Single-nucleus and spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated with neoadjuvant treatment

William L. Hwang, Karthik A. Jagadeesh, Jimmy A. Guo, Hannah I. Hoffman, Payman Yadollahpour, Jason W. Reeves, Rahul Mohan, Eugene Drokhlyansky, Nicholas Van Wittenberghe, Orr Ashenberg, Samouil L. Farhi, Denis Schapiro, Prajan Divakar, Eric Miller, Daniel R. Zollinger, George Eng, Jason M. Schenkel, Jennifer Su, Carina Shiau, Patrick Yu, William A. Freed-Pastor, Domenic Abbondanza, Arnav Mehta, Joshua Gould, … Aviv Regev ✉ + Show authors
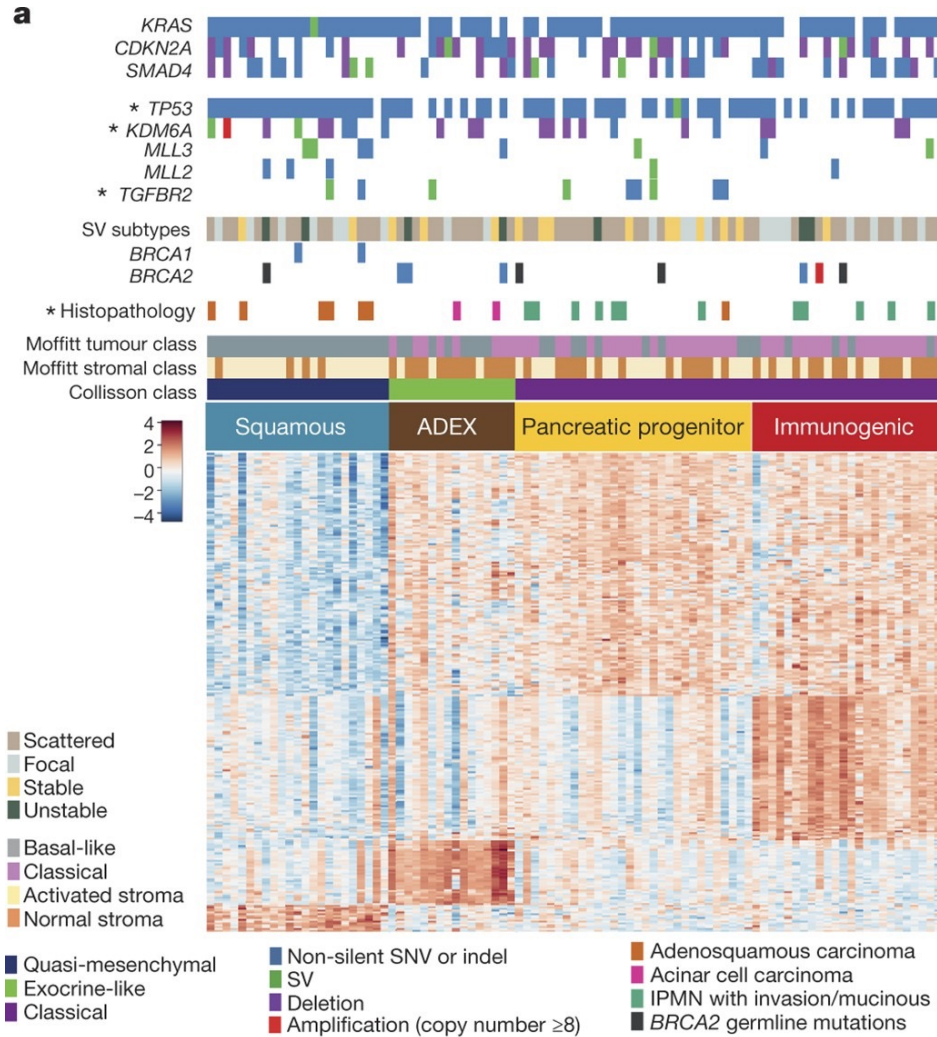
2022/10/26　抄読会

呼吸器外科　末吉担当

Background
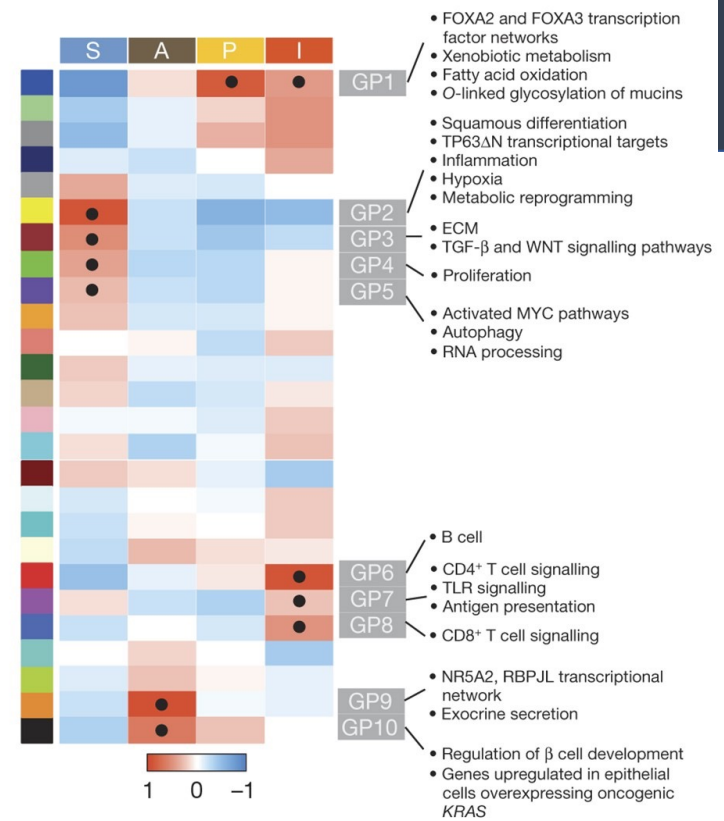
- Pancreatic ductal adenocarcinoma (PDAC)
  - classical/epithelial
  - basal-like/squamous/quasi-mesenchymal

- scRNAseq is challenging to apply to PDAC
  - intrinsic nuclease activity
  - desmoplastic stroma

