Applications of Single Cell Sequencing

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Disclosures

Consultant/Honoraria: Merck, AstraZeneca, Illumina, Chrysalis Biomedical Advisors, Canada Pension Plan Investments, PACT Therapeutics

Research funding: Roche/Genentech imCORE

Inventor on patents filed by University Health Network "Hybrid-capture sequencing for determining immune cell clonality"

Director of an academic research core offering single cell profiling (10X Genomics Certified Service Provider, www.pmgenomics.ca)

Learning Objectives

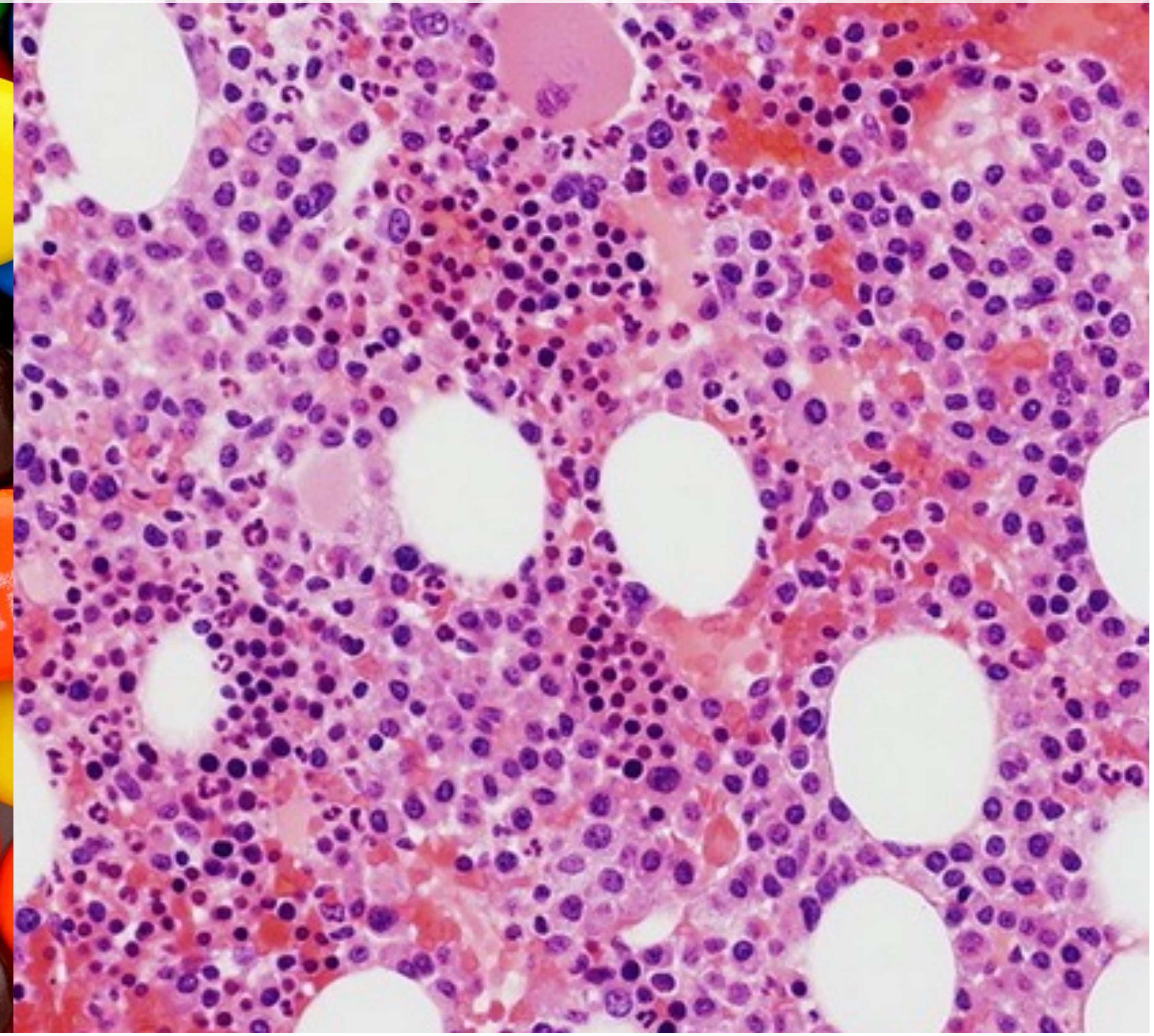
1) Understand the conceptual shift in moving from bulk to single cell profiling

2) Become acquainted with types, parameters and trade-offs of various single cell technologies

3) Using cancer as an example, be exposed to scientific questions and experimental designs utilizing single cell analysis

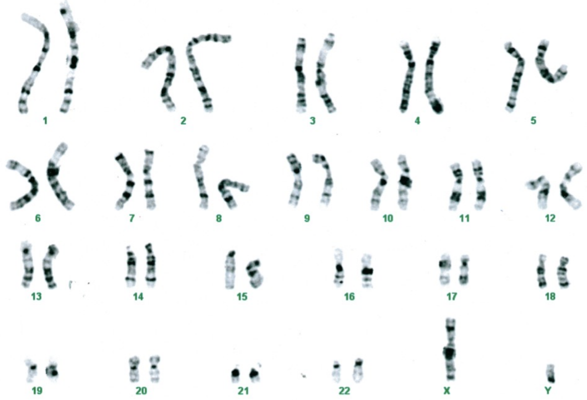
4) Appreciate new scientific and translational opportunities enabled by integrative single cell molecular profiling

We are all made of cells: tissues consist of immune, stromal & many other cell types that interact physically and functionally

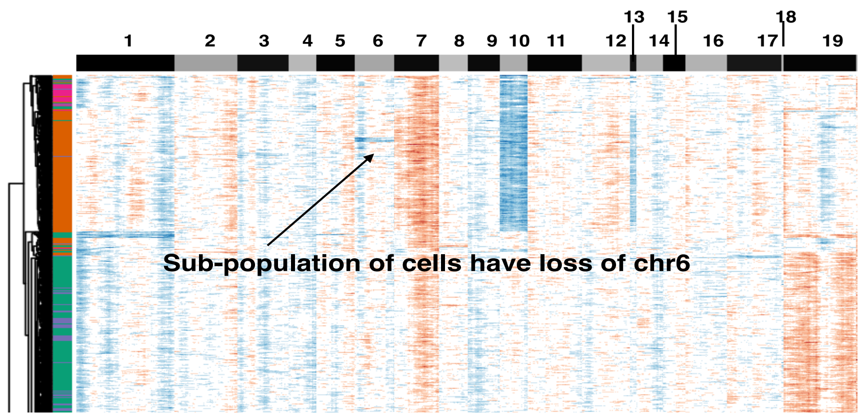


Single cell analysis is not new…the revolution is in the scale, completeness, & quantitative nature of genomic technologies

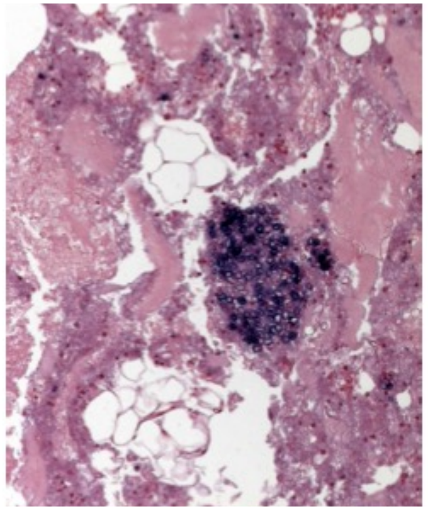
One karyotype in one cell



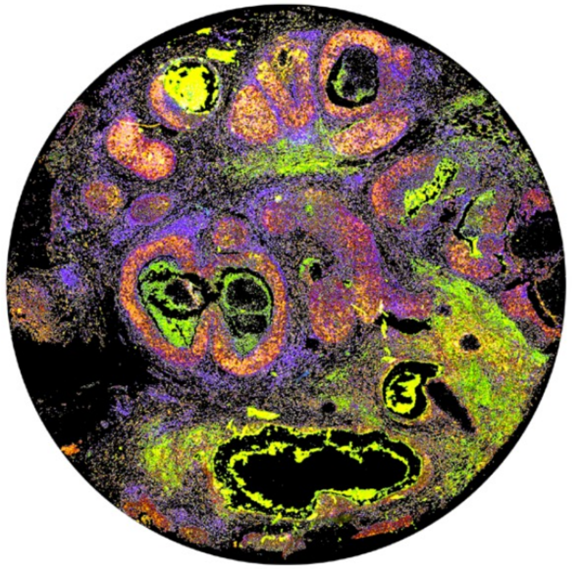
Quantification of all chromsomes in all cells



