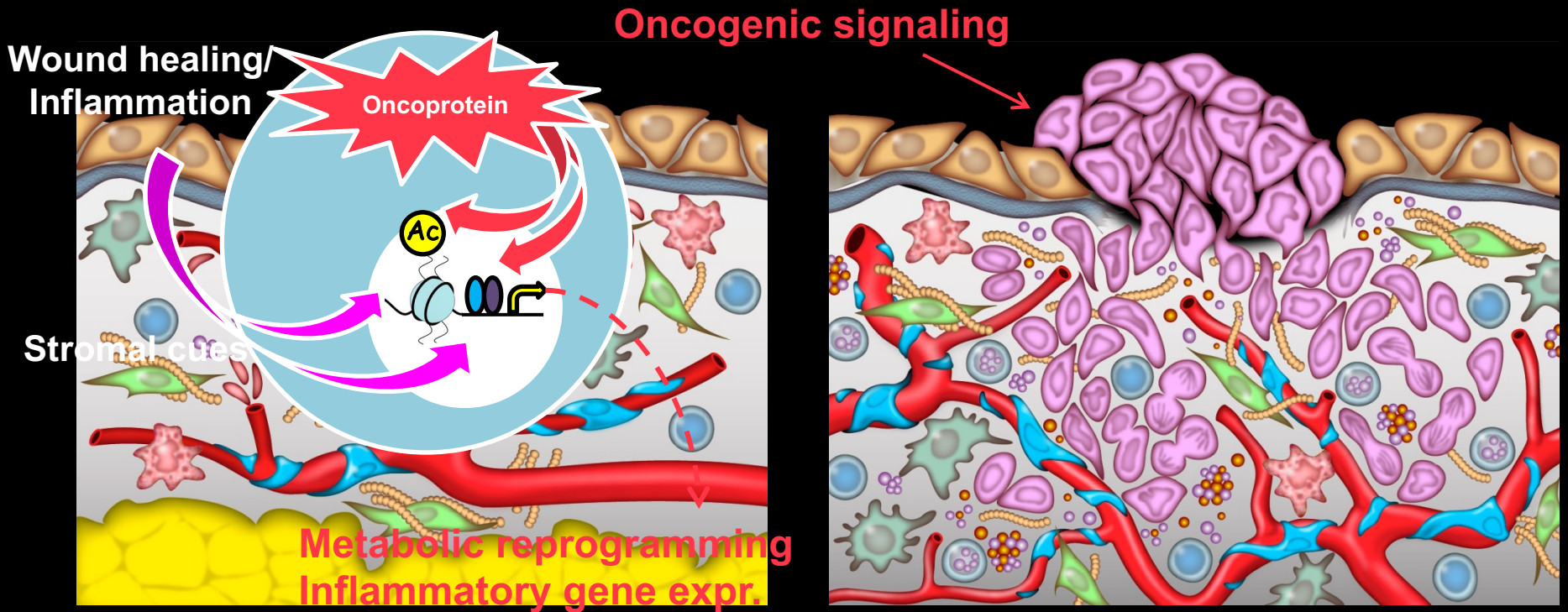


Growth-permissive microenvironmental changes accompany tumorigenesis

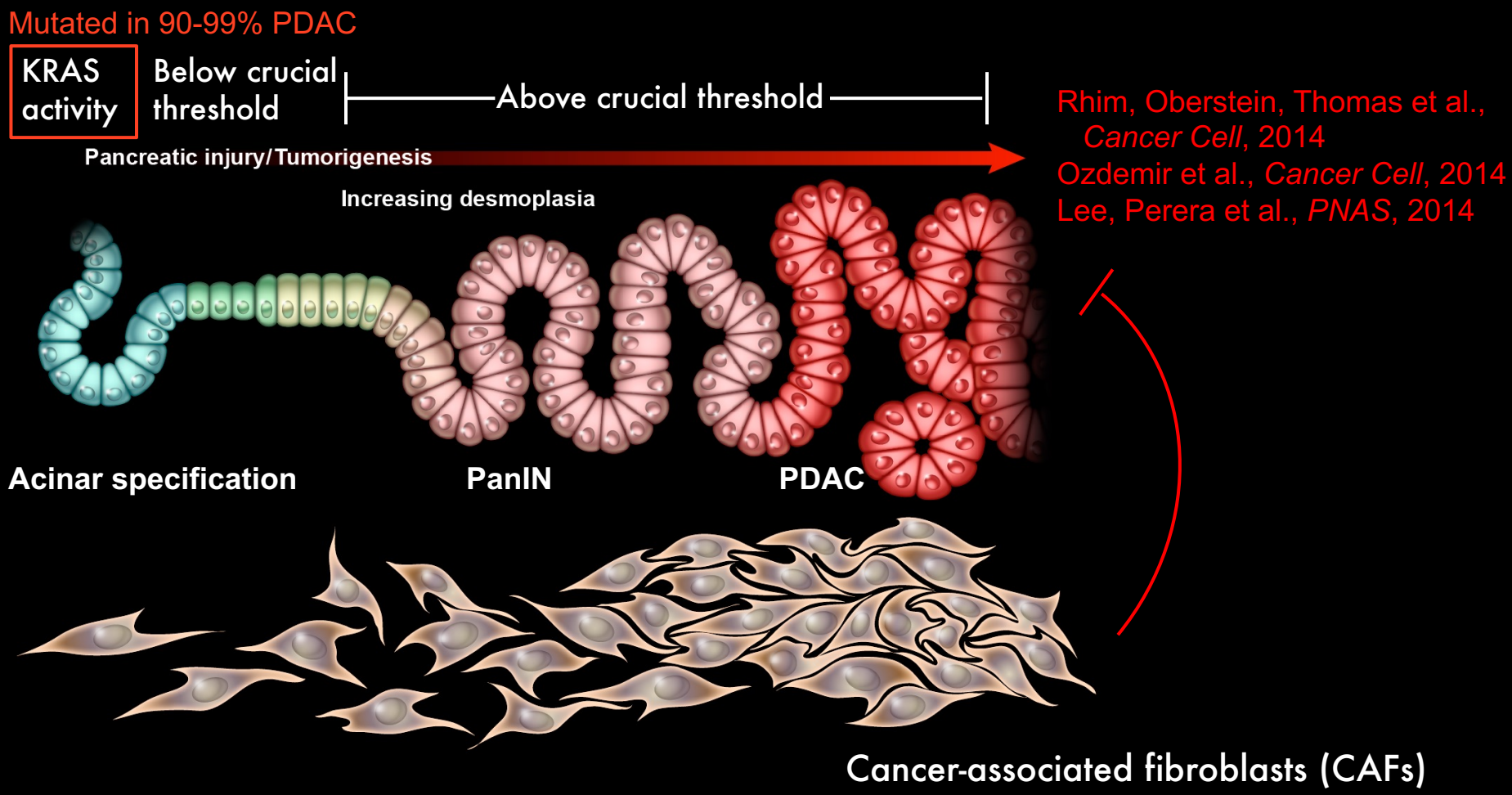
Wound-healing reaction

Tumor microenvironment

Metabolic reprogramming
Growth factors
Inflammation
Fibrosis



Pancreatic ductal adenocarcinoma (PDAC) features KRAS activation and a prominent desmoplastic stroma

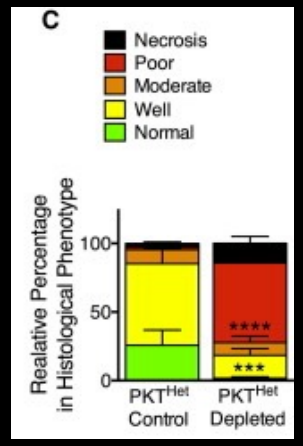


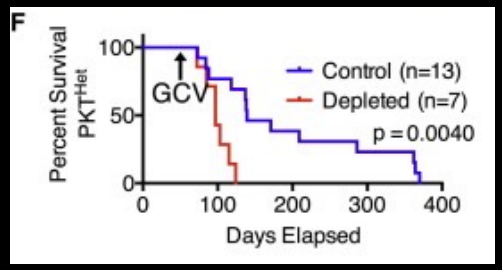
Rhim, Oberstein, Thomas et al., *Cancer Cell*, 2014
Ozdemir et al., *Cancer Cell*, 2014
Lee, Perera et al., *PNAS*, 2014

Adapted from Liot S et al., *Front Immunol*, 2021

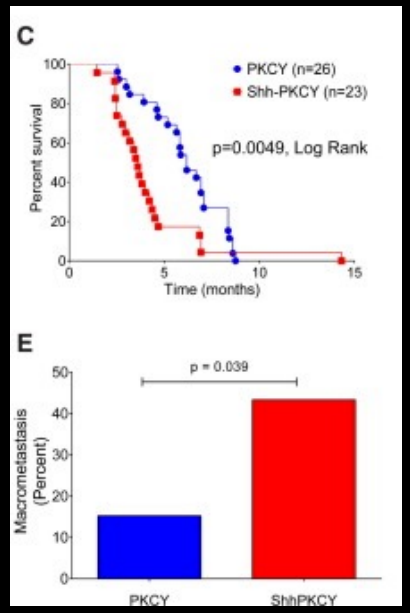
Stromal CAF ablation makes PDAC outcome worse

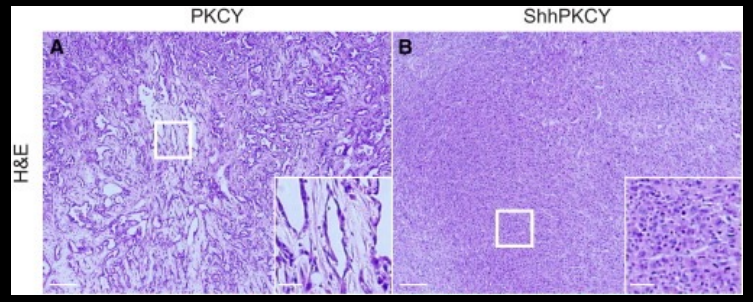
Kalluri lab: αSMA-tk +/- gangiclovir

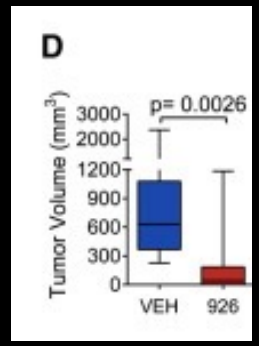




Stanger/Olive labs: Genetic and pharmacologic (early) Shh inhibition

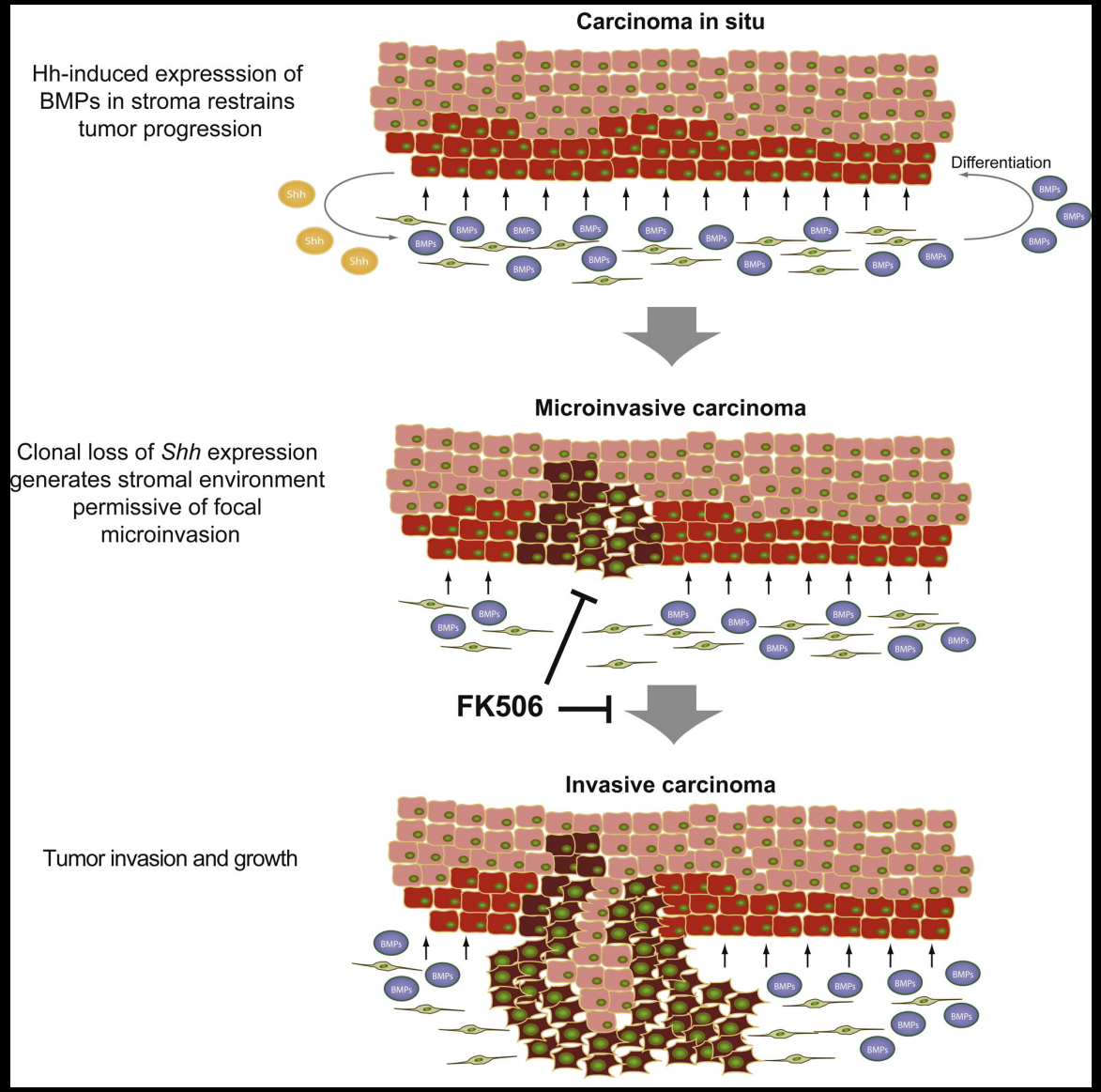






Higher mean vessel density, better perfusion with Shh inhibition (consistent with Lee, Perera et al., Olive et al., others)

CAFs also restrain bladder cancer progression



Shin et al., *Cancer Cell*, 2014

Pancreatic ductal adenocarcinoma (PDAC) features KRAS activation and a prominent desmoplastic stroma

Mutated in 90-99% PDAC

KRAS activity

Below crucial threshold

Above crucial threshold

Pancreatic injury/Tumorigenesis

Increasing desmoplasia

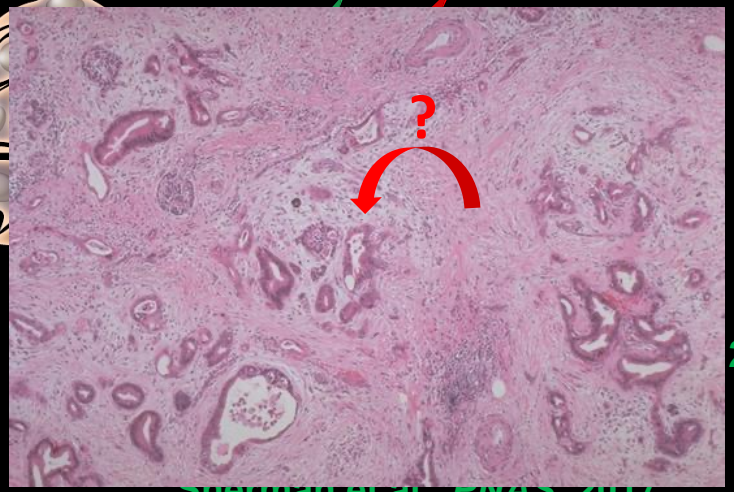
Acinar specification

PanIN

PDAC

X

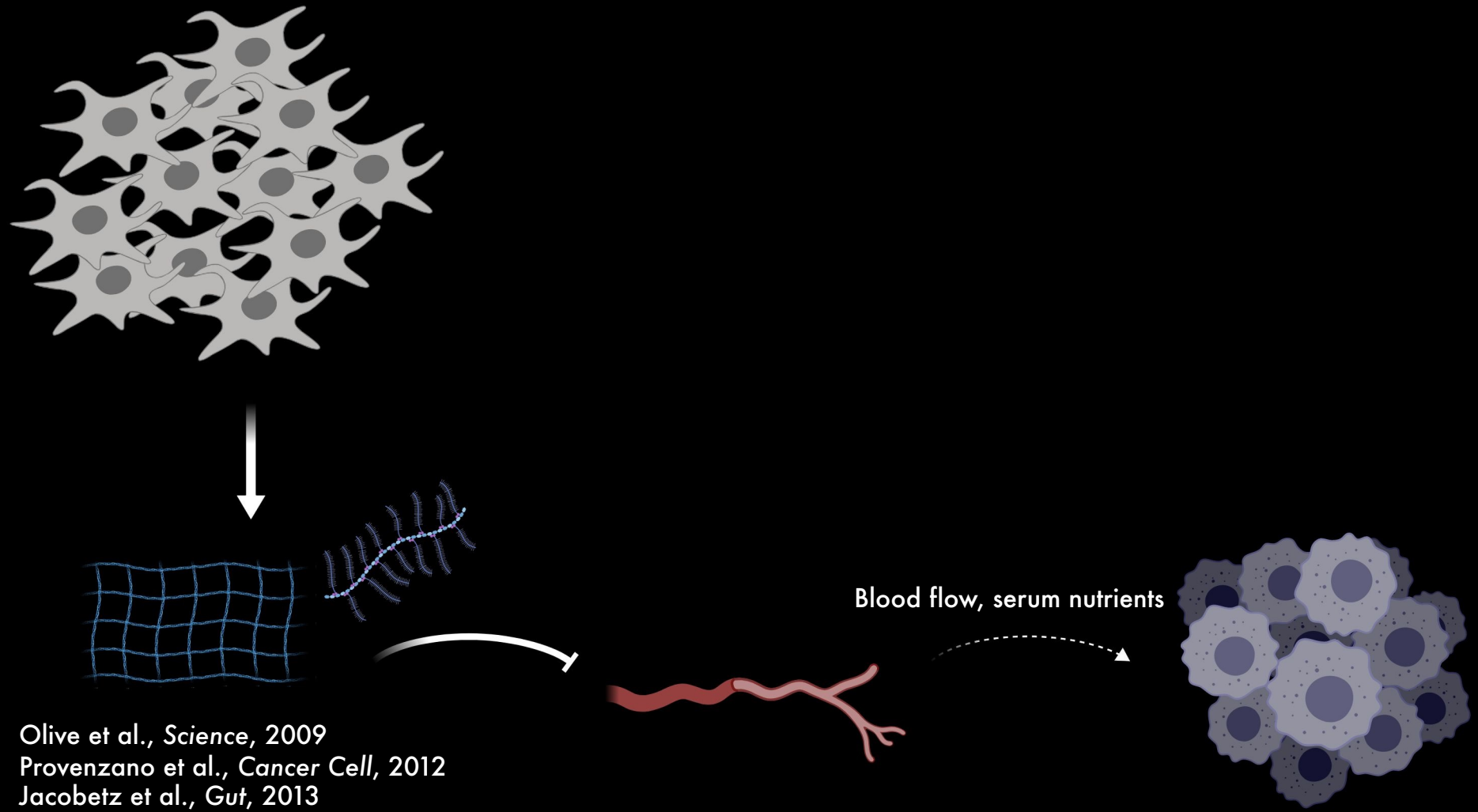
Rhim et al., *Cancer Cell*, 2014
Ozdemir et al., *Cancer Cell*, 2014
Lee et al., *PNAS*, 2014



