T A P E Lab

CANCER RESEARCH UK | Cancer Institute UCL

Wellcome SC Biology 2022:

Stromal and Oncogenic Regulation of Colonic Stem Cells Revealed by Single-cell Analysis of Heterocellular Organoids

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Intrinsic and Extrinsic Cues as Cell Fate Regulators

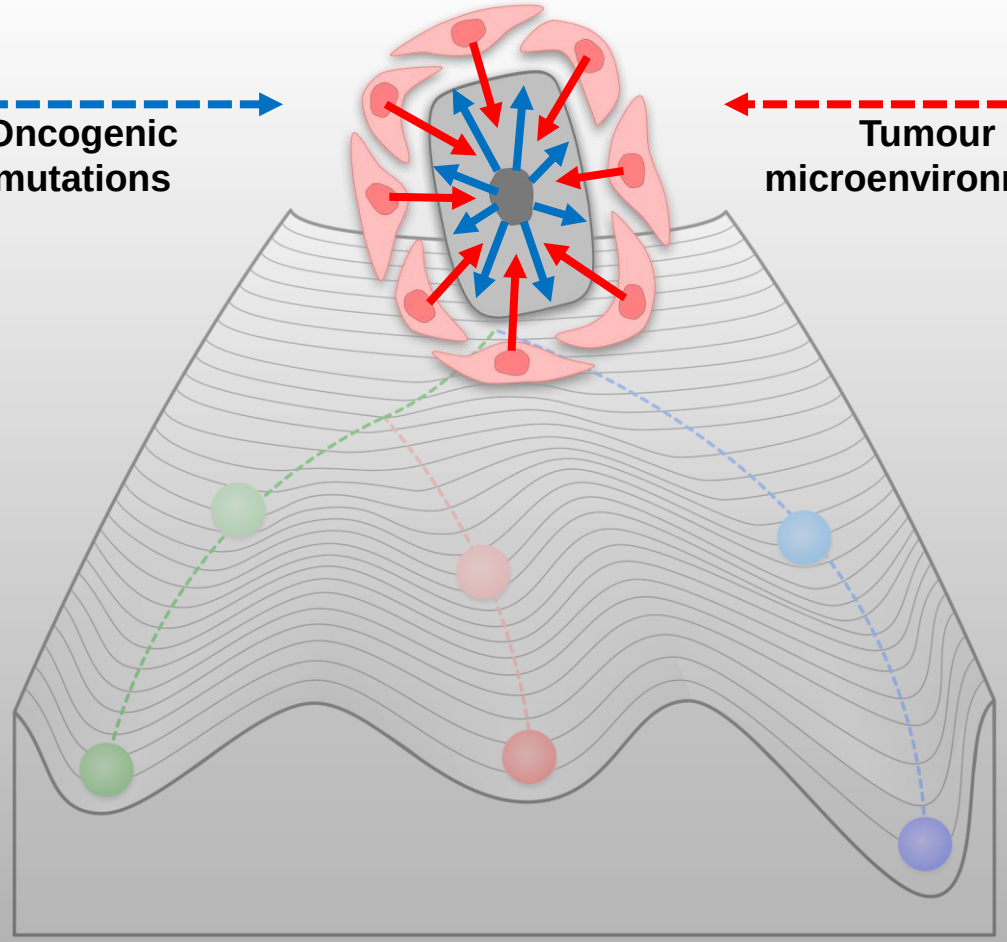
Cancer cell

Intrinsic cues

- Mutations
- Epigenetic state
- Transcriptional networks

…

Oncogenic mutations



Tumour microenvironment

Extrinsic cues

- Mechanics
- Metabolites
- Growth Factors

…

How do oncogenes and the TME regulate cell fate in CRC?

Single-cell Technologies to Study CRC Organoids

Colorectal Cancer (CRC)

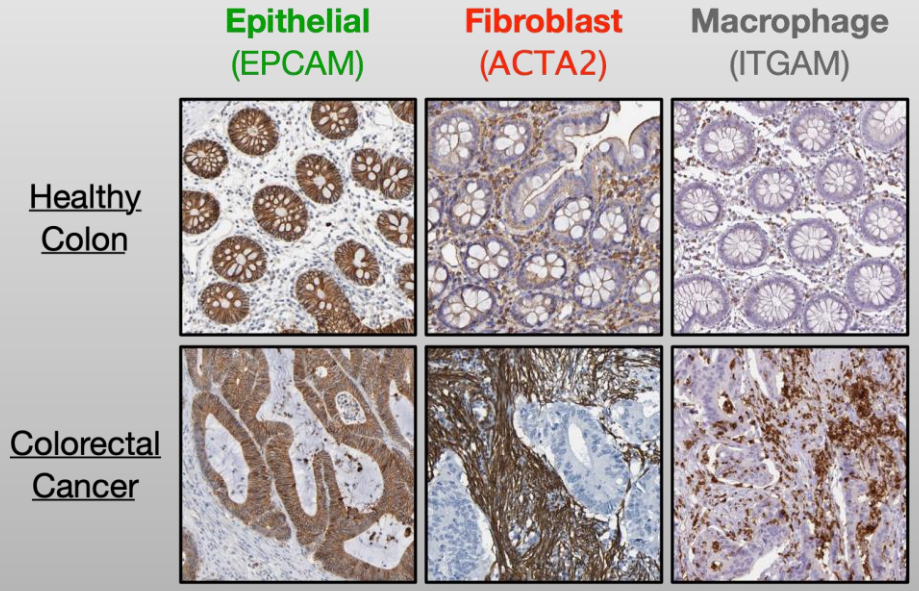
- 800.000 deaths/year
- Tumour microenvironment (TME)

Organoids as models

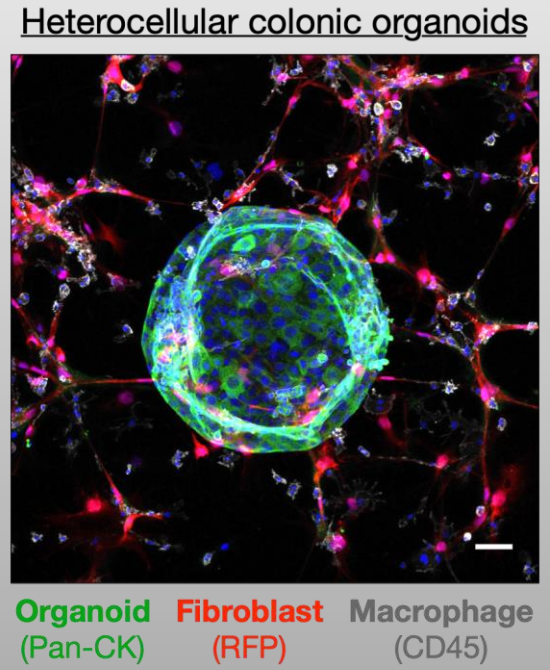
- 3D *in vitro* cultures
- Heterocellular