- Single-nucleus RNA sequencing (snRNA-seq)
  - compatible with frozen samples
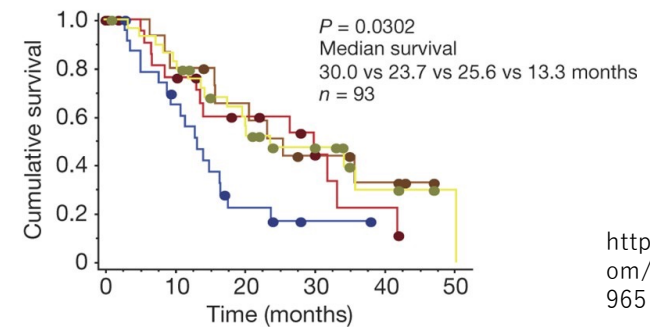 - better recovery of malignant and stromal cells
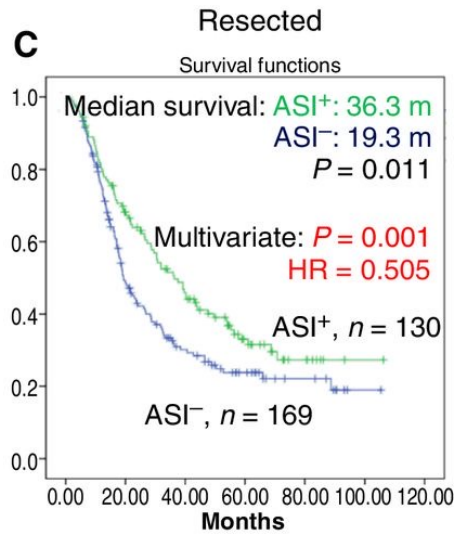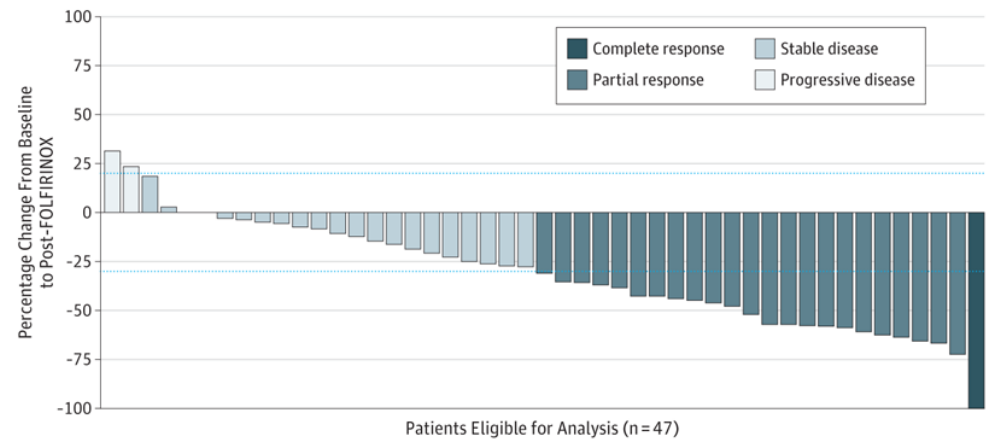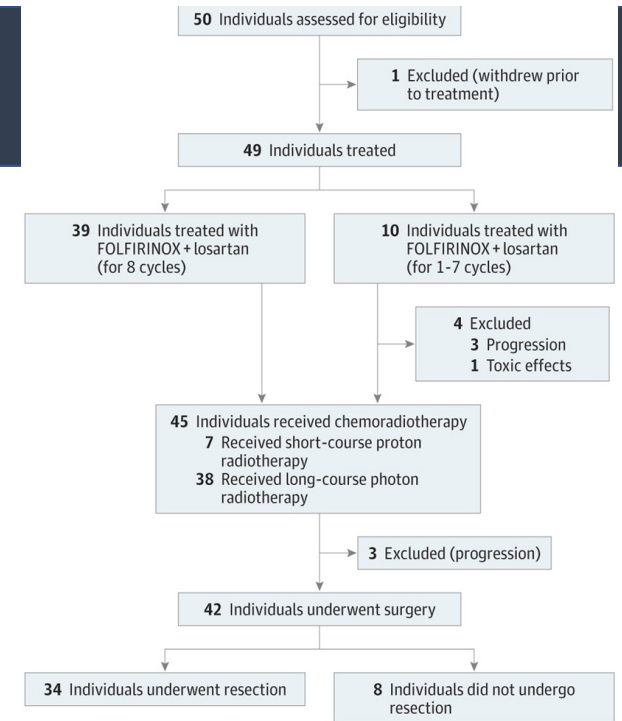
PDAC subtypes

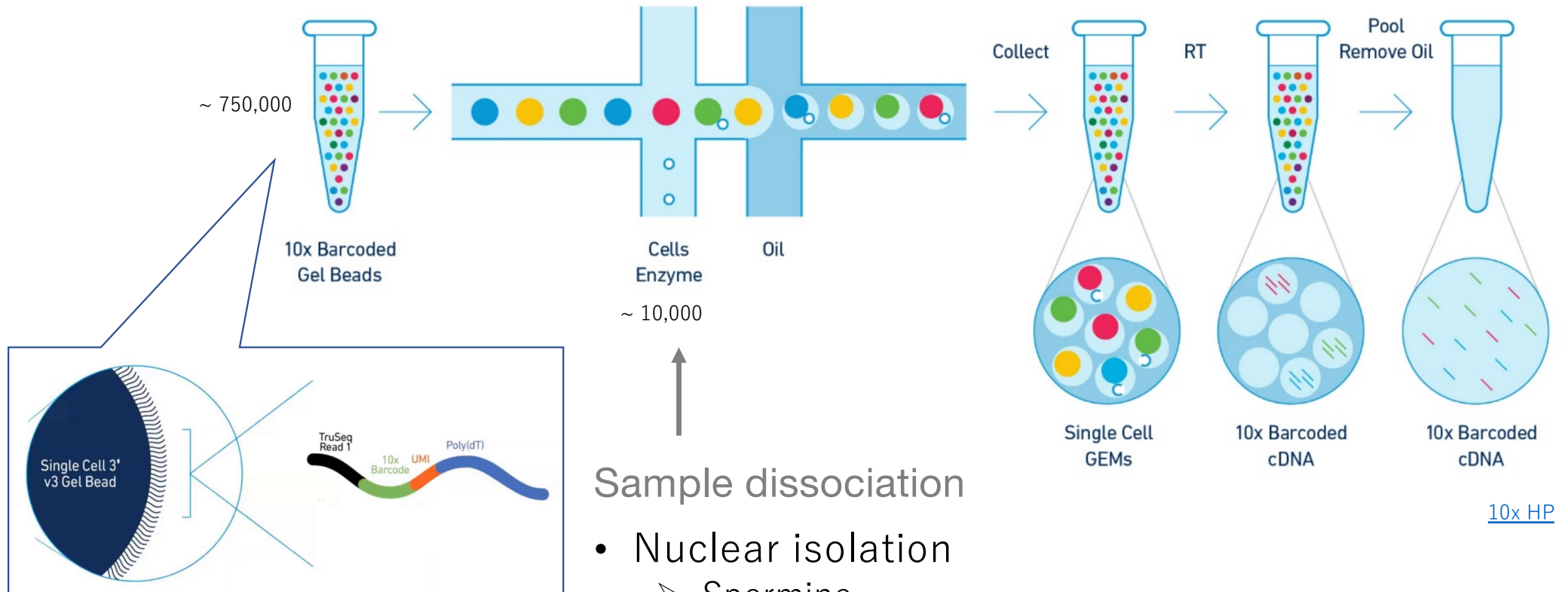https://www.nature.com/articles/nature16965

Angiotensin systemic inhibitor (ASI) use and PDAC

Resected

Survival functions

Median survival: ASI+: 36.3 m
ASI−: 19.3 m
P = 0.011

Multivariate: P = 0.001
HR = 0.505

ASI+, n = 130

ASI−, n = 169

69% R0 resection

10.1001/jamaoncol.2019.0892

doi.org/10.1158/1078-0432.CCR-17-0256

Single cell/nucleus sequence

~ 750,000

10x Barcoded Gel Beads

Single Cell 3' v3 Gel Bead

TruSeq Read 1 · 10x Barcode · UMI · Poly(dT)