2012

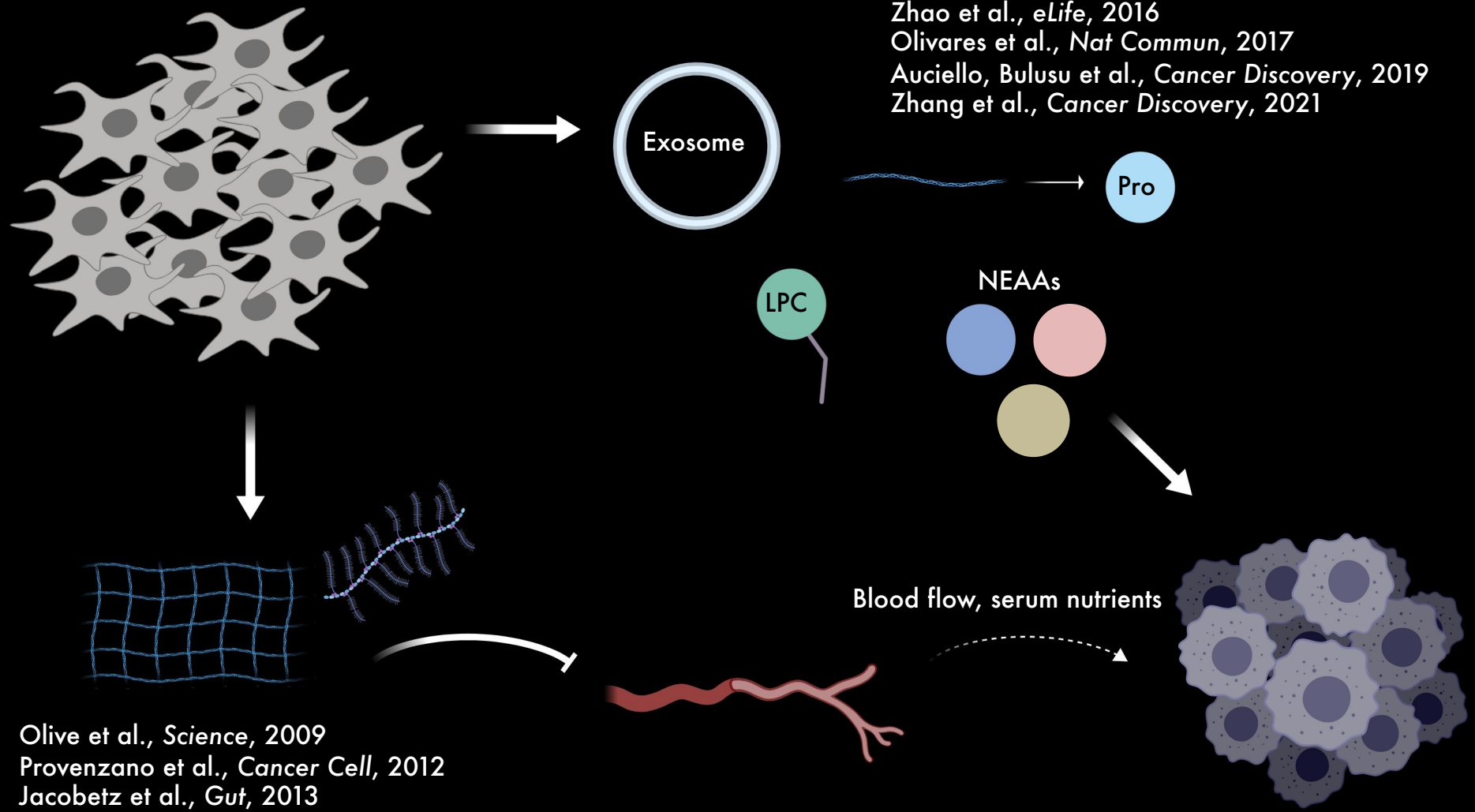
Adapted from Morris JP et al., *Nat Rev Cancer*, 2010

Model 1: Evolutionary CAF functions are both pro- *and* anti-tumorigenic



Olive et al., *Science*, 2009
Provenzano et al., *Cancer Cell*, 2012
Jacobetz et al., *Gut*, 2013
Lee, Perera et al., *PNAS*, 2014
Rhim, Oberstein, Thomas et al., *Cancer Cell*, 2014
Kamphorst, Nofal, Commisso et al., *Cancer Res*, 2015

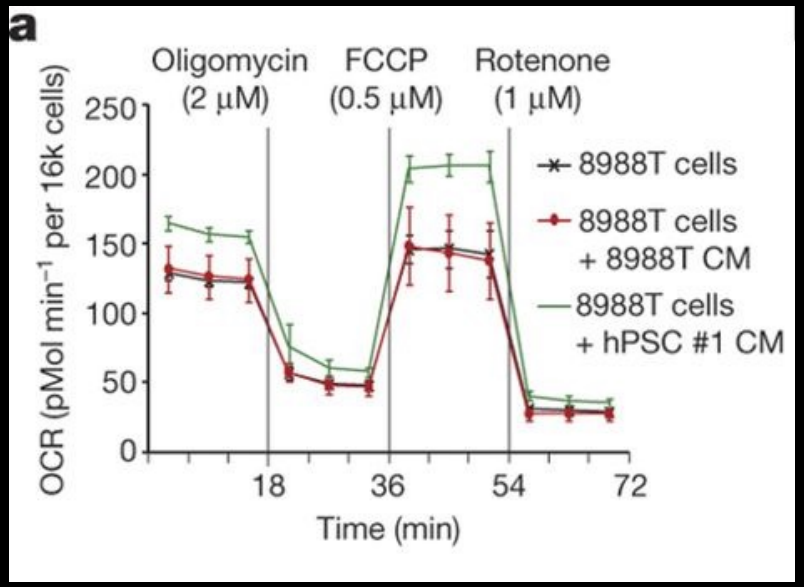
Model 1: Evolutionary CAF functions are both pro- *and* anti-tumorigenic

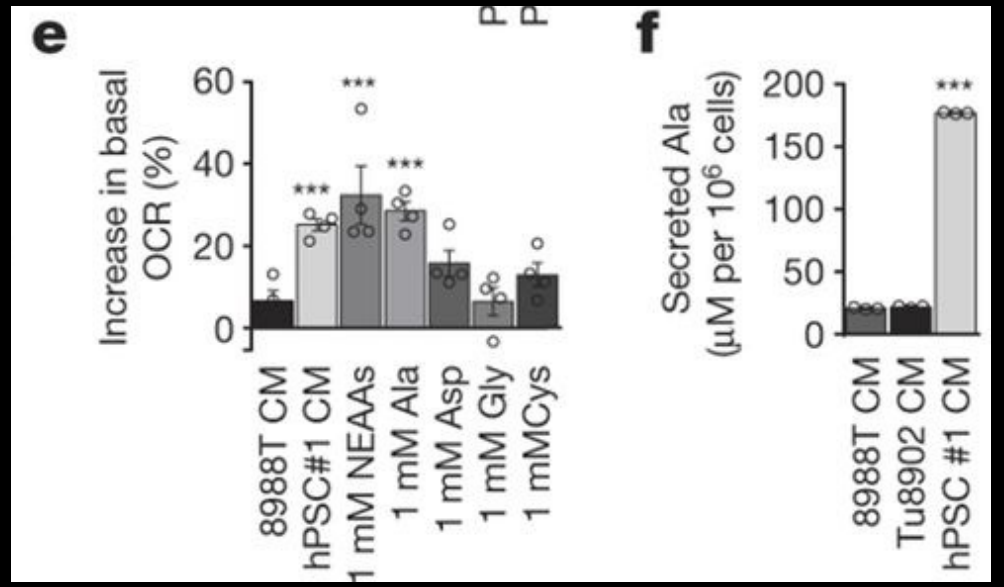


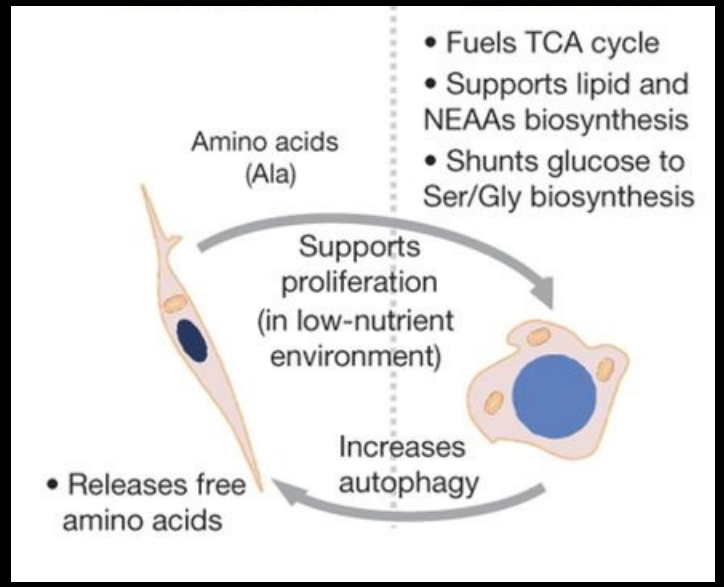
Sousa et al., *Nature*, 2016
Zhao et al., *eLife*, 2016
Olivares et al., *Nat Commun*, 2017
Auciello, Bulusu et al., *Cancer Discovery*, 2019
Zhang et al., *Cancer Discovery*, 2021

Olive et al., *Science*, 2009
Provenzano et al., *Cancer Cell*, 2012
Jacobetz et al., *Gut*, 2013
Lee, Perera et al., *PNAS*, 2014
Rhim, Oberstein, Thomas et al., *Cancer Cell*, 2014
Kamphorst, Nofal, Commisso et al., *Cancer Res*, 2015

CAFs as modulators of PDAC metabolism

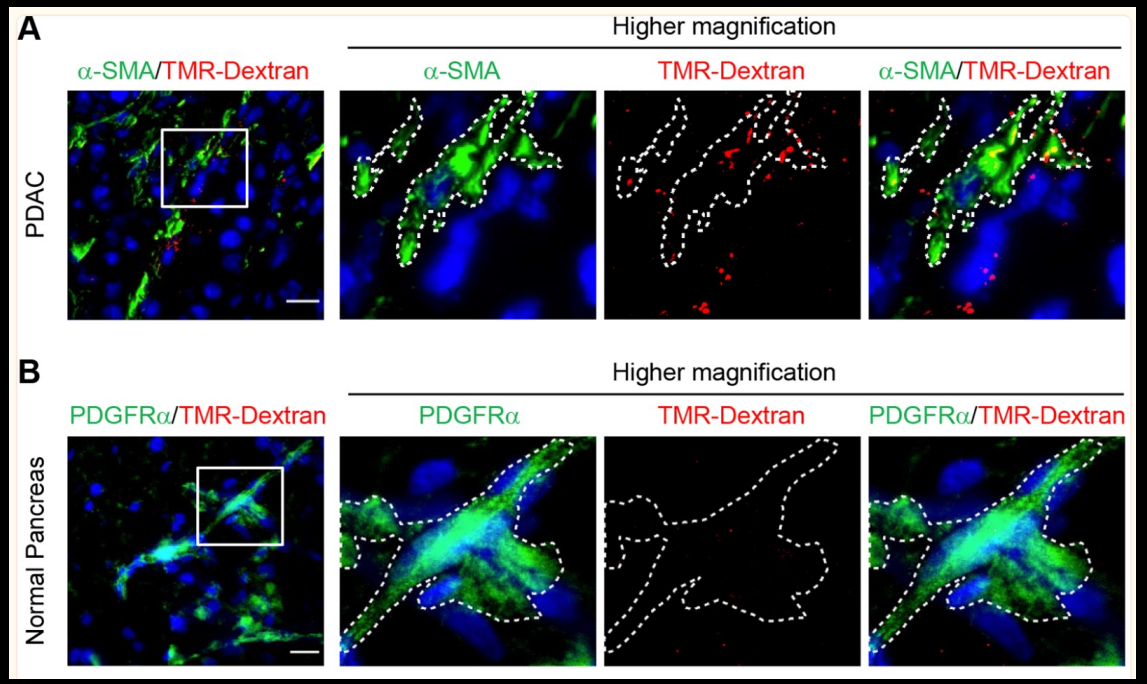




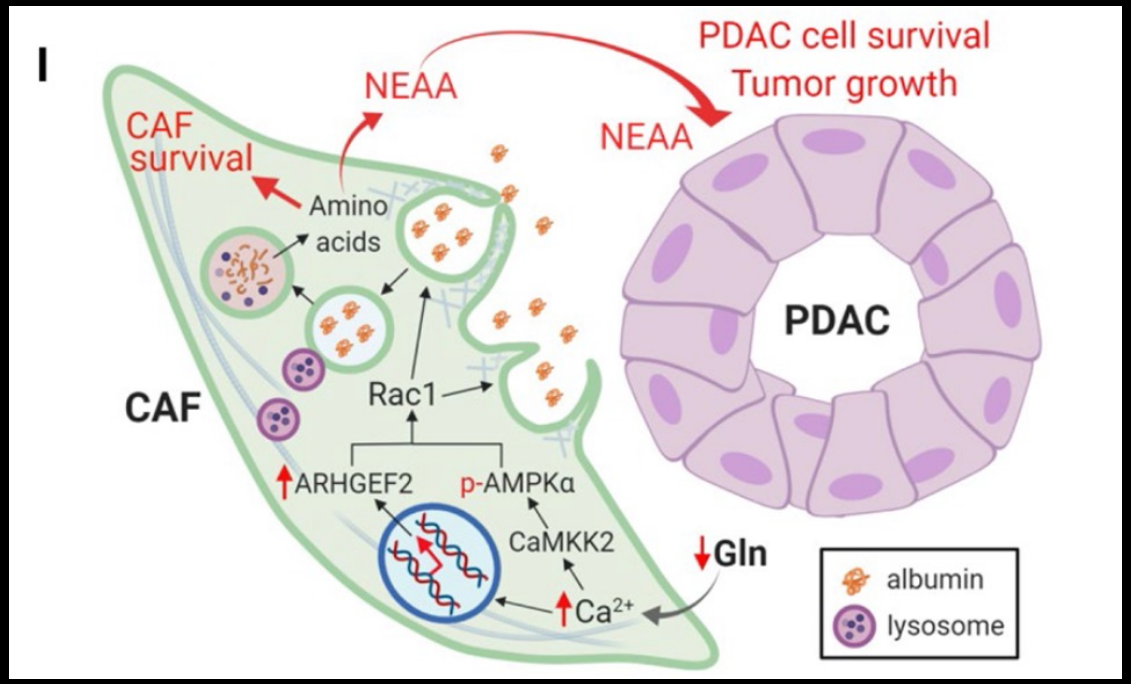


Sousa et al., *Nature*, 2016

CAFs as modulators of PDAC metabolism



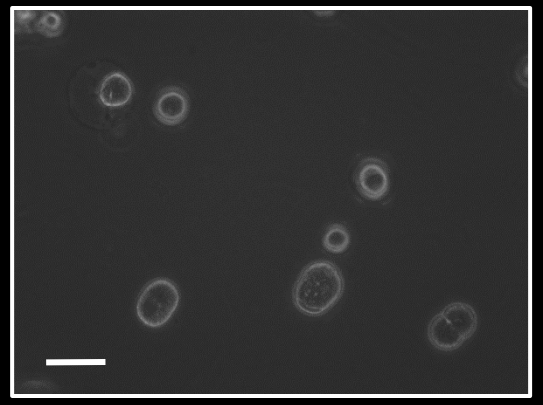
Zhang et al., *Cancer Discovery*, 2021



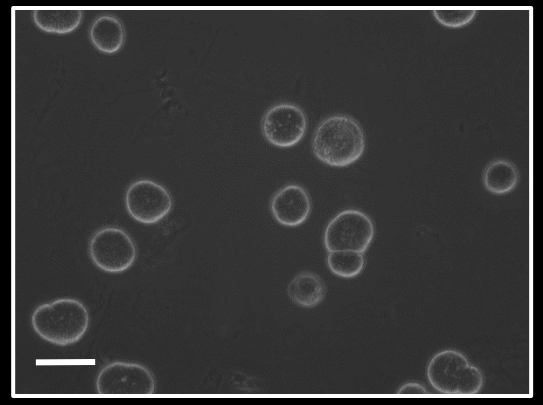
Stromal macropinocytosis

Astromal vs. stromal cultures enable genomic studies of tumor-stroma crosstalk

Astromal conditions
Growth substrate: PEG hydrogel
Medium: DMEM



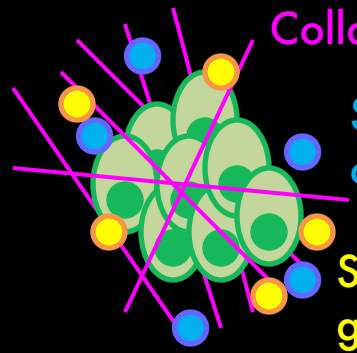
Stromal conditions
Growth substrate: PEG hydrogel + Collagens
Medium: Stroma-conditioned medium