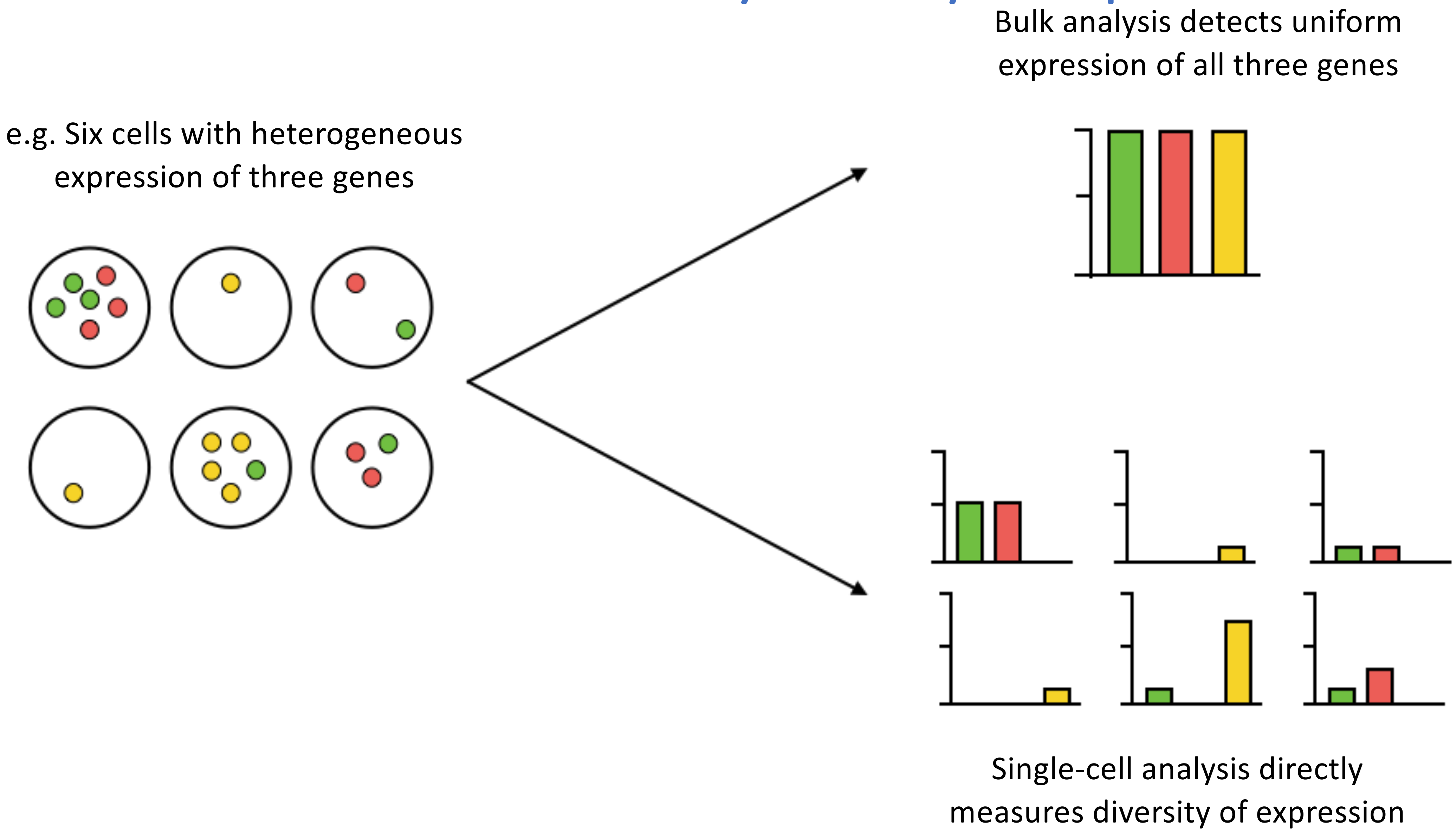
In situ hybridization of one transcript



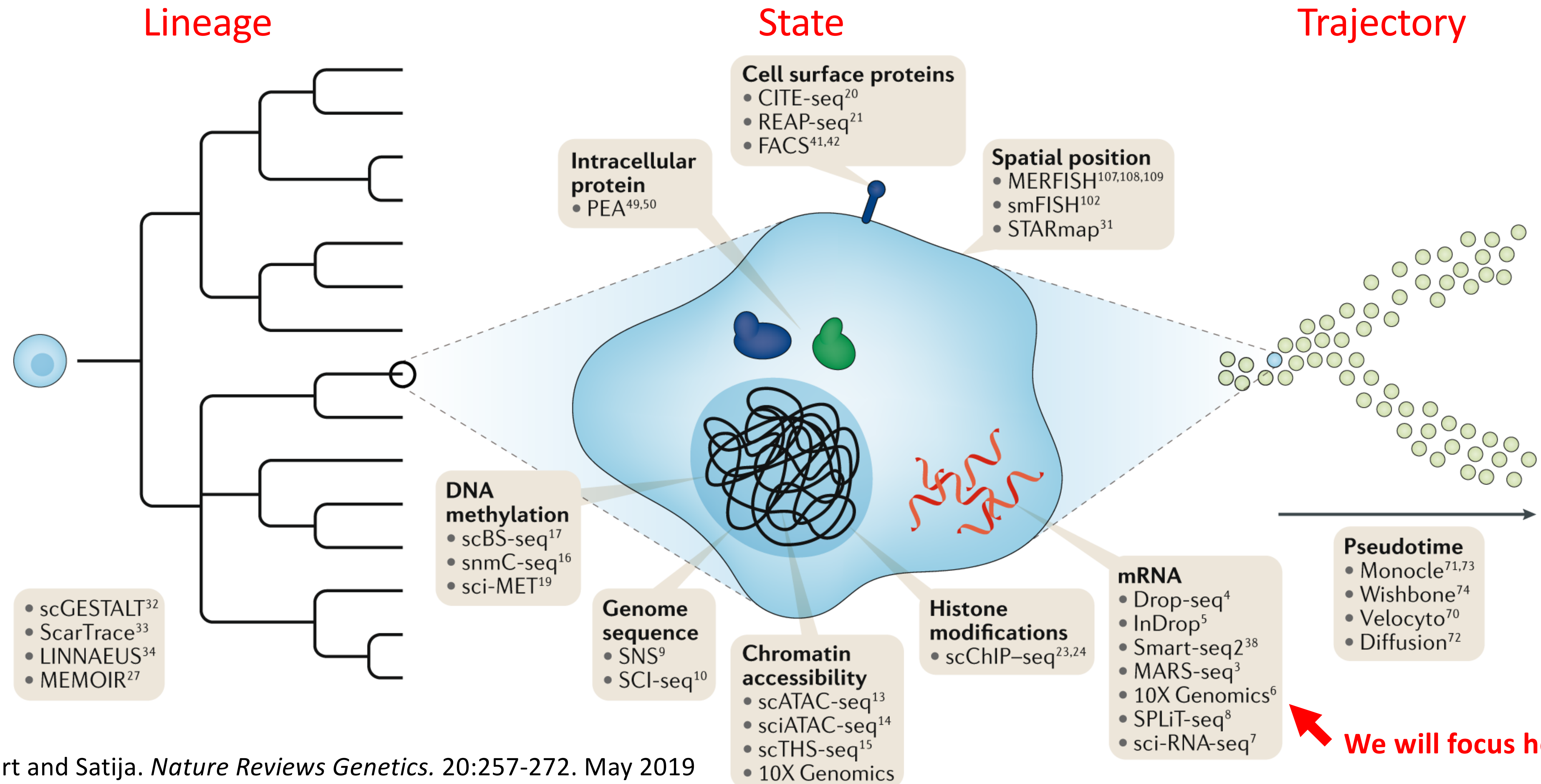
Visualization of 1,000s of genes expressed in all cells



Single-cell analysis reveals heterogeneity in molecular profiles at resolution bulk analysis may not permit



“A wide variety of single-cell methods have now been developed to measure a broad range of cellular parameters”