Single-cell technologies

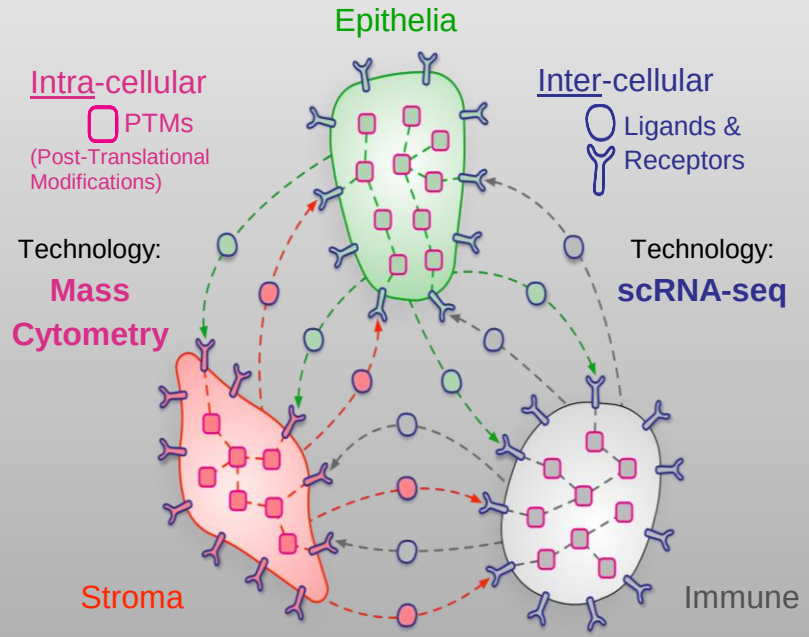
- Heterogeneity
- Cell-cell communications



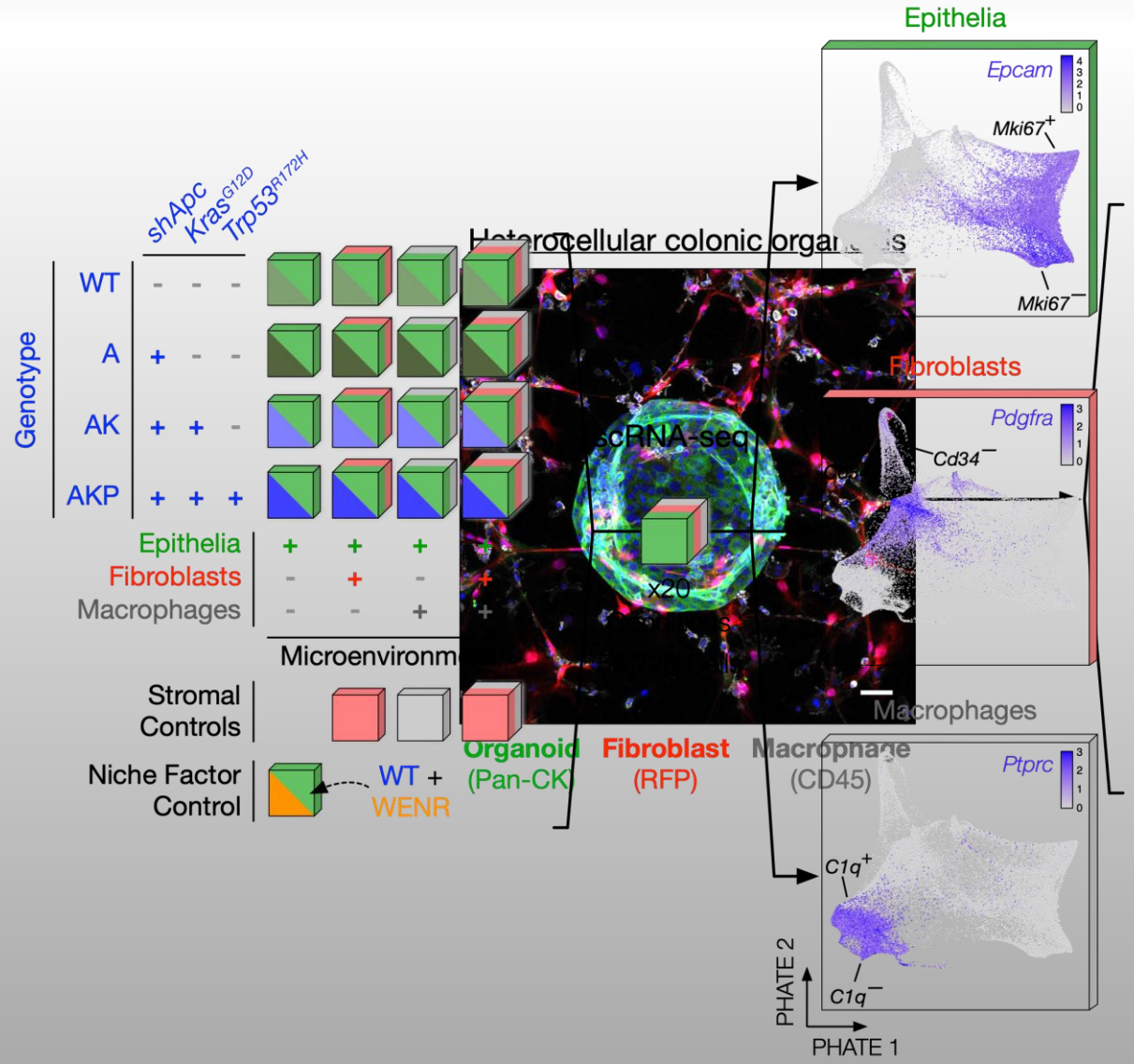
Human Protein Atlas 2022



Qin et al. 2020



Multivariate scRNA-seq of CRC-TME Organoids

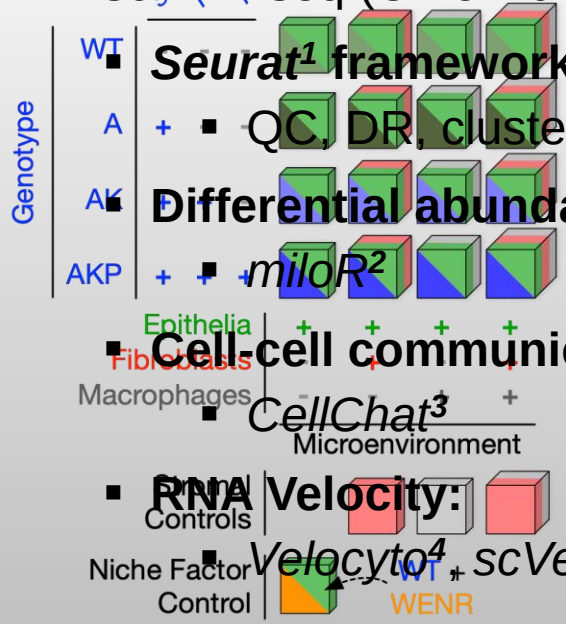


Intrinsic cues

Analysis:

scRNA-seq (Chromium 10X)