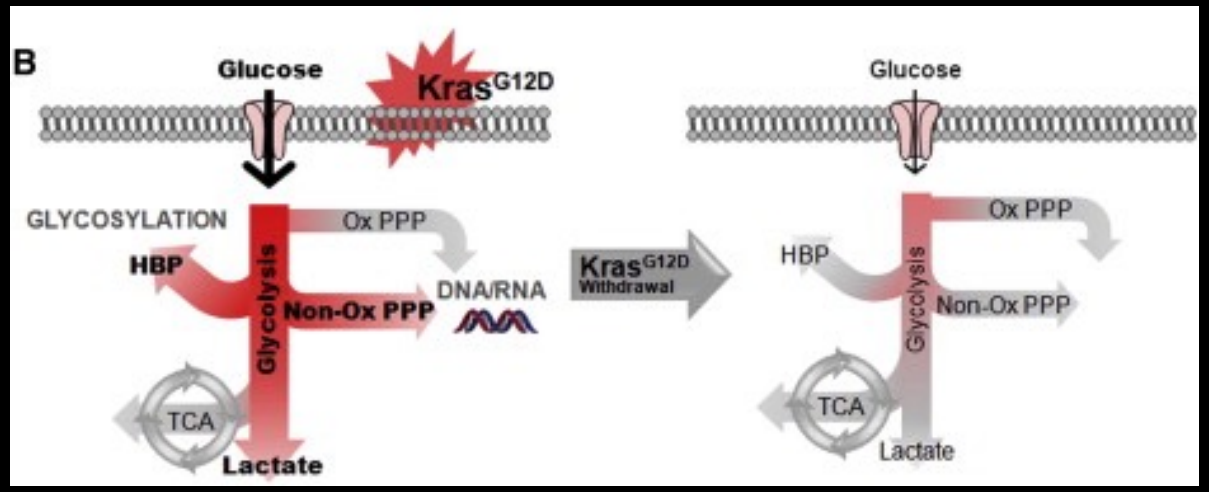
Functional genomics, metabolomics
(3 different Kras mutant cell lines used)

Astromal conditions

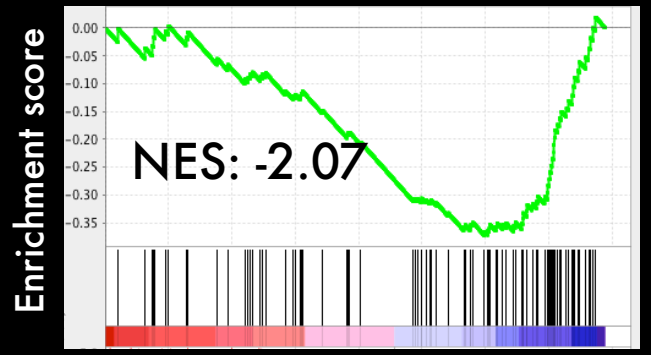
Stromal conditions



Stromal inputs are functionally complementary to oncogenic Kras



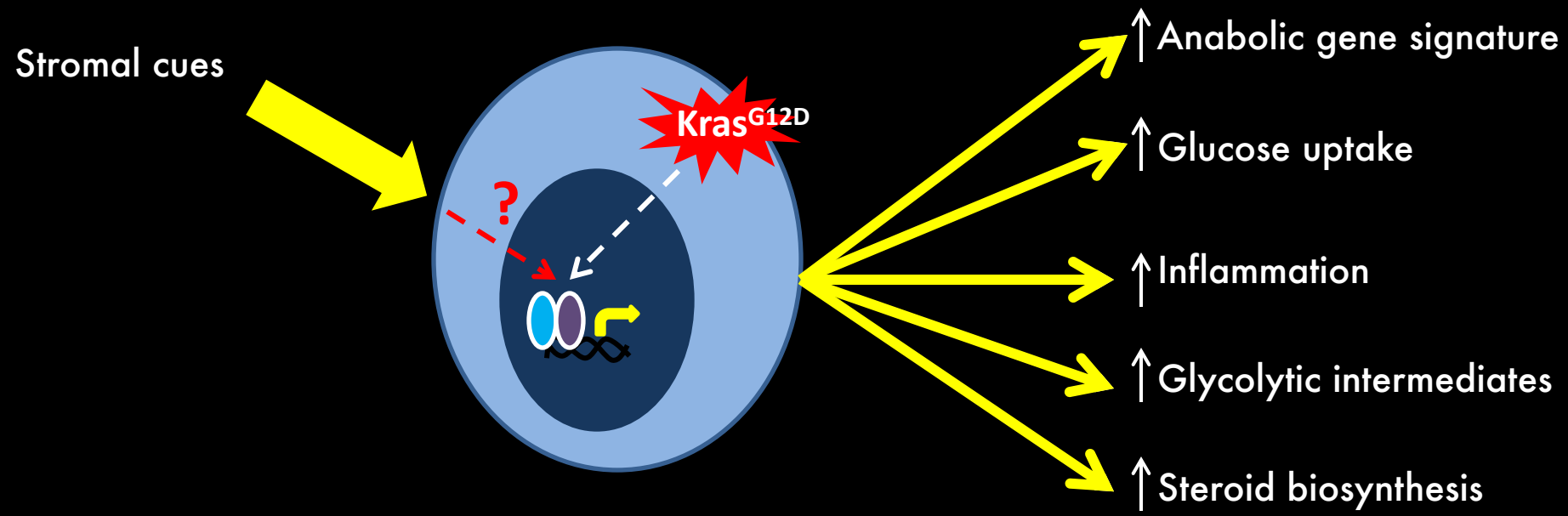
Ying, Kimmelman, Lyssiotis et al., *Cell*, 2012



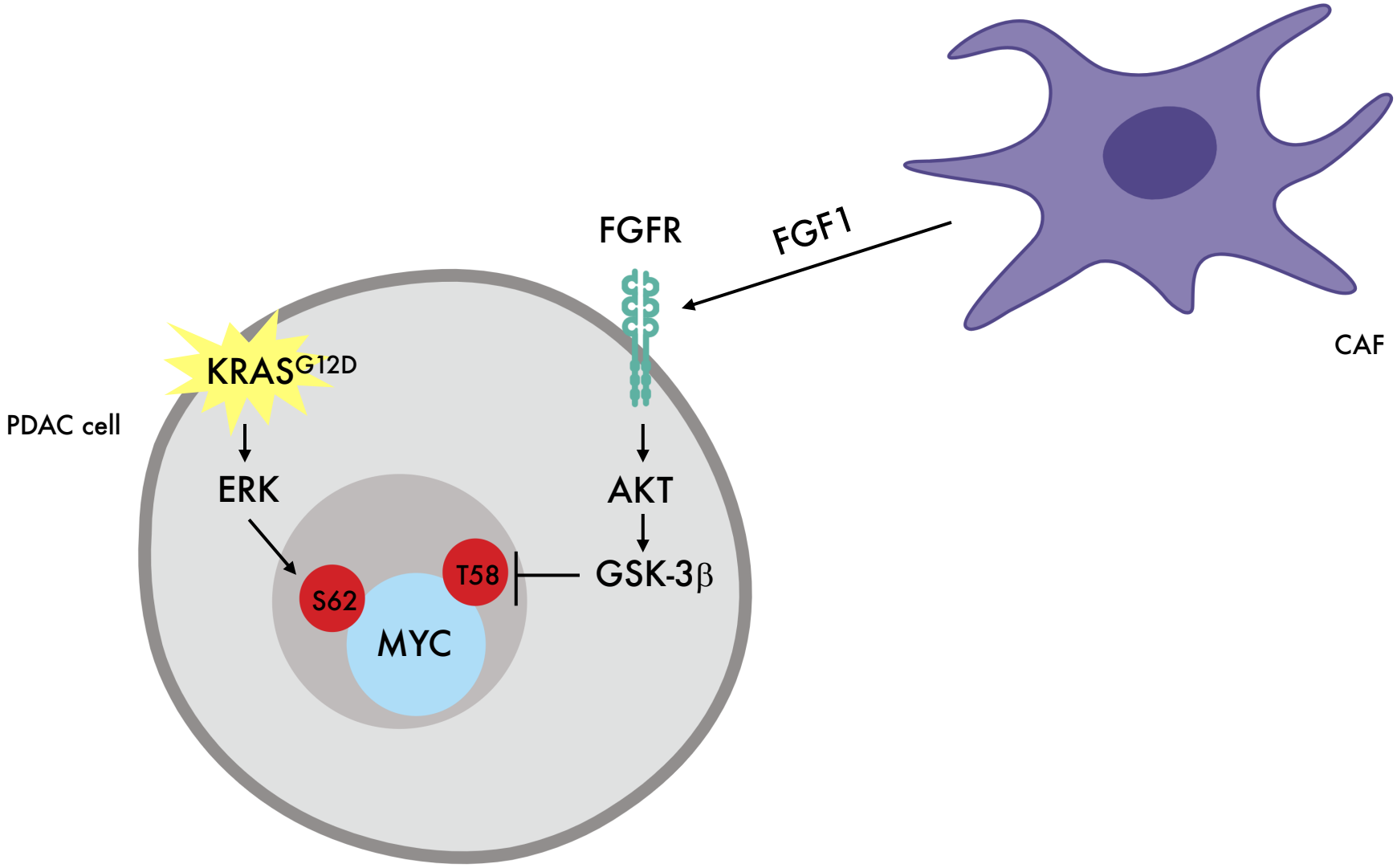
Sherman et al., *PNAS*, 2017

Enrichment plot:
Genes downregulated upon KrasG12D extinction are enriched among stroma-inducible genes.
(↑KrasG12D enriched in ↑Stromal)

Stromal inputs are functionally complementary to oncogenic Kras

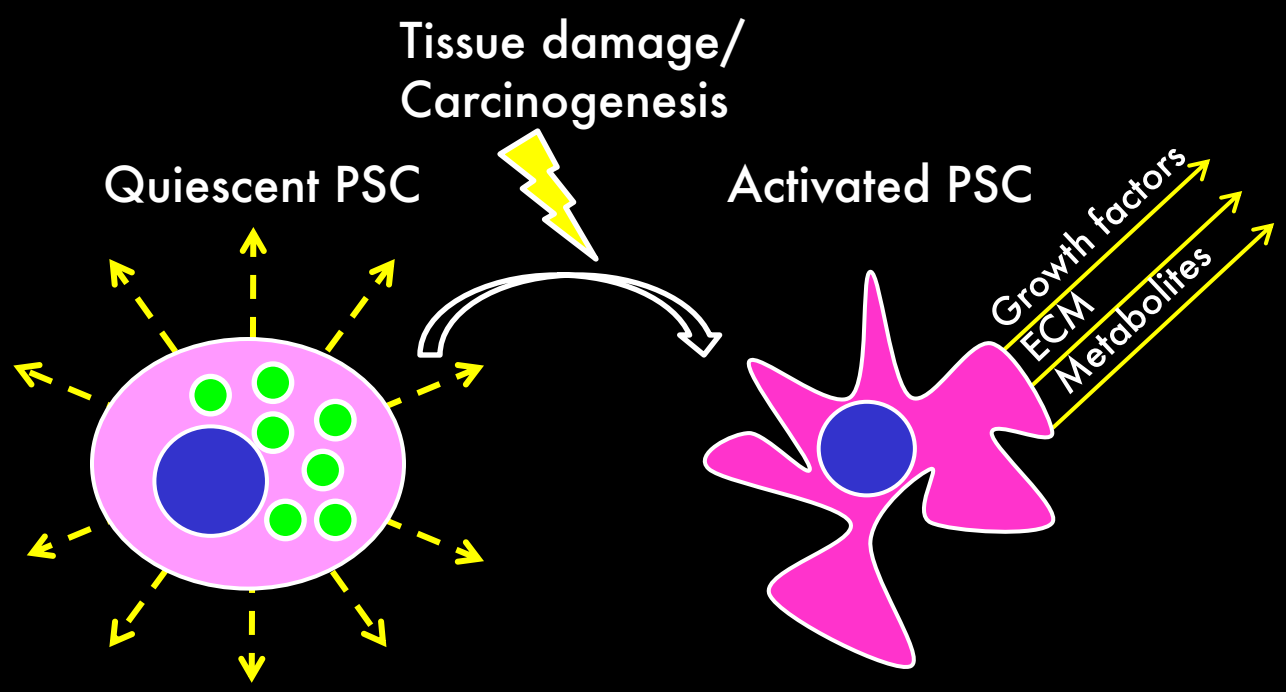


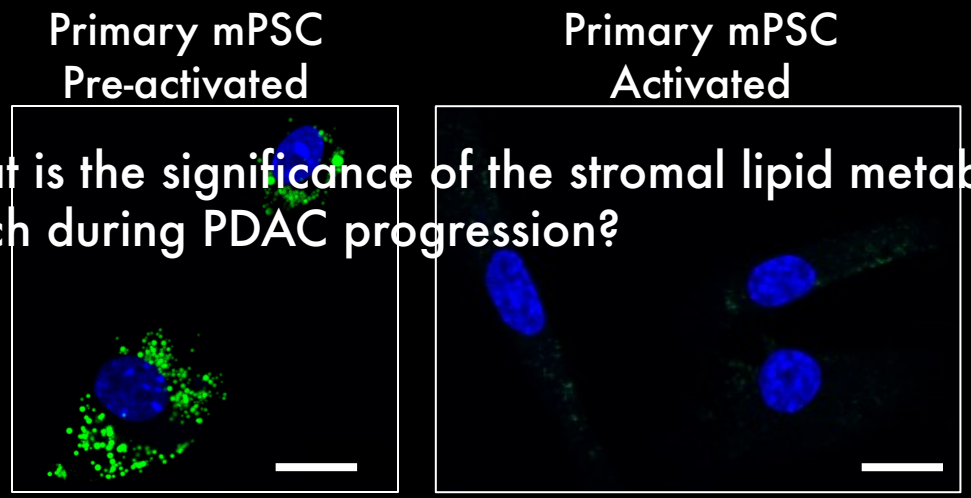
Cooperative regulation of MYC by cell-autonomous and microenvironmental mechanisms



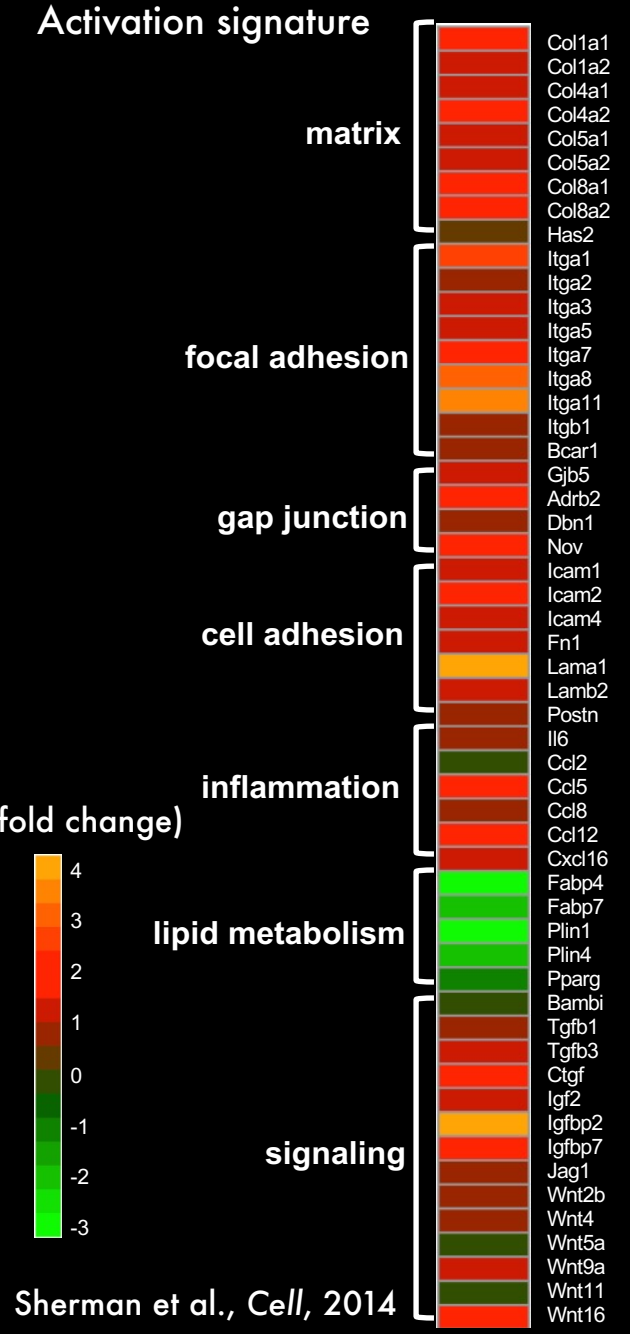
Bhattacharyya et al., *J Exp Med*, 2020

Pancreatic stellate cells (PSCs) give rise to CAFs in the PDAC microenvironment



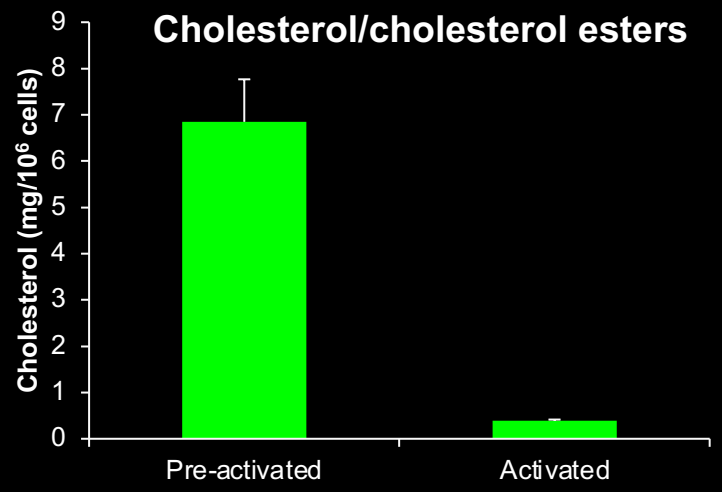


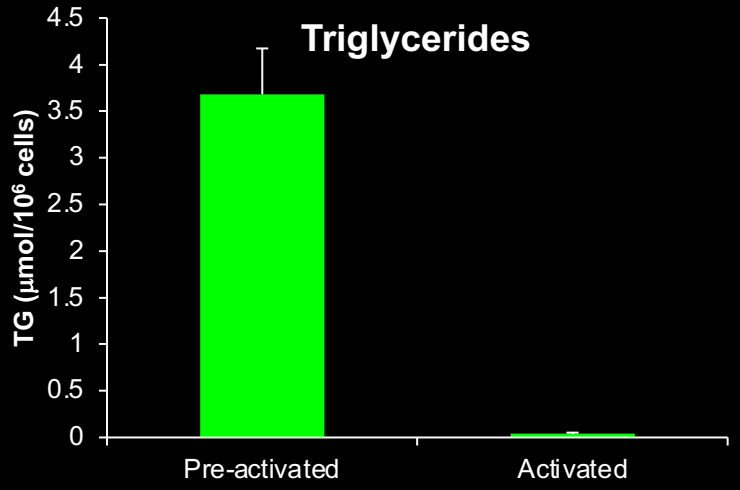
What is the significance of the stromal lipid metabolic switch during PDAC progression?