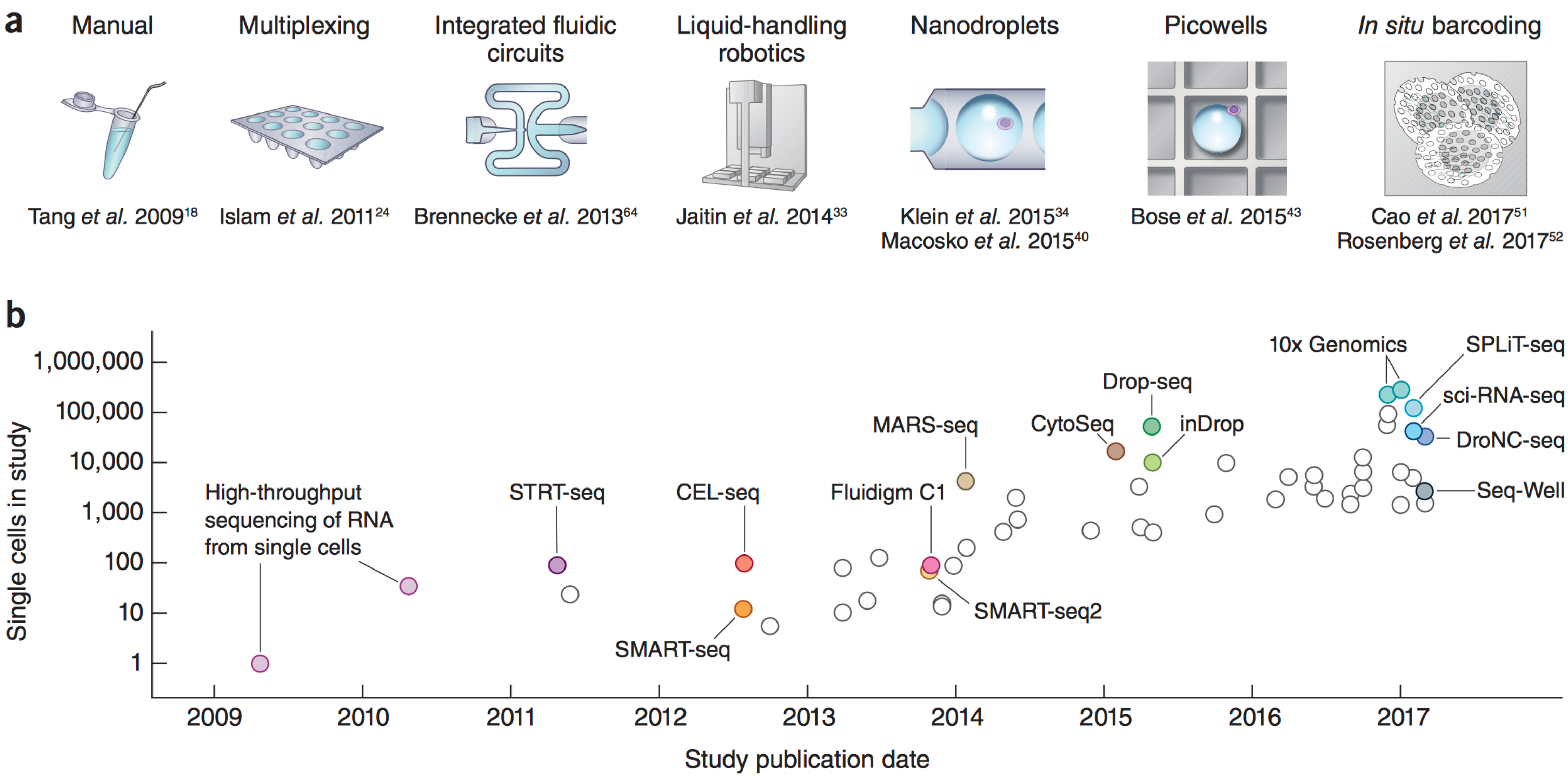


We will focus here

Stuart and Satija. *Nature Reviews Genetics.* 20:257-272. May 2019

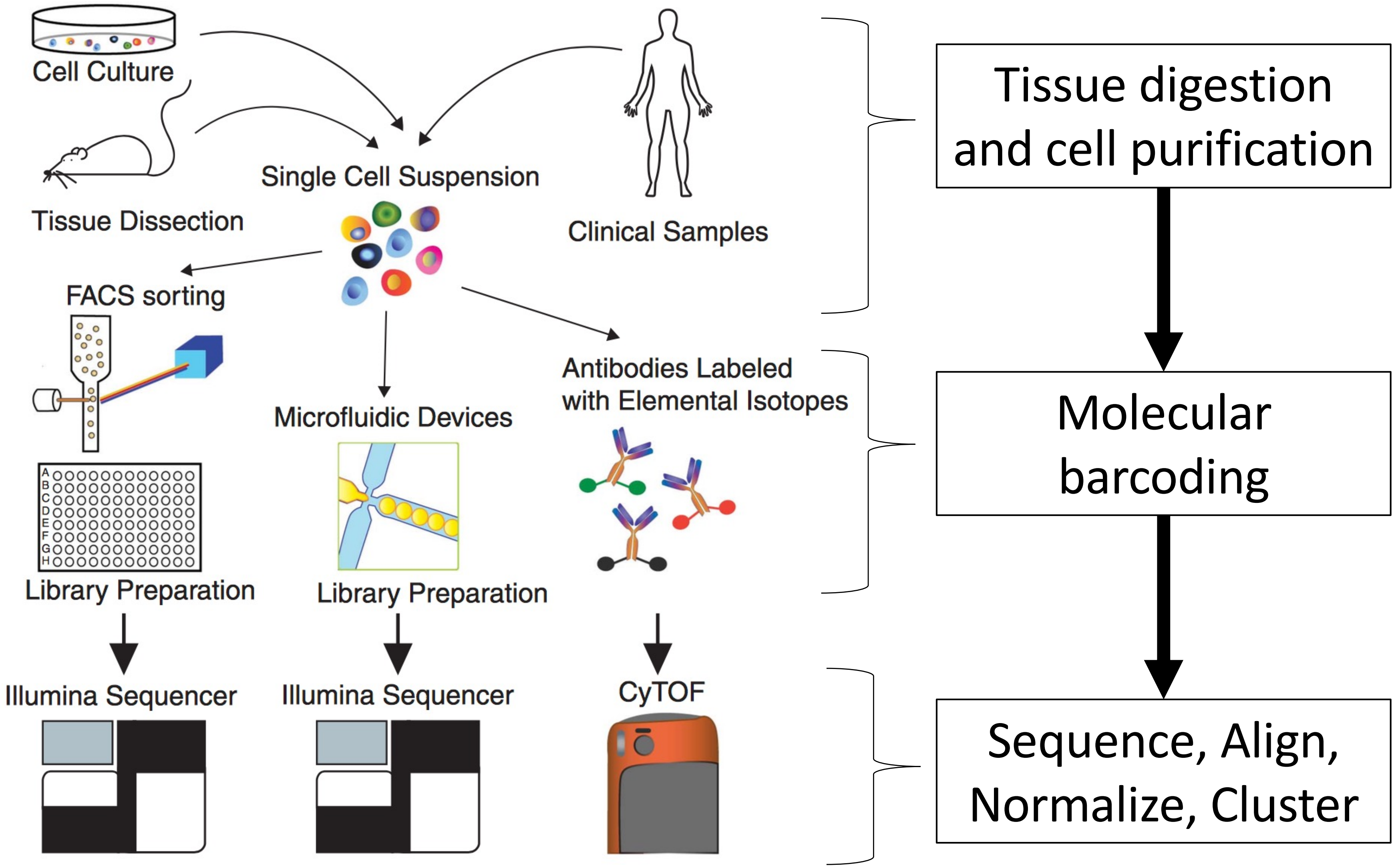
Considerations and capabilities for generation of single cell data

“Exponential scaling of single-cell RNA-seq in the past decade”



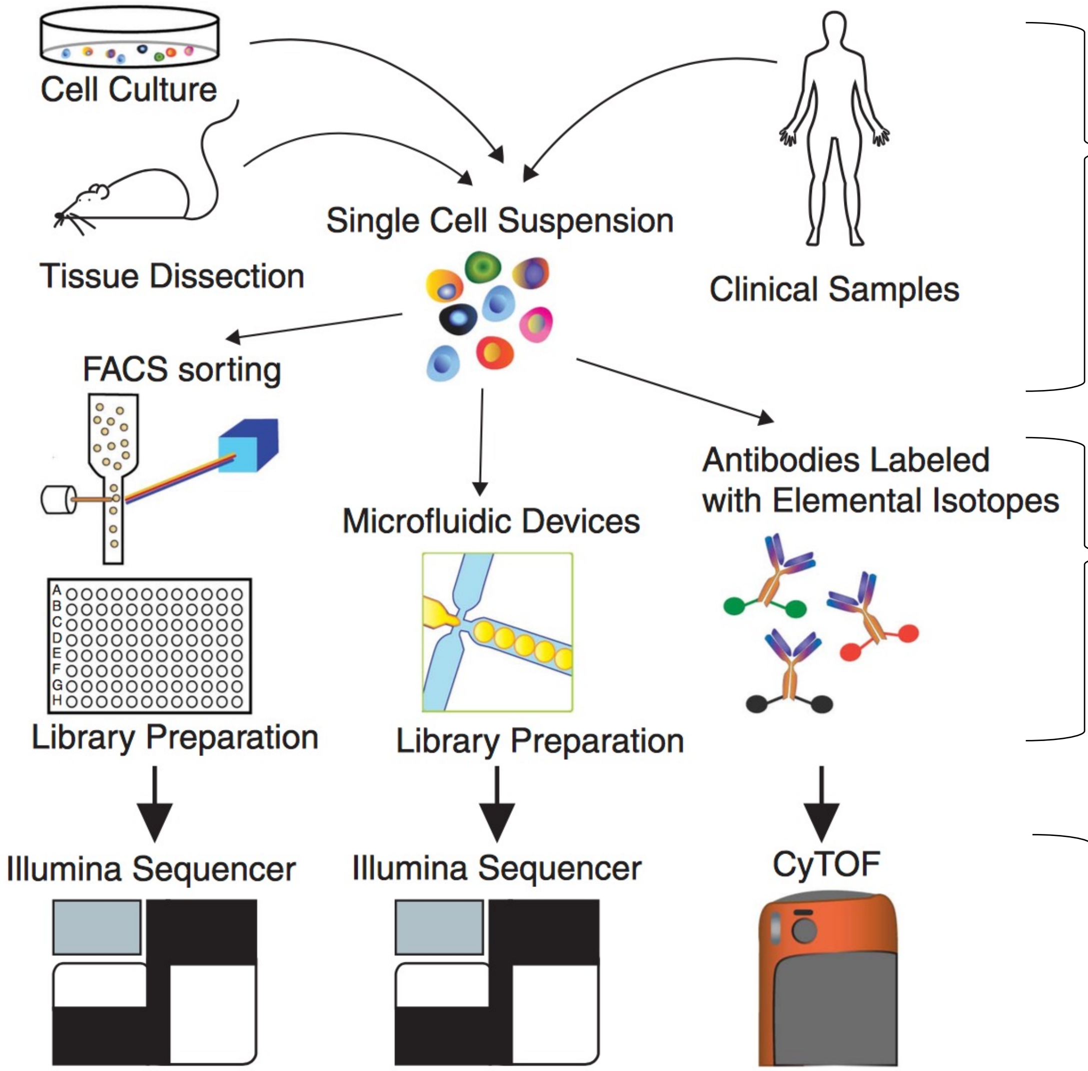
Svensson, Vento-Tormo, and Teichmann. Nat Protoc. 2018 Apr;13(4):599-604.