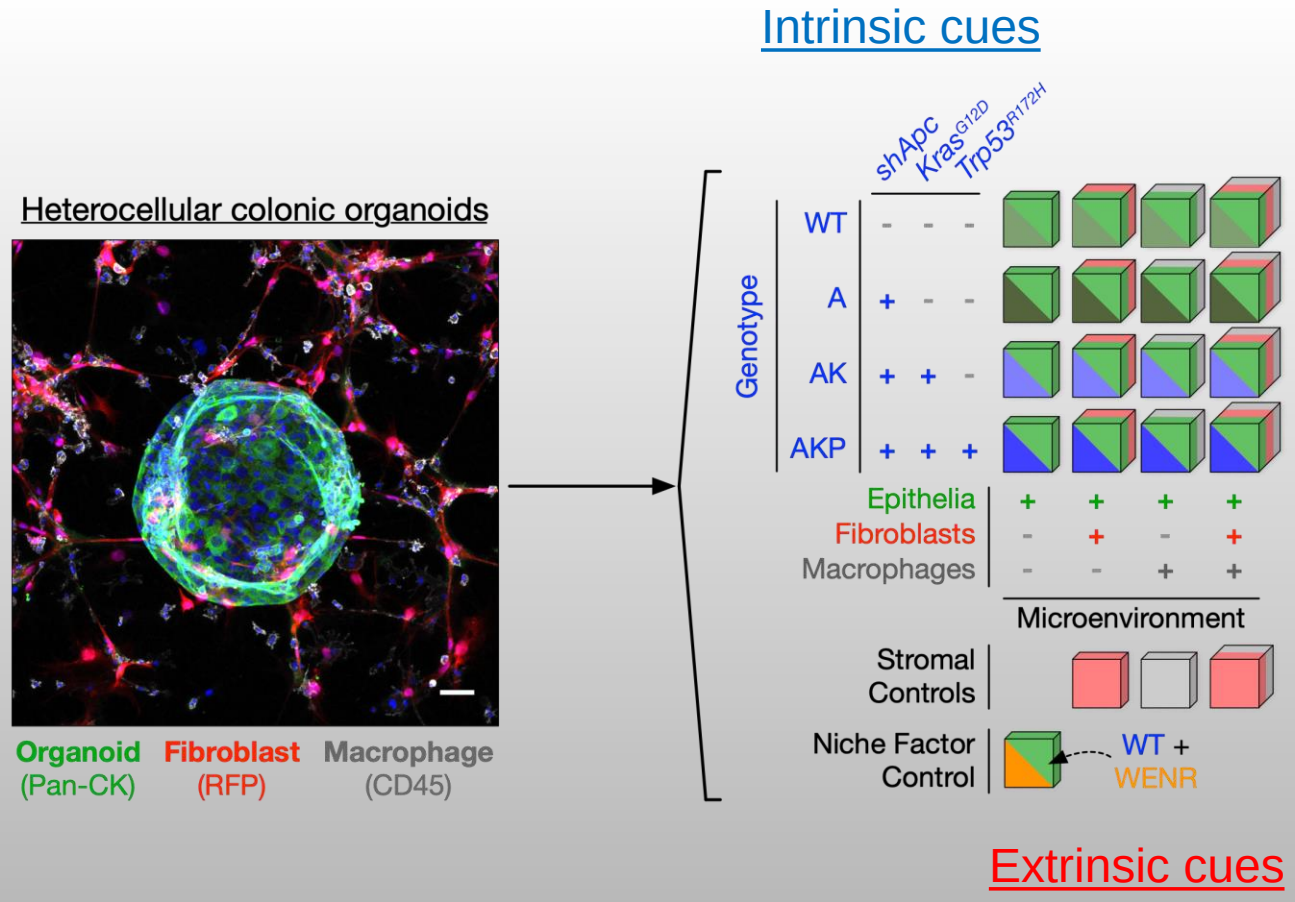
- ***Seurat*[1] framework**:
  - QC, DR, clustering, and DE
- **Differential abundance:**
  - *miloR*[2]
- **Cell-cell communication:**
  - *CellChat*[3]
- **RNA Velocity:**
  - *Velocyto*[4], *scVelo*[5], and *CellRank*[6].



Extrinsic cues

1.- *Hao et al. 2021*
2.- *Dann et al. 2021*
3.- *Jin et al. 2021*
4.- *La Manno et al. 2018*
5.- *Bergen et al. 2020*
6.- *Lange et al. 2022*