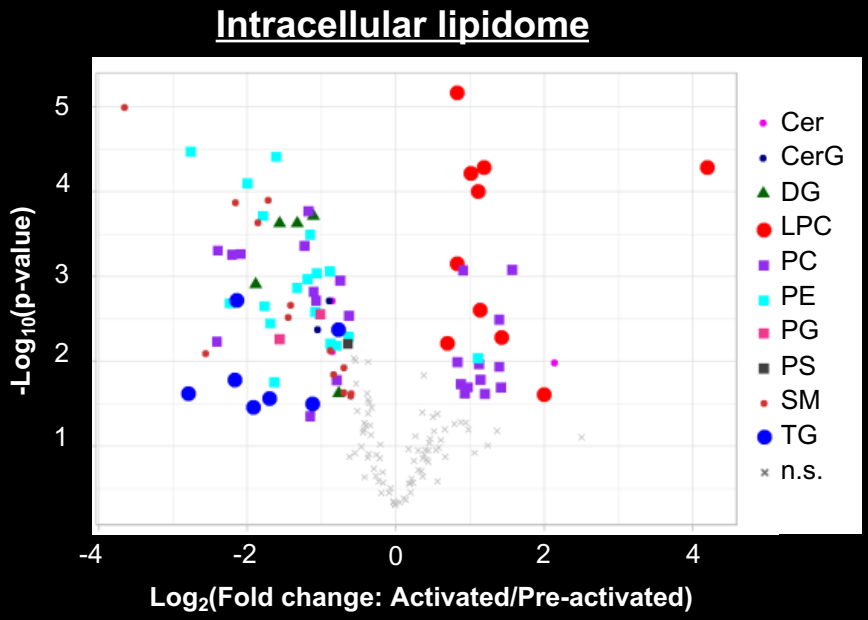


Sherman et al., *Cell*, 2014

Intracellular lipids in the PC-LPC axis change dramatically during PSC activation







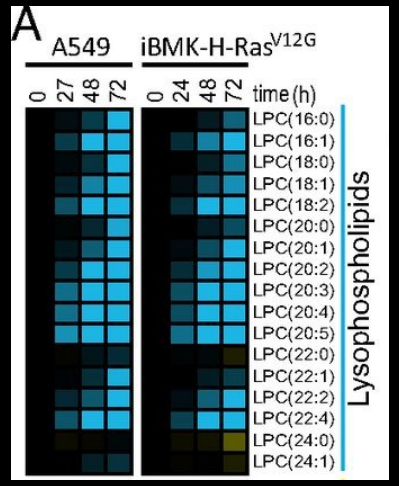
Intracellular lipids in the PC-LPC axis change dramatically during PSC activation

PNAS

Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids

Jurre J. Kamphorst[a,b,1], Justin R. Cross[c,1], Jing Fan[a,b], Elisa de Stanchina[d], Robin Mathew[e,f], Eileen P. White[e,f,g], Craig B. Thompson[c,2], and Joshua D. Rabinowitz[a,b,e,2]

[a]Lewis-Sigler Institute for Integrative Genomics and [b]Department of Chemistry, Princeton University, Princeton, NJ 08544; [c]Cancer Biology and Genetics Program and [d]Antitumor Assessment Core Facility, Memorial Sloan Kettering Cancer Center, New York, NY 10065; [e]The Cancer Institute of New Jersey, New Brunswick, NJ 08903; [f]Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854; and [g]Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ 08854



Ras-transformed cells "eat" lysophospholipids (preferred substrate) and scavenge FAs from them to support proliferative expansion.

Are activated PSCs programmed to release/secrete lysophospholipids?

Intracellular lipids in the PC-LPC axis change dramatically during PSC activation

PNAS

Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids

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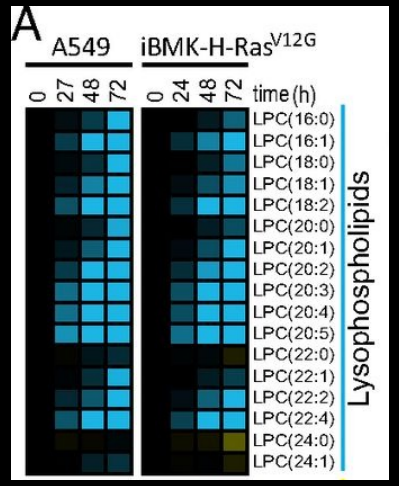
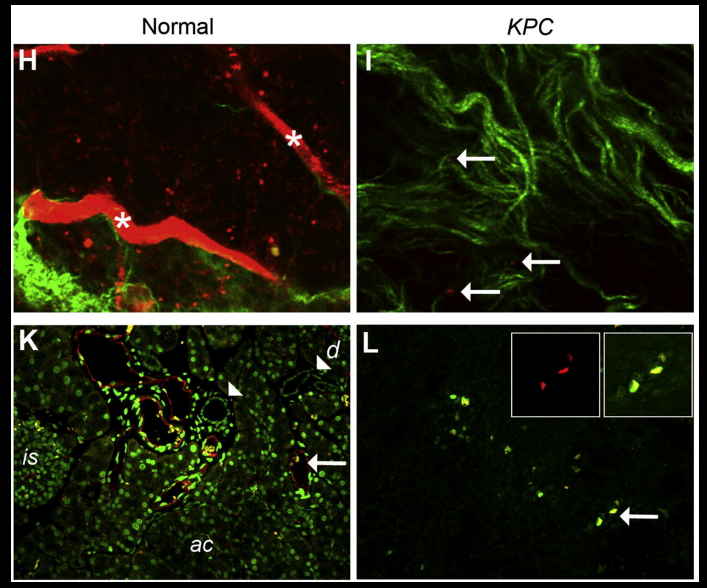


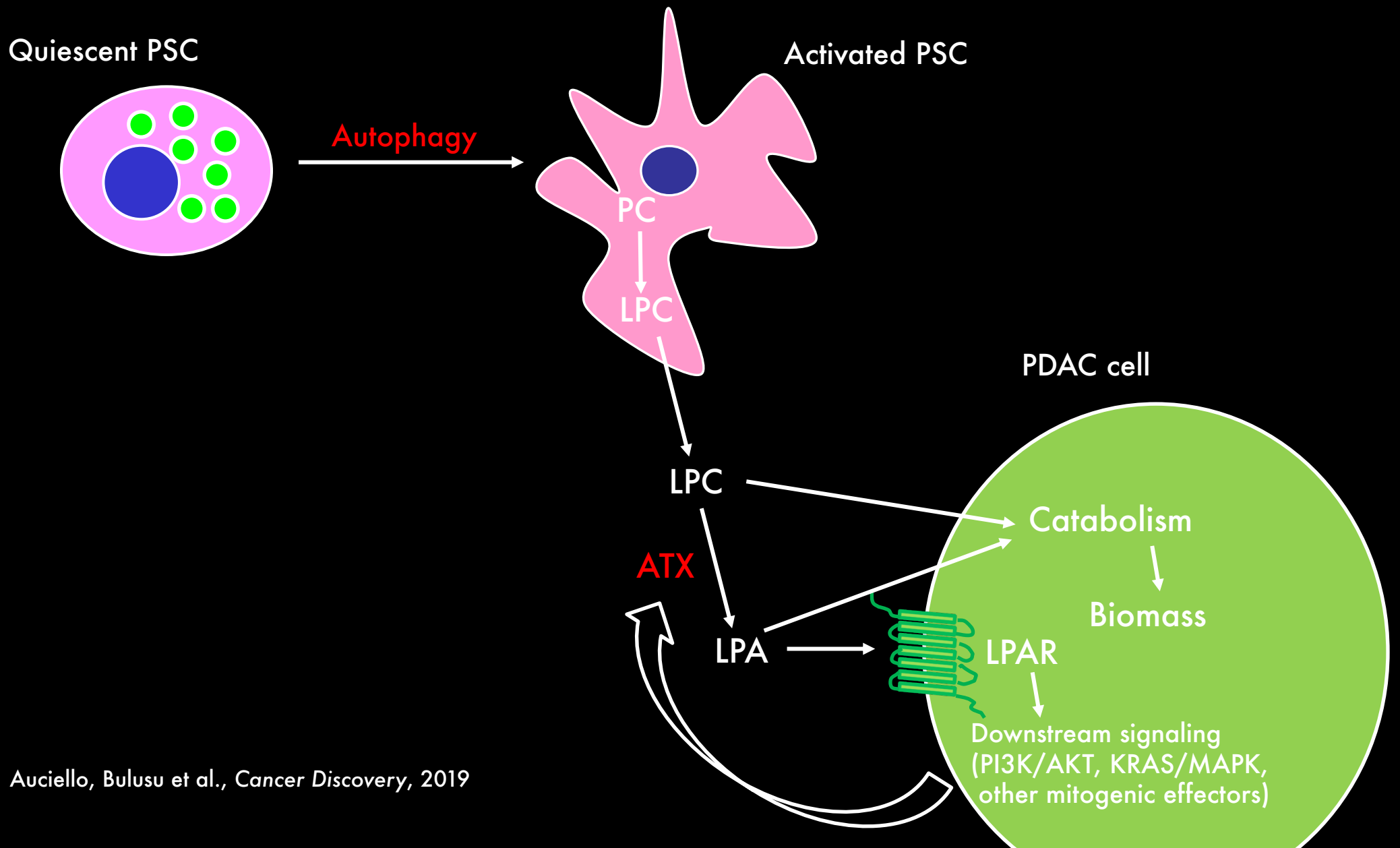


Lectin
Collagen (SHG)

Lectin
Doxorubicin

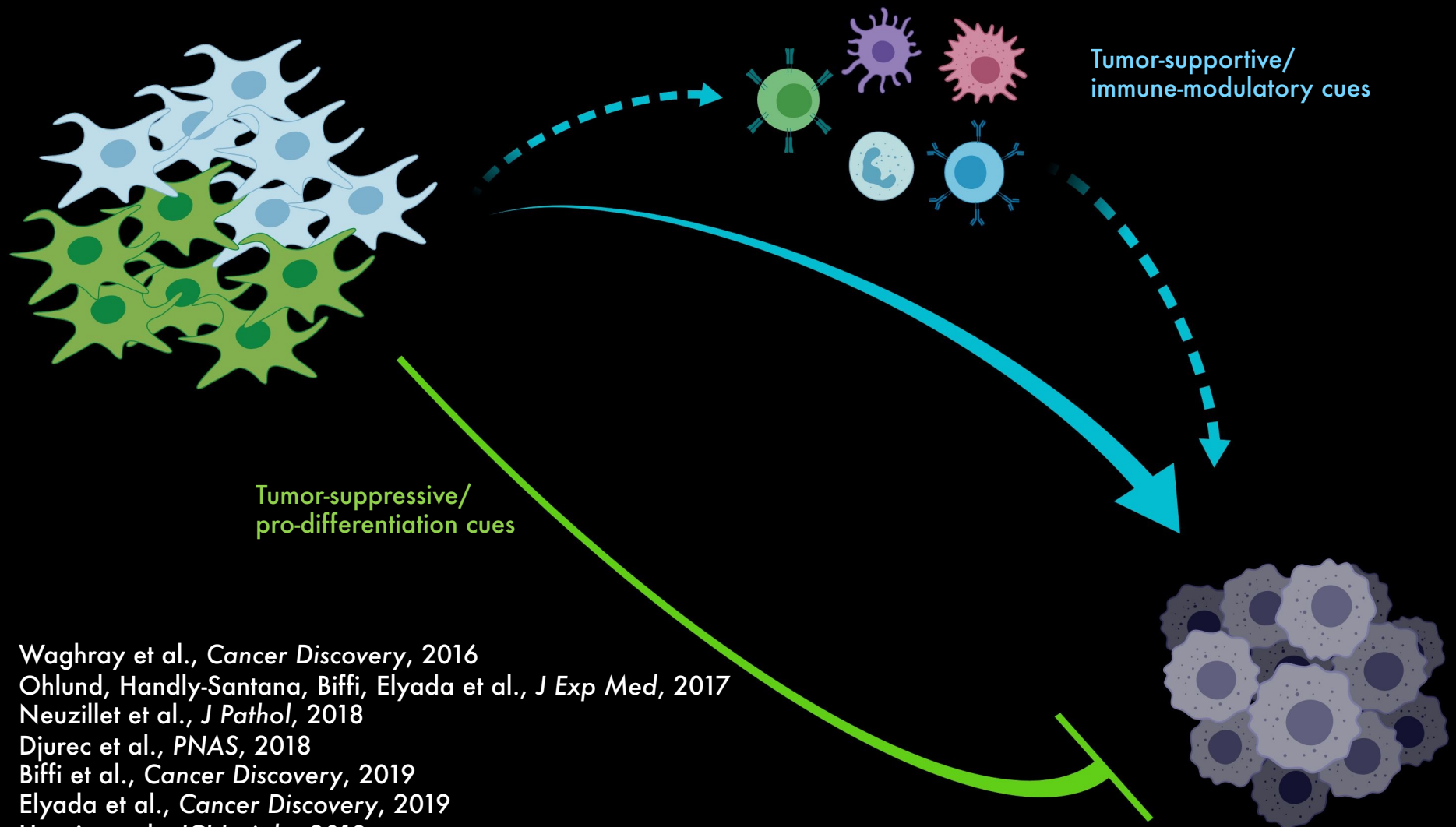
Provenzano et al., *Cancer Cell*, 2012

Working Model



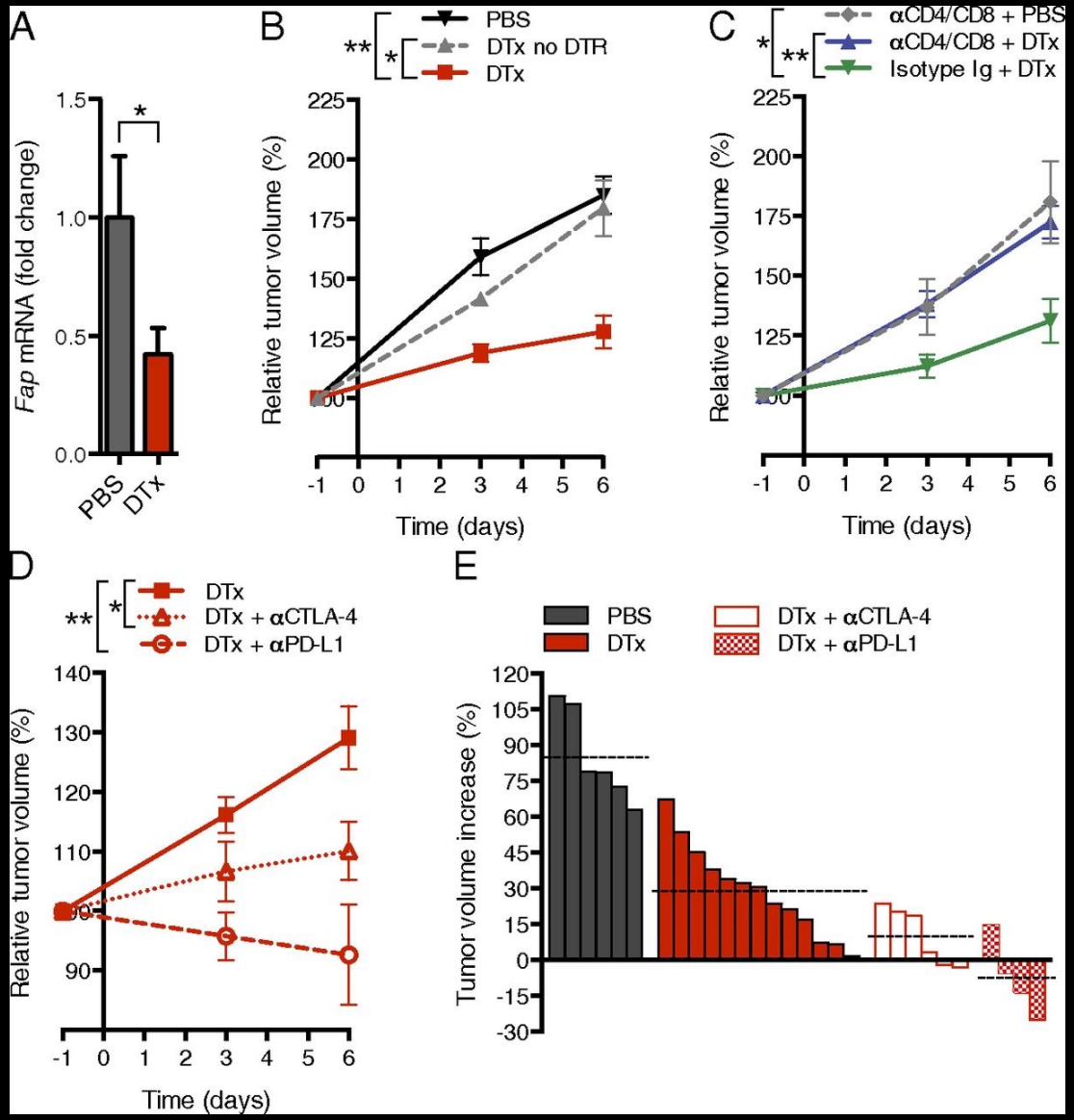
Auciello, Bulusu et al., *Cancer Discovery*, 2019

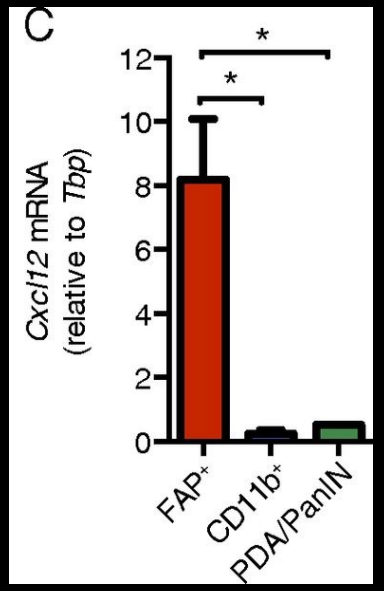
Model 2: CAFs are heterogeneous, including distinct pro- and anti-tumorigenic subsets