Multiple pathways and technology options to analyze 100s-100,000s of single cells from a variety of sources



Proserpio and Lönnberg. Immunol Cell Biol. 2016 Mar;94(3):225-9.

Multiple pathways and technology options to analyze 100s-100,000s of single cells from a variety of sources



Tissue digestion and cell purification

Bulk profiling?
Isolate rare populations?
Split and sequence?

↓

Molecular barcoding

Full-length transcripts?
End-reads?
Combination?

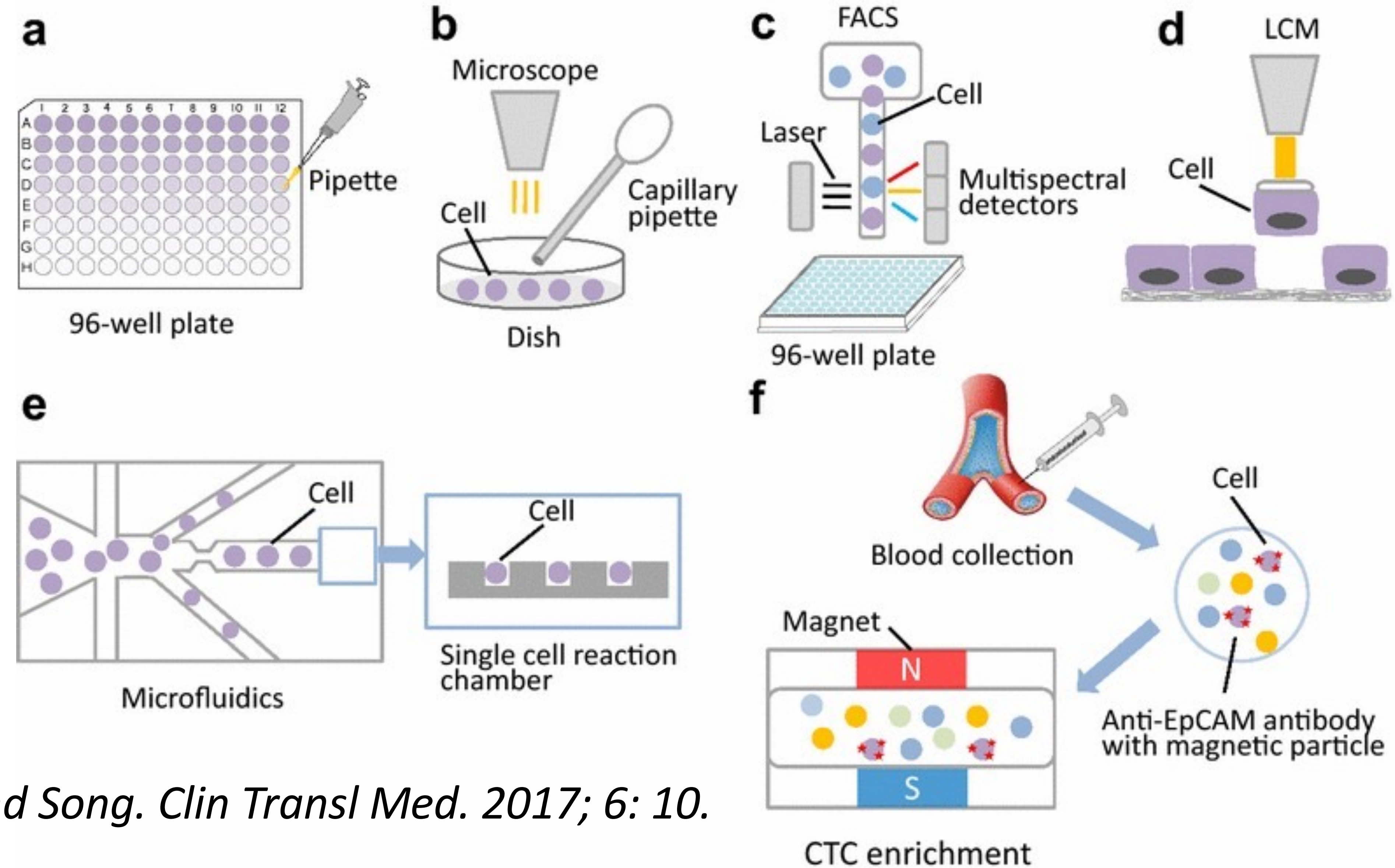
↓

Sequence, Align, Normalize, Cluster

CellRanger, Seurat, Biscuit, custom analyses

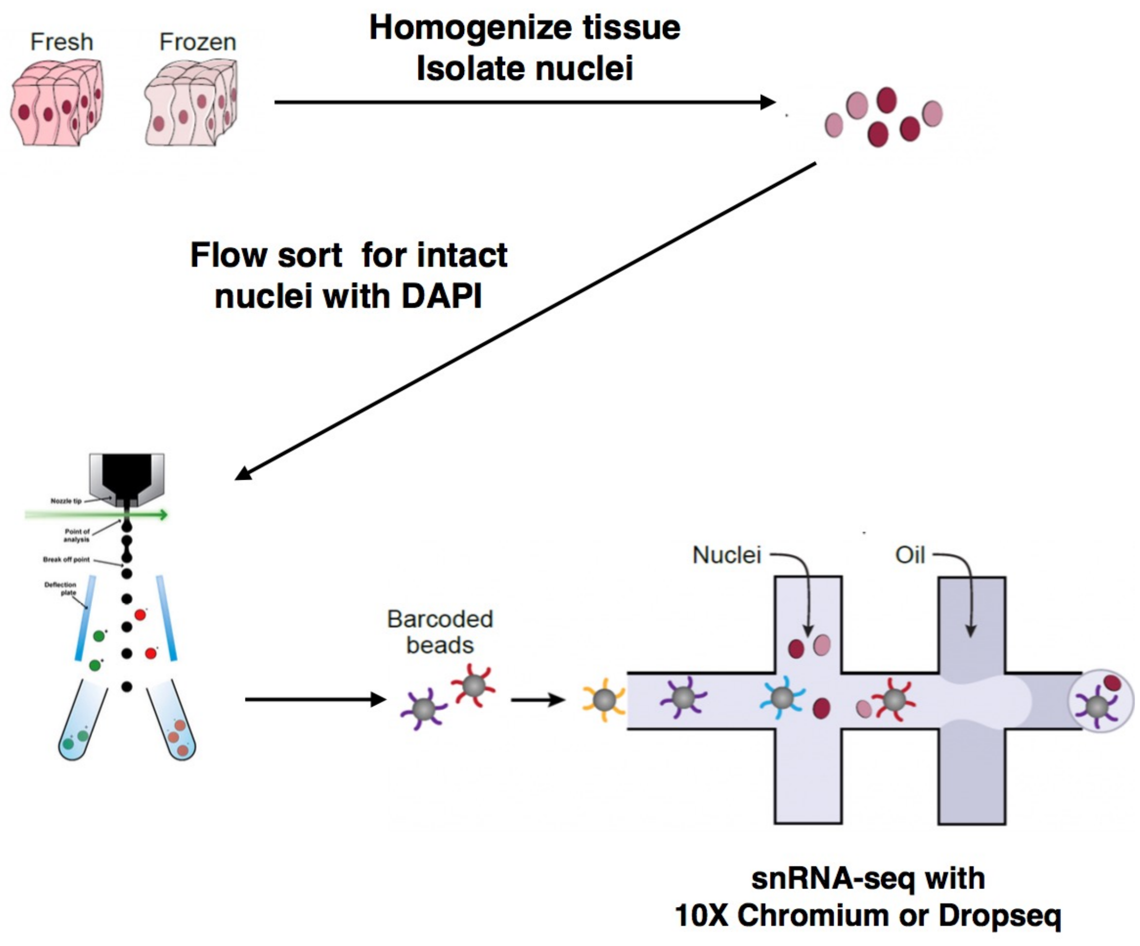
Proserpio and Lönnberg. Immunol Cell Biol. 2016 Mar;94(3):225-9.

Numerous methods to isolate single cells, some more scalable than others



Wang and Song. Clin Transl Med. 2017; 6: 10.