Cells Enzyme · Oil

~ 10,000

Collect · RT · Pool Remove Oil

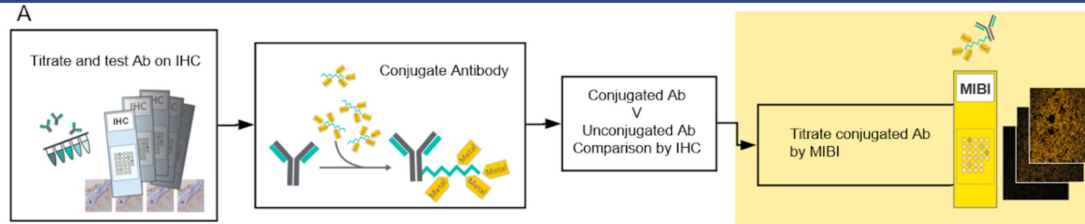Single Cell GEMs · 10x Barcoded cDNA · 10x Barcoded cDNA

10x HP

Sample dissociation

- Nuclear isolation
  - Spermine

- Cell isolation
  - Collagenase

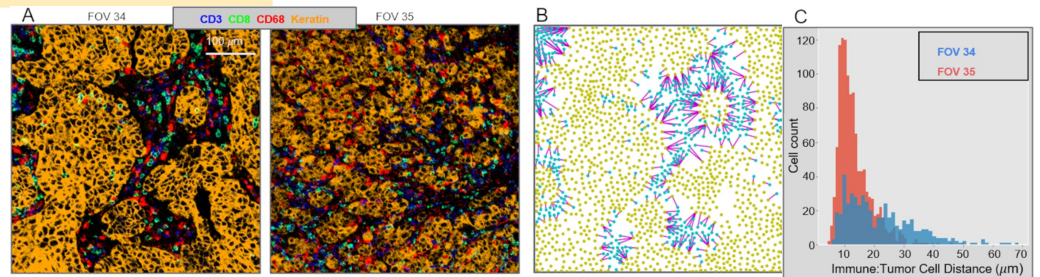# Multiplexed ion beam imaging (MIBI)

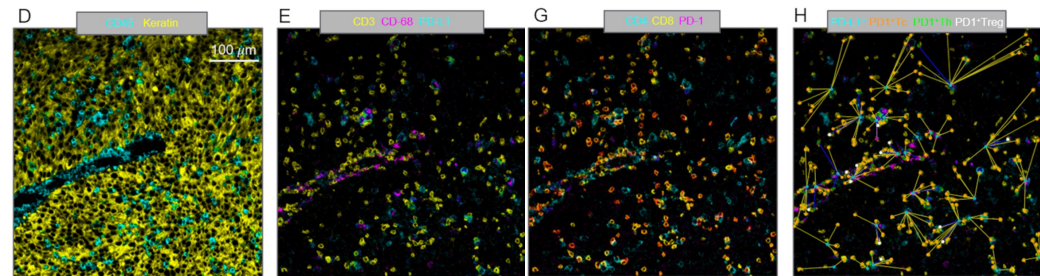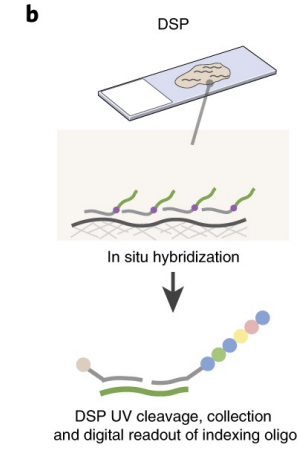Antibody validation isotope labeling

IHC staining and Tof-MASS
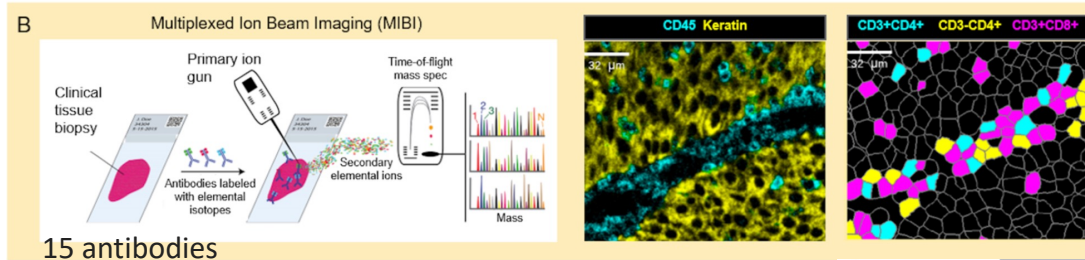
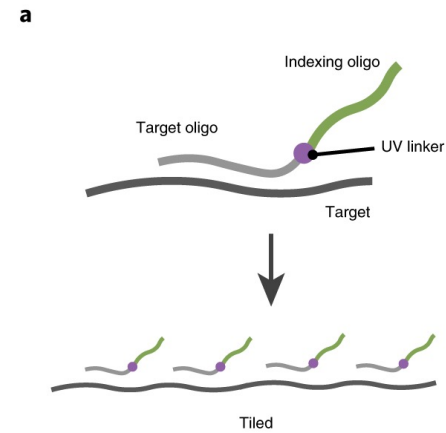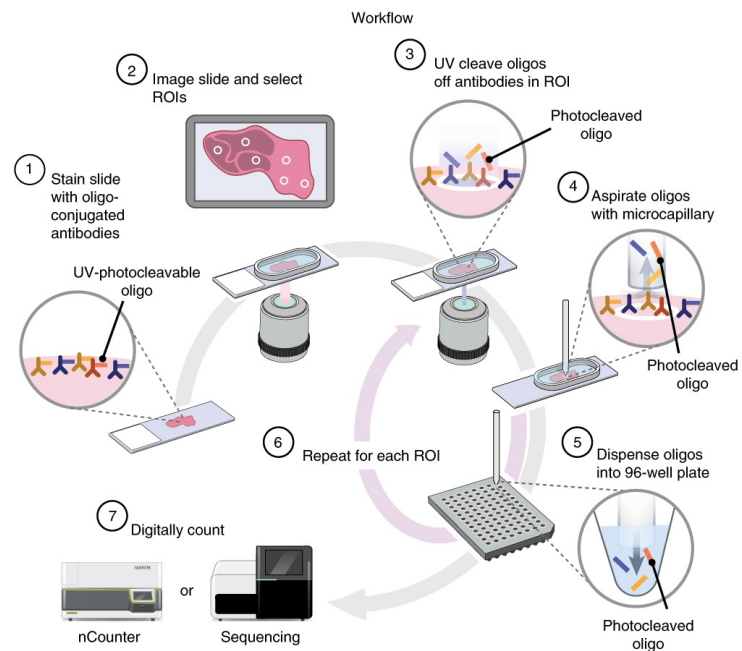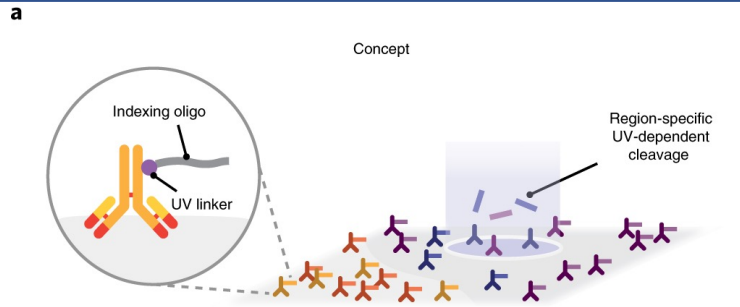Distance of Keratin$^+$ – CD45$^+$ cells in ovarian serous carcinoma
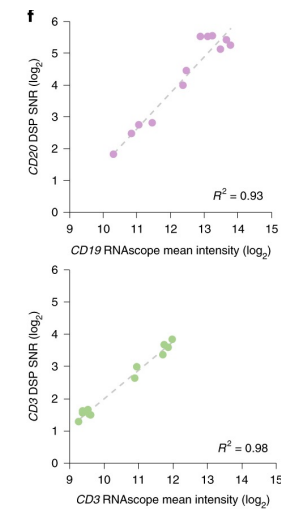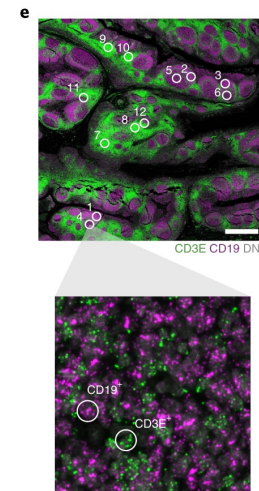
Distance of PDL1$^+$ – PD1$^+$ cells in urothelial carcinoma

Digital spatial profiling (DSP) with NanoString GeoMx whole-transcriptome atlas (WTA)

(Nat Biotechnol 2020)

WTA; 18,269 genes

Results

Fig. 1: snRNA-seq of untreated and treated PDAC captures representative diversity of cell types.
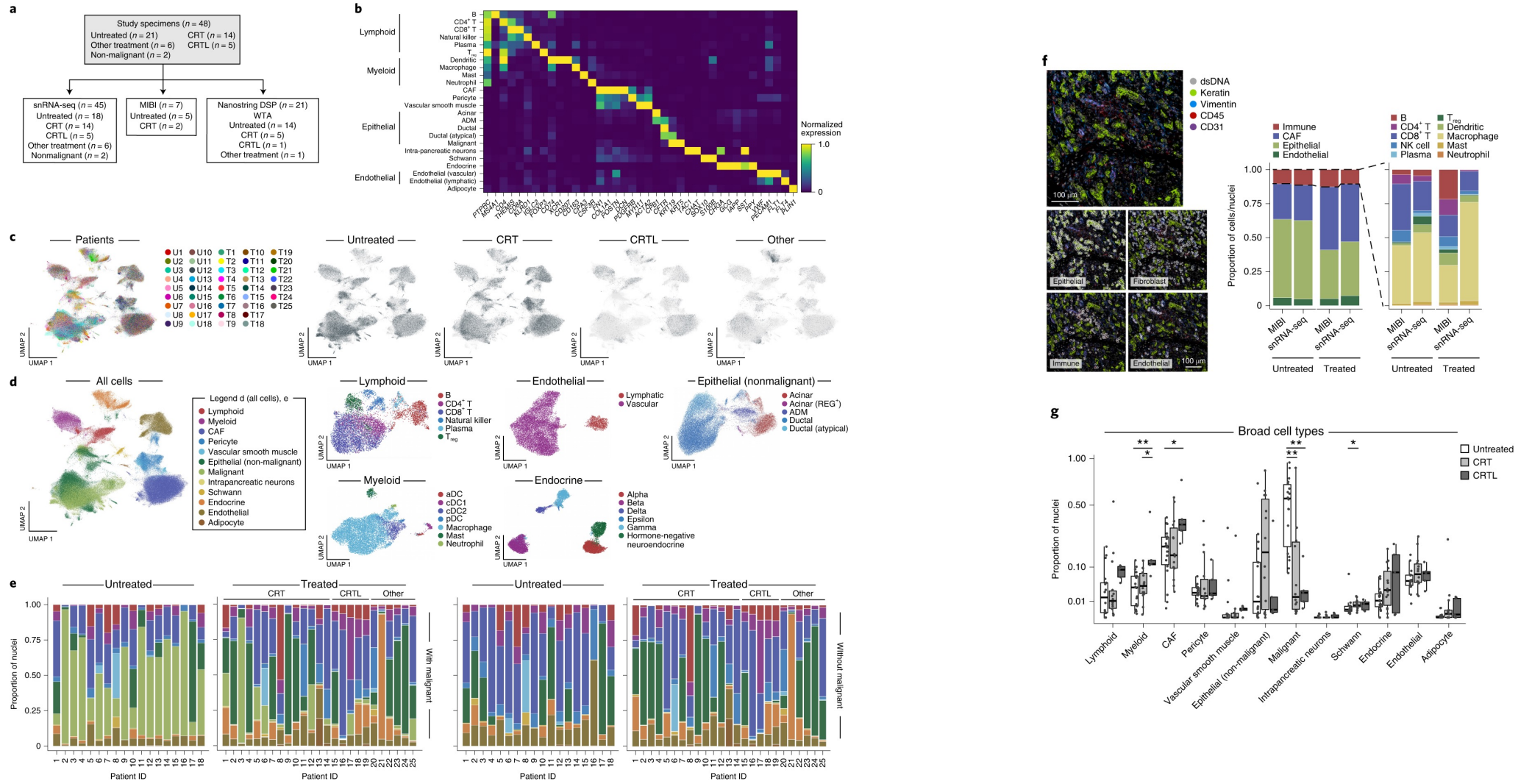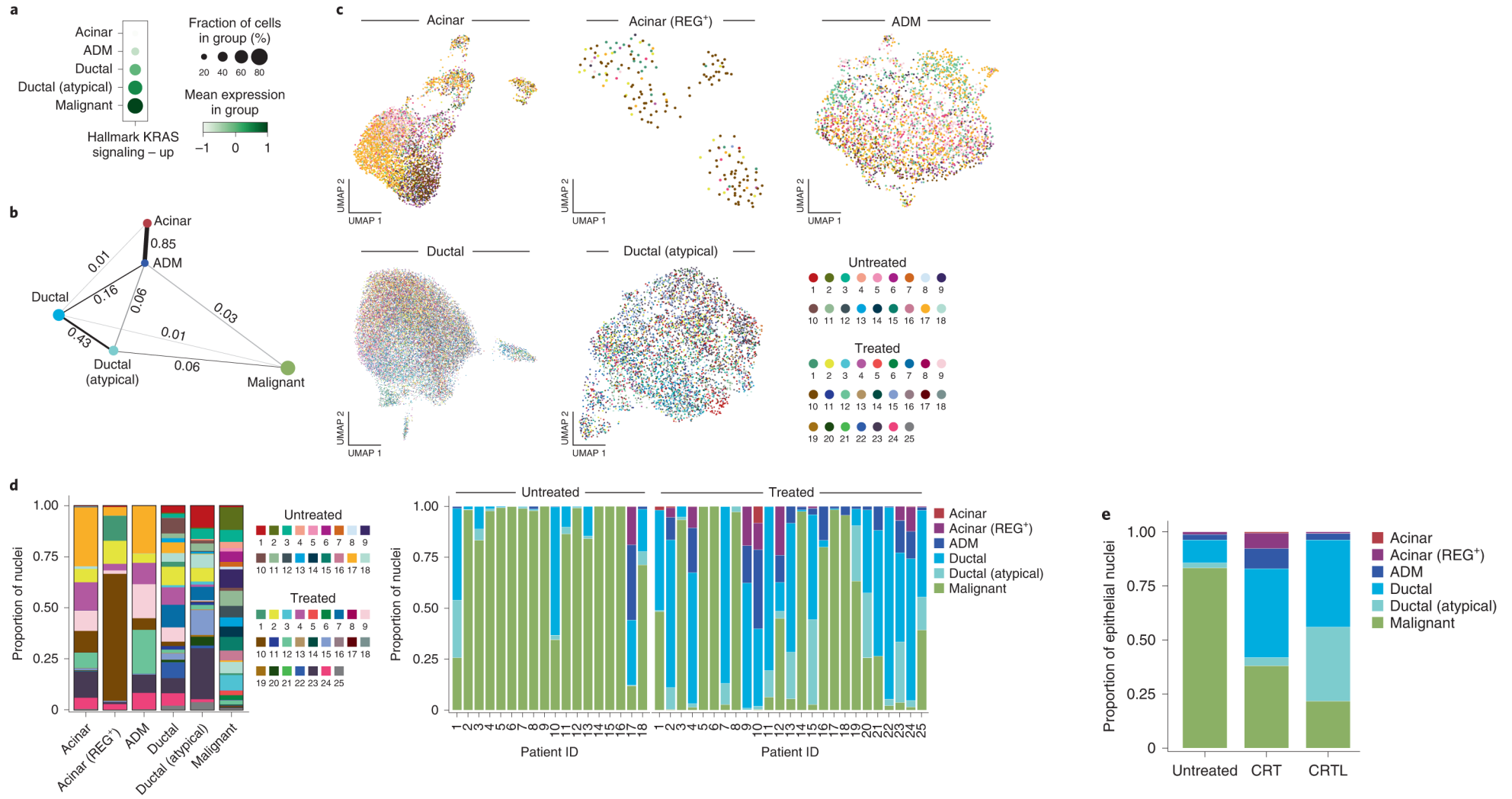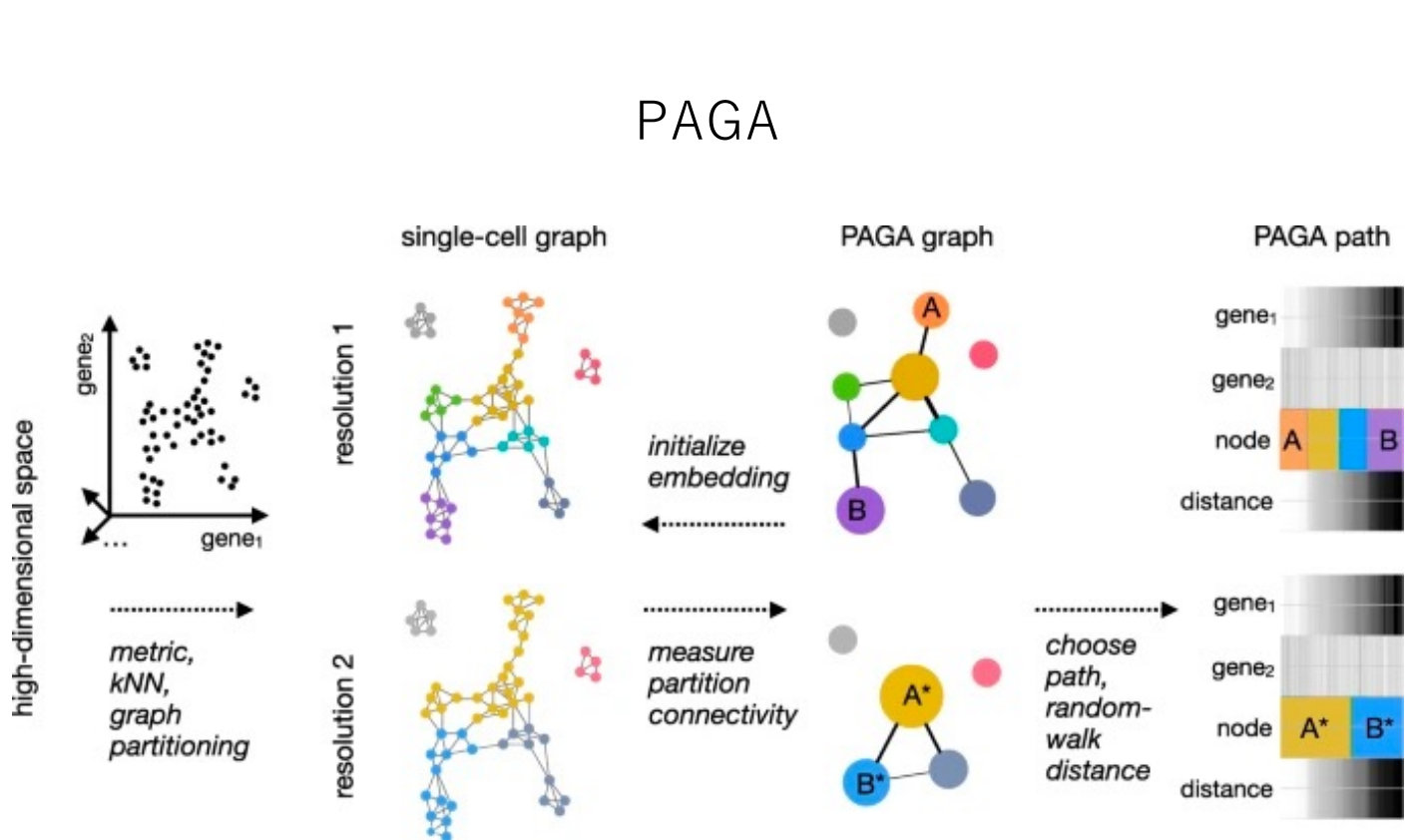
Fig. 2: Epithelial cell-type composition and inferred pseudotemporal trajectory includes putative acinar–ductal metaplasia and atypical ductal intermediates.
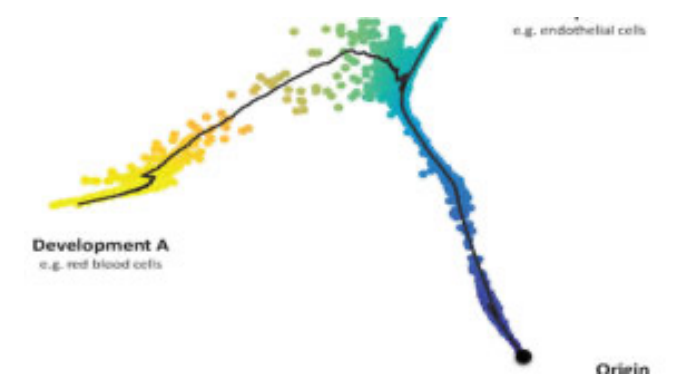
背景追加. Partition-based graph abstraction (PAGA)

PAGA

Pseudotime
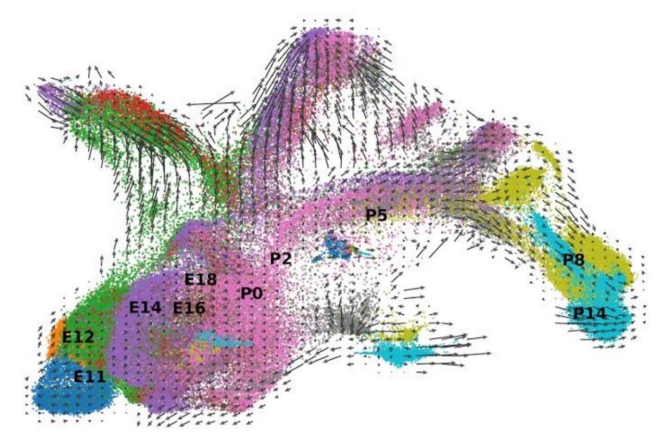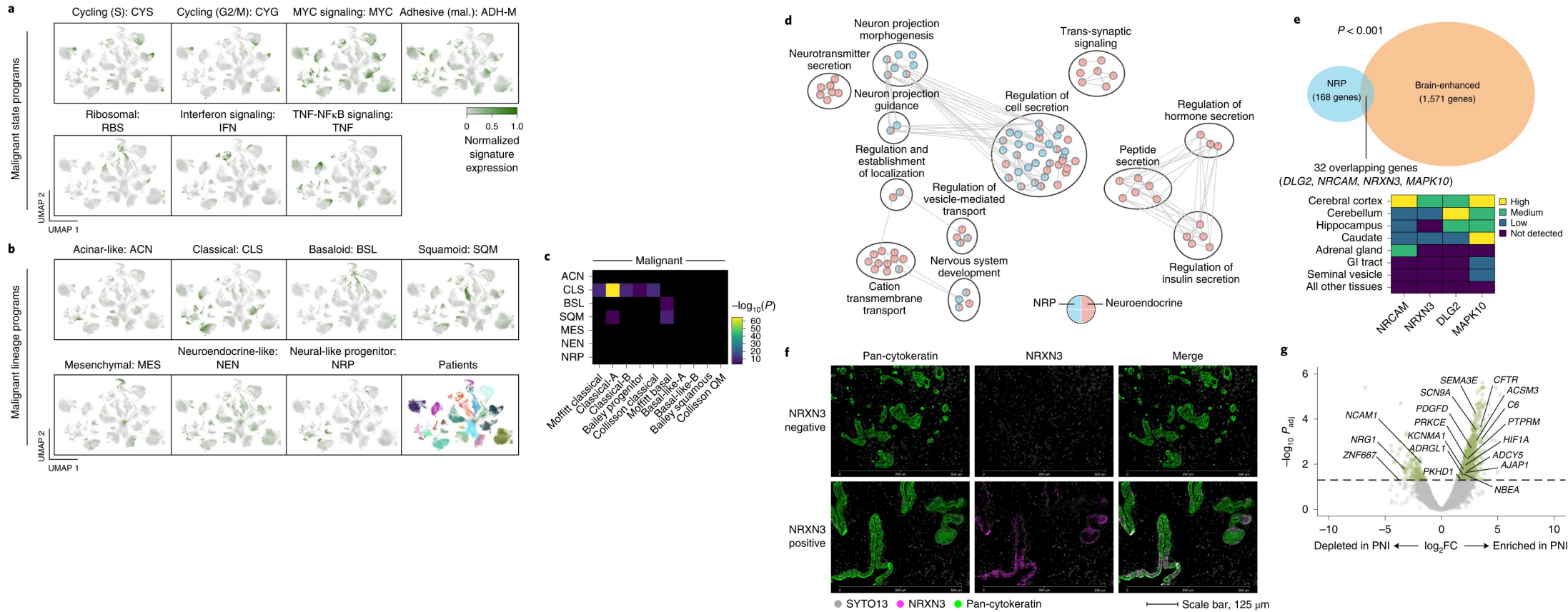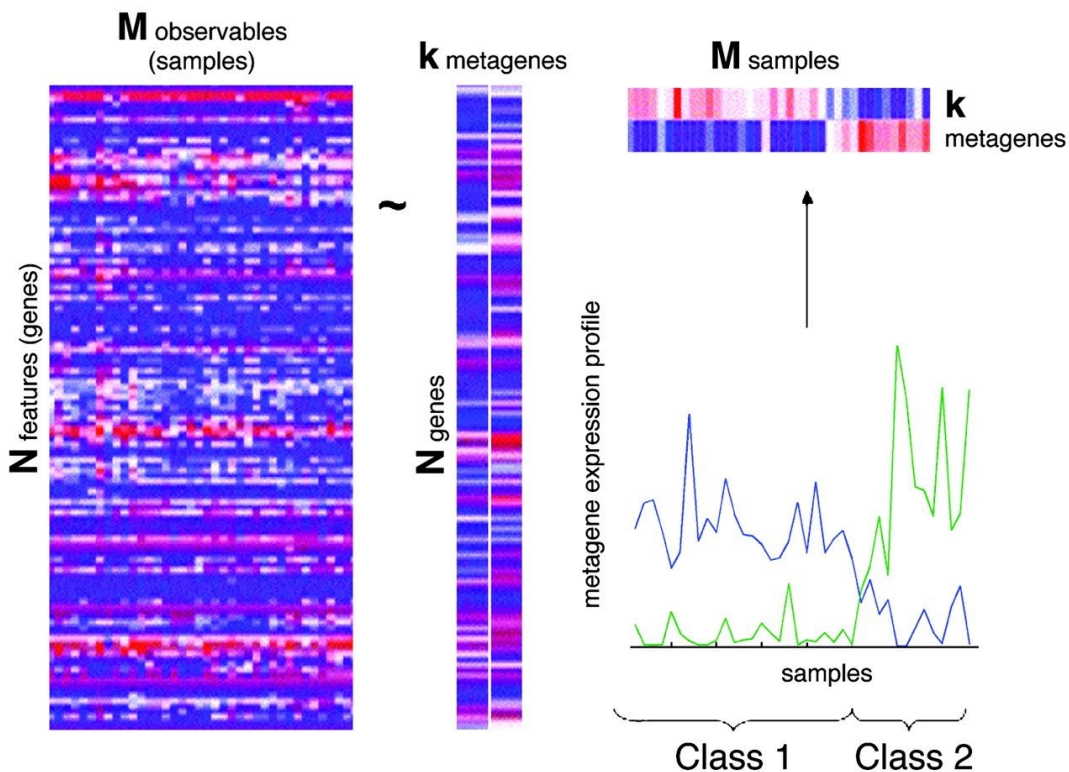
RNA velosity

Genome Biol. 2019

Fig. 3: Molecular stratification of malignant cells in PDAC reveals a neural-like progenitor (NRP) program that is associated with the perineural invasion (PNI).

