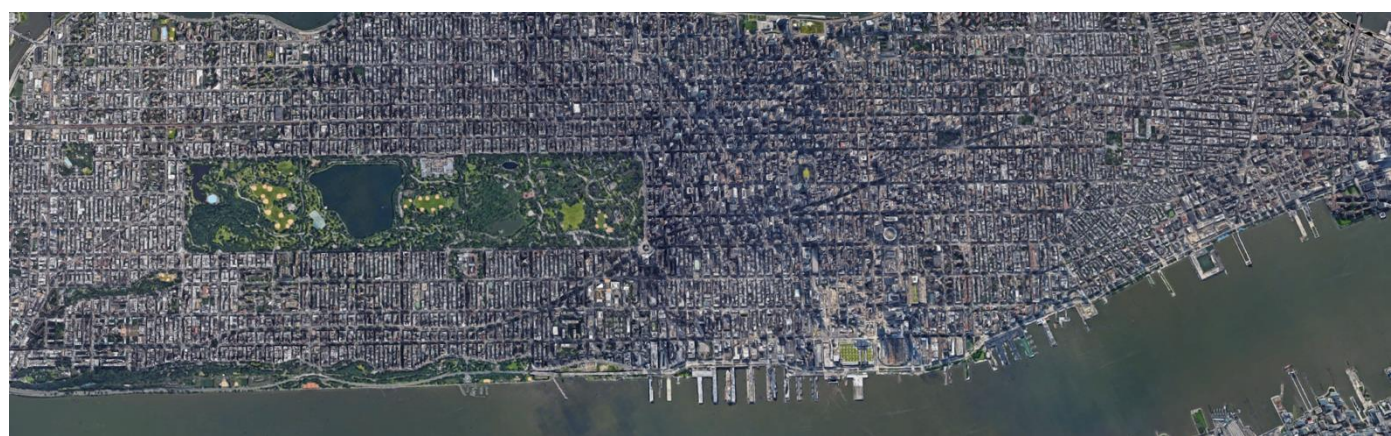




Utilizing Perturb-map to identify genetic determinants of the tumor microenvironment

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Precision Immunology Institute

Icahn School of Medicine at Mount Sinai

**Mount
Sinai**



*Icahn Genomics
Institute*

Disclosures



Scientific Advisory Board, Licensed patent

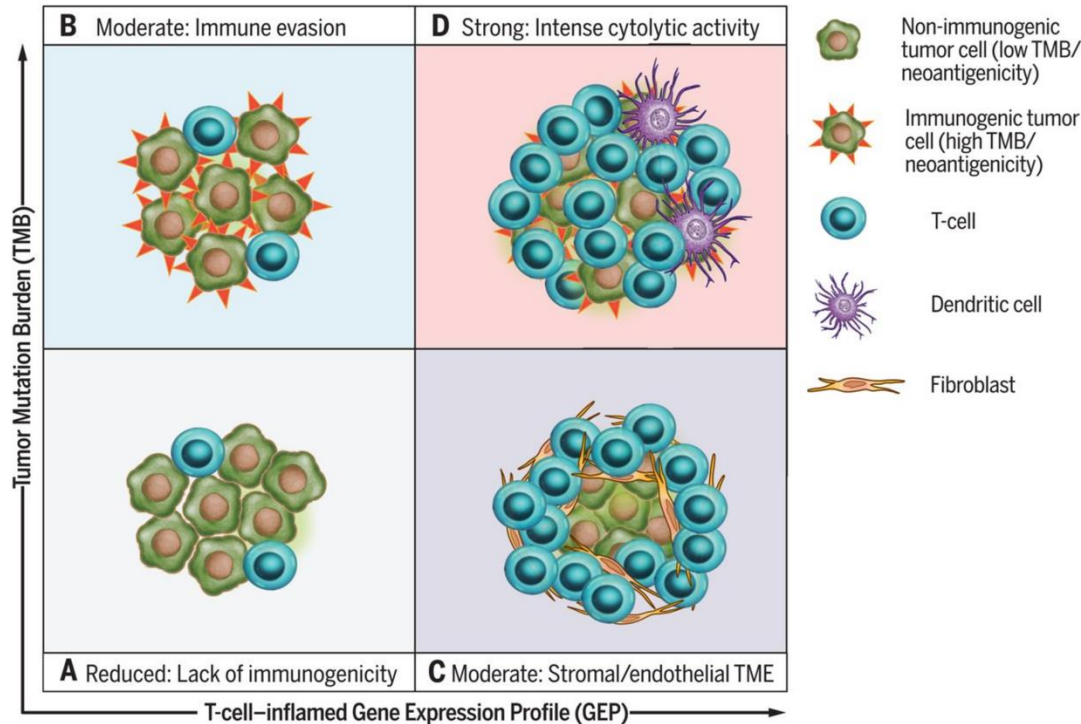


Licensed patent

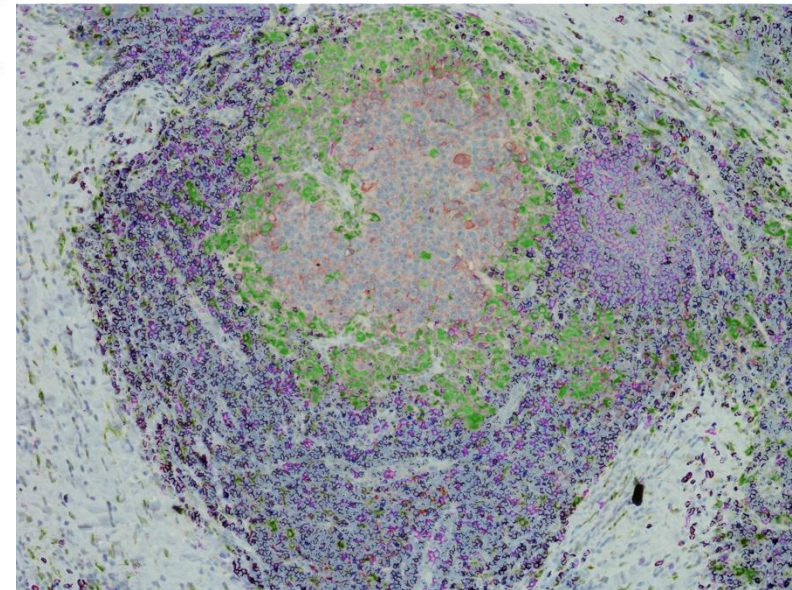


Licensed patent

Tumor immune composition is a major determinant of cancer outcome and response to immunotherapy



Cristescu et al, Science 2018



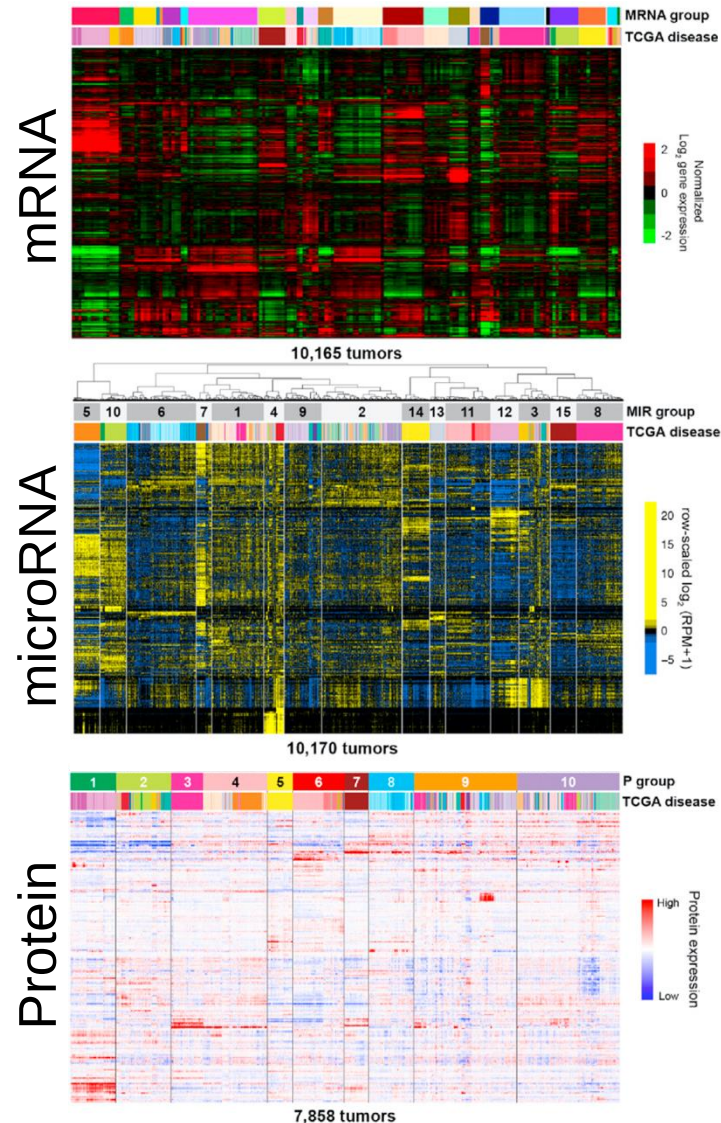
PD-L1 CD68 DC-LAMP CD20 CD3 FoxP3

Remark et al, Science Immunol 2016

How is the cancer controlling local immune composition?
(e.g. recruitment of macrophages, exclusion of T cells?)

Major changes in gene expression underlie tumor biology

Challenge: What genes control TME composition and architecture?



- 1000s of genes differentially expressed in tumors
- Determining each genes contribution to different aspects of tumor biology is one of the major goals of the modern era of cancer biology
- Which genes are used by cancer cells to evade the immune system and control the local TME?

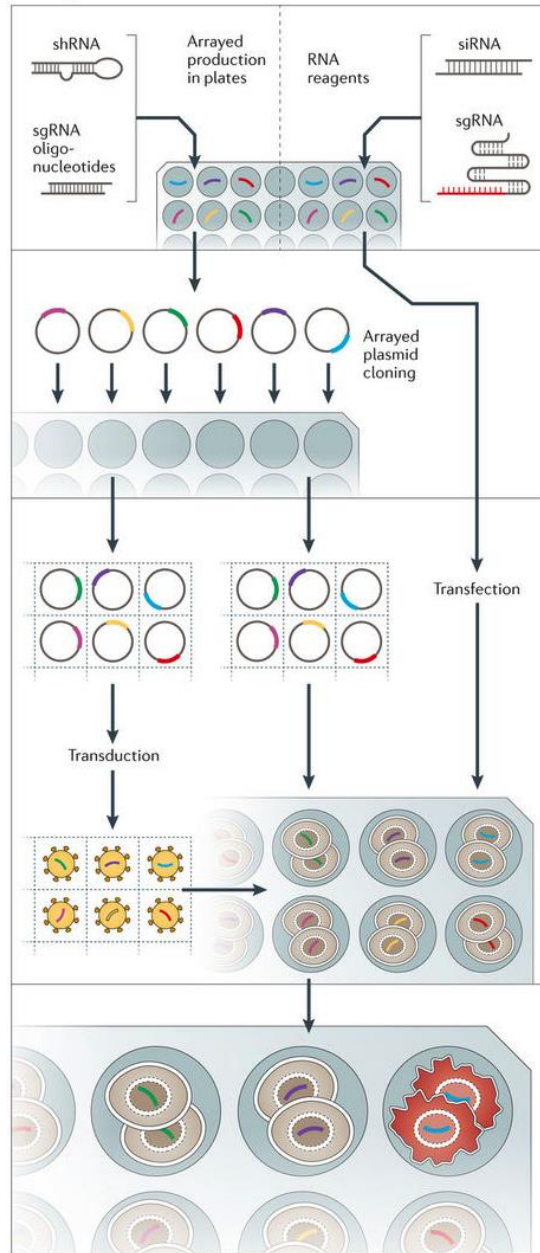
Reagent Synthesis

Library Construction

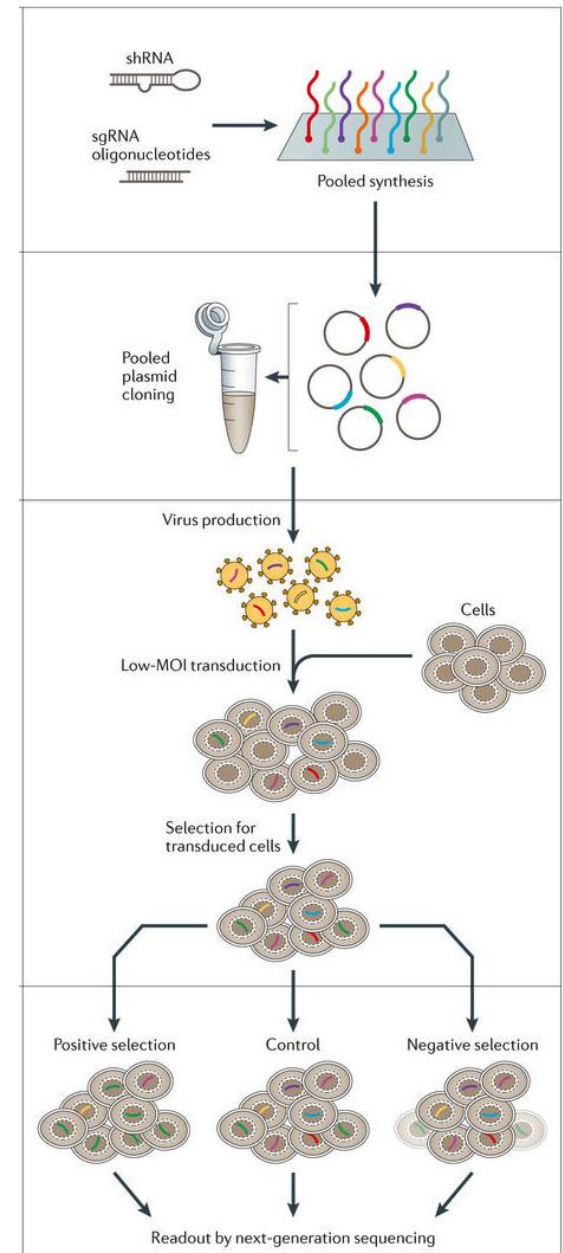
Cell Targeting

Screen Readout

Arrayed Screen



Pooled Screen

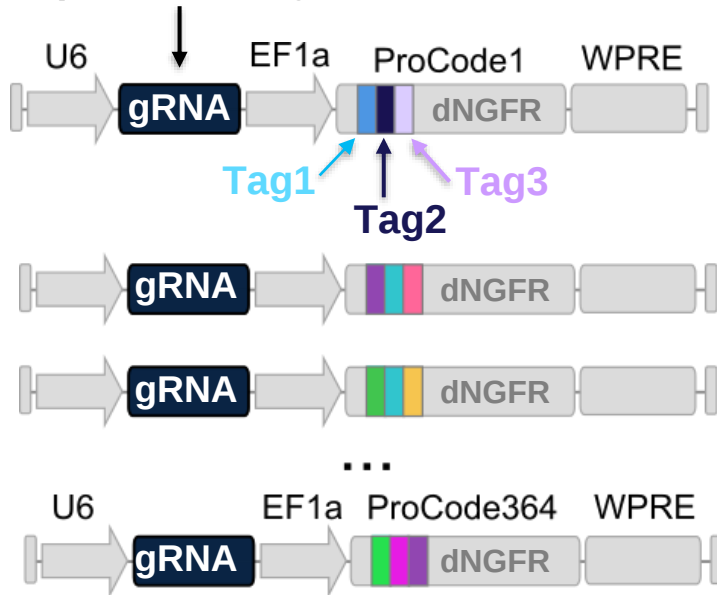


How can we identify the genetic determinants of cancer immunity in vivo at scale?

- CRISPR screens are limited to cell autonomous effects.
- Cell extrinsic phenotypes can not be assessed by current functional genomics approaches (e.g. cell recruitment, etc.).
- The function of whole classes of genes can not be assessed by current functional genomics approaches (e.g. cytokines, chemokines, etc.).
- We need CRISPR genomics methods that retain the spatial information.

Protein Barcodes (Pro-Codes) – a CRISPR barcoding system for high-dimensional phenotypic screens at a single cell resolution

Unique CRISPR gRNA

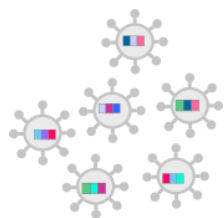
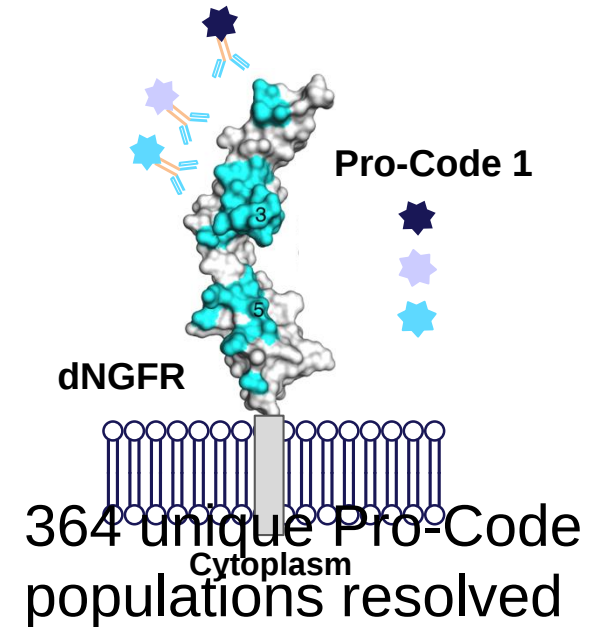


$$C(n, r) = \frac{n!}{r!(n - r)!}$$

n (tags)
 r (positions)
 C (Pro-Codes)

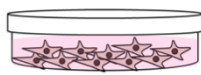
$$C(14, 3) = 364$$

14 tags 3 spots =
364 Pro-Codes



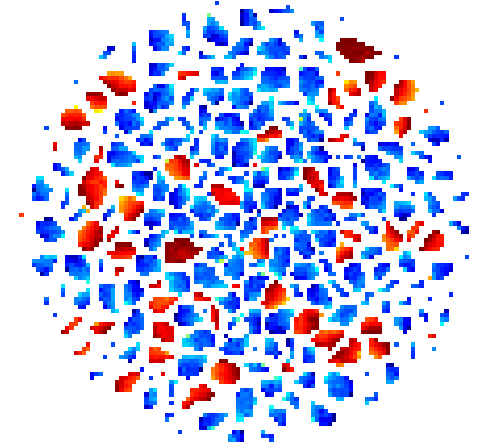
Pro-Code/
CRISPR library

Transduce
(single copy)



Stain for Pro-
Codes (~10 Tags)
+
Phenotypic
markers
(10 – 25 proteins)

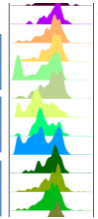
- FACS
- CyTOF



Protein Barcodes (Pro-Codes) – a CRISPR barcoding system for high-dimensional phenotypic screens at a single cell resolution

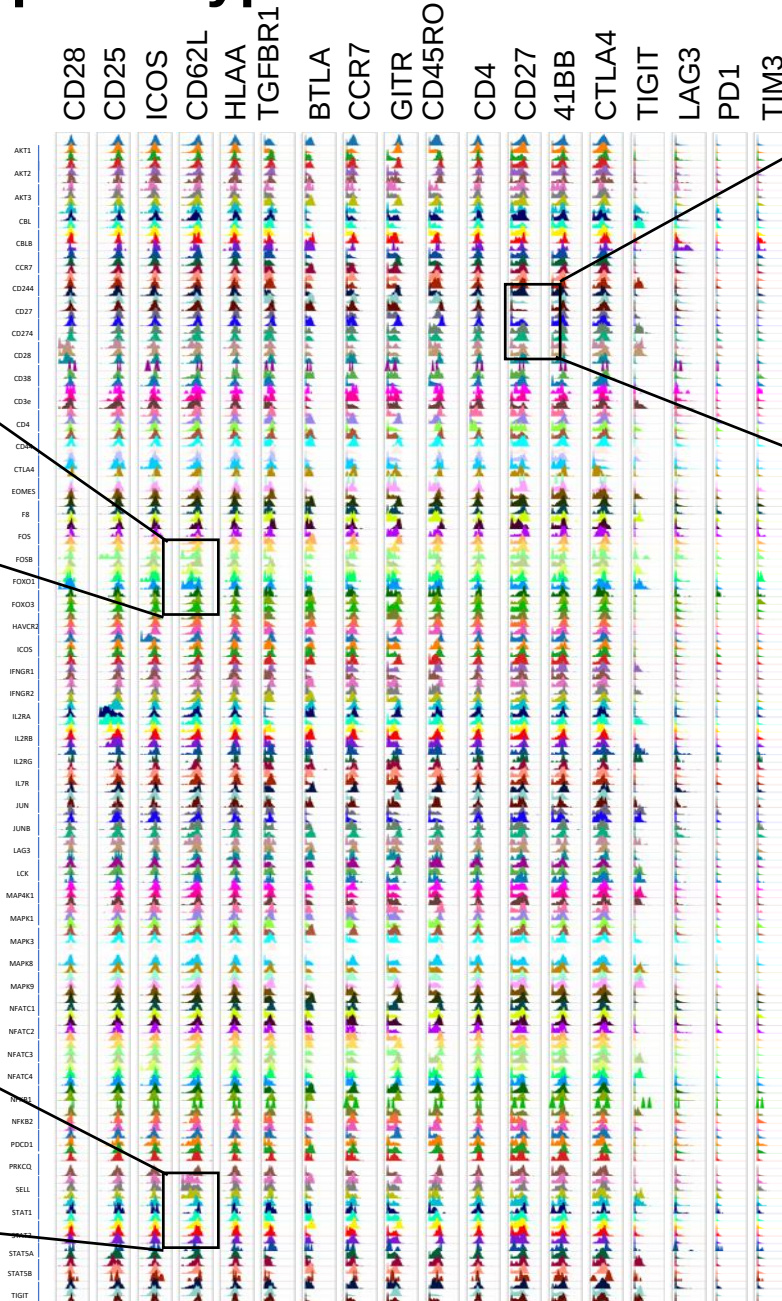
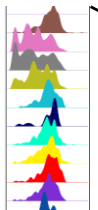
990 (x3) genotype-phenotype connections from a single sample

gFOSB
gFOXO1
gFOXO3



ProCode/
CRISPR

gSELL
gSTAT1
gSTAT3

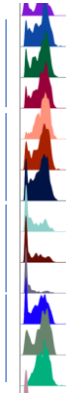


gCCR7

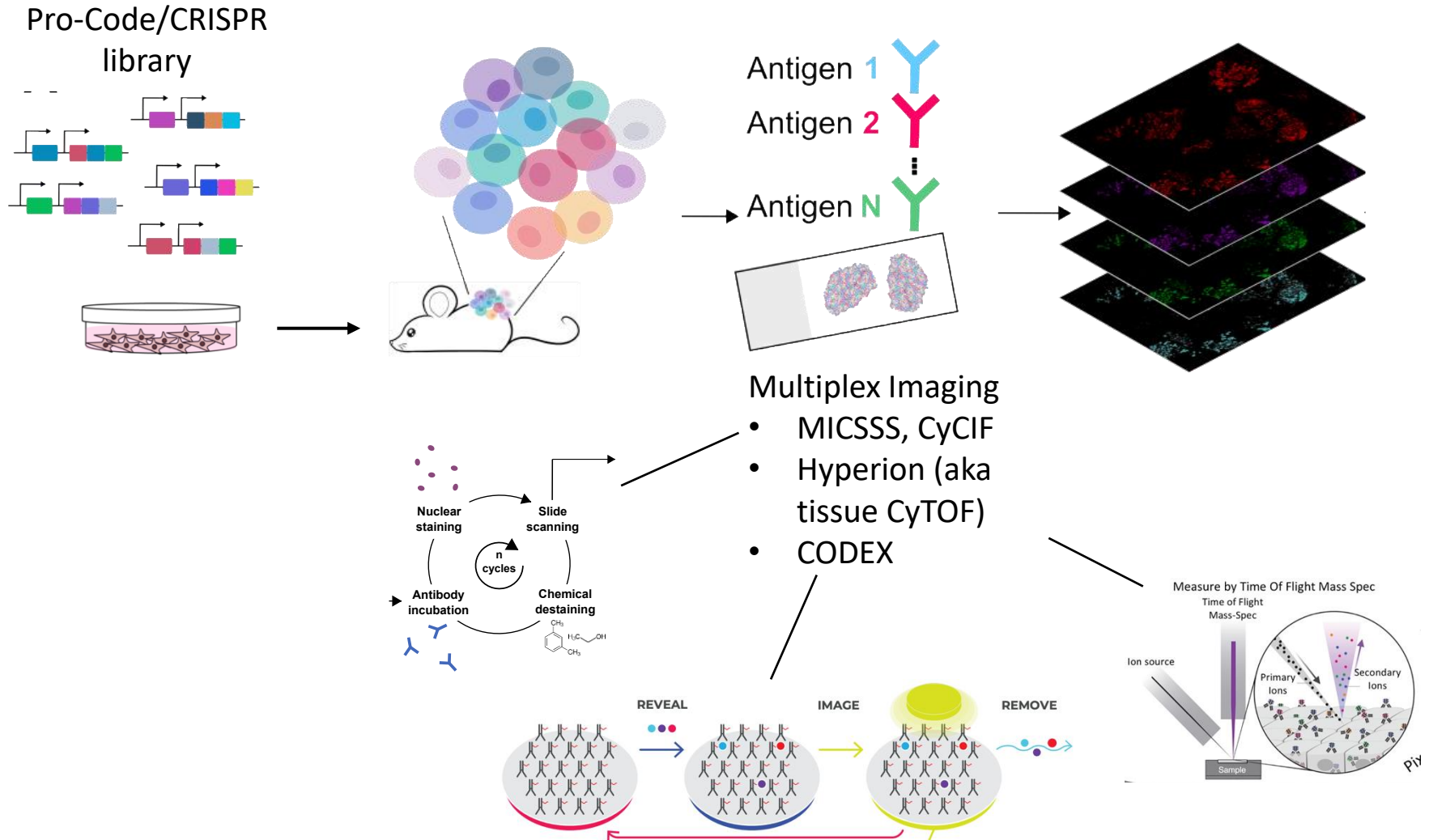
gCD244

gCD27

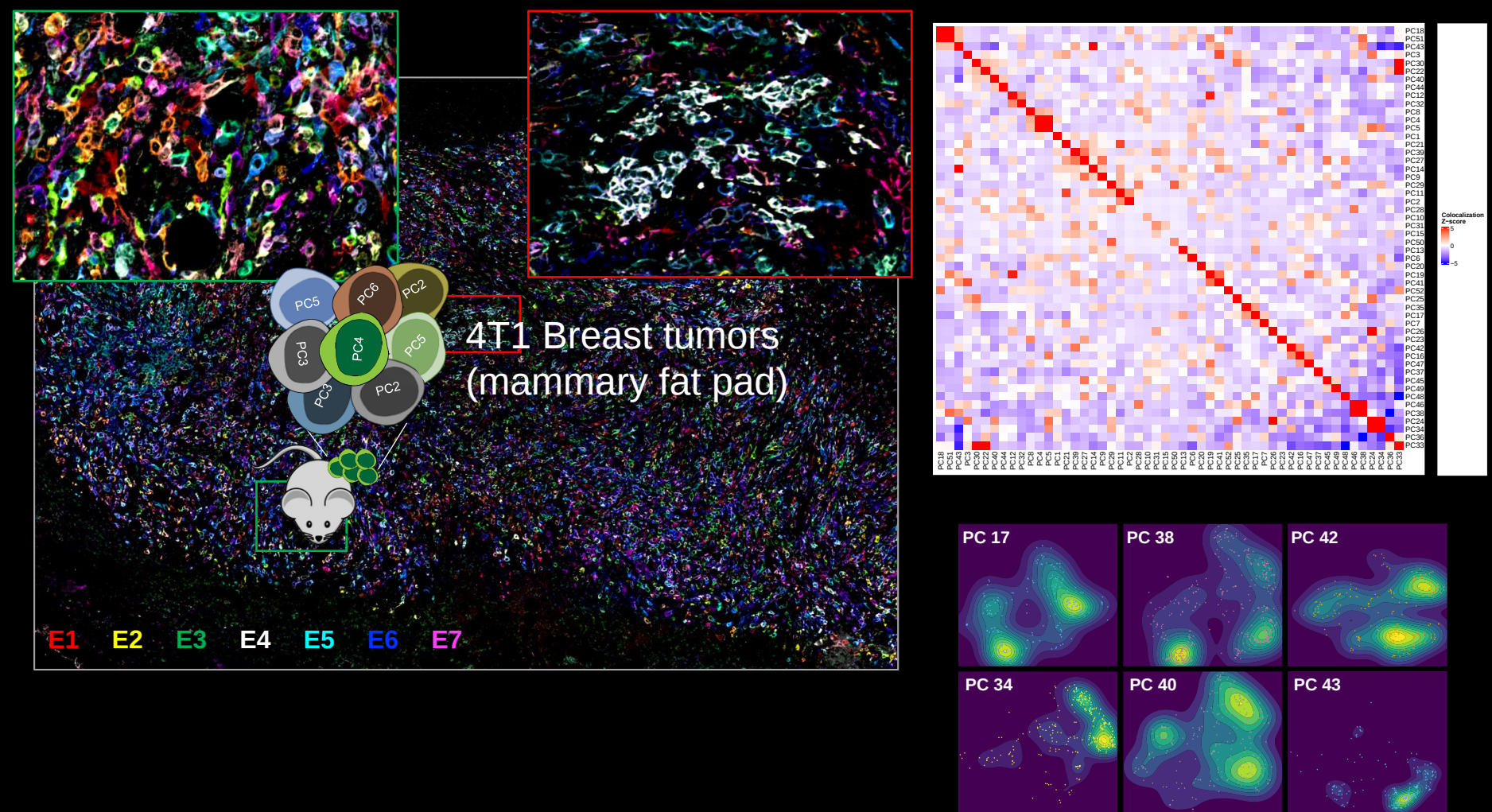
gCD274



Multiplex imaging of Pro-Codes to spatially map gene perturbations in situ

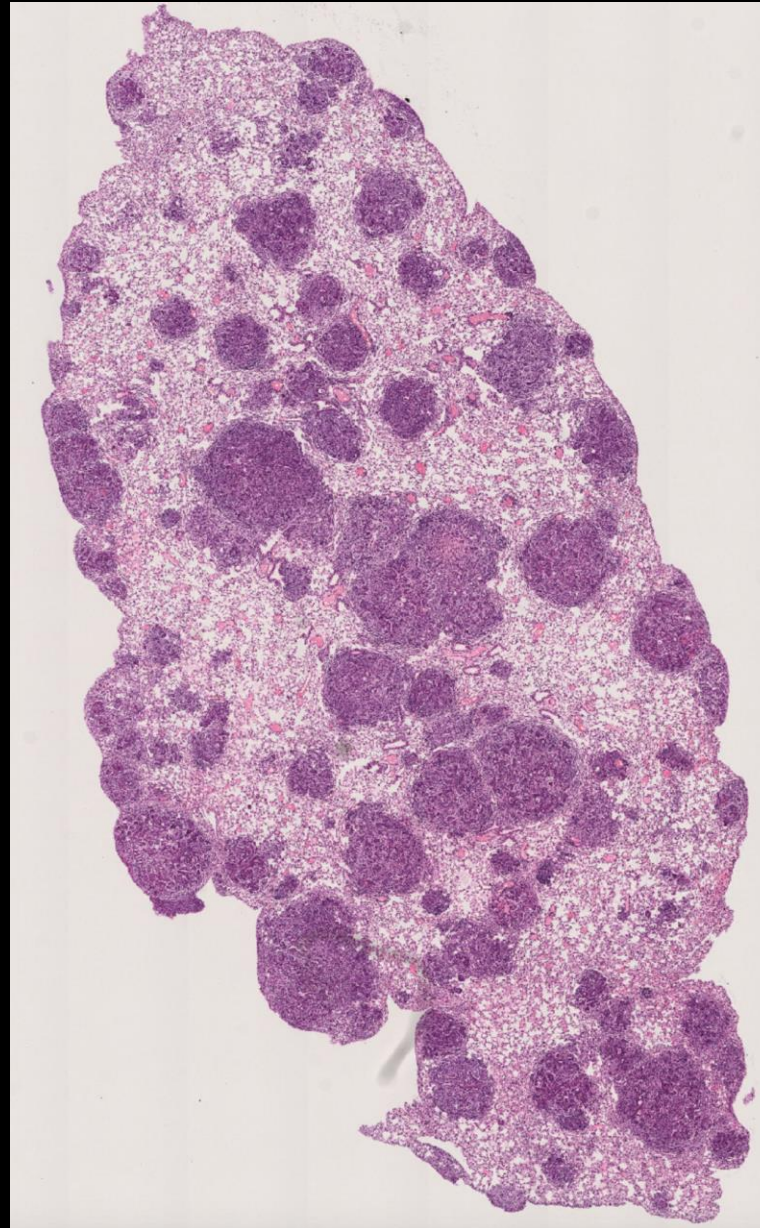
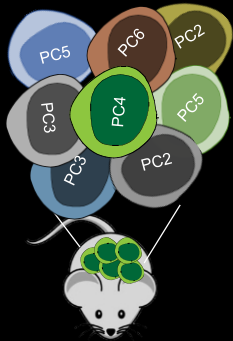


Pro-Code spatial mapping of 120 Pro-Code-labeled 4T1 breast tumors reveals high heterogeneity in spatial clonality



Spatial mapping of Pro-Code-labeled KP lung tumors lesions

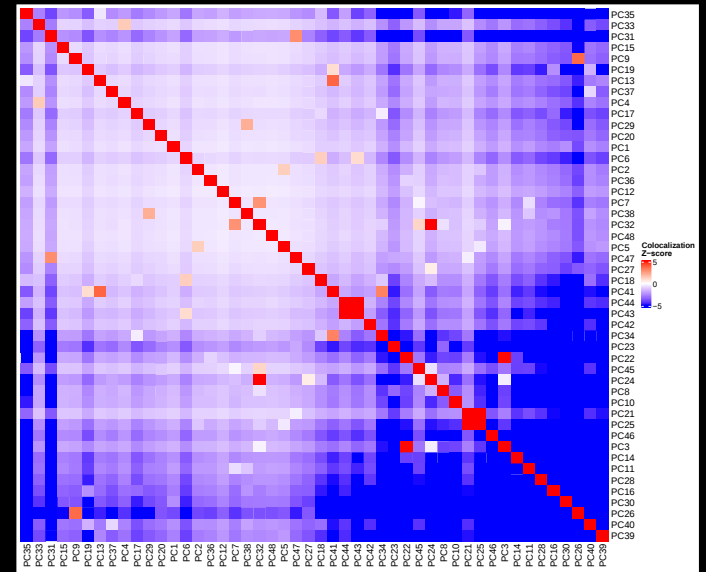
Kras^{G12D},P53^{-/-} (KP)
Lung Cancer
i.v. injection (lung)



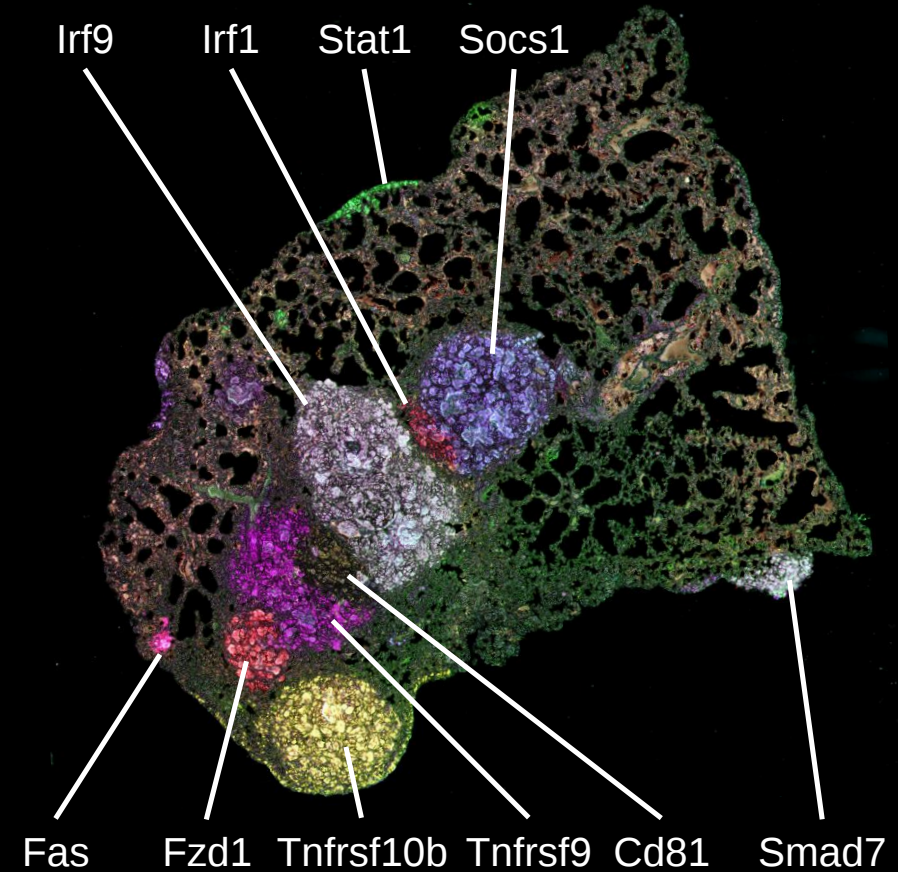
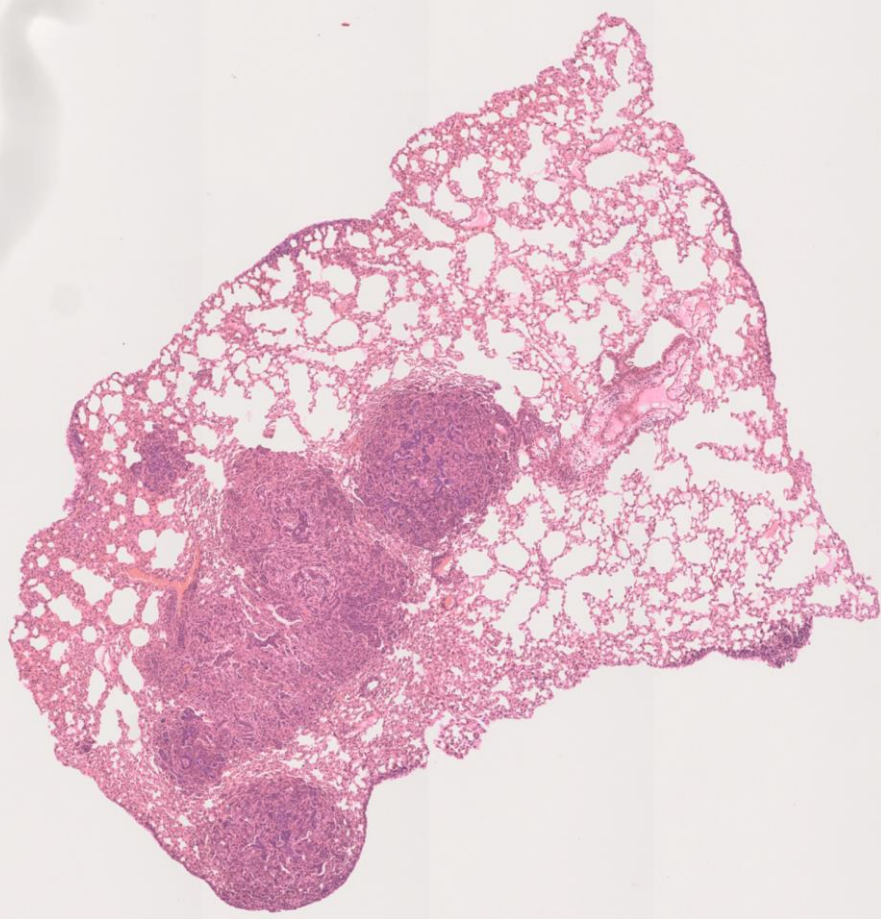
Pro-Code spatial mapping of KP lung tumors reveals lesions are highly clonal



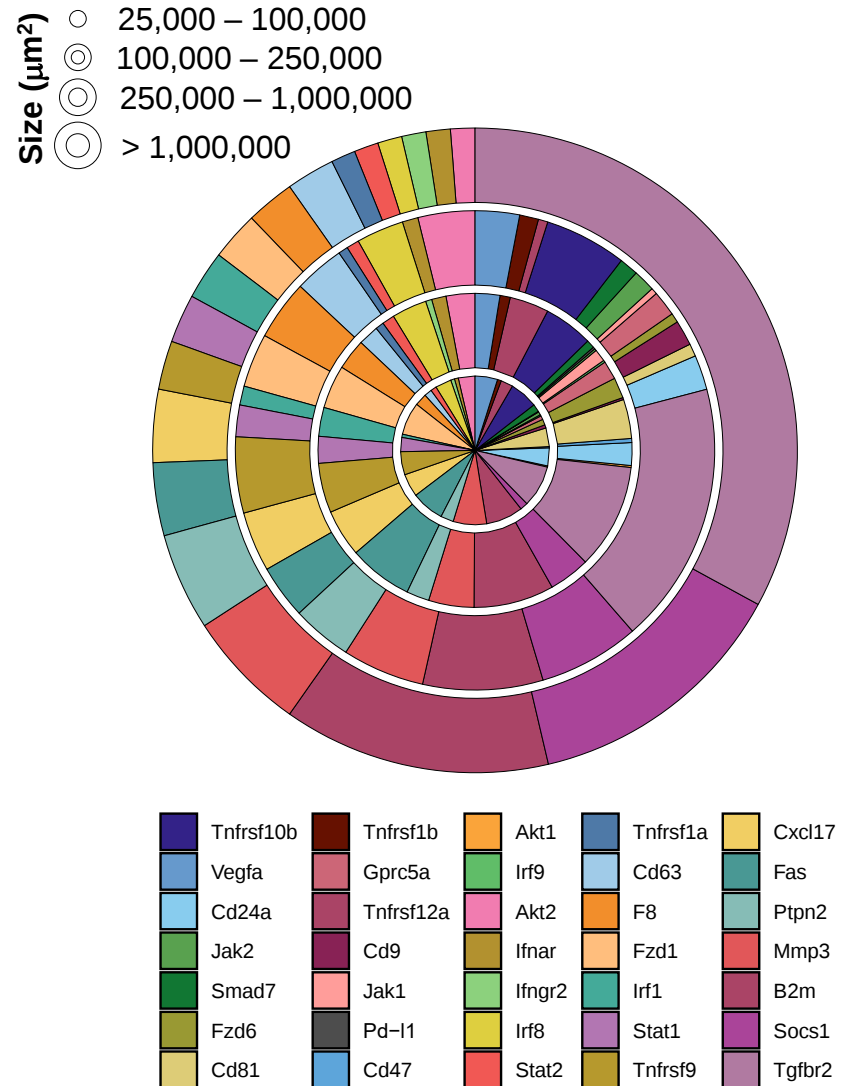
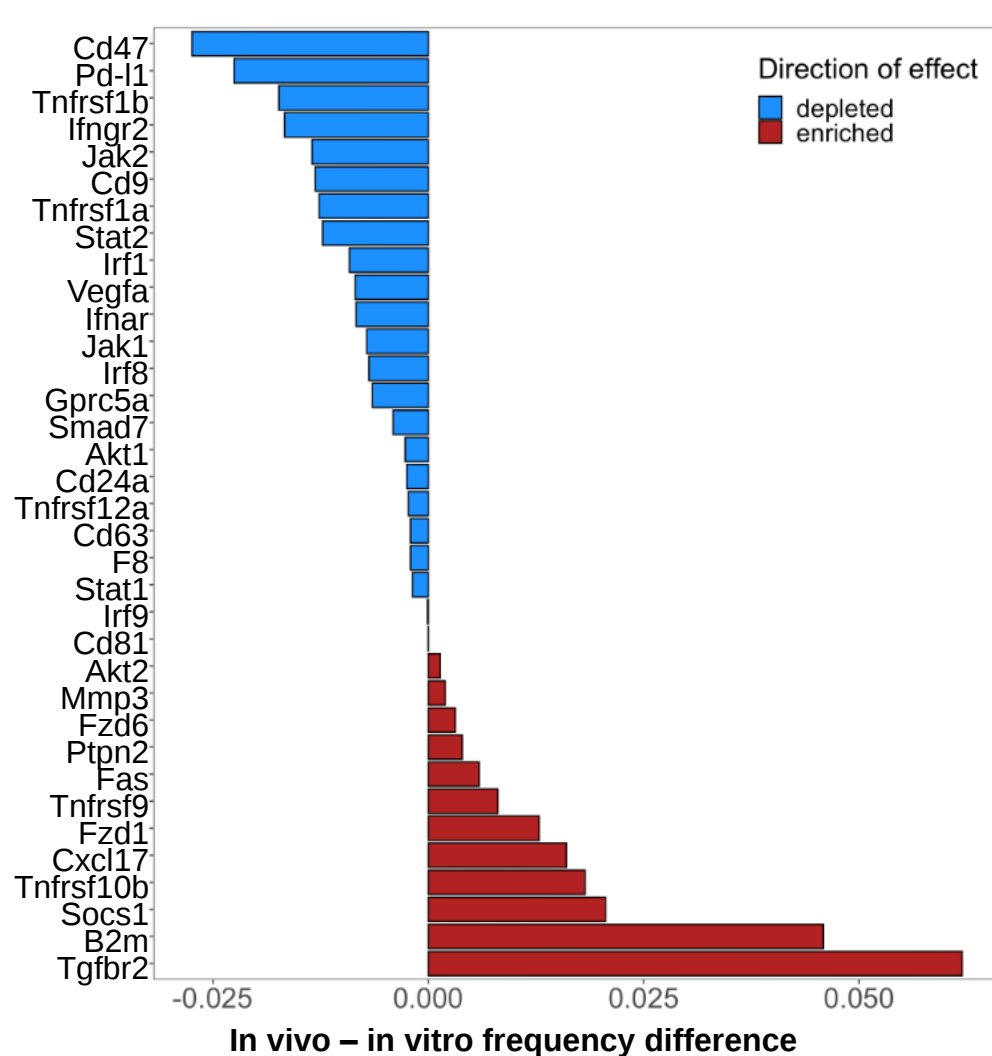
~100 - 200
lung tumor
lesions/mouse



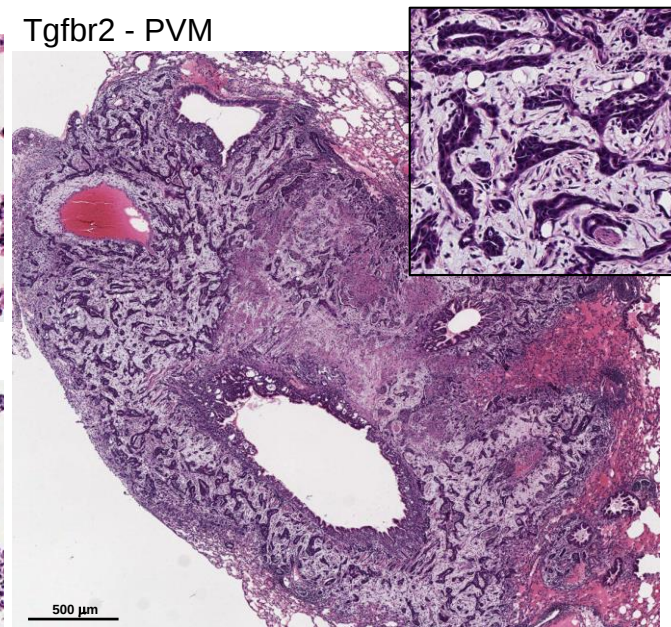
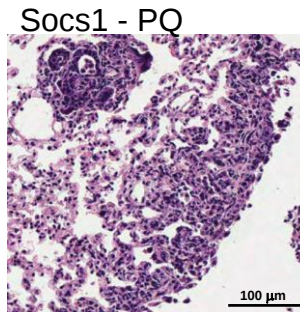
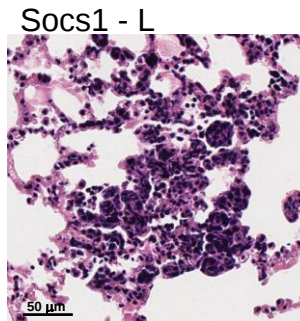
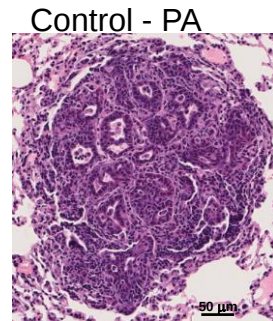
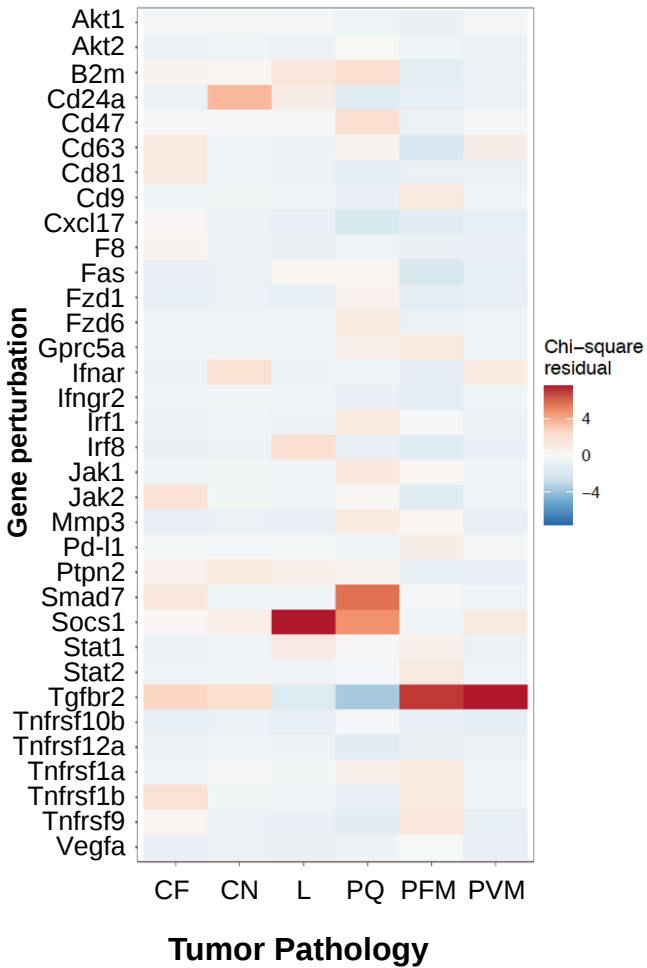
Spatial Mapping of Pro-Code/CRISPR Tumor Lesions



Loss of IFN γ signaling activity diminishes tumor growth, whereas loss of TGF β R2 enhances tumor growth

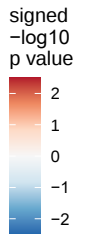
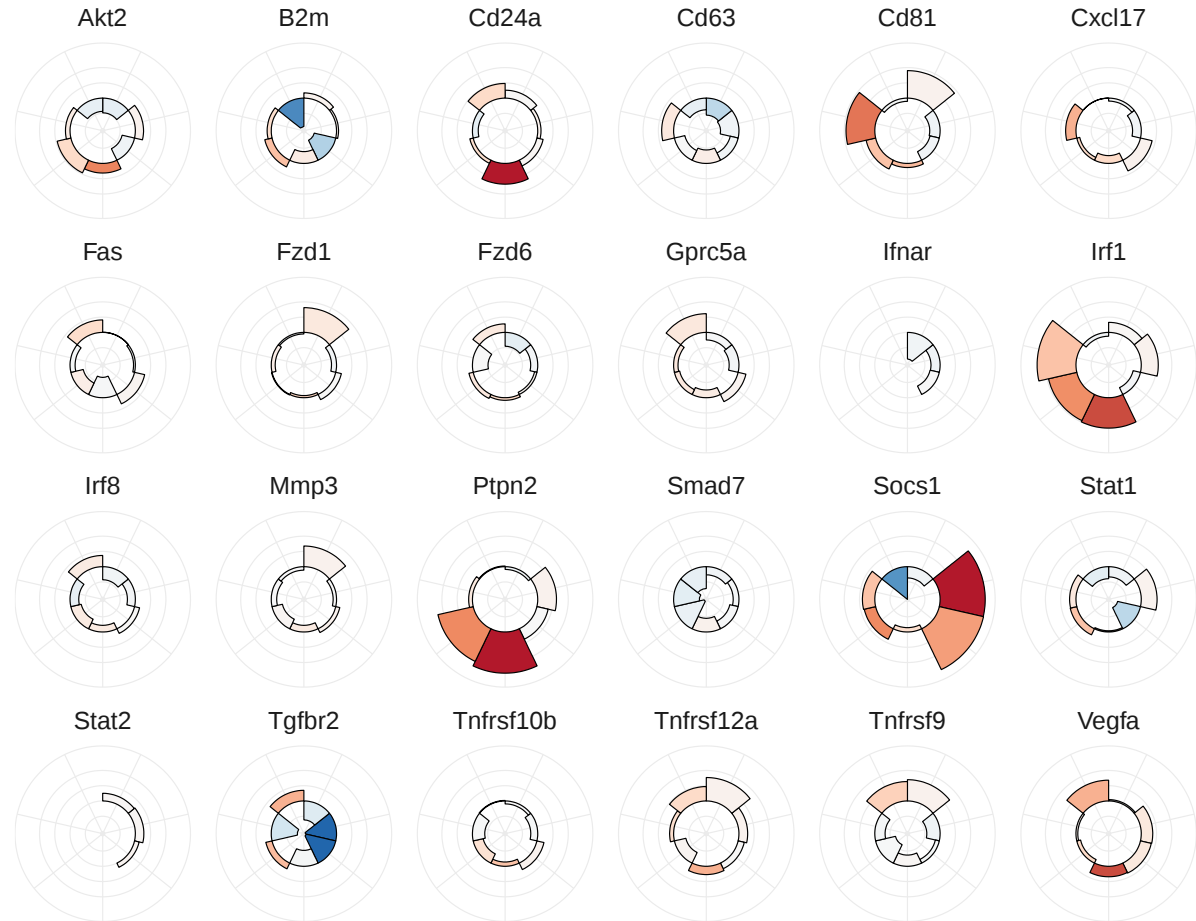
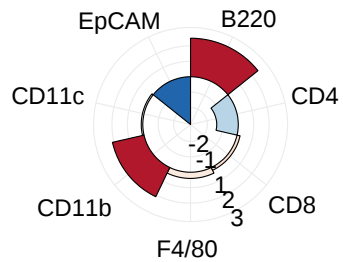
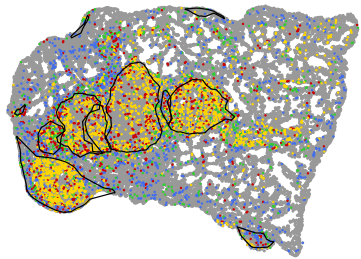
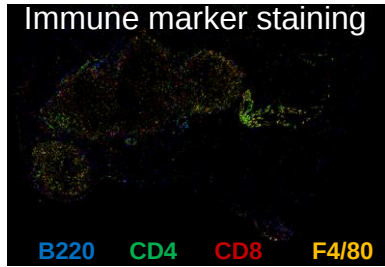
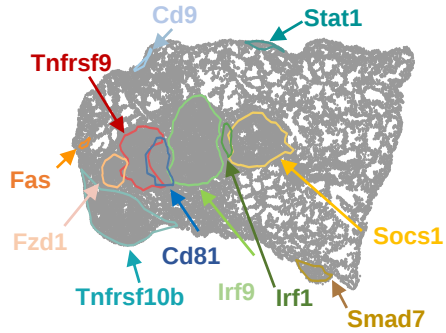


Loss of Socs1 and TGF β R2 results in remodeling of tumor architecture



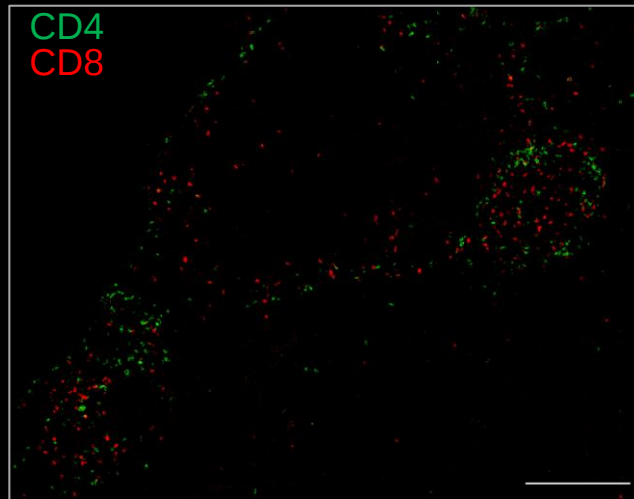
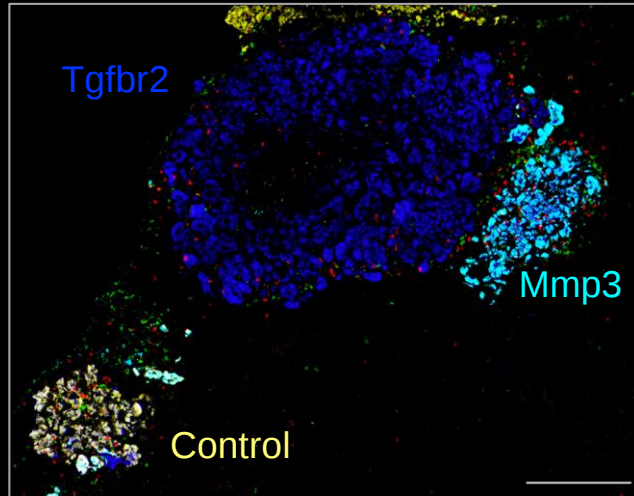
CF=central fibrotic; CN=central necrotic; L=lepidic;
 PQ= poorly differentiated pleural plaque;
 PFM=fibro-mucinous; PVM=perivascular mucinous

Cancer cell gene loss influence on tumor immune composition

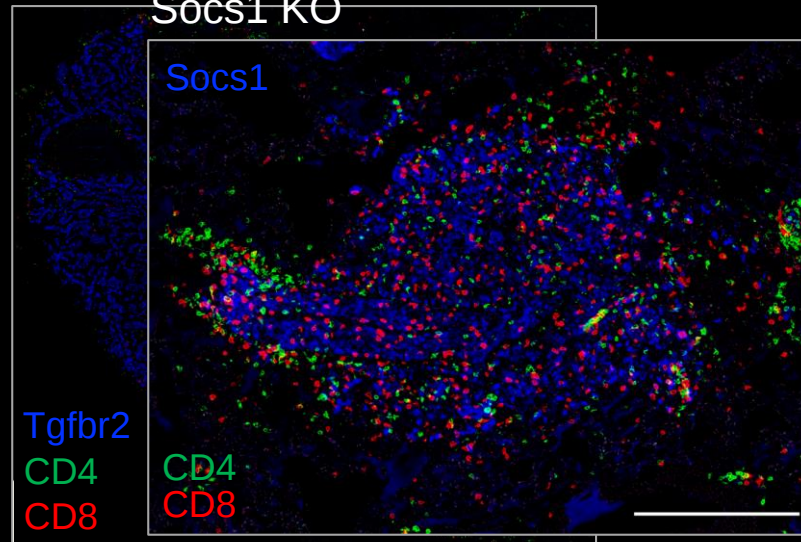


Tgfbr2 loss leads to cold tumors while Socs1 loss leads to hot tumors

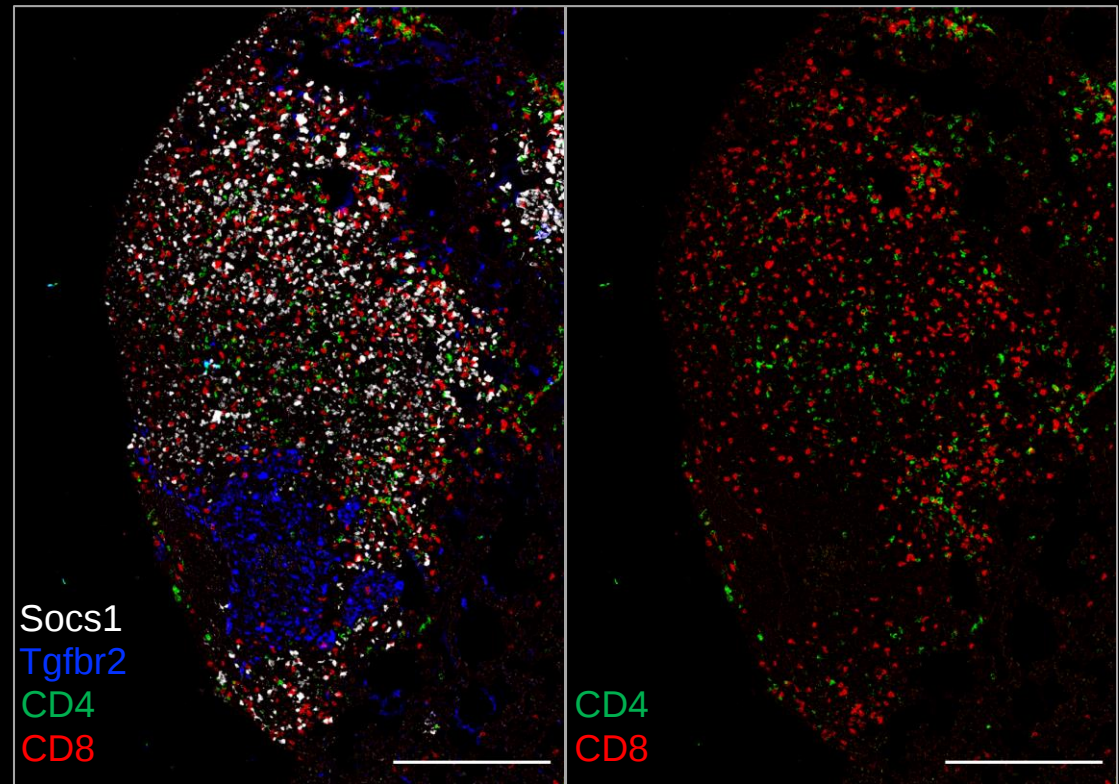
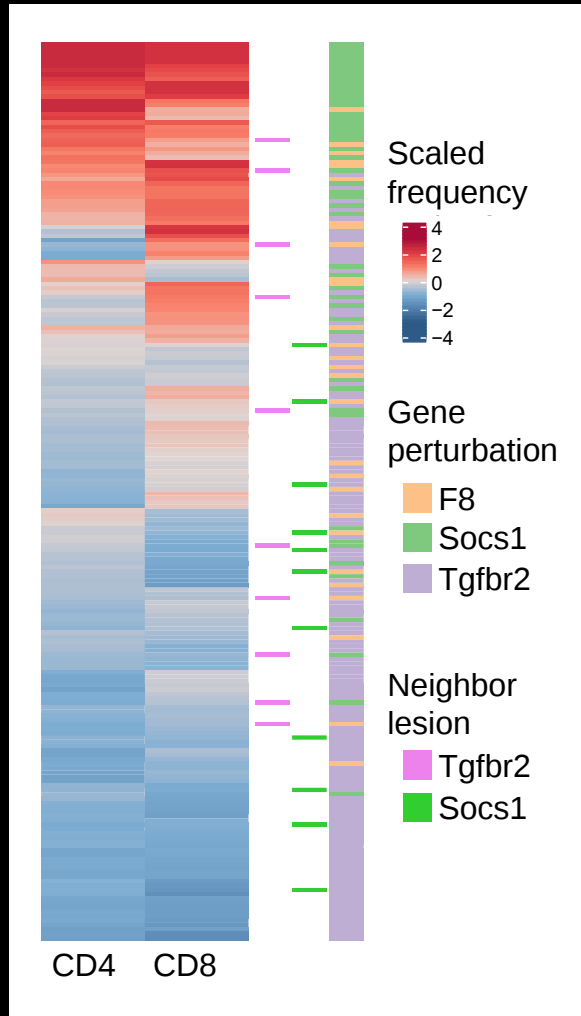
Tgfbr2 KO



Socs1 KO



Sub-clonal immune composition is highly local



Genetically-distinct subclonal differences in immune composition are being discovered in human tumors

Spatially resolved analyses link genomic and immune diversity and reveal unfavorable neutrophil activation in melanoma

Mitra et al. Nature Communications 2020

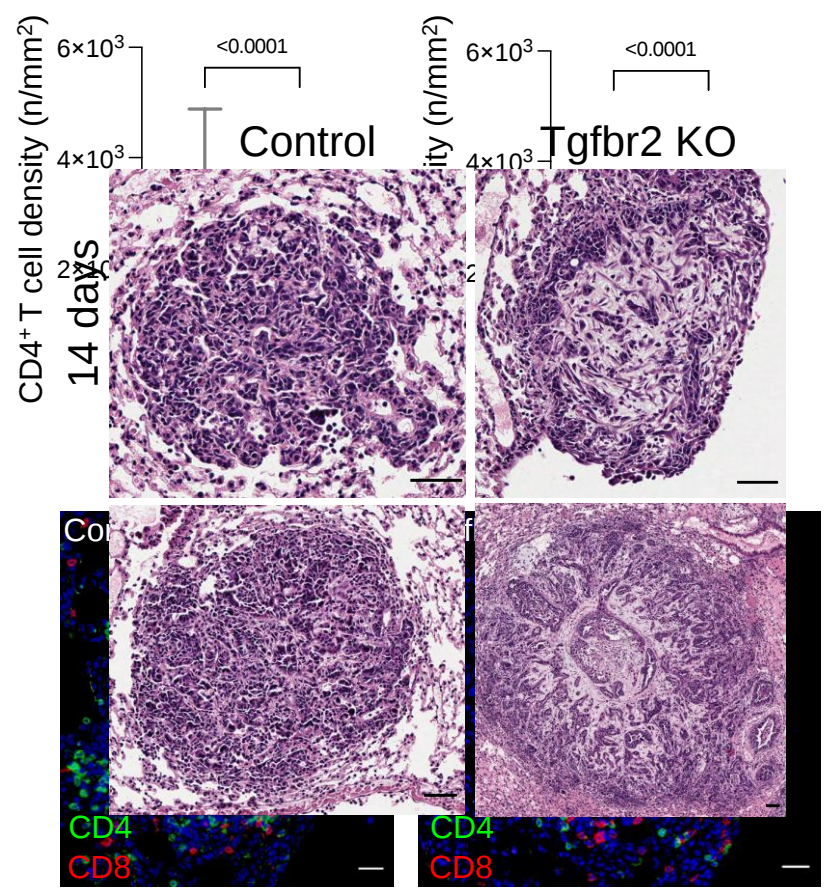
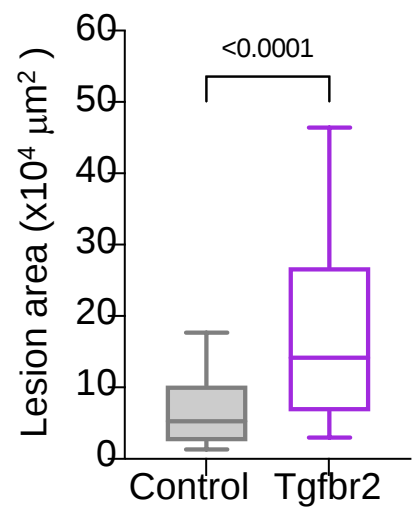
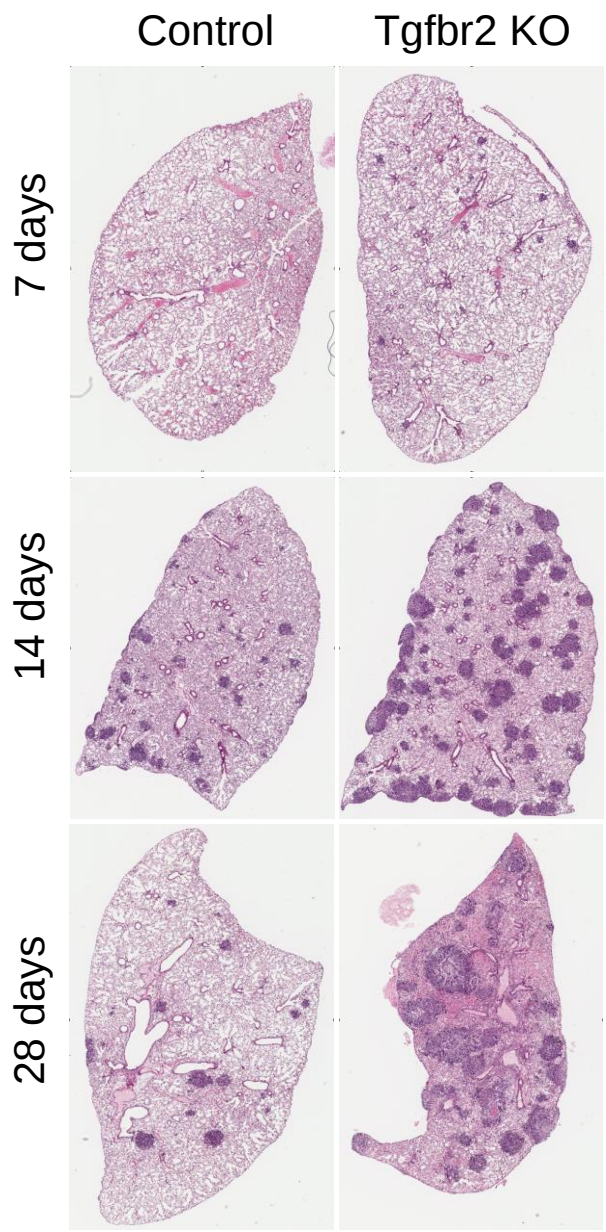
“We identify spatially distinct evolution of copy number alterations influencing local immune composition. Sub-regions with chromosome 7 gain display a relative lack of leukocyte infiltrate ... and associated with lack of response to IO across three clinical cohorts”

Spatial genomics maps the structure, character and evolution of cancer clones

Lomakin et al. BioRxiv 2021

“Distinct transcriptional, histological and immunological features distinguish coexistent genetic clones”

Tgfr2 loss promotes lung tumor aggression and remodels the TME



Tgfbr2 loss associated with more aggressive human lung adenocarcinoma

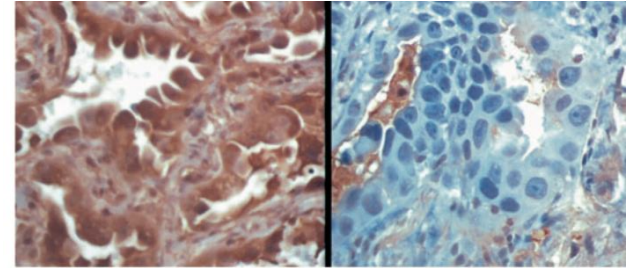
Lung Adenocarcinoma Global Profiling Identifies Type II Transforming Growth Factor- β Receptor as a Repressor of Invasiveness

Borcuk et al. Am J Respir Crit Care Med 2005

TGF β R2
staining (brown)

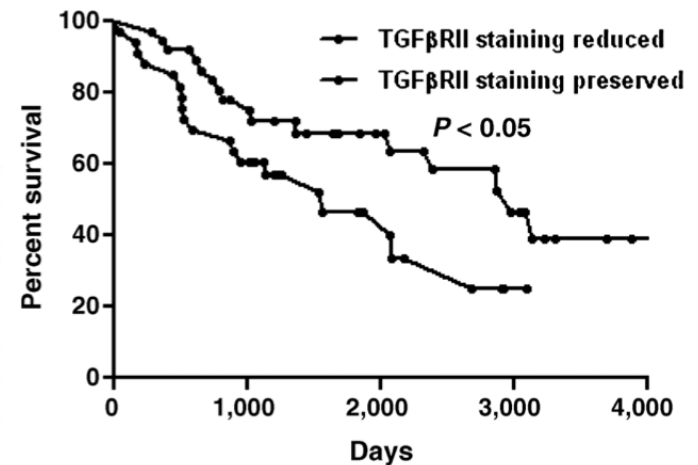
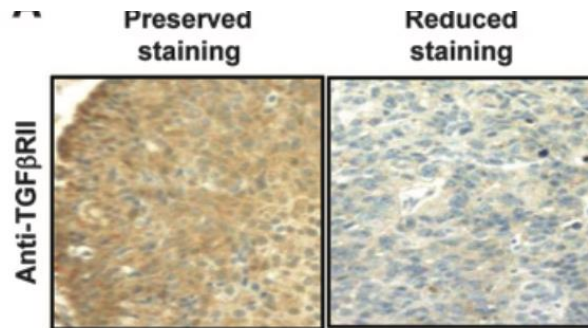
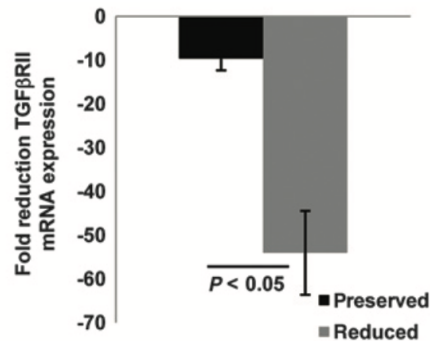
Noninvasive
bronchioloalveolar
carcinoma

Invasive
adenocarcinoma

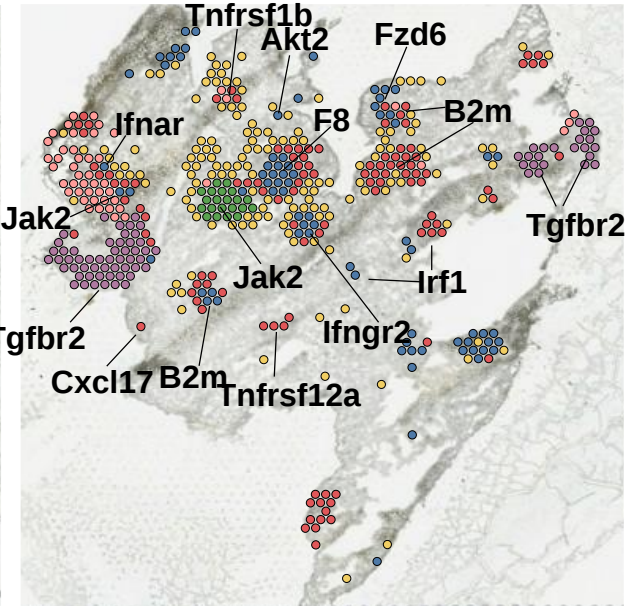
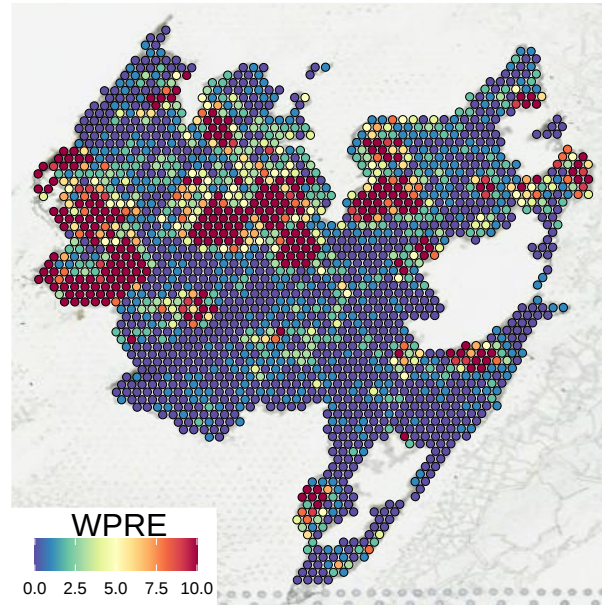
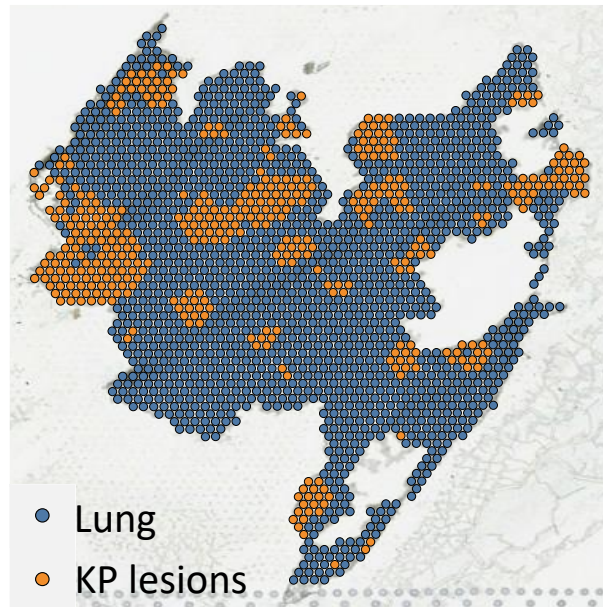


Loss of Transforming Growth Factor Beta Type II Receptor Increases Aggressive Tumor Behavior and Reduces Survival in Lung Adenocarcinoma and Squamous Cell Carcinoma

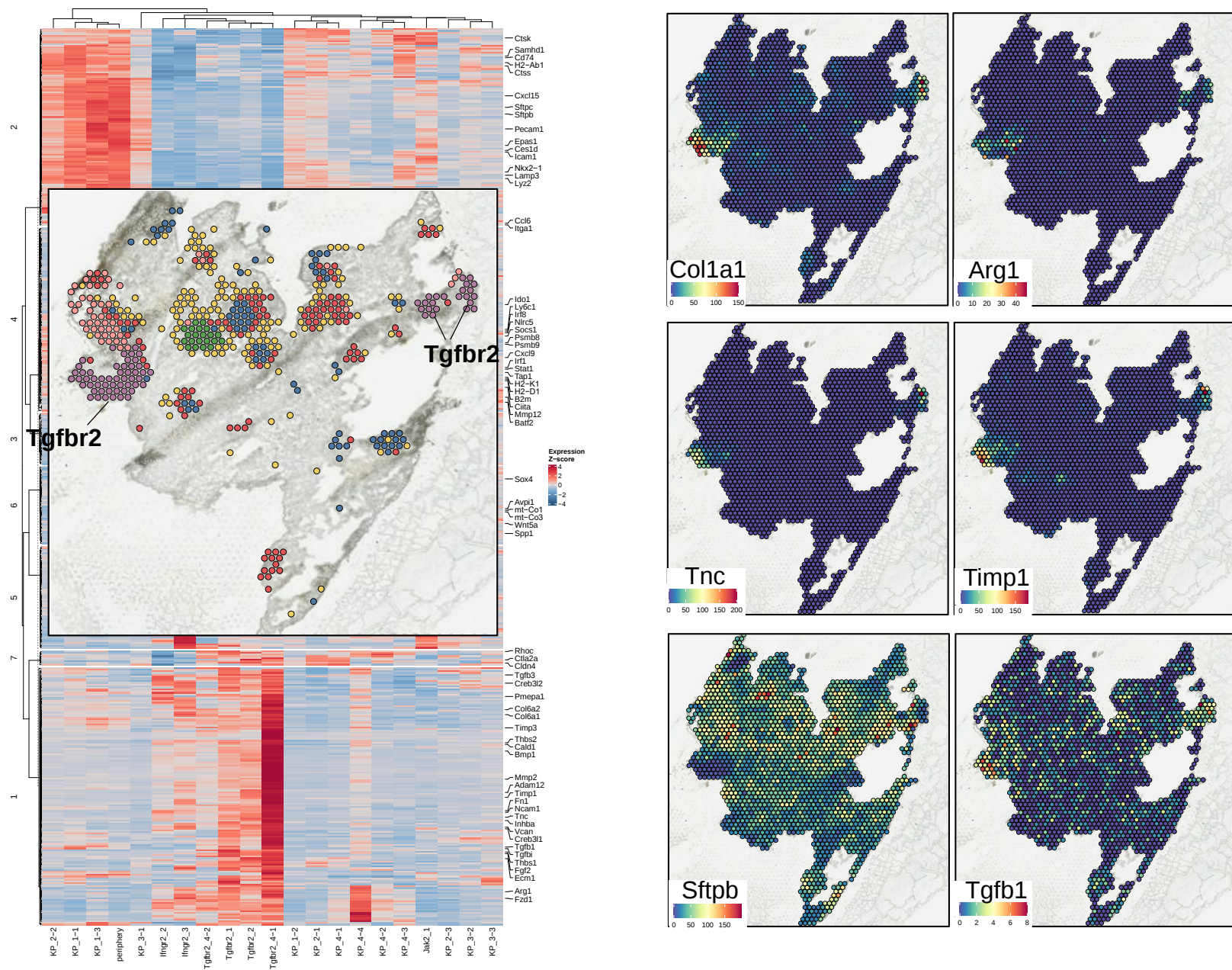
Malkoski et al. Clin Can Res 2011



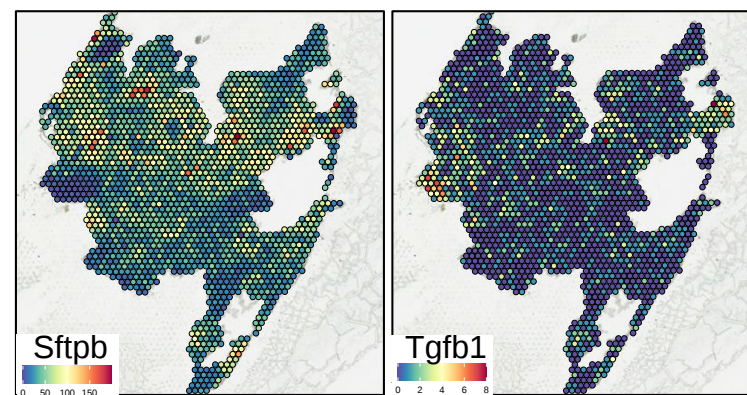
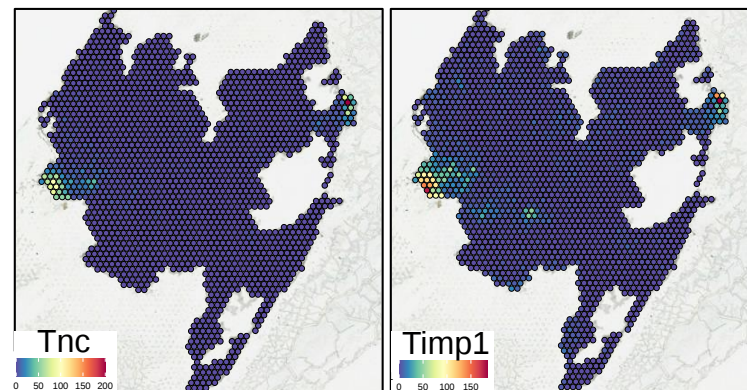
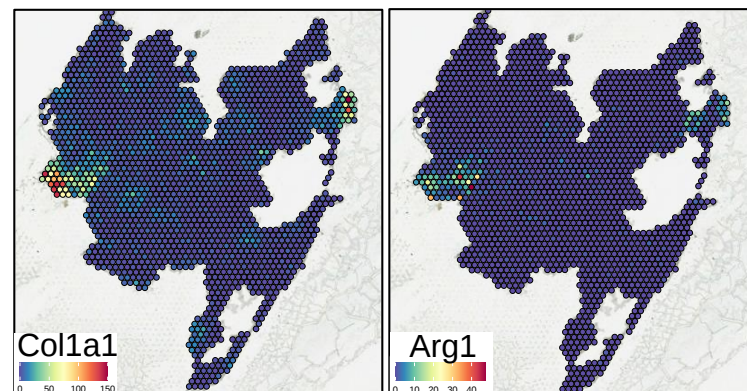
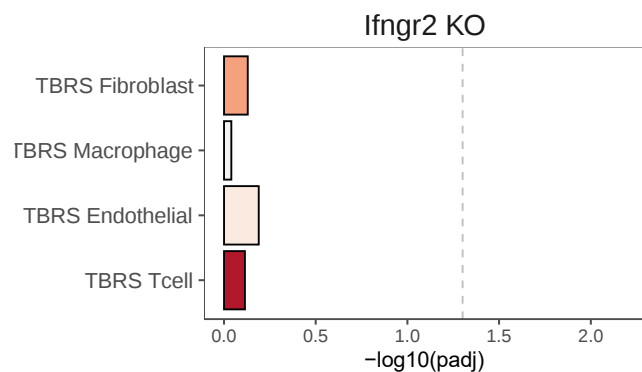
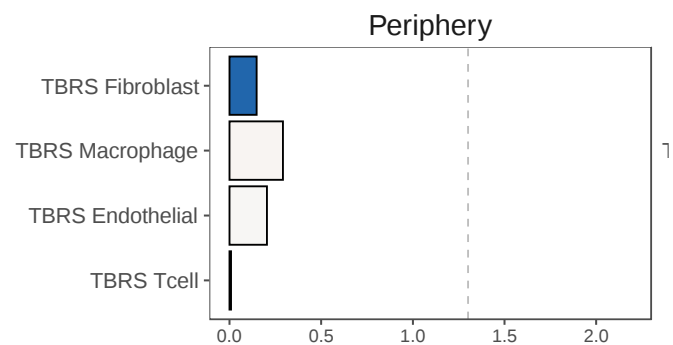
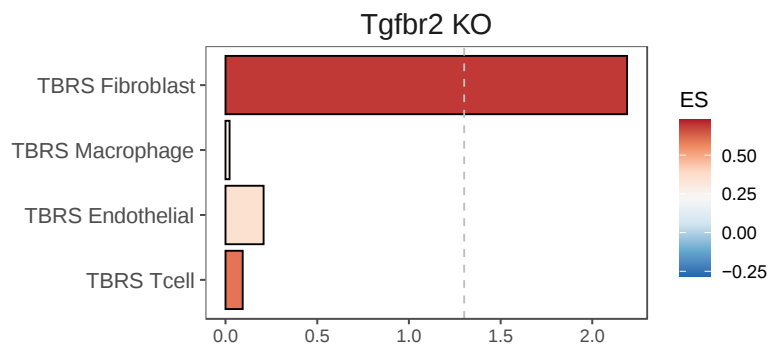
Perturb-map spatial transcriptomic analysis



Tgfb β 2 loss increases Tgfb and associates with TGF β stromal signature



Tgfb β loss increases Tgfb and associates with TGF β stromal signature



Summary

- Established Perturb-map: a platform for spatially resolved functional genomic screens within tissue.
- Perturb-map found genes positively regulating IFN γ signaling (Ifngr2, Jak2, Irf1, Stat2, Socs1) negatively regulated tumor growth.
- Loss of Socs1 on lung cancer cells promoted tumor growth, de-differentiation & altered localization, and enhanced T cell infiltration.
- Loss of Tfbp2 on lung cancer cells promoted tumor growth, TME conversion to a fibro-mucinous and T cell excluded state.
- Tfbp2 loss leads to increased TGF β pathway activation in the tumor
→ likely via upregulated TGF β and increased bioavailability through receptor loss on cancer cells.

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Eun Park
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Miriam Merad
Adeeb Rahman
Sacha Gnjatich
Guray Akturk
Mark Buckup
Vladimir Roudko
Dana Pe'er



INNOVATIVE MOLECULAR
ANALYSIS TECHNOLOGIES

Mount Sinai
Precision
Immunology
Institute
(PrIISM)