Single nuclei sequencing of snap frozen cells overcomes viability problem in primary tumours



(Habib et al., 2017)

Permits use of frozen, banked samples

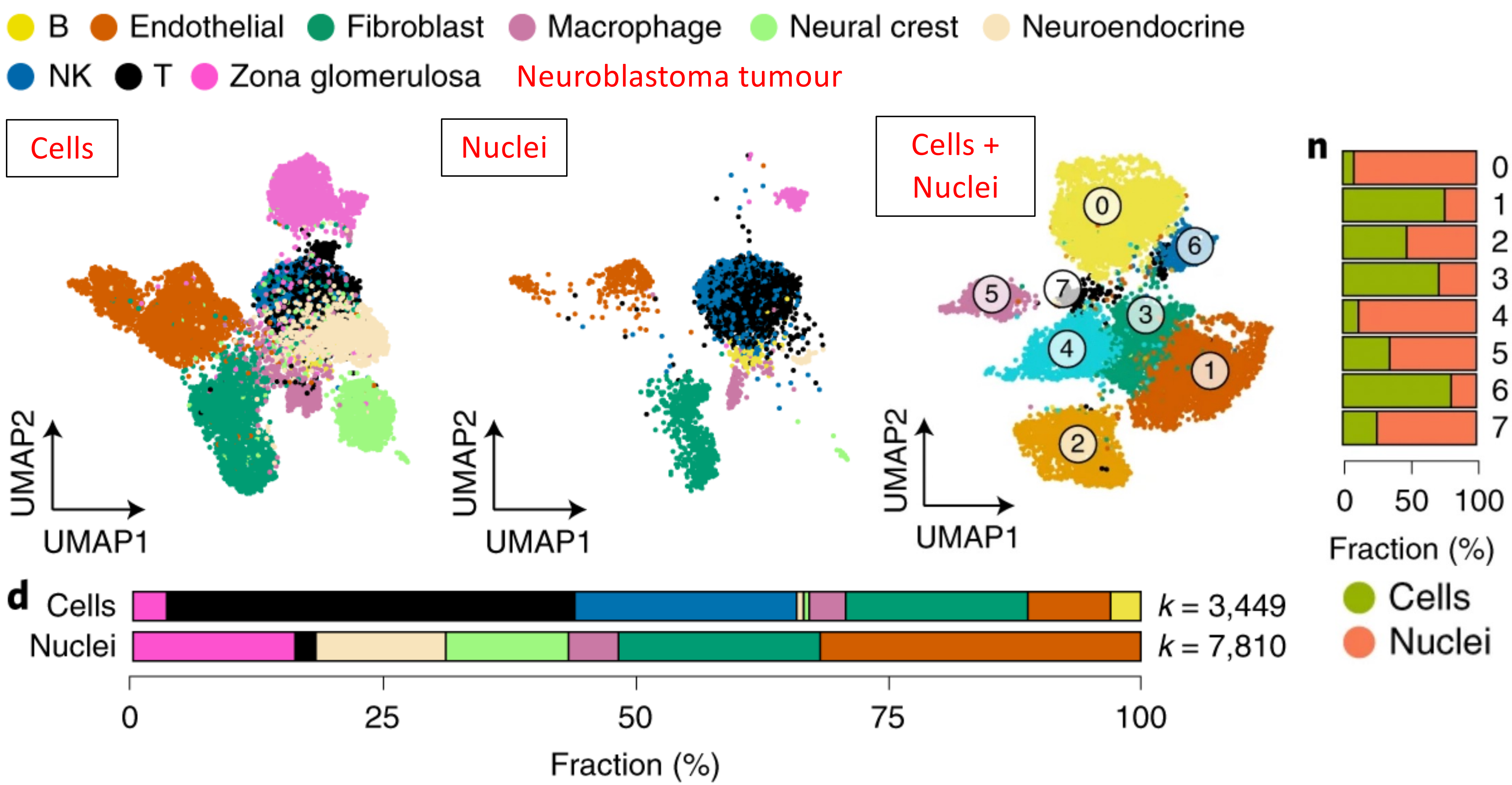
Avoids potential dissociation/processing-induced transcriptional shifts and cell-type depletion

Lower transcript and gene counts compared to live cell cytoplasmic RNA-seq

Increased mapping to ironic regions compared to mature cytoplasmic mRNA

Able to recapitulate clusters and cell-type marker genes using nuclei

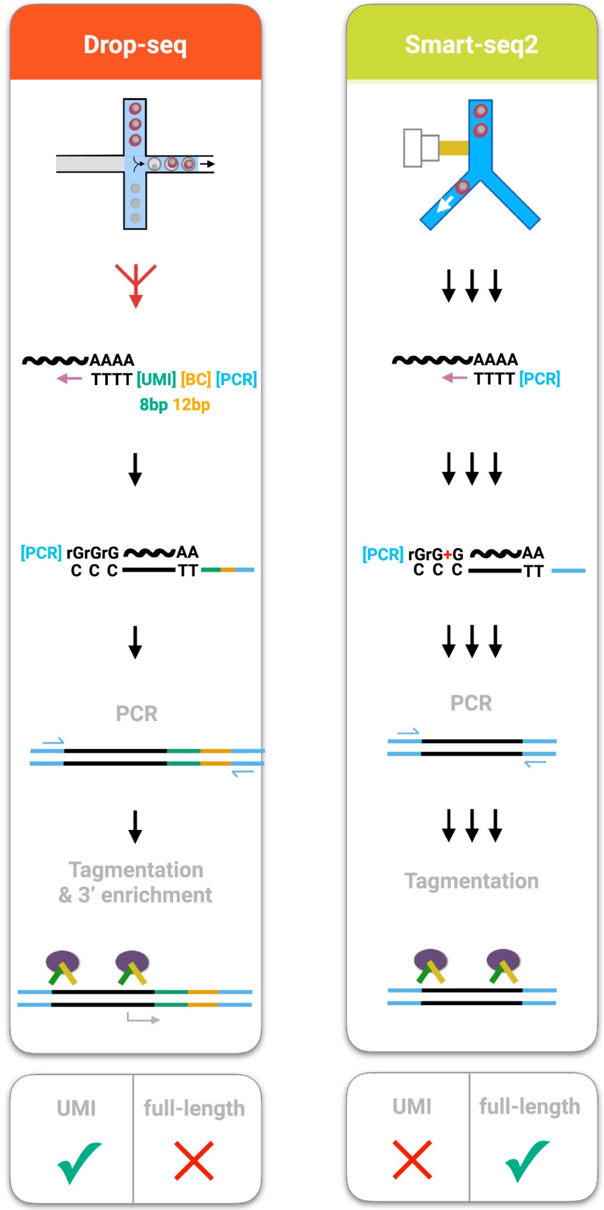
Nuclei RNA sequencing now routine: use frozen tissues but drawbacks of no cytoplasm, fewer transcripts, more introns, no cell enrichment



Slyper et al. *Nat Med* 26, 792–802 (2020). A single-cell and single-nucleus RNA-Seq toolbox for fresh and frozen human tumors.

Two commonly-employed RNA-seq strategies: 10X Genomics End-reads versus Smart-Seq2 Full-length transcripts

10x Genomics Chromium
$2-4/cell including sequencing
100–100,000 cells
3′- or 5′-tag method in droplets
Tagmentation, 3′ or 5′ enrichment, Illumina sequencing

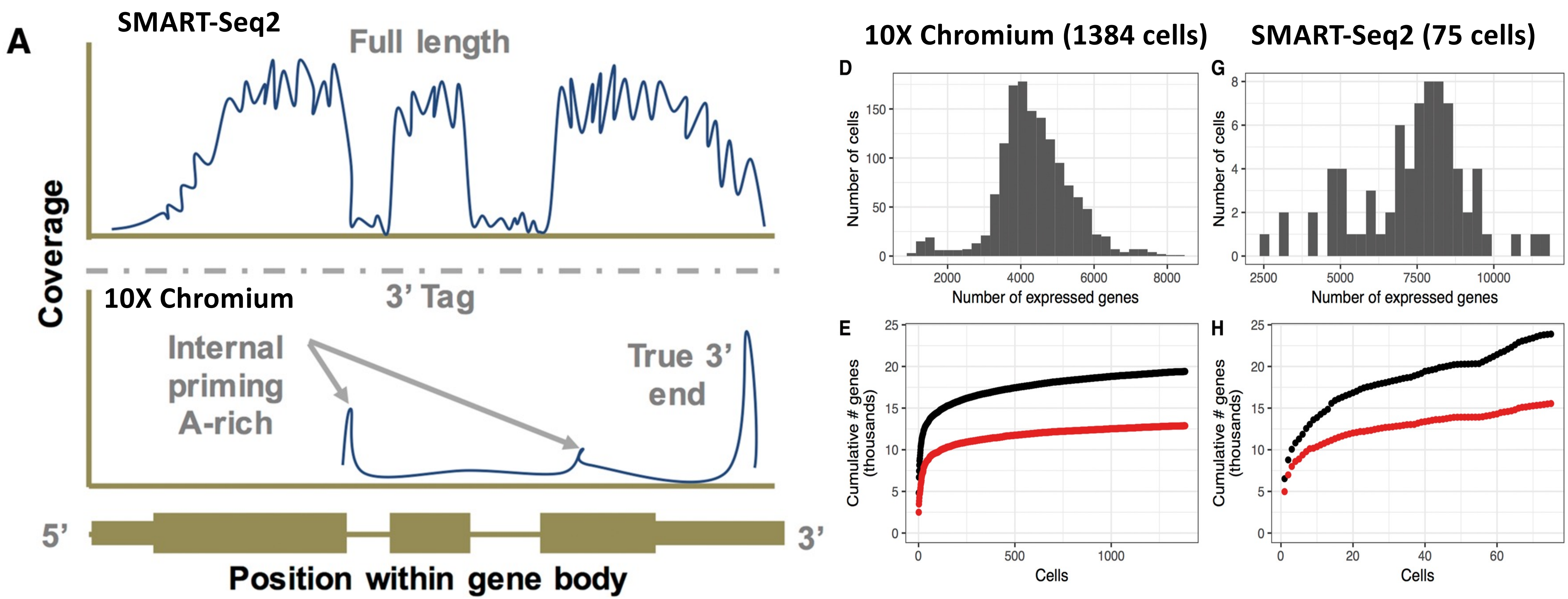


SmartSeq2
$28-69/cell including sequencing
96–384 cells
Full length capture in plates
Tagmentation, Illumina sequencing

Figure modified from:
Ziegenhain et al. Mol Cell. 2017 Feb 16;65(4):631-643.e4.