Multivariate scRNA-seq of CRC-TME Organoids



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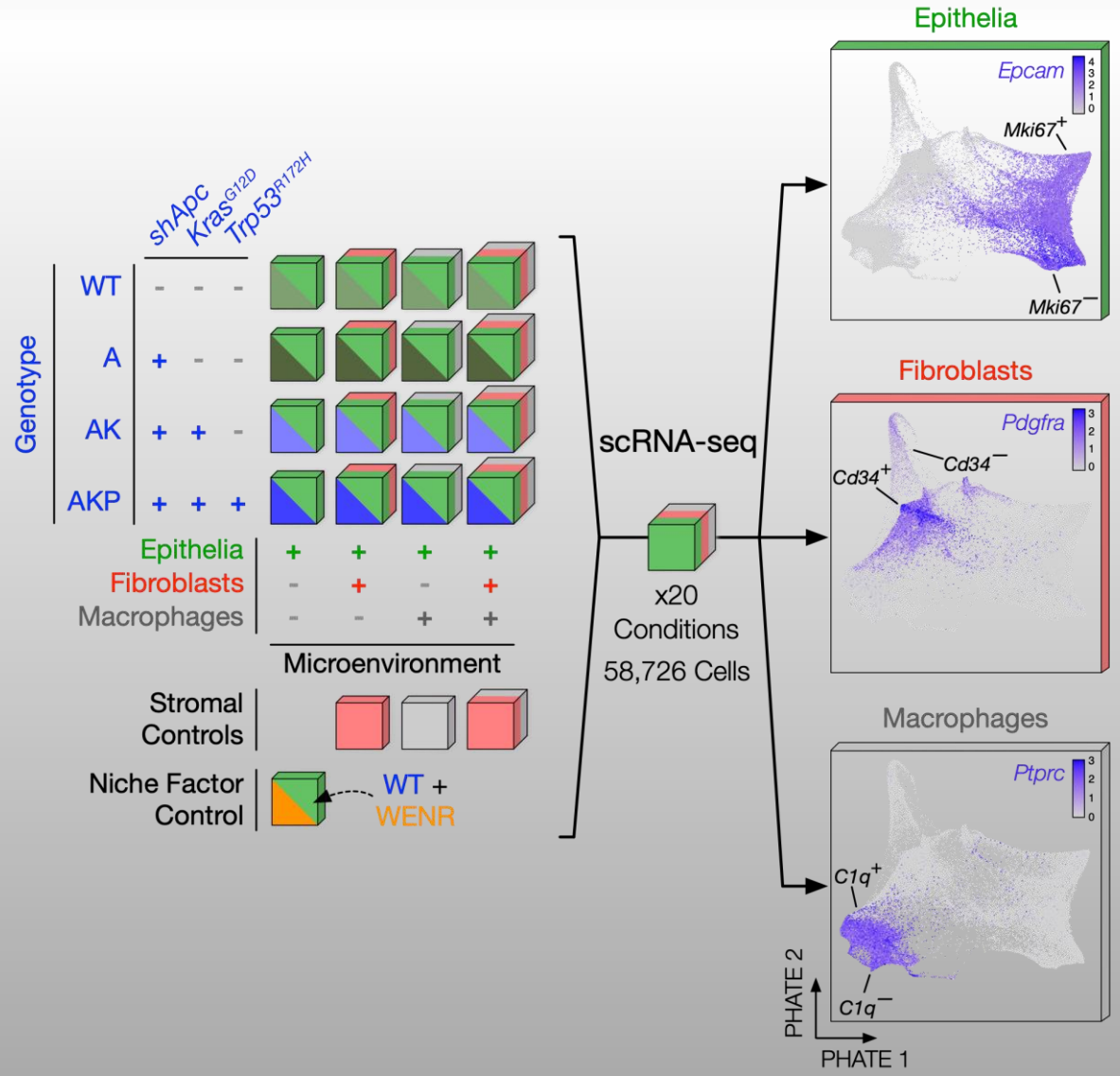

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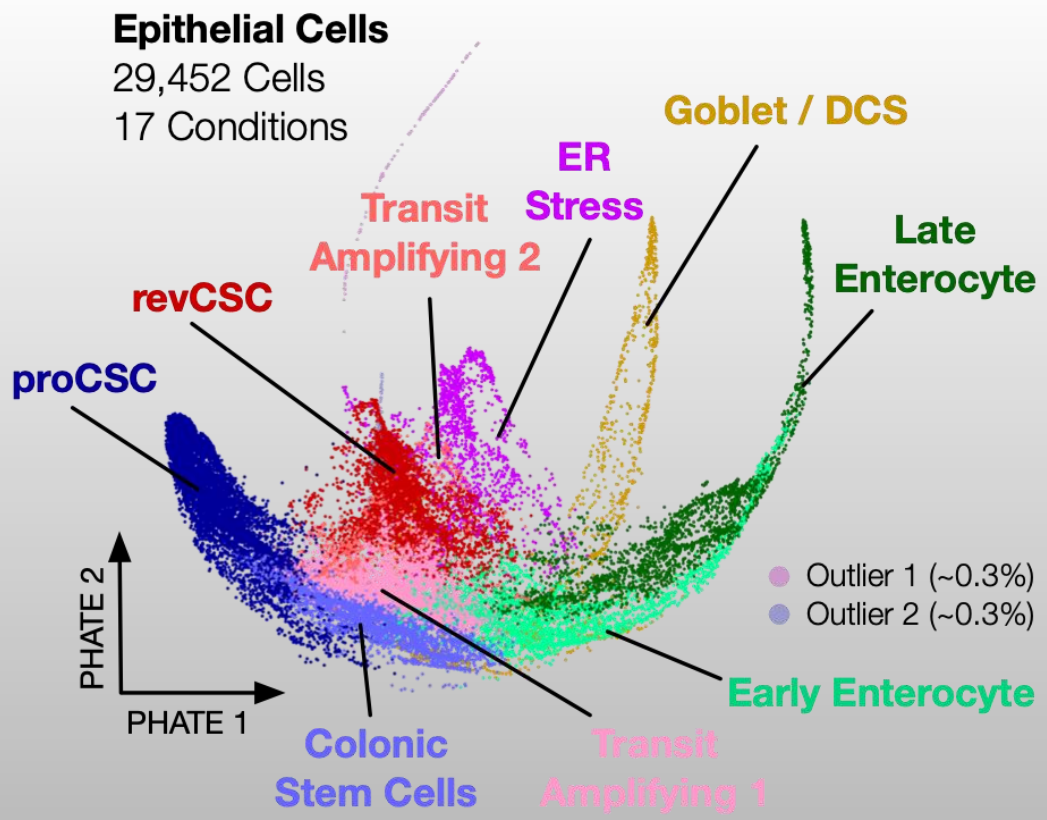
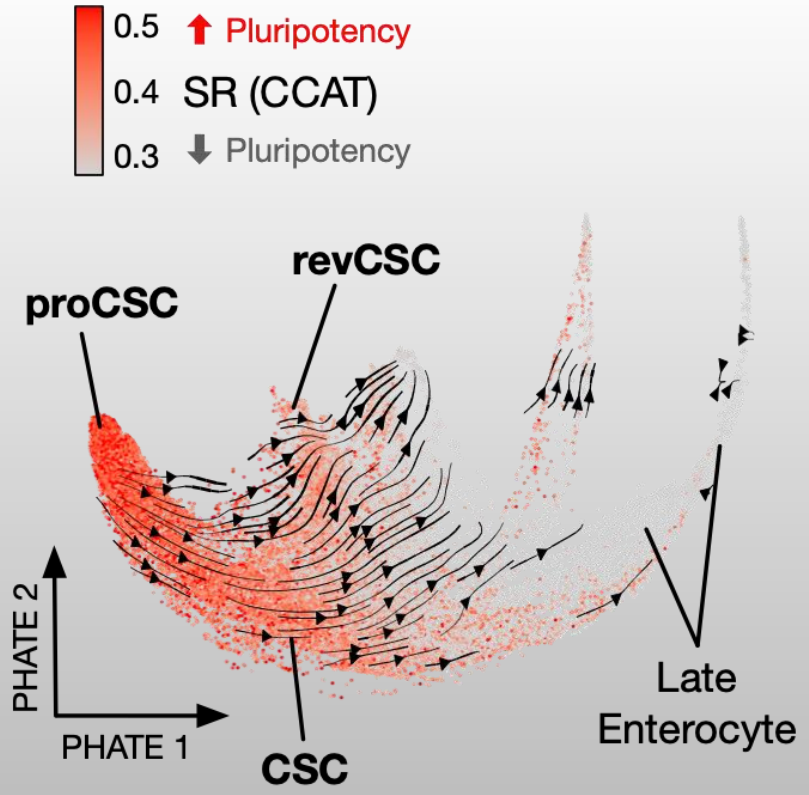
scRNA-seq Captures Colonic Epithelia Differentiation