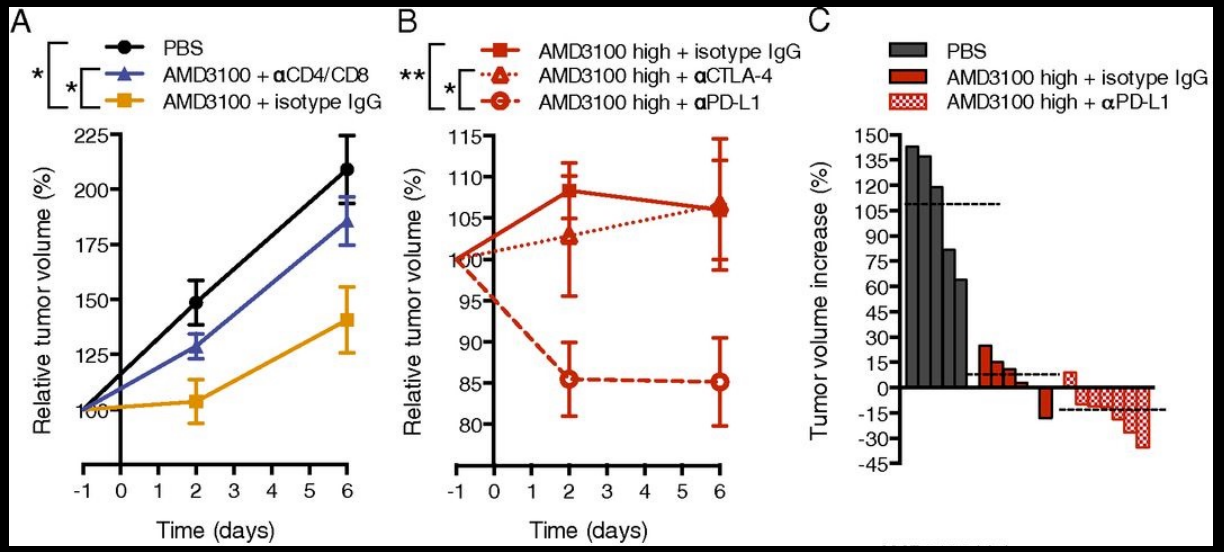


Waghray et al., *Cancer Discovery*, 2016
Ohlund, Handly-Santana, Biffi, Elyada et al., *J Exp Med*, 2017
Neuzillet et al., *J Pathol*, 2018
Djurec et al., *PNAS*, 2018
Biffi et al., *Cancer Discovery*, 2019
Elyada et al., *Cancer Discovery*, 2019
Hosein et al., *JCI Insight*, 2019
Dominguez et al., *Cancer Discovery*, 2020
Hutton et al., *Cancer Cell*, 2021

Immune modulation by PDAC CAFs

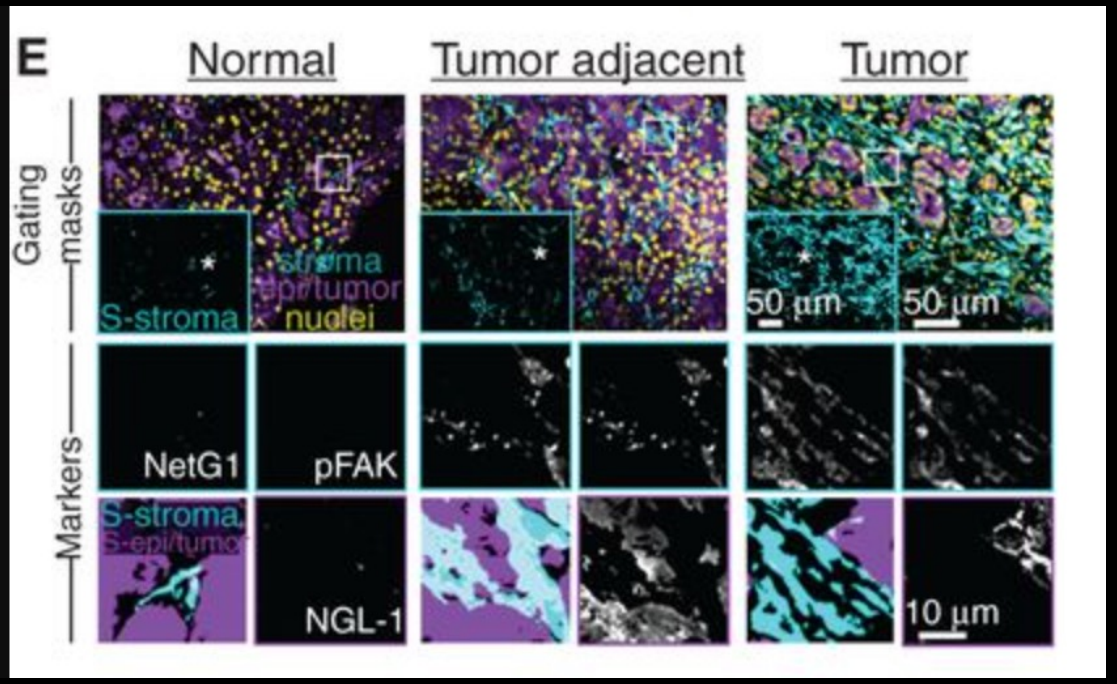




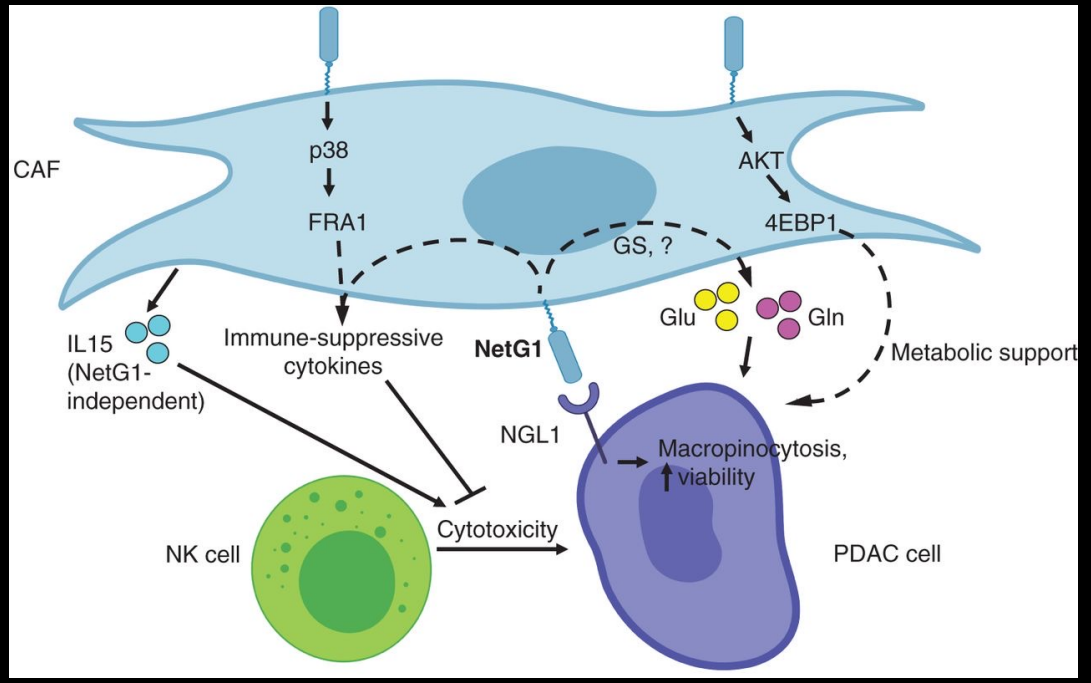


Feig et al., *PNAS*, 2013

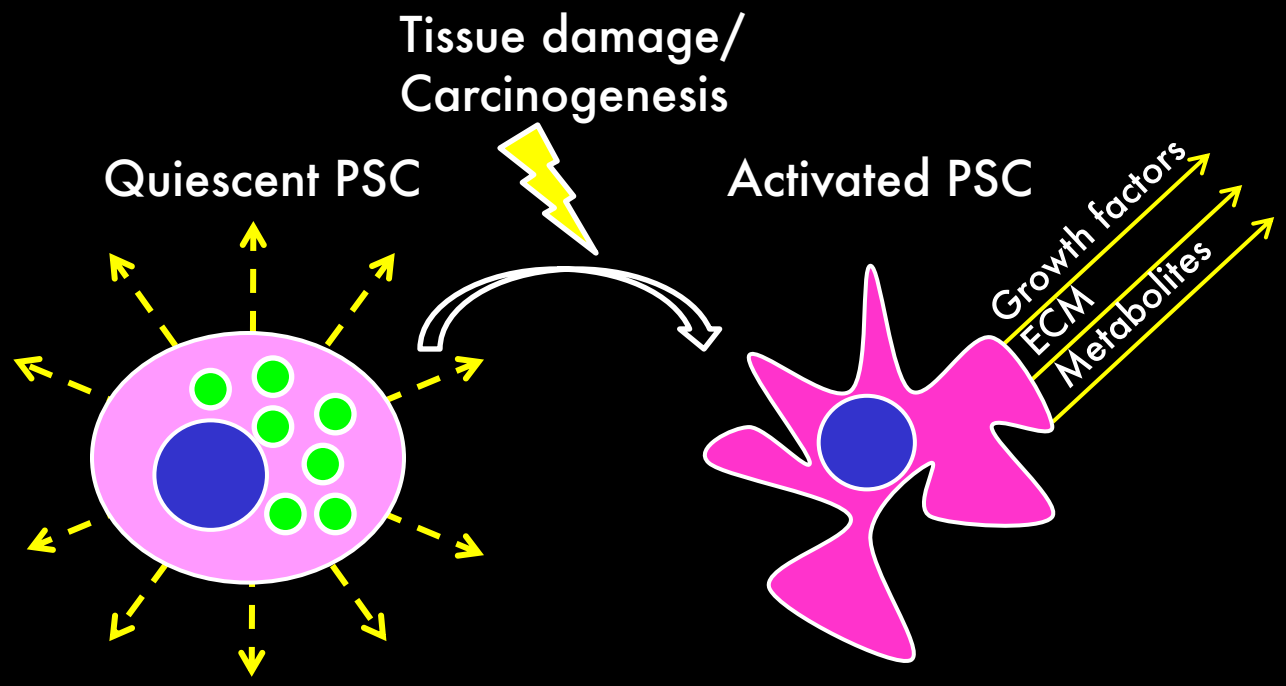
Immune (and metabolic) modulation by PDAC CAFs



Francescone, Vendramini-Costa et al., *Cancer Discovery*, 2021



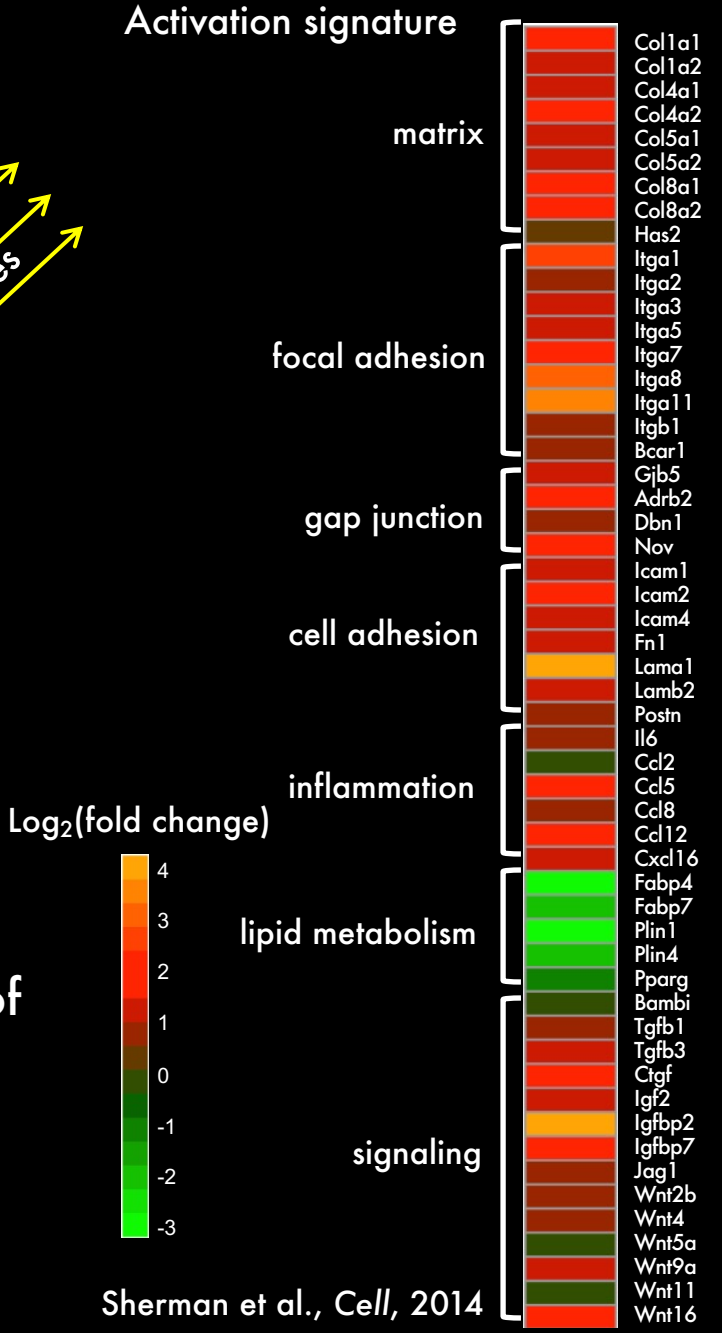
Pancreatic stellate cells (PSCs) give rise to a PDAC CAF phenotype



Primary mPSC
Pre-activated

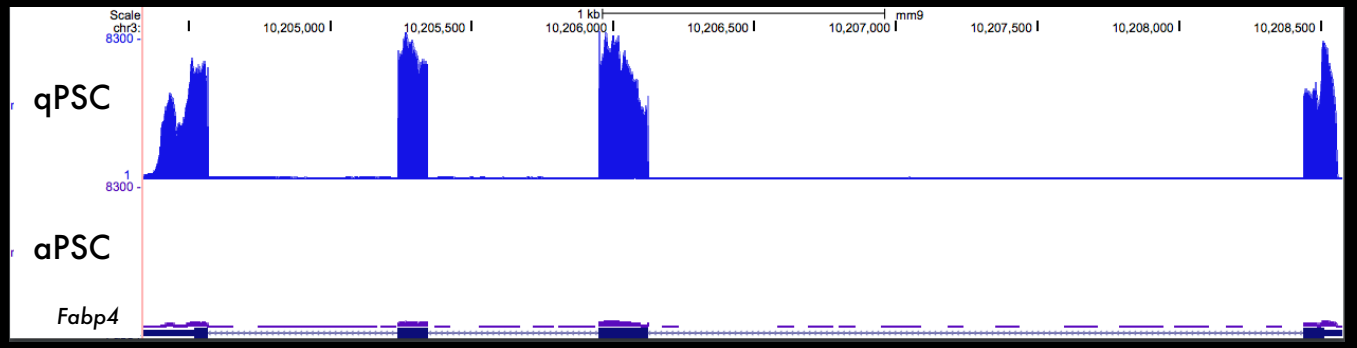
Primary mPSC
Activated

Can we leverage the unique lipid-storing origins of PSC-derived CAFs to track these cells *in vivo*?



Sherman et al., *Cell*, 2014

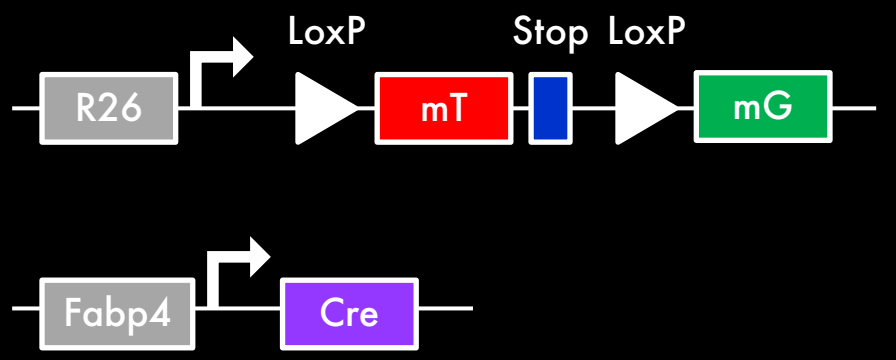
"Adipocyte-specific" Cre driver to track and isolate PSCs and their derivative CAFs in vivo



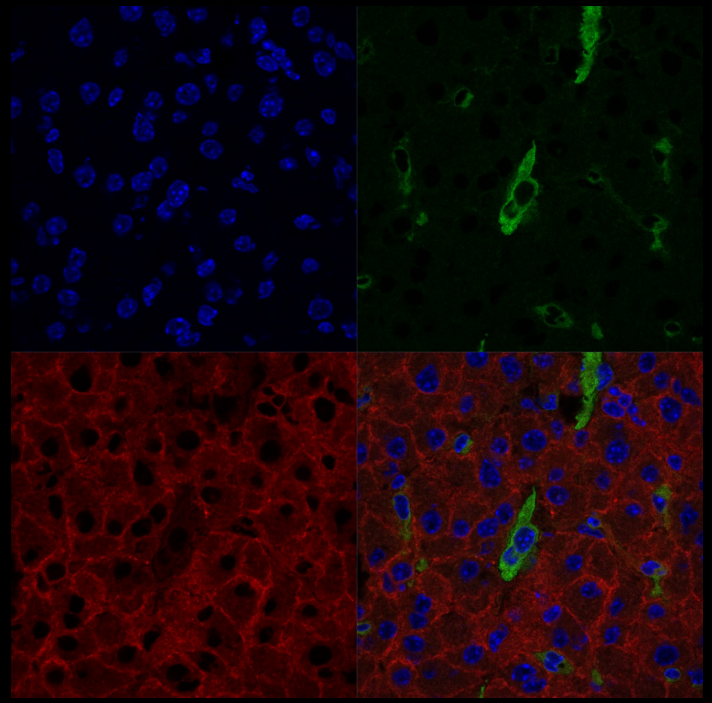
Fabp4-Cre (a.k.a. aP2-Cre) to induce recombination of floxed alleles in PSCs?

Fabp4-Cre pervasively and specifically labels PSCs within the pancreas

Cre⁻ host tissues: tdTomato
Cre⁺ cells and progeny: EGFP

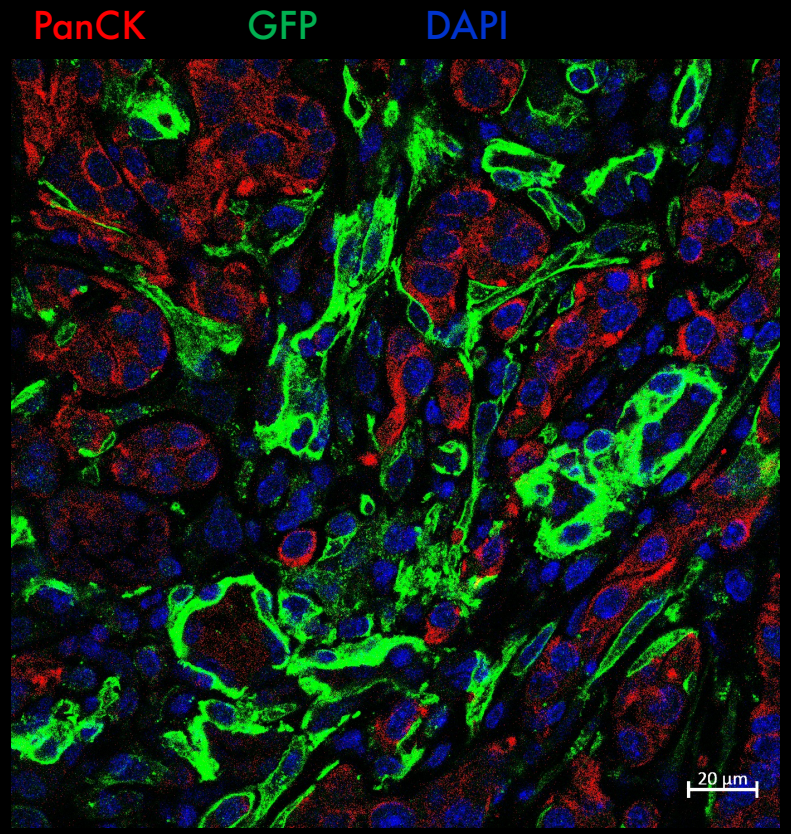


Normal pancreas



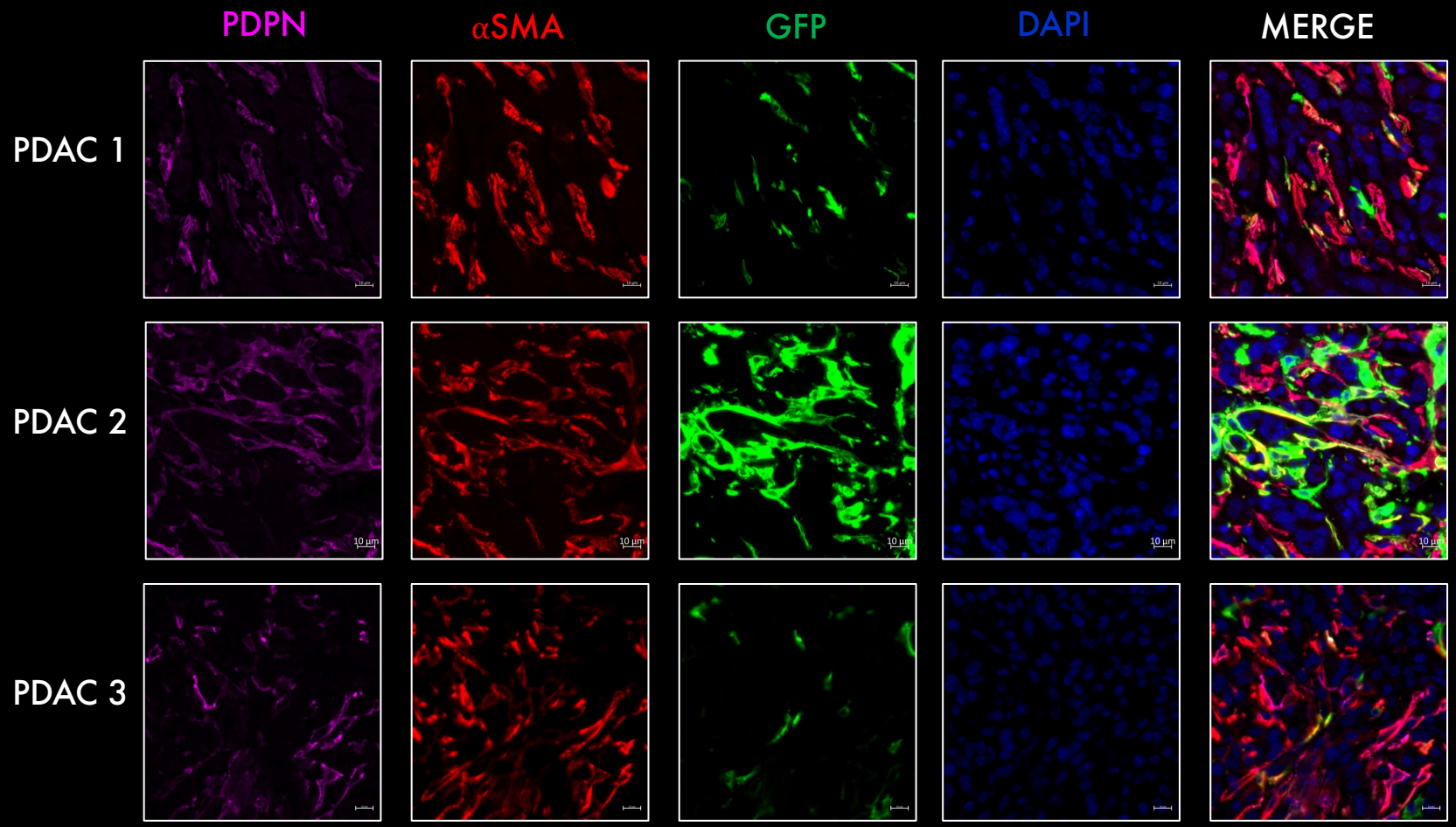
Validation of specificity and pervasiveness by flow cytometry, IHC, and density centrifugation + culture (Helms et al., *Cancer Discovery*, 2022)

PSCs give rise to aSMA-positive, PDPN-positive CAFs



KPC PDAC in *Rosa26*^{mTmG}; *Fabp4-Cre* host

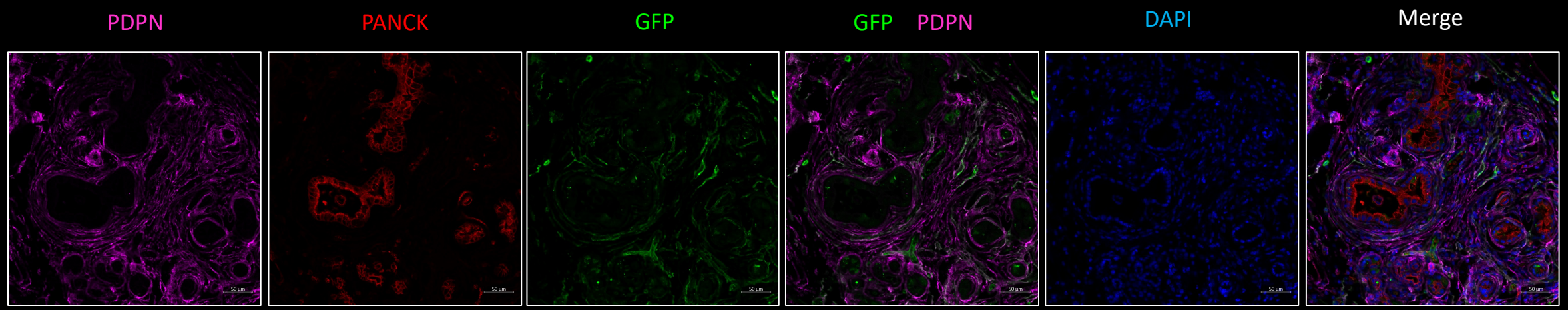
PSCs give rise to a subset of PDAC CAFs



KPC PDAC in *Rosa26*^{mTmG}; *Fabp4-Cre* host

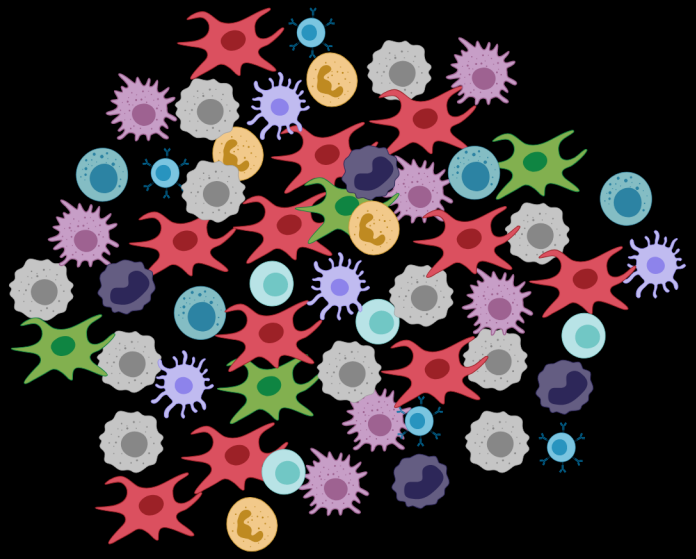
Premalignant lesions?
Primary vs metastatic microenvironments?

PSC-derived CAFs are a subset of PDAC CAFs

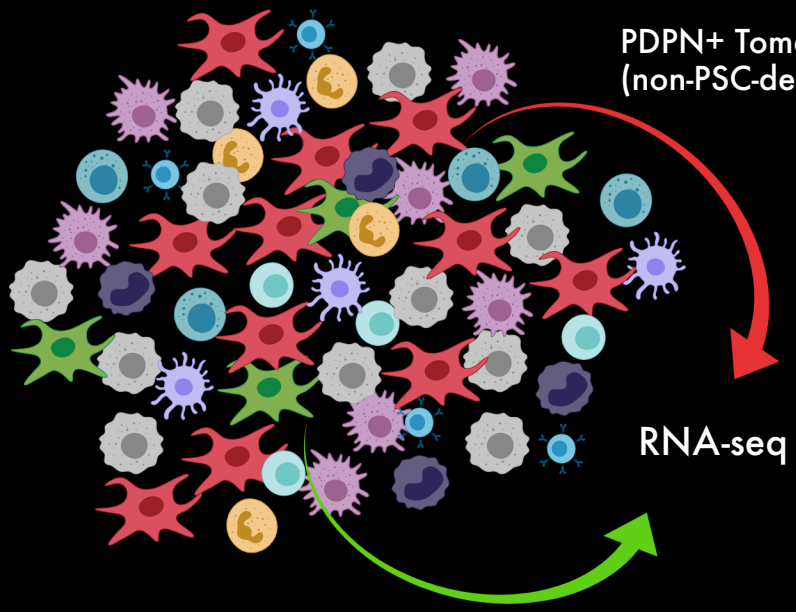


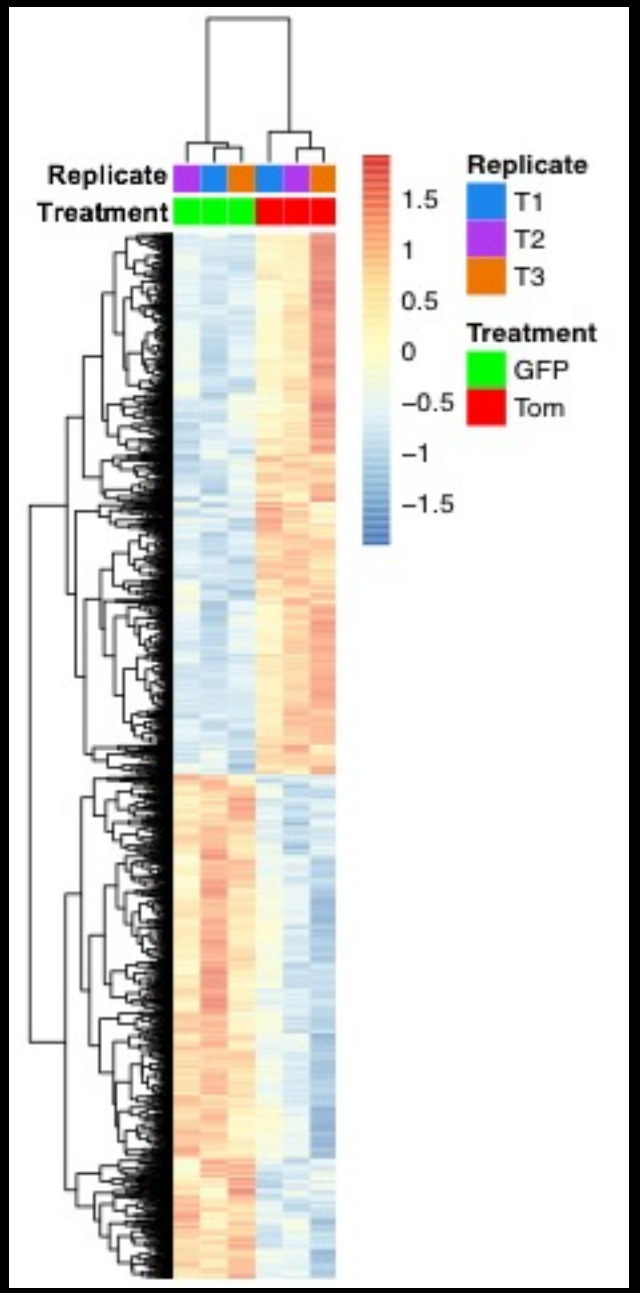
Kras$^{FSF\text{-}G12D/+}$;Trp53$^{FRT/+}$;Pdx1-FlpO;Rosa$^{mTmG/+}$;Fabp4-Cre

RNA-seq enables analysis of PSC-derived vs non-PSC-derived CAFs



RNA-seq enables analysis of PSC-derived vs non-PSC-derived CAFs





No difference in *Acta2*, *Fap*, *Pdpn*

RNA-seq enables analysis of PSC-derived vs non-PSC-derived CAFs

Upregulated in PSC-derived CAFs (>2-fold, p adj < 0.05):

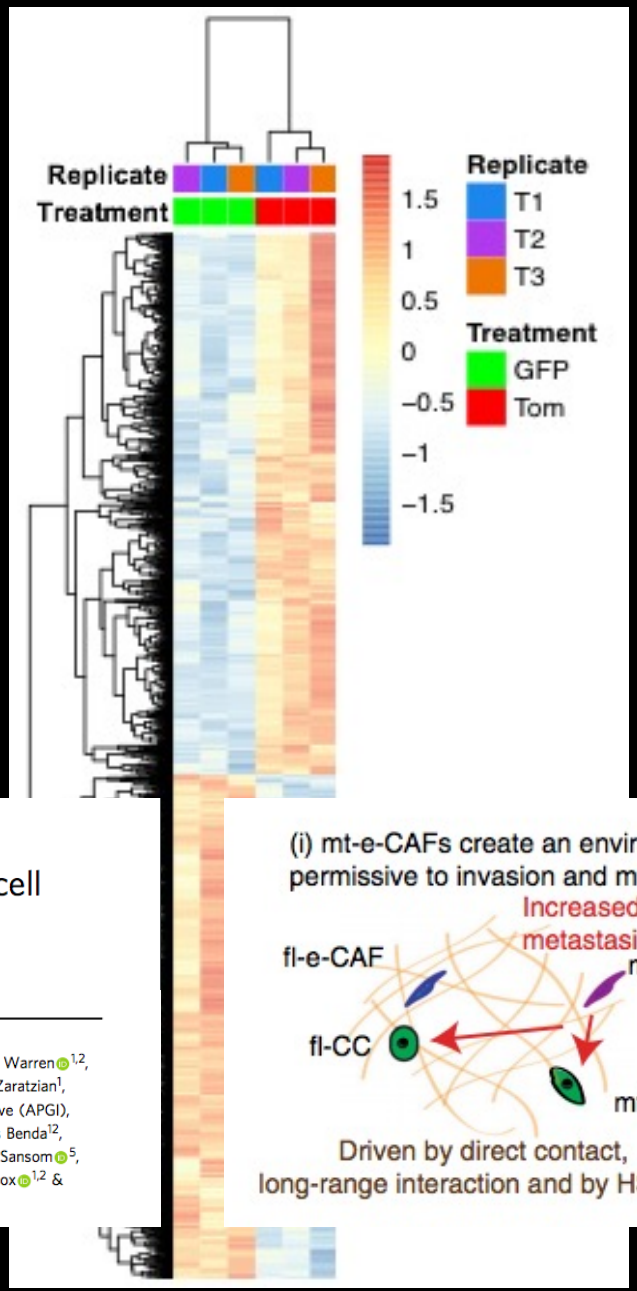
Cell adhesion	$p = 8.7 \times 10^{-21}$
ECM-receptor interaction	$p = 3.2 \times 10^{-15}$
Axon guidance	$p = 5.3 \times 10^{-9}$

Gene categories of interest:

Trafficking/transport (like LECS, but not LECs! No CD31+/GFP+ cells):
Tie1
Lyve1
Flt4
Icam2
Cadherins/protocadherins

Matrix components/tissue stiffness:
Frem1
Tenascins
Laminins
Multimerins
Perlecan

Neural cues:
Sema6c
Nrp2
Slit1/2
Robo1/2/4



ARTICLE

https://doi.org/10.1038/s41467-019-10968-6 OPEN

CAF hierarchy driven by pancreatic cancer cell p53-status creates a pro-metastatic and chemoresistant environment via perlecan

Claire Vennin[1,2,3], Pauline Mélénec[1,2], Romain Rouet[1,2], Max Nobis[1,2], Aurélie S. Cazet[1,2], Kendelle J. Murphy[1,2], David Herrmann[1,2], Daniel A. Reed[1,2], Morghan C. Lucas[1,2], Sean C. Warren[1,2], Zehra Elgundi[4], Mark Pinese[1,2], Gabriella Kalna[5], Daniel Roden[1,2], Monisha Samuel[6], Anaiis Zaratzian[1], Shane T. Grey[1,2], Andrew Da Silva[1], Wilfred Leung[1,2,7], Australian Pancreatic Genome Initiative (APGI), Suresh Mathivanan[6], Yingxiao Wang[8], Anthony W. Braithwaite[9,10,11], Daniel Christ[1,2], Ales Benda[12], Ashleigh Parkin[1,2], Phoebe A. Phillips[13,14], John M. Whitelock[4], Anthony J. Gill[1,15,16,17], Owen J. Sansom[5], David R. Croucher[1,2], Benjamin L. Parker[18], Marina Pajic[1,2], Jennifer P. Morton[5], Thomas R. Cox[1,2] & Paul Timpson[1,2]

PSC-derived CAF depletion during PDAC progression

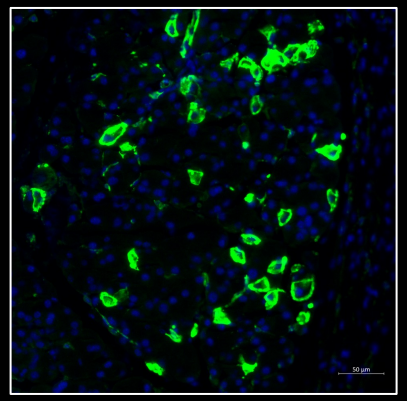
For functional studies, use iDTR; Rosa26^{mTmG}; Fabp4-Cre

Two limitations of the model to overcome:

1) Adipocytes
2) CD45+/GFP+ cells recruited into TME (not found in normal pancreas)

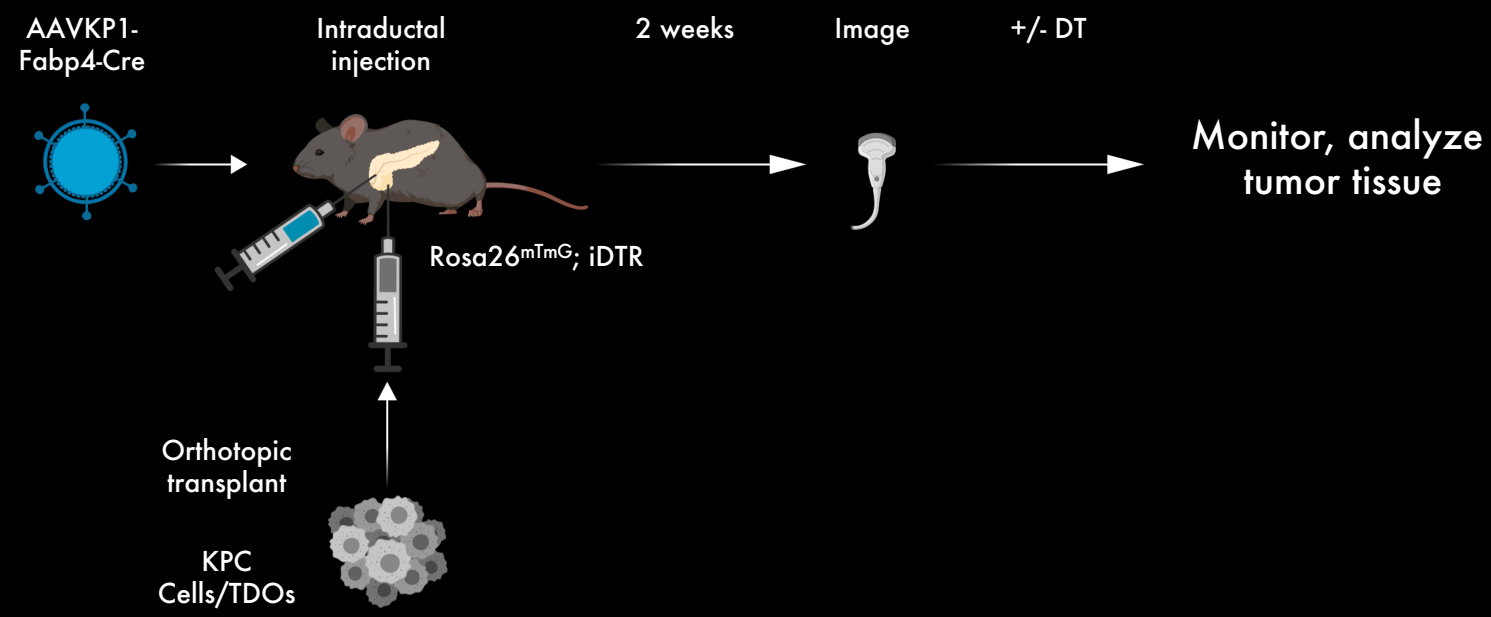
Solution:

Viral Fabp4-Cre injection into pancreas via retrograde ductal injection (adaptation of Winslow/Grompe lab protocols) → spatial and temporal control of Cre expression



Rosa26^{mTmG} + AAVKP1-Fabp4-Cre

PSC-derived CAF depletion during PDAC progression



Outcome parameters (enroll at 3mm, tx until humane endpoint):

-Tumor growth

-Survival

Tissue parameters (enroll at 6mm, tx 5 days):

-Tumor stiffness (w/ Sunil Hingorani)

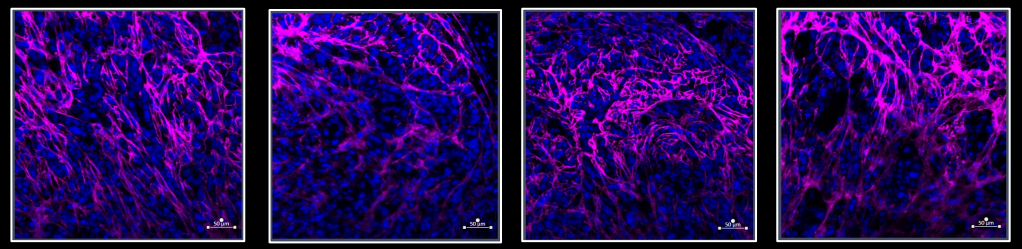
-Spatial regulation of nerve cells (w/ Dan Marks) and leukocytes (w/ Lisa Coussens)

PSC-derived CAFs regulate PDAC tissue stiffness

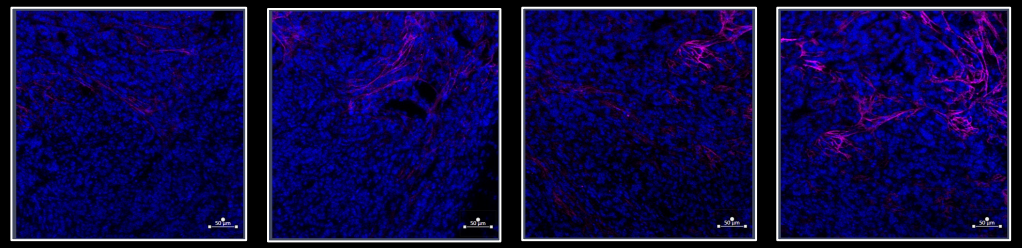
Short-term depletion in established tumors:

Tenascin C DAPI

Control

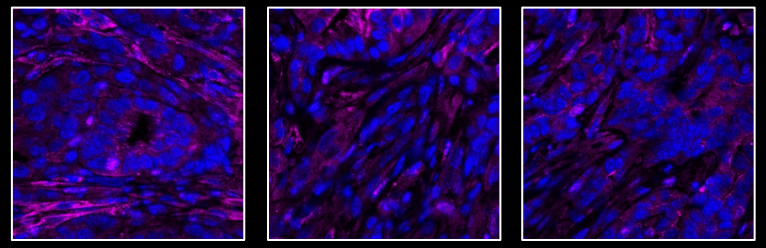


PSC-depleted

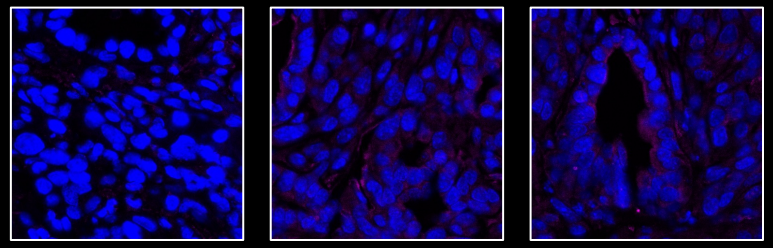


pMLC2 DAPI

Control

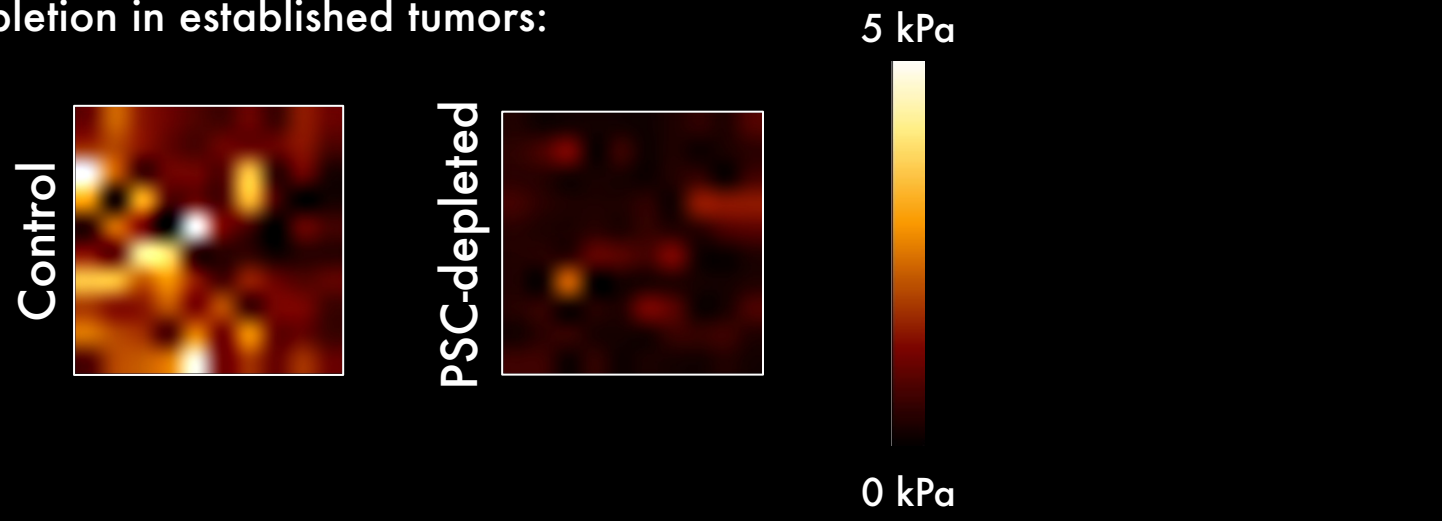


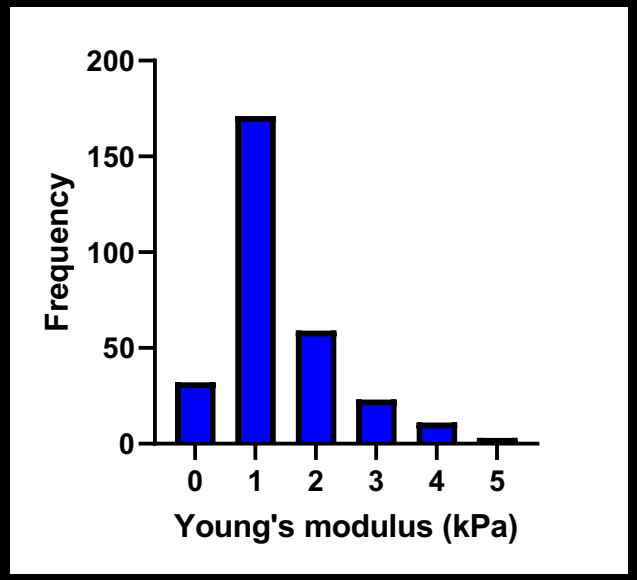
PSC-depleted

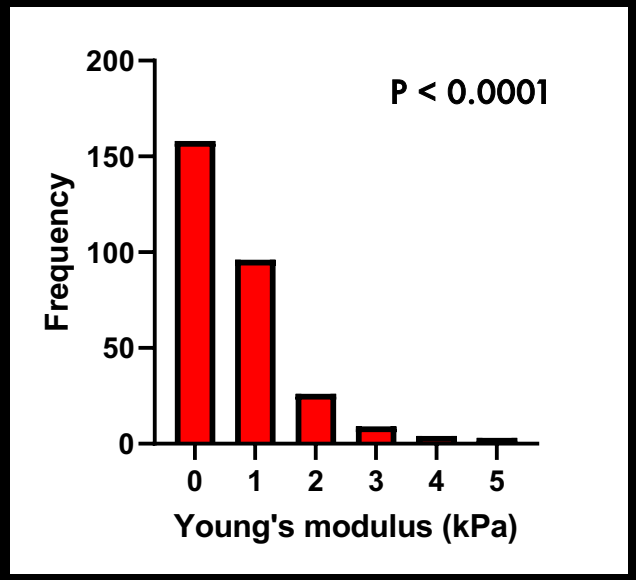


PSC-derived CAFs regulate PDAC tissue stiffness

Short-term depletion in established tumors:

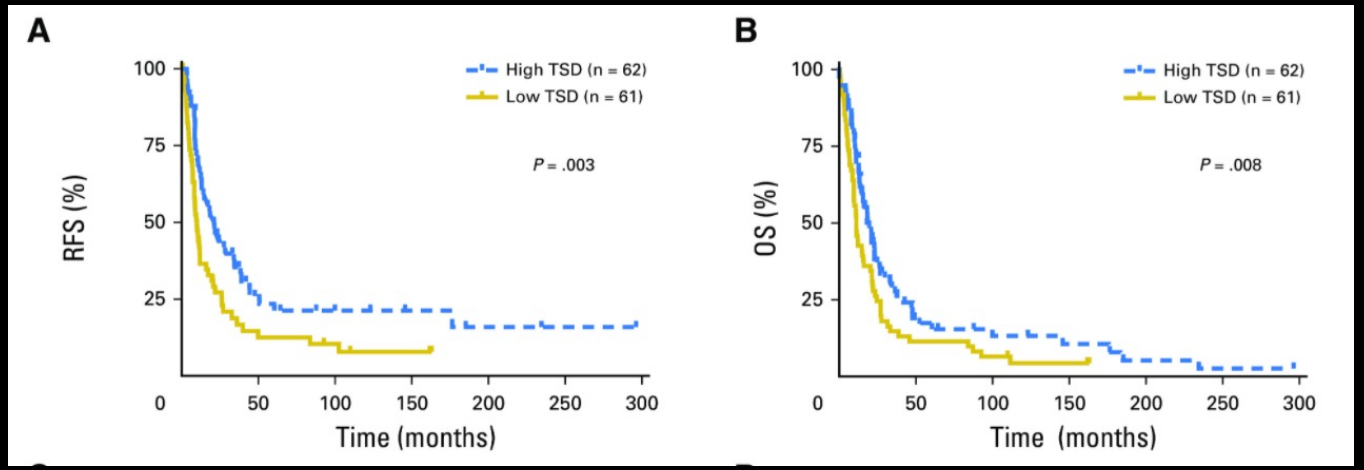




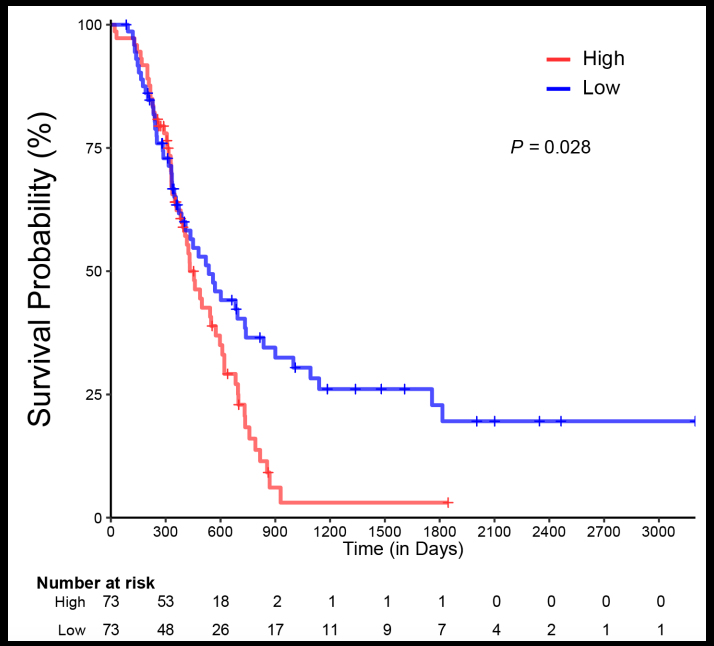


Atomic force microscopy by Chris DuFort and Sunil Hingorani, FHCRC

Non-redundant ECM factors from PSC-derived CAFs associate with poor prognosis



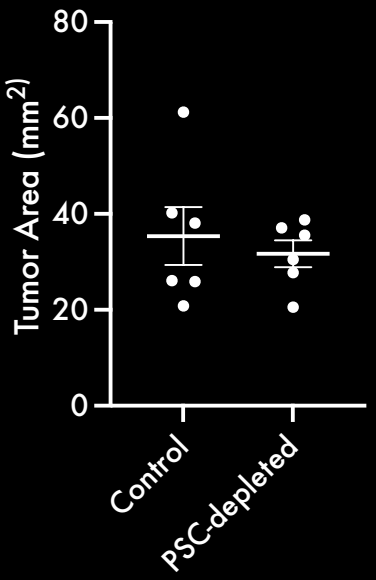
Torphy et al., JCO Precis Oncol, 2018

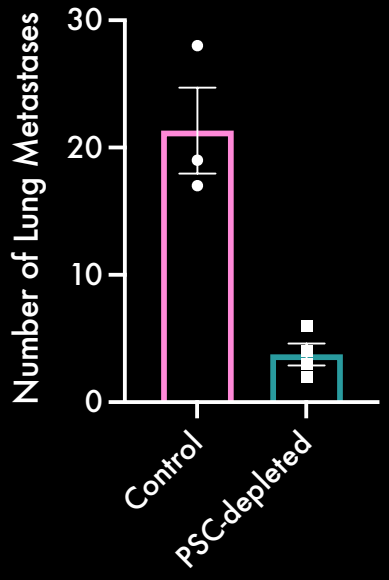


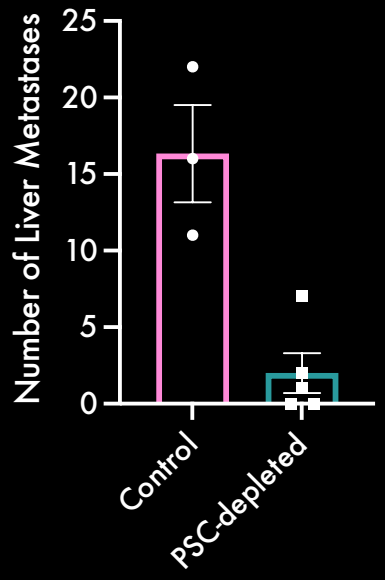
Upper vs. lower quartile, PSC-specific ECM signature

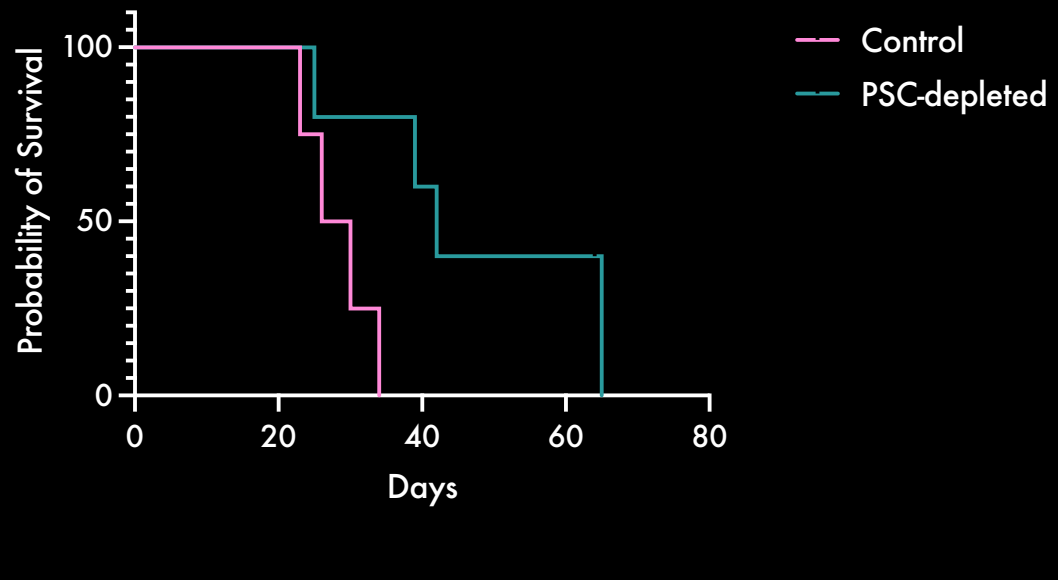
PSC-derived CAFs promote metastatic progression

Enrollment at 3mm tumor diameter, on study until humane endpoint:

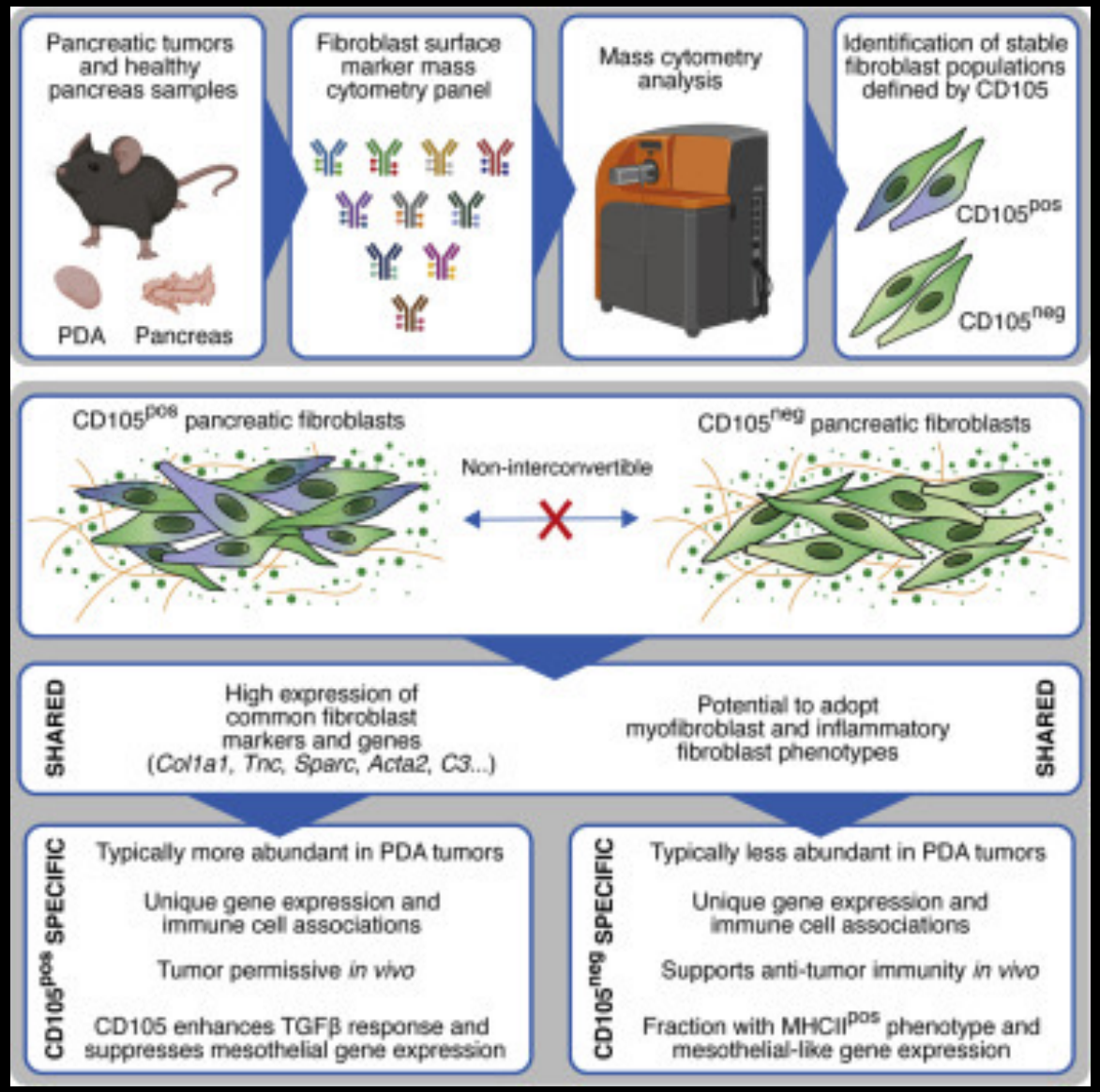




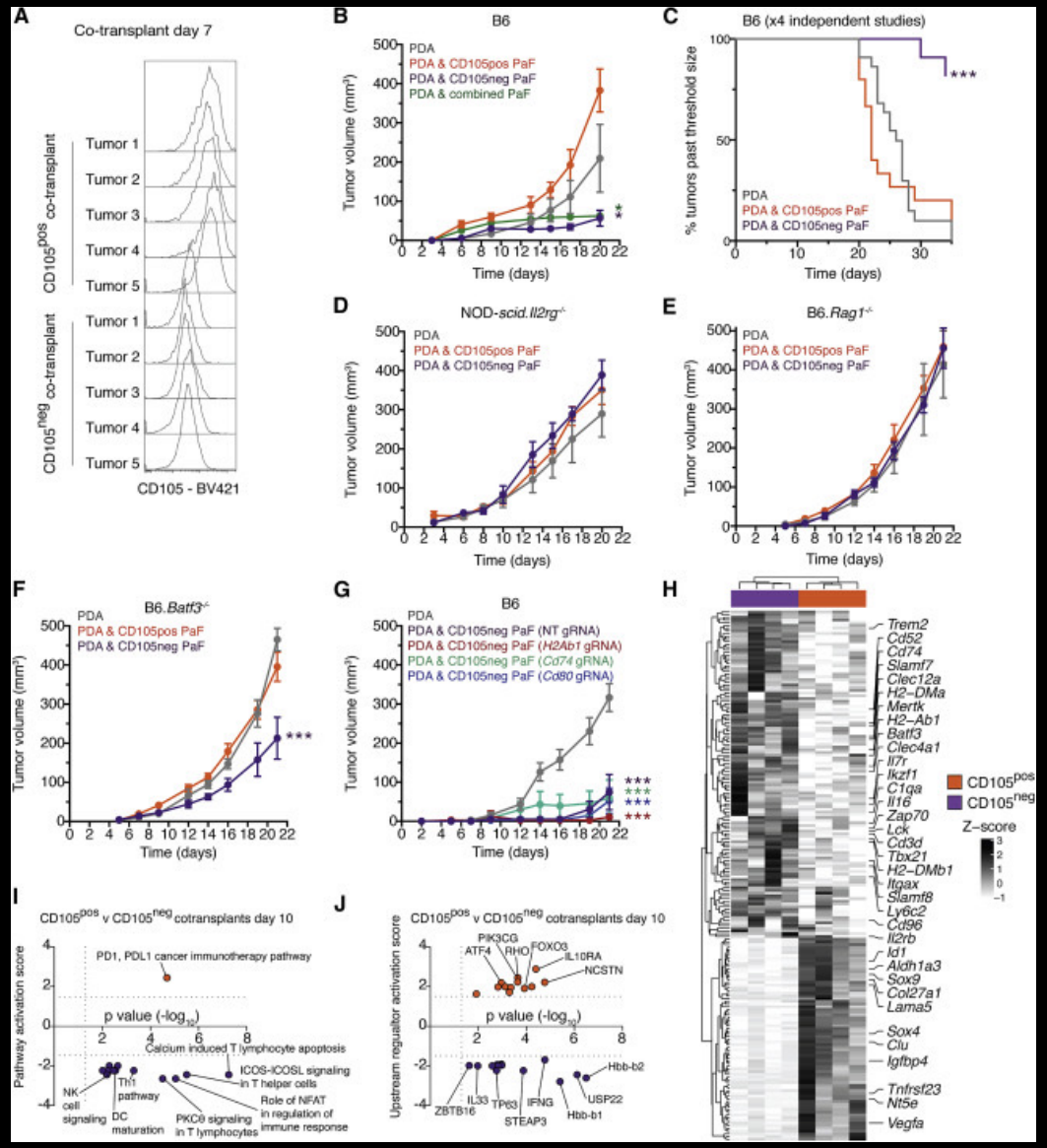




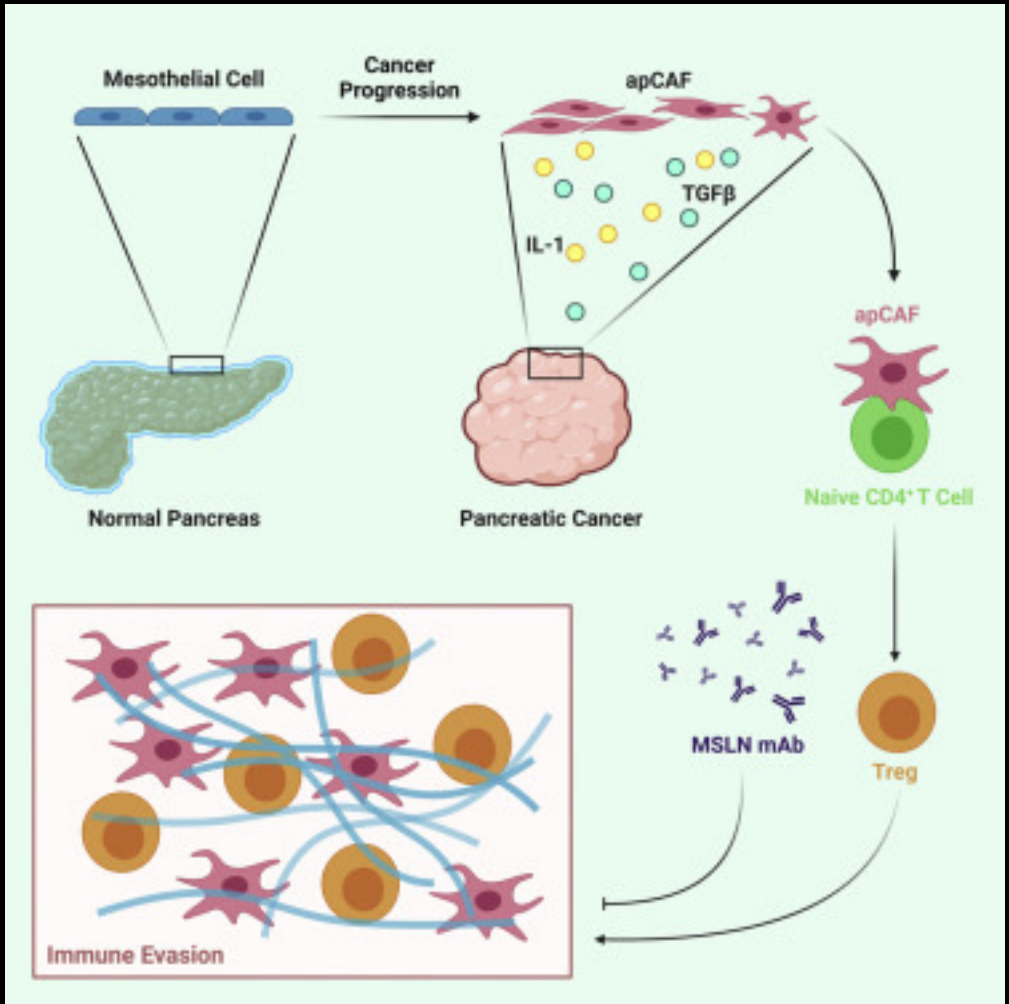
CD105+ CAFs support while CD105- CAFs suppress PDAC progression



Hutton et al., *Cancer Cell*, 2021



Mesothelial cell-derived apCAFs promote immune evasion

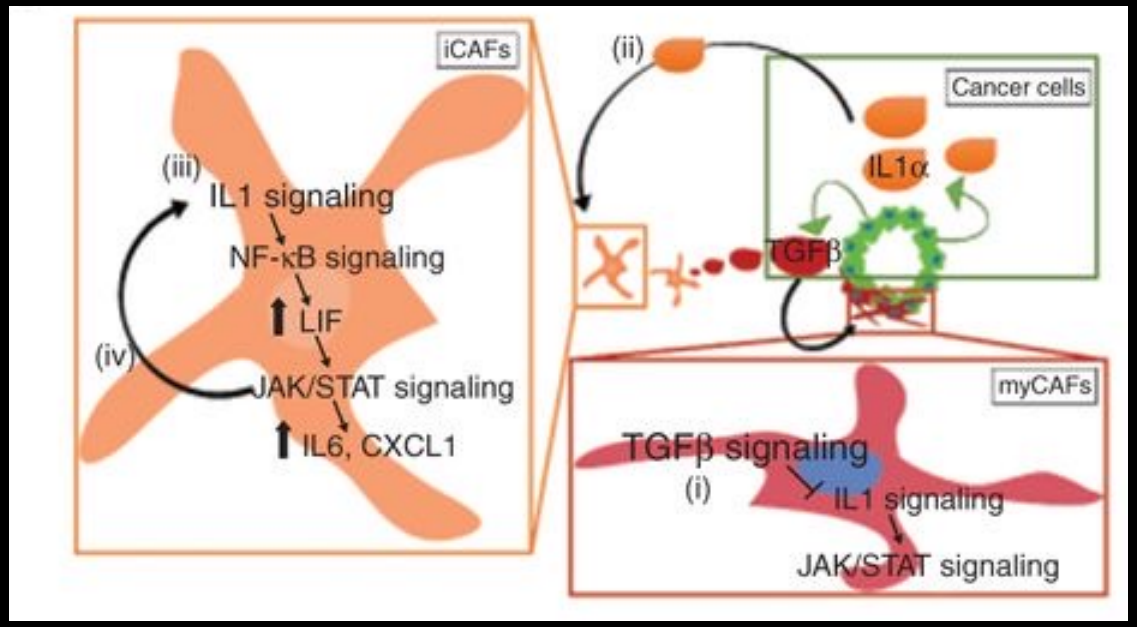


Huang et al., *Cancer Cell*, 2022

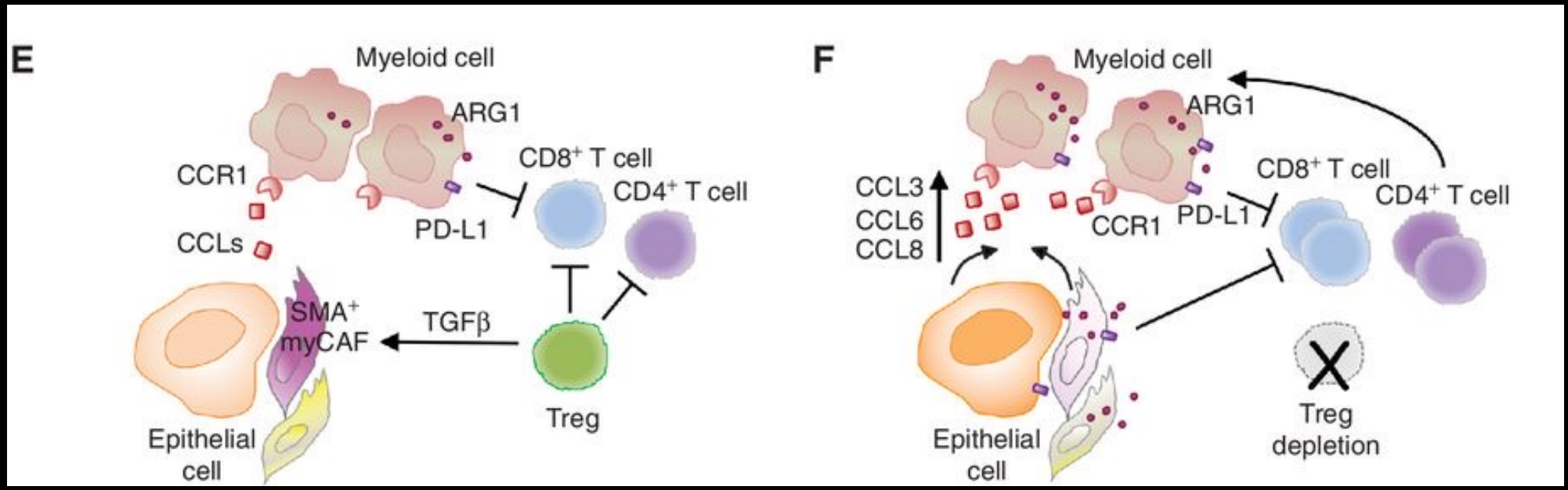
*Huang et al. suggest antigen-specific mechanism of immune suppression by MHCII^{+} apCAFs

*Four sub-populations of CD105^{-} CAFs in PDAC, 2 pos for MHCII per Hutton et al.; perhaps MHCII-negative, non-mesothelial populations promote anti-tumor immunity

Key determinants of CAF heterogeneity



Biffi et al., *Cancer Discovery*, 2019



Zhang et al., *Cancer Discovery*, 2020