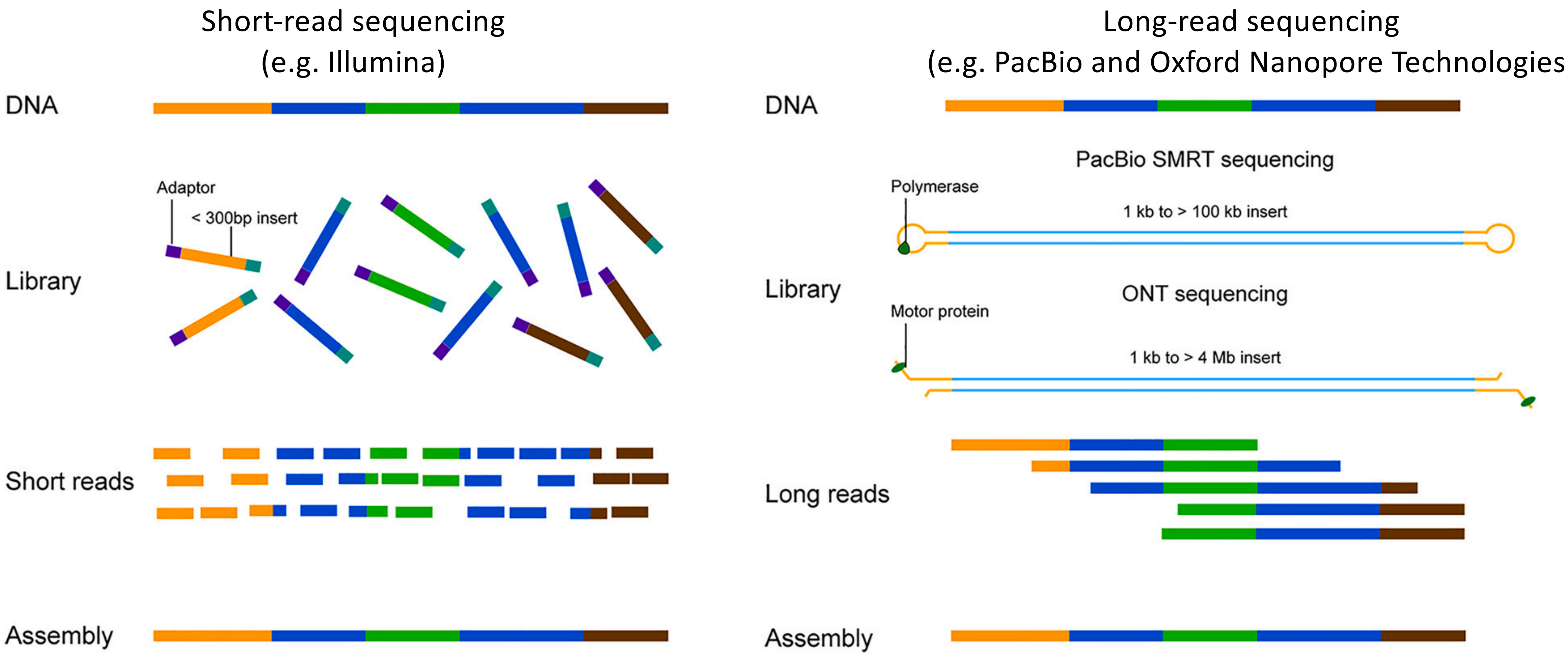
Baran-Gale, Chandra, and Kirschner K. Brief Funct Genomics. 2017 Nov 8.

Experimental design balancing transcript coverage, number of genes detected, and library complexity



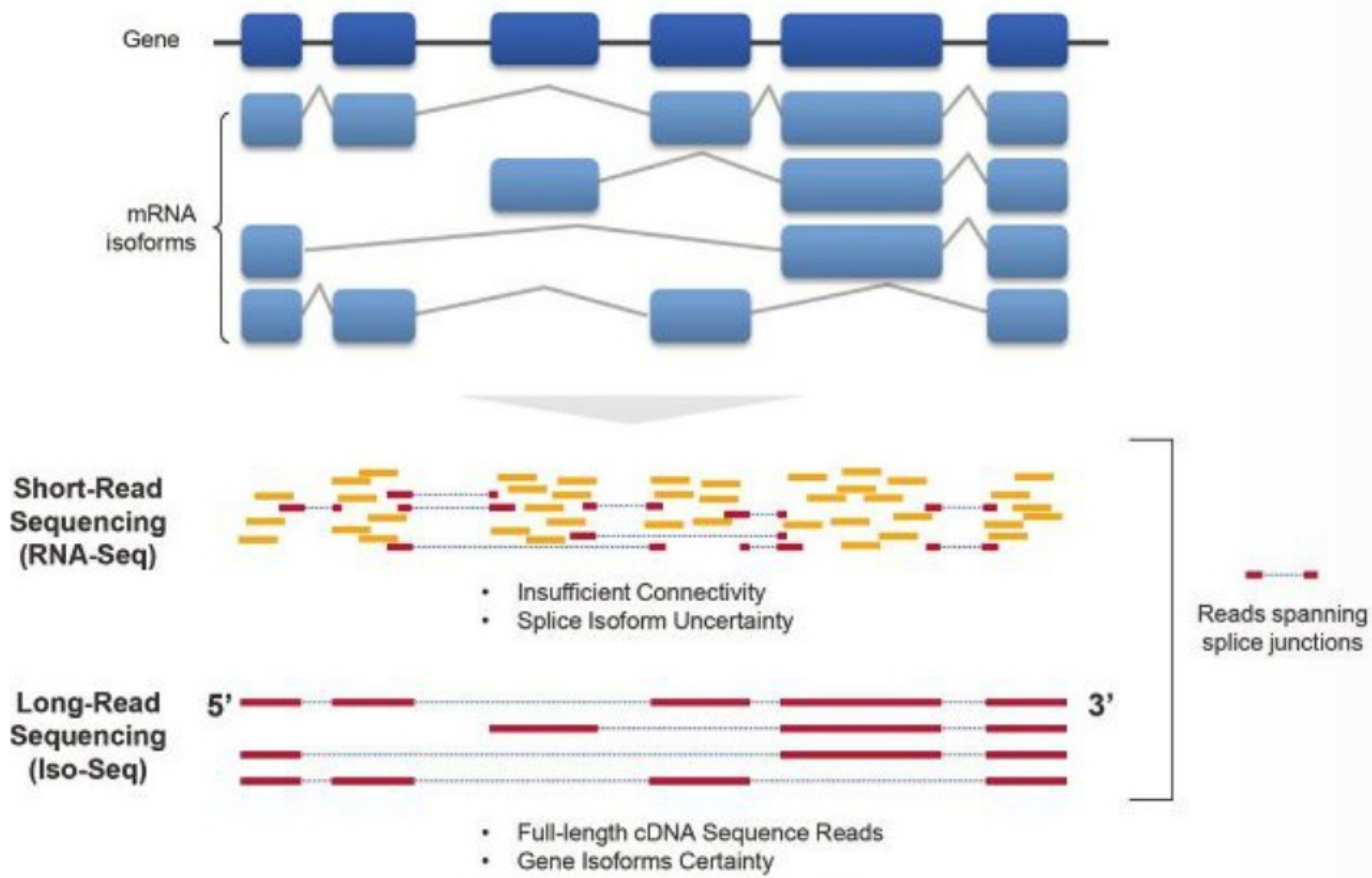
Baran-Gale, Chandra, and Kirschner K. Brief Funct Genomics. 2017 Nov 8.