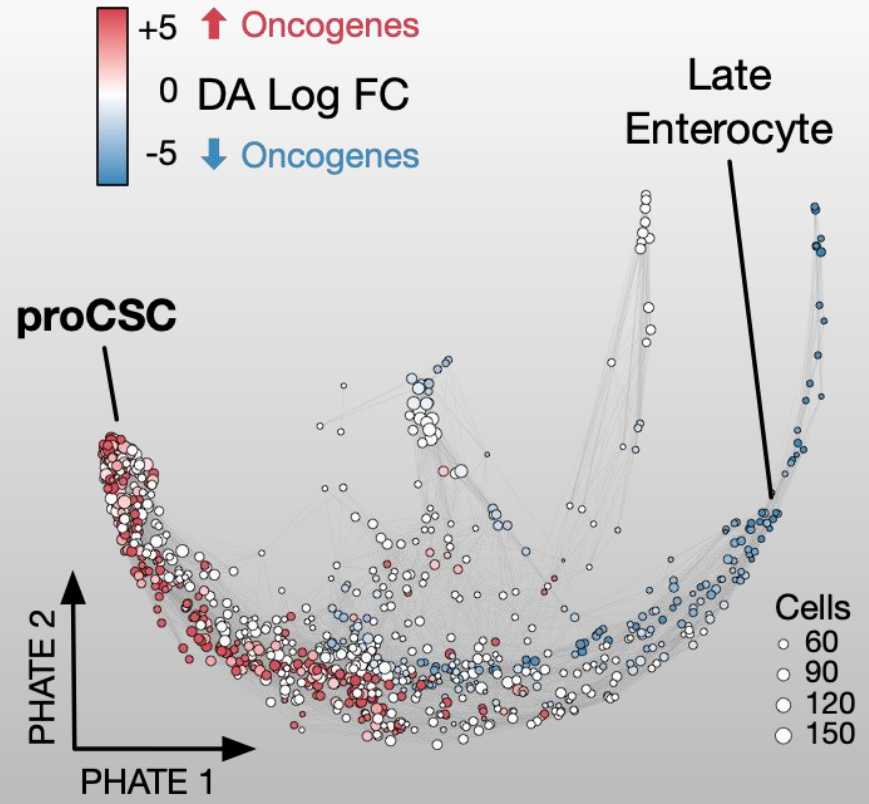


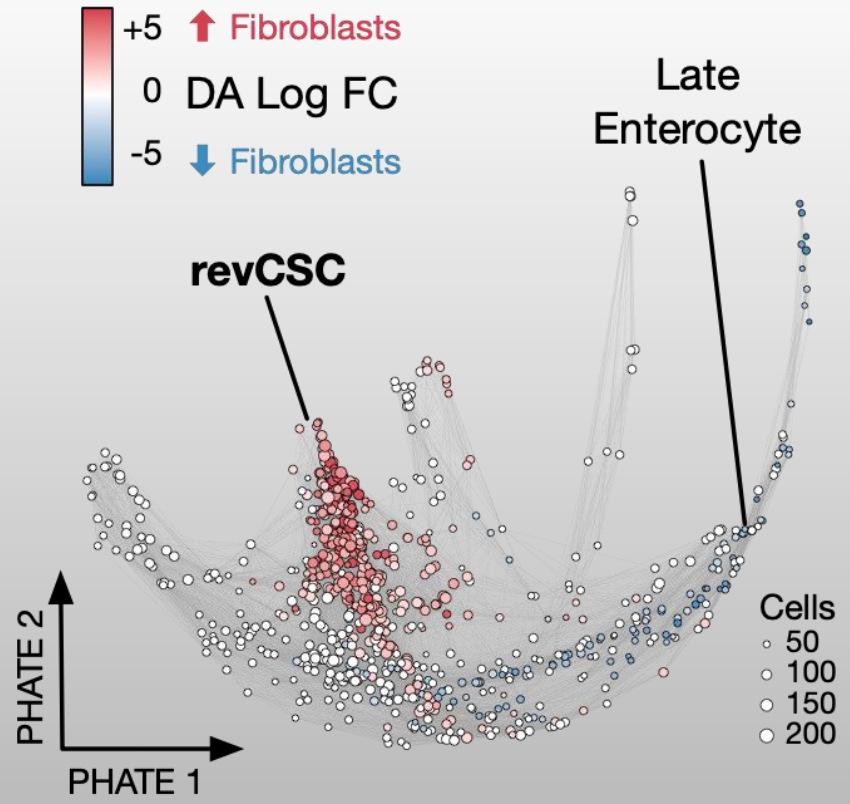
SCENT & CCAT: ***Teschendorff and Enver 2017***

- Recapitulation of colon epithelia dynamics
- Heterogenous Colonic Stem Cell (CSC) compartment

Oncogenes and Fibroblasts Differentially Regulate CSC

Differential abundance analysis



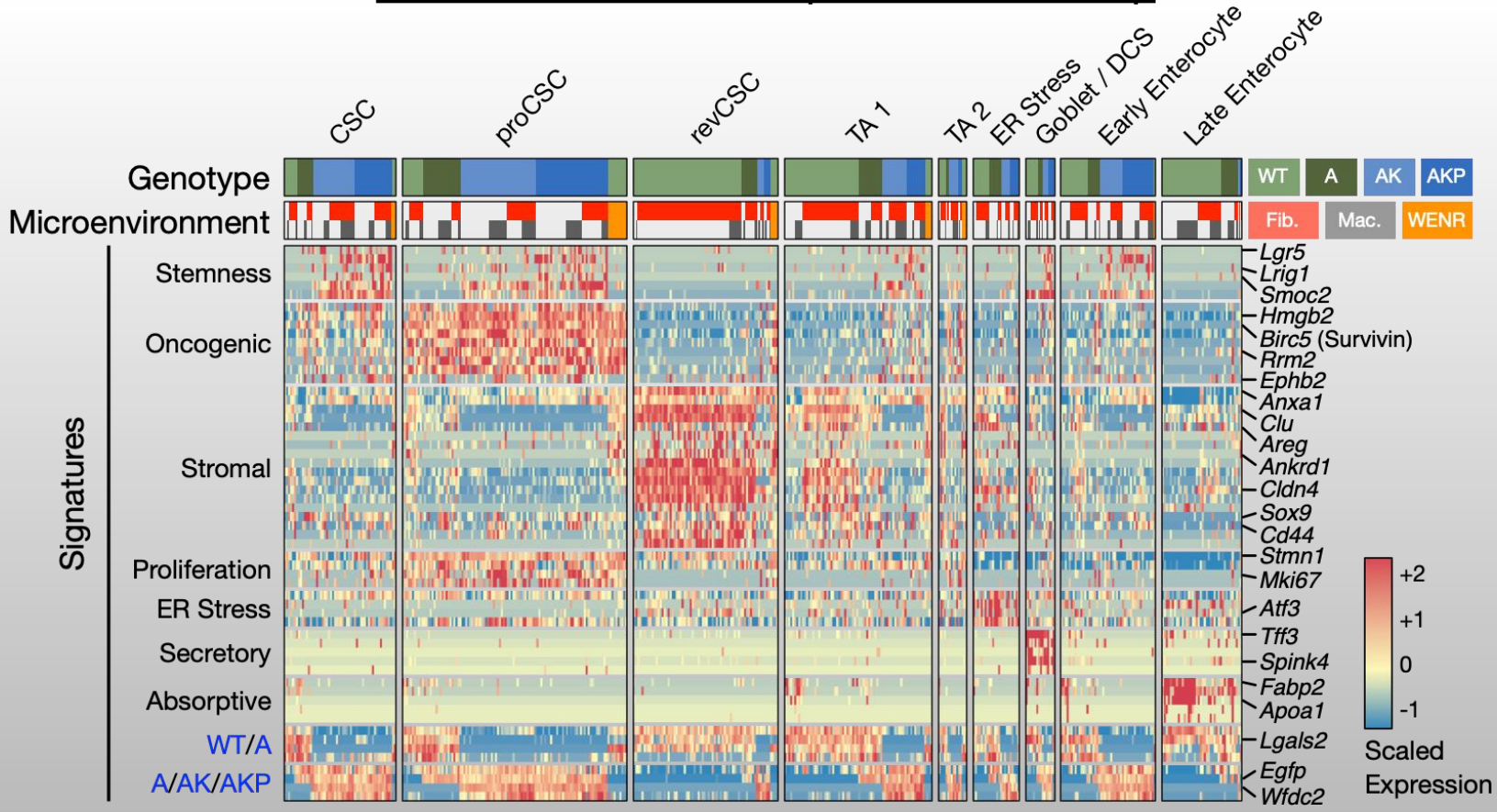


Oncogenes -> proliferative CSCs (proCSC)
DA test: AK/AKP vs WT (monocultures)