非負値行列因子分解
＝元の行列を、非負値行列の掛け算形式で、分解する

$$A \text{ (rank M)} \sim W \quad H \text{ (rank k=2)}$$

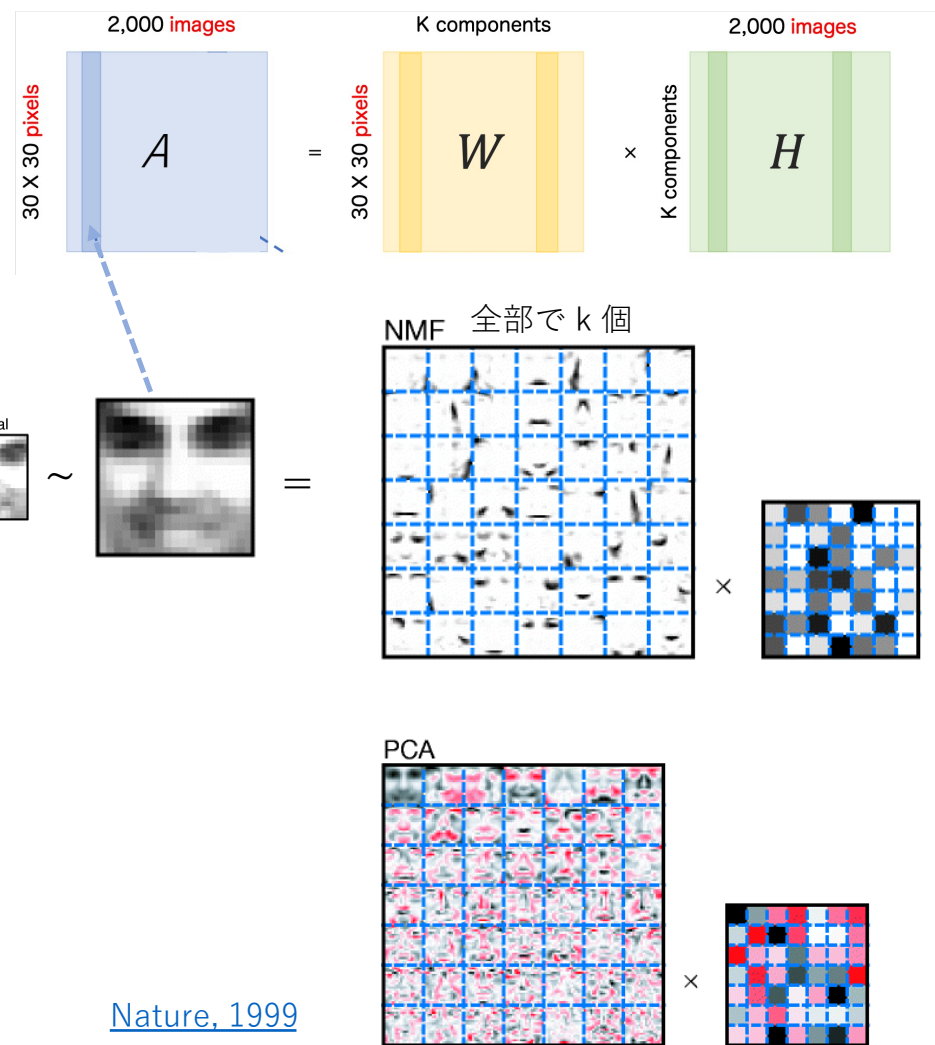
PNAS, 2004

全部で k 個

NMF

PCA

Nature, 1999

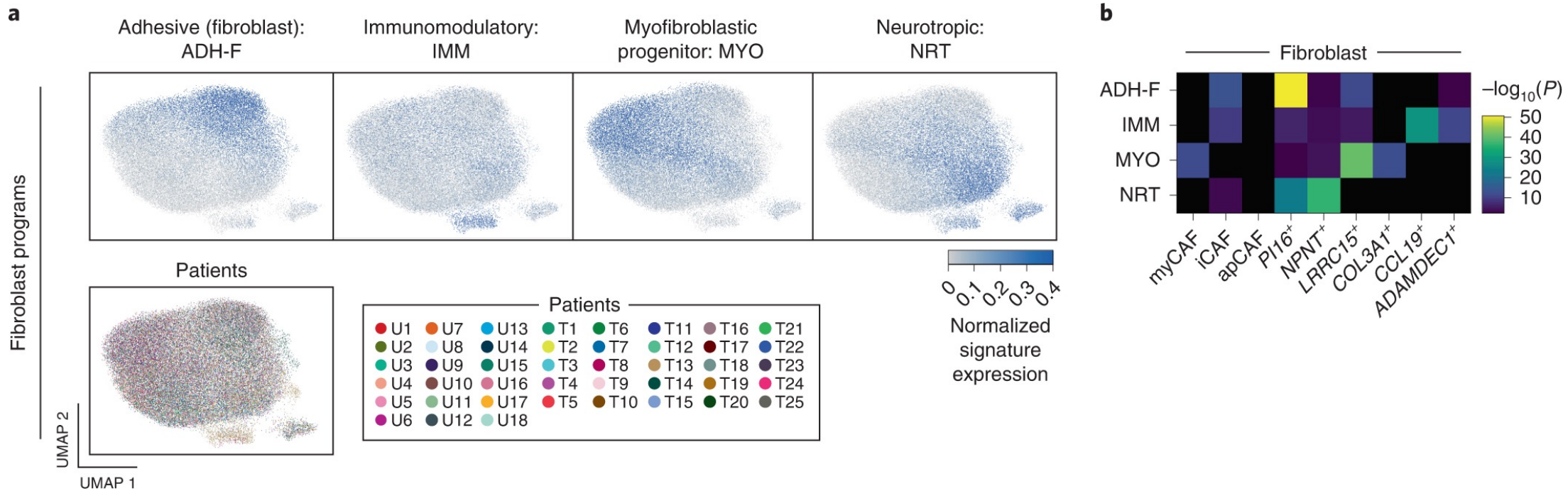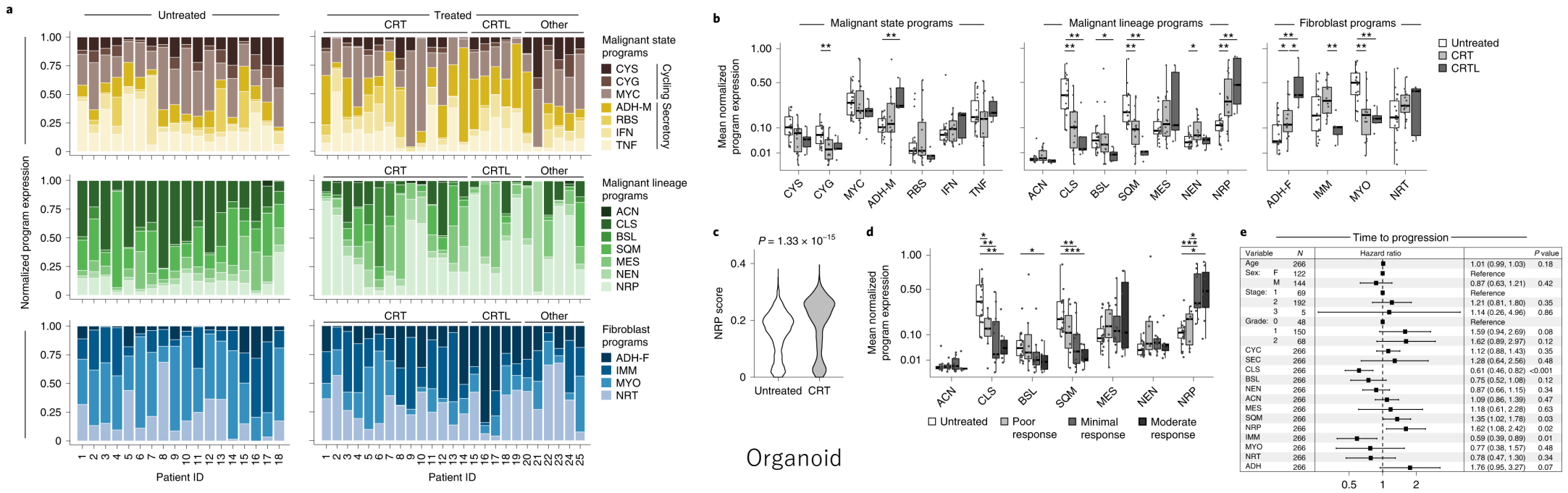Fig. 4: Molecular stratification of CAFs in PDAC.

Fig. 5: The NRP program is enriched in residual tumor and patient-derived organoids after cytotoxic therapy and is associated with poor clinical outcomes.

Fig. 6: Spatial mapping of malignant and CAF programs reveals program-specific associations with intra- versus intertumor heterogeneity.
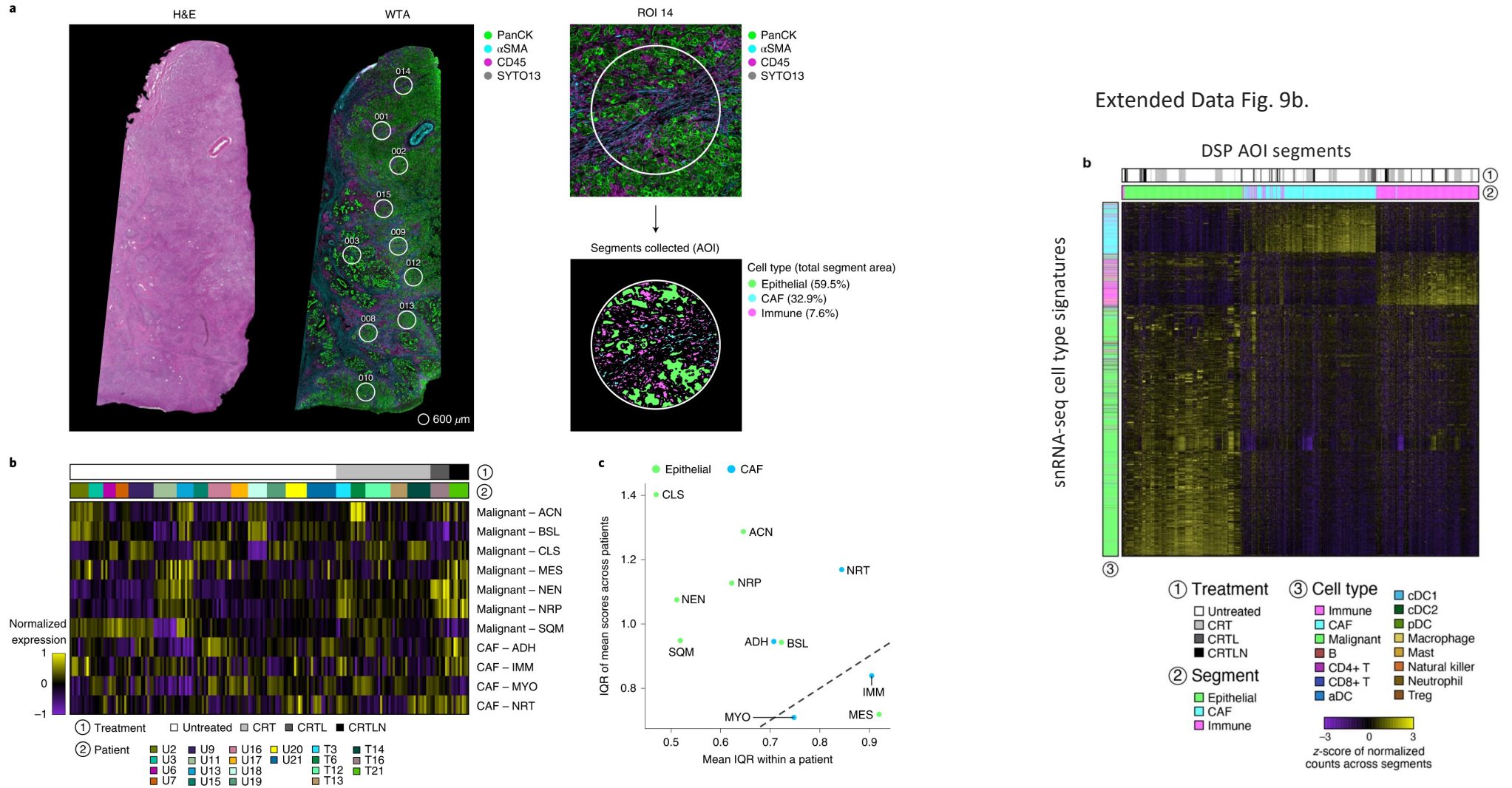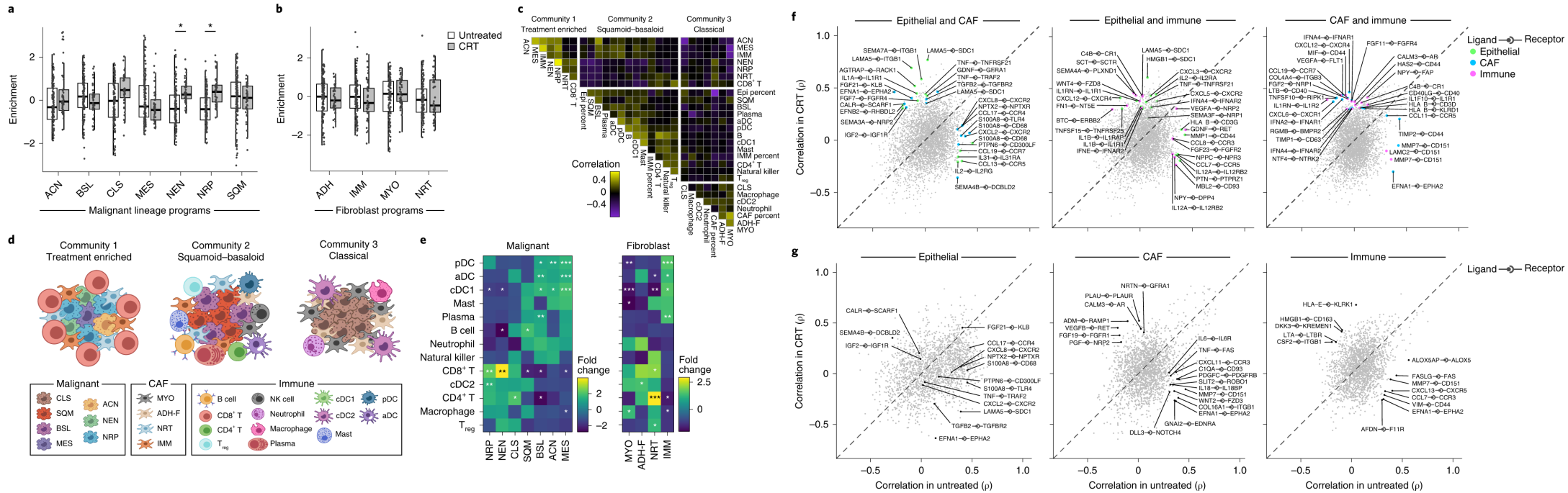
Fig. 7: Spatial analysis of malignant programs, CAF programs and immune cell composition reveals three distinct multicellular communities and treatment-associated receptor–ligand interactions.

まとめ

- scRNAseq を使って、細胞単離困難な組織を解析した
- scRNAseq では recovery rate が高いため、細胞構成をケモの有無で比較することができた
- NMF 解析で得た Metagene を、「細胞状態」や「腫瘍サブタイプ」などの生物学的なカテゴリに分類して、Programとして定義した
- Program と空間情報を結びつけ、ROI内での相関を取る
  - コミュニティを同定
- リガンド受容体ペアと空間情報を結びつけ、AOI内・AOI間で相関を取る
  - オートクライン・パラクラインを同定

以上です

Fig. 8: Refined molecular taxonomy of PDAC.