Long-read sequencing technologies are applicable to DNA and RNA libraries barcoded at the single cell level



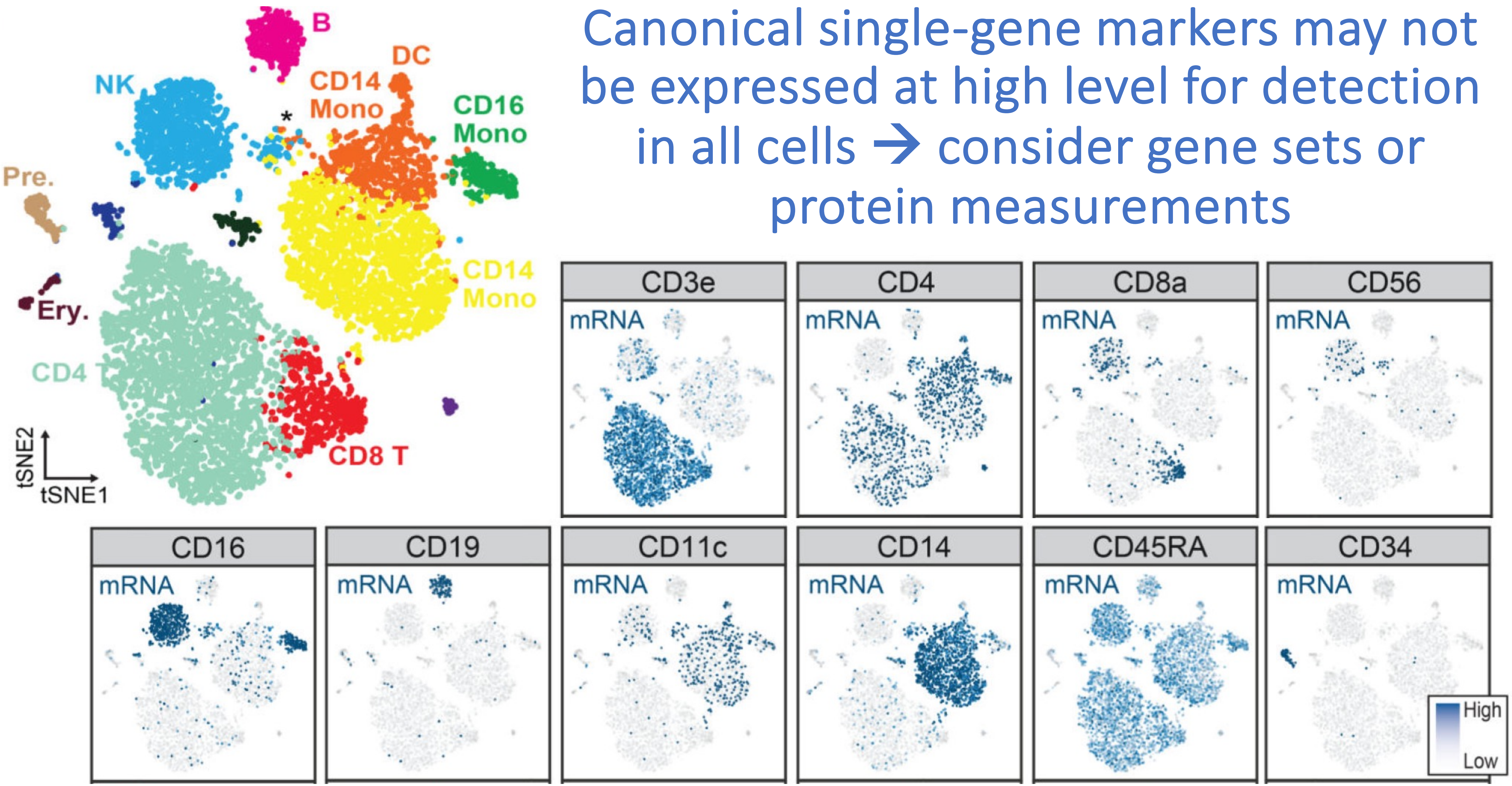
Chen and He. *Medical Review.* https://doi.org/10.1515/mr-2021-0013

Long-read sequencing technologies can enable complete reconstruction of transcript isoforms at the single cell level



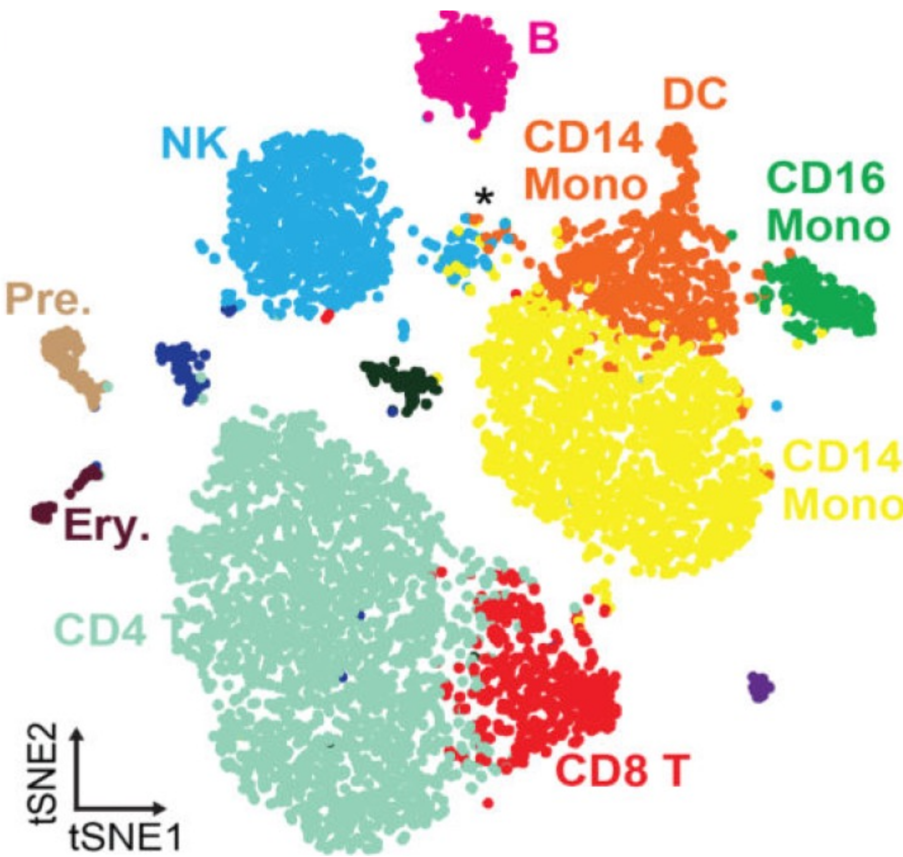
Chen and He. *Medical Review.* https://doi.org/10.1515/mr-2021-0013
https://www.ddw-online.com/full-length-isoform-sequencing-iso-seq-yields-a-more-comprehensive-view-of-gene-activity-1586-201608/

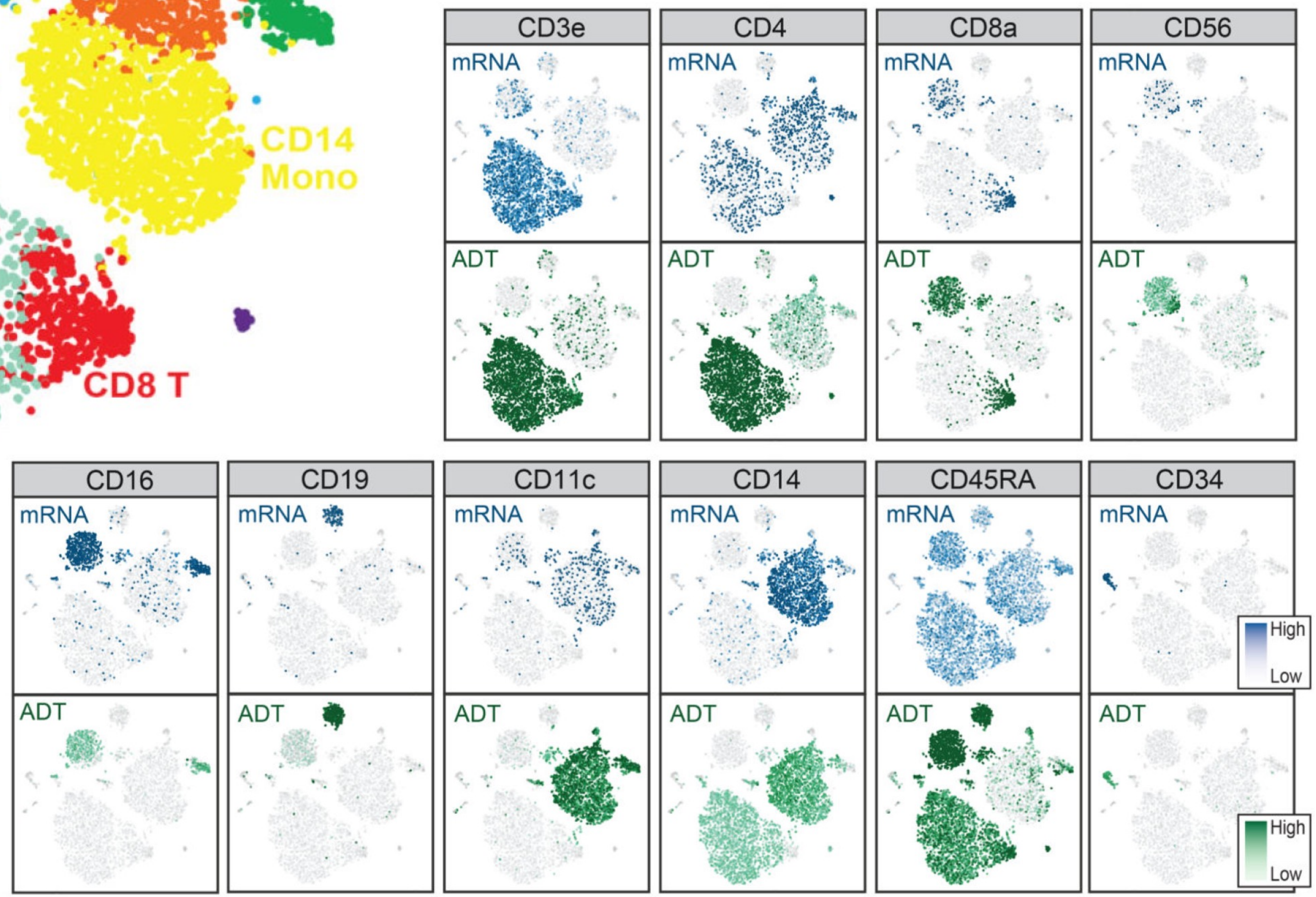
Canonical single-gene markers may not be expressed at high level for detection in all cells → consider gene sets or protein measurements

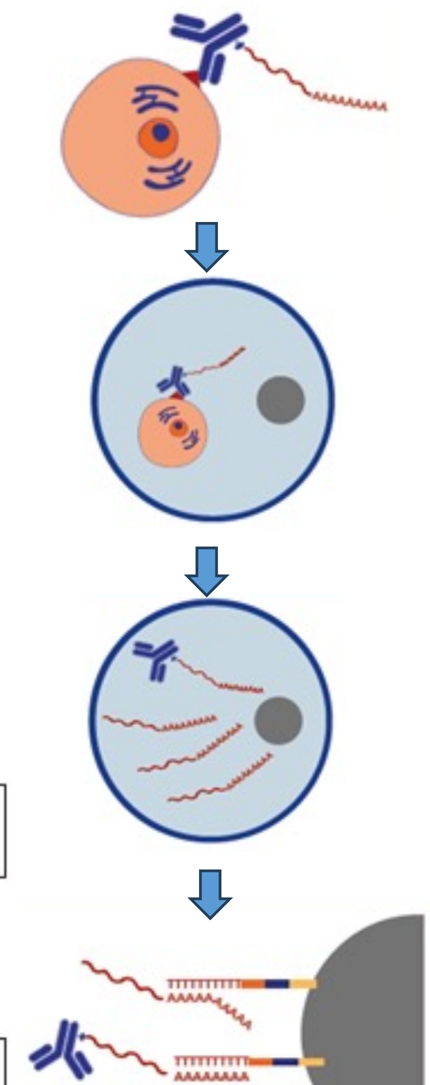


Modified from Stoeckius et al. Nat Methods. 2017 Sep;14(9): 865-868.

Protein & RNA expression can be measured simultaneously by CITE-seq (Cellular Indexing of Transcriptomes & Epitopes by Sequencing)





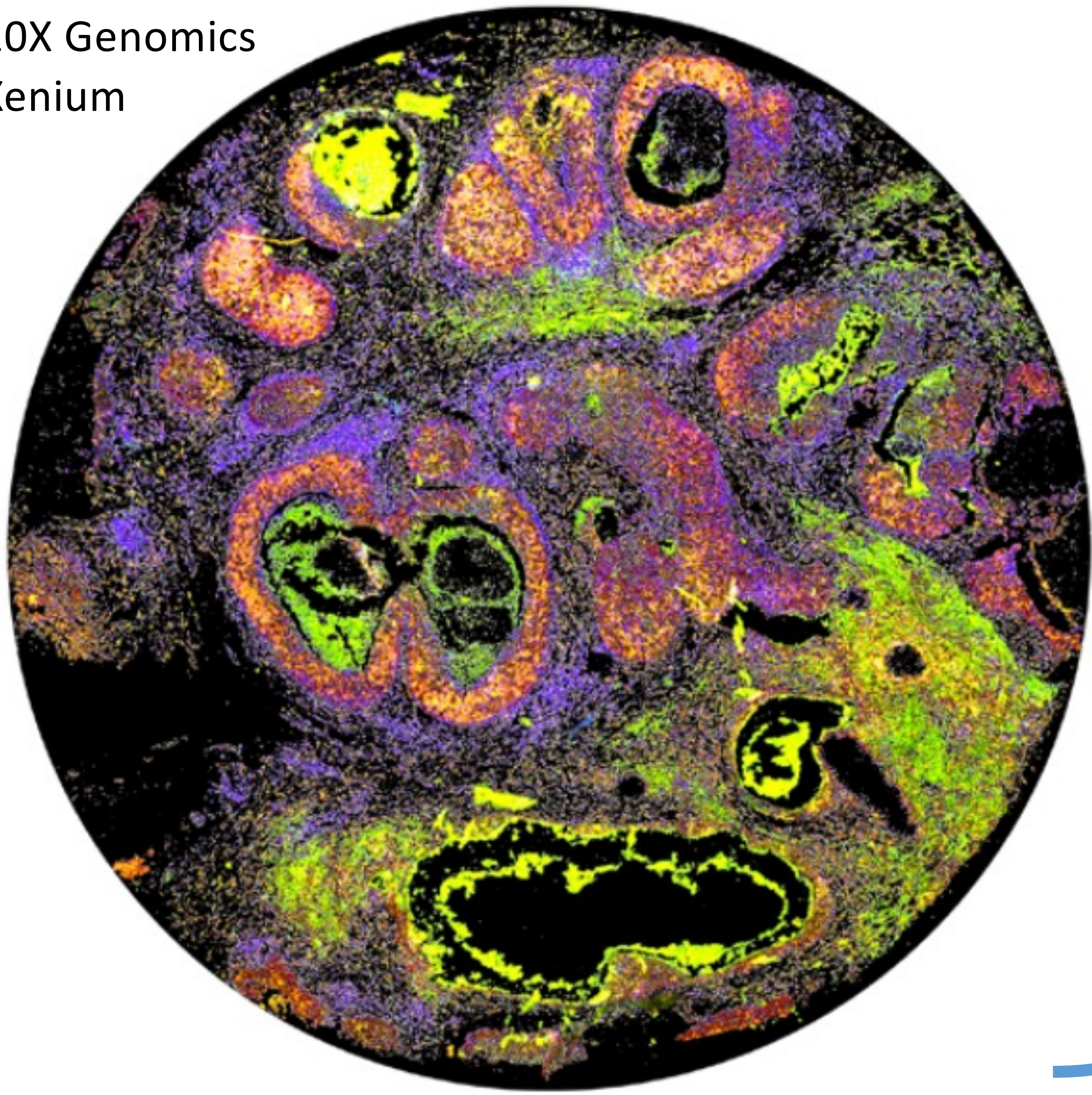


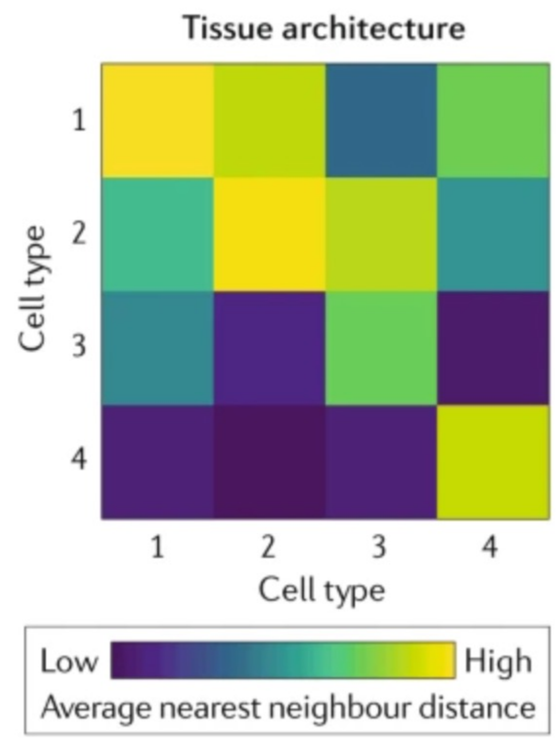
alphathera.com

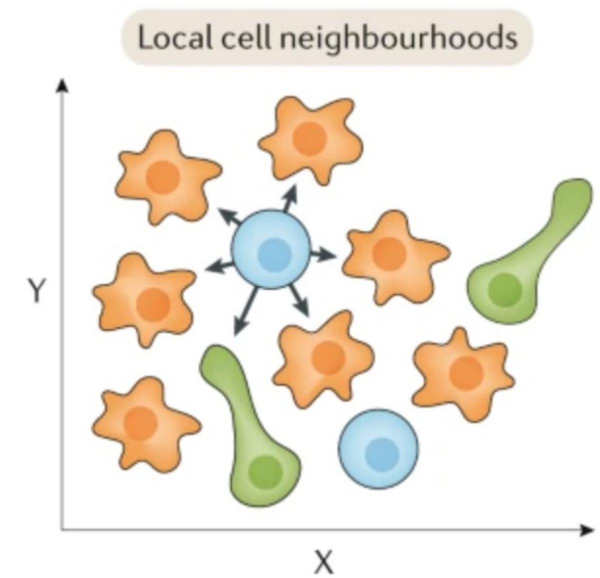
Stoeckius et
Nat Metho
2017 Sep;14
865-868.

New spatial technologies enable additional cellular metadata describing physical distances between cell types and cell states

10X Genomics
Xenium