Fibroblasts -> Revival CSCs (revCSC)
DA test: WT+Fibroblasts vs WT monoculture

Oncogenes and Fibroblasts Differentially Regulate CSC

Curated differential expression heatmap

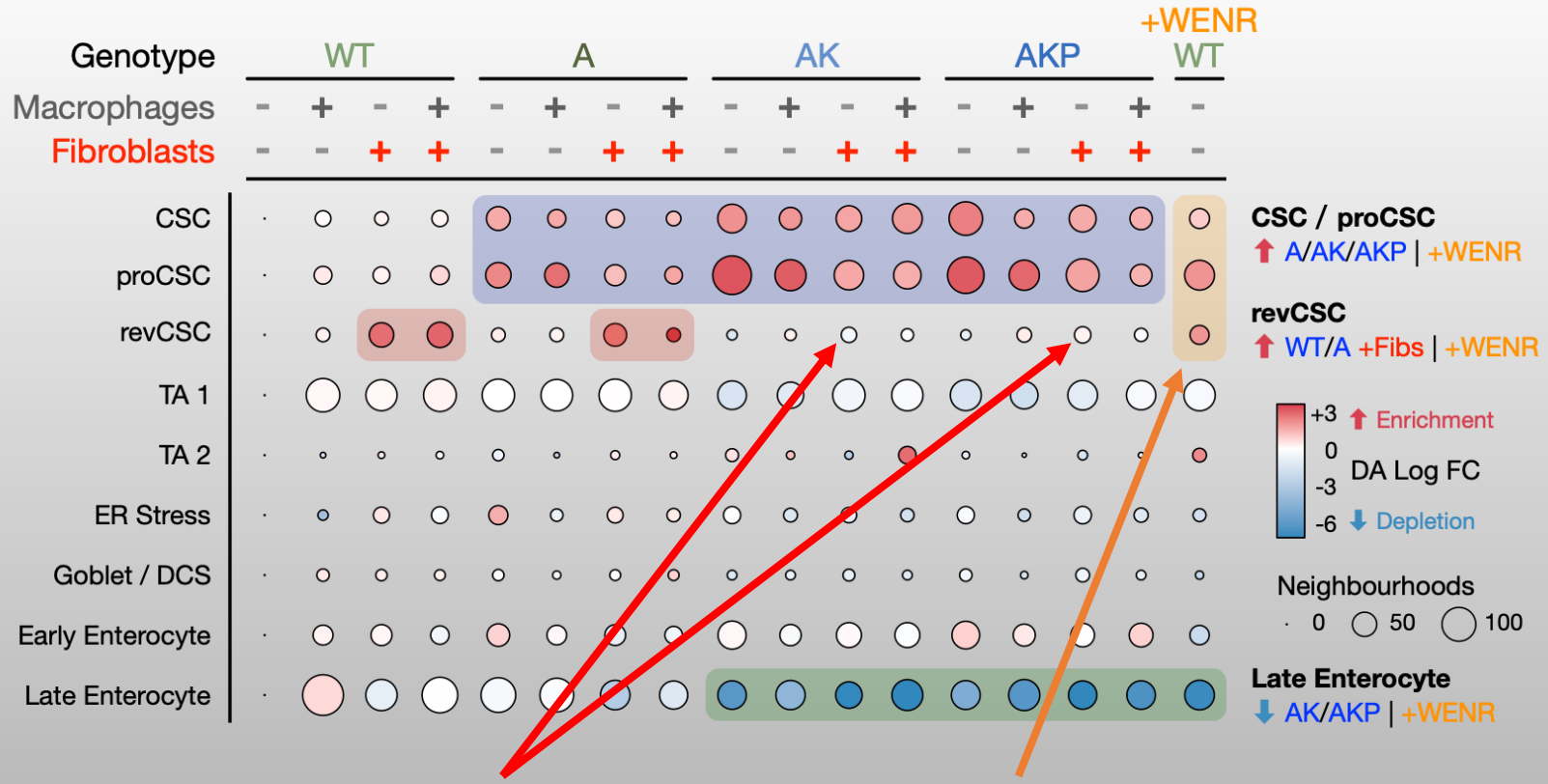


Oncogenes -> proliferative CSCs (proCSC)
Lgr5 and *Birc5* positive

Fibroblasts -> Revival CSCs (revCSC)
Less proliferative and *Clu* positive

Oncogenic Entrapment of the Colonic Epithelia

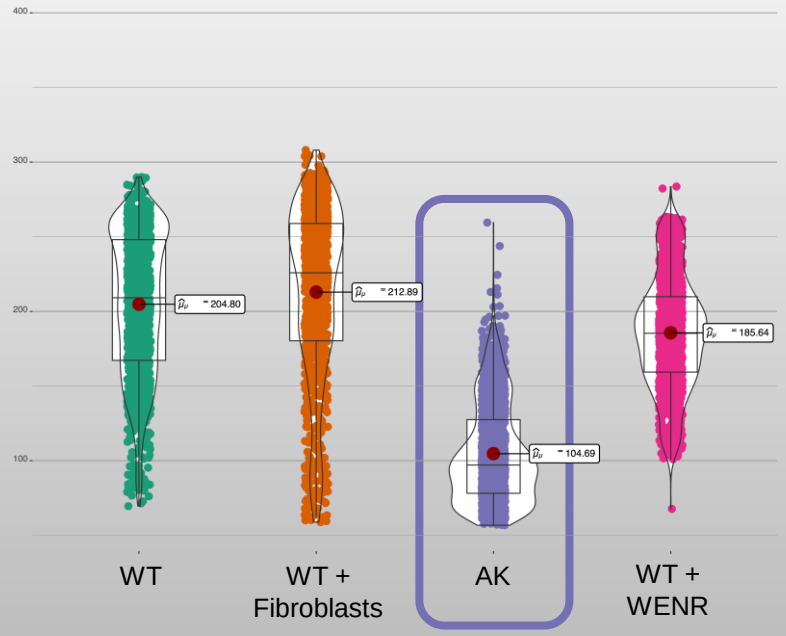
Summary of DA results across conditions



Loss of fibroblast regulation in AK and AKP organoids

WENR upregulates all CSC states in WT organoids

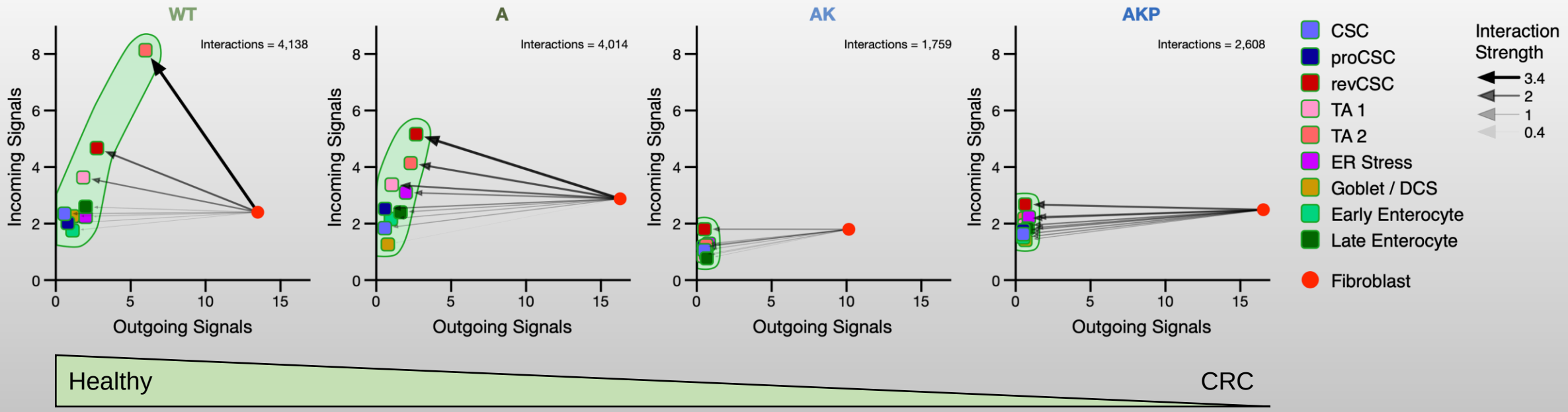
RNA velocity length



CRC organoids show the lowest differentiation rates

Oncogenic Entrapment of the Colonic Epithelia

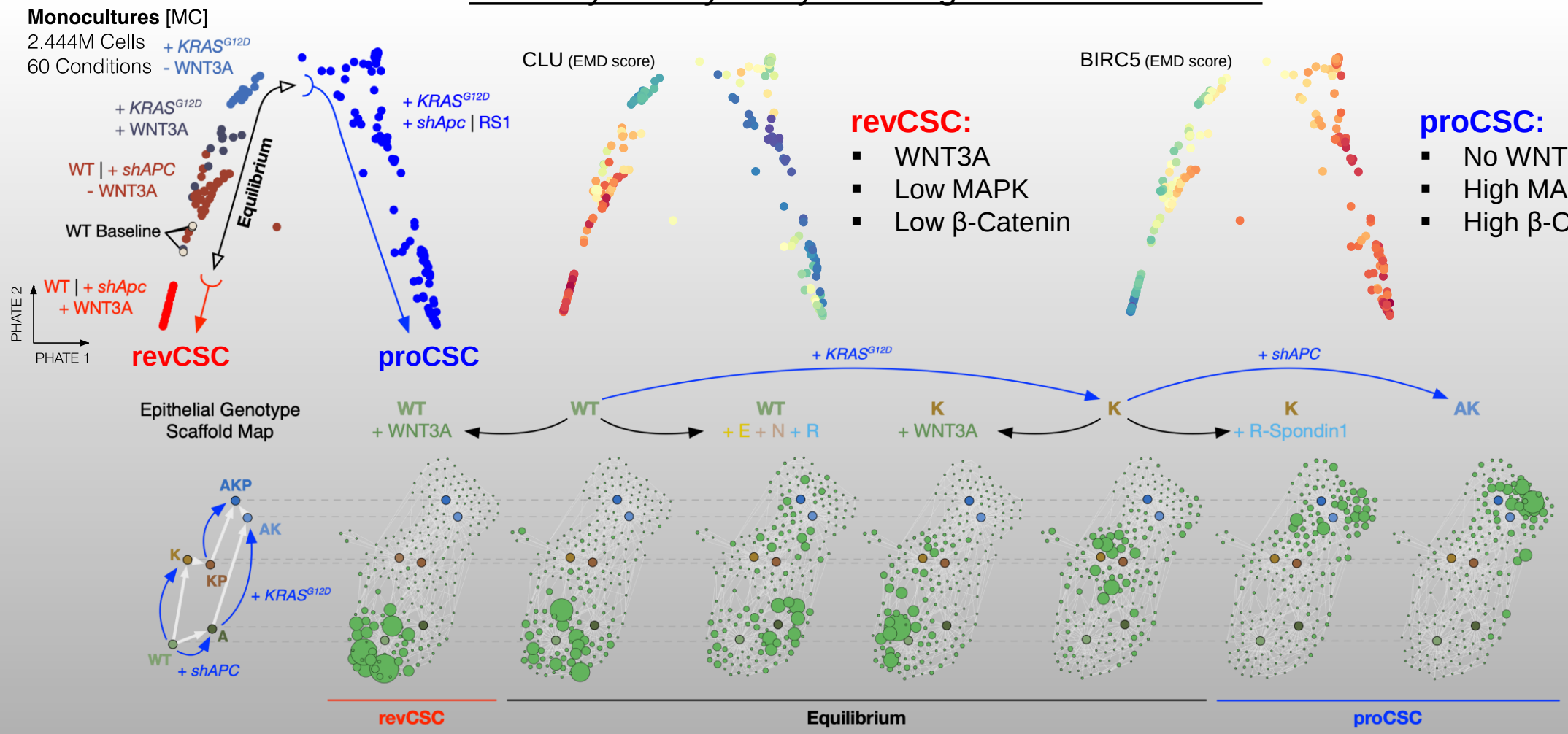
Cell-cell communication analysis of organoid+fibroblast co-cultures



Oncogenes erode fibroblast to epithelia communication

Exogenous Ligands Model CSC Regulation

Mass Cytometry analysis of organoid monocultures

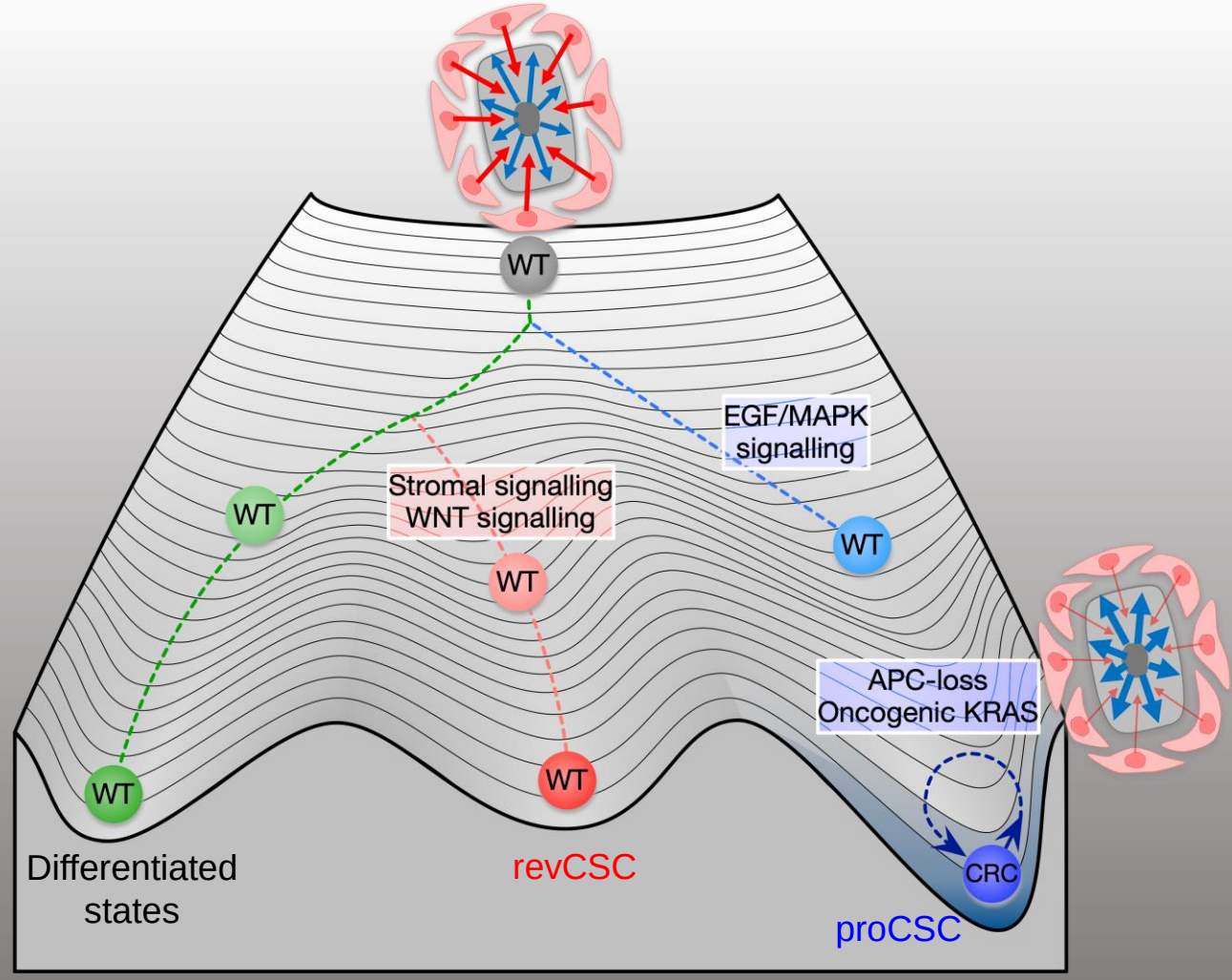


Oncogenes and ME navigate a shared regulatory landscape

Credit to Dr Xiao Qin

Summary

- Differentially regulated CSC states
  - Intrinsic oncogenic cues -> proCSC
  - Extrinsic stromal cues -> revCSC
- Oncogenic entrapment
  - Oncogenes erode stromal regulation
  - Lowered differentiation rates in AK/AKP
- Intrinsic and extrinsic cues navigate a shared regulatory landscape
  - EGF and WNT as regulators of proCSC and revCSC fates



Acknowledgements

Accessibility:
Talk transcript

The Tape Lab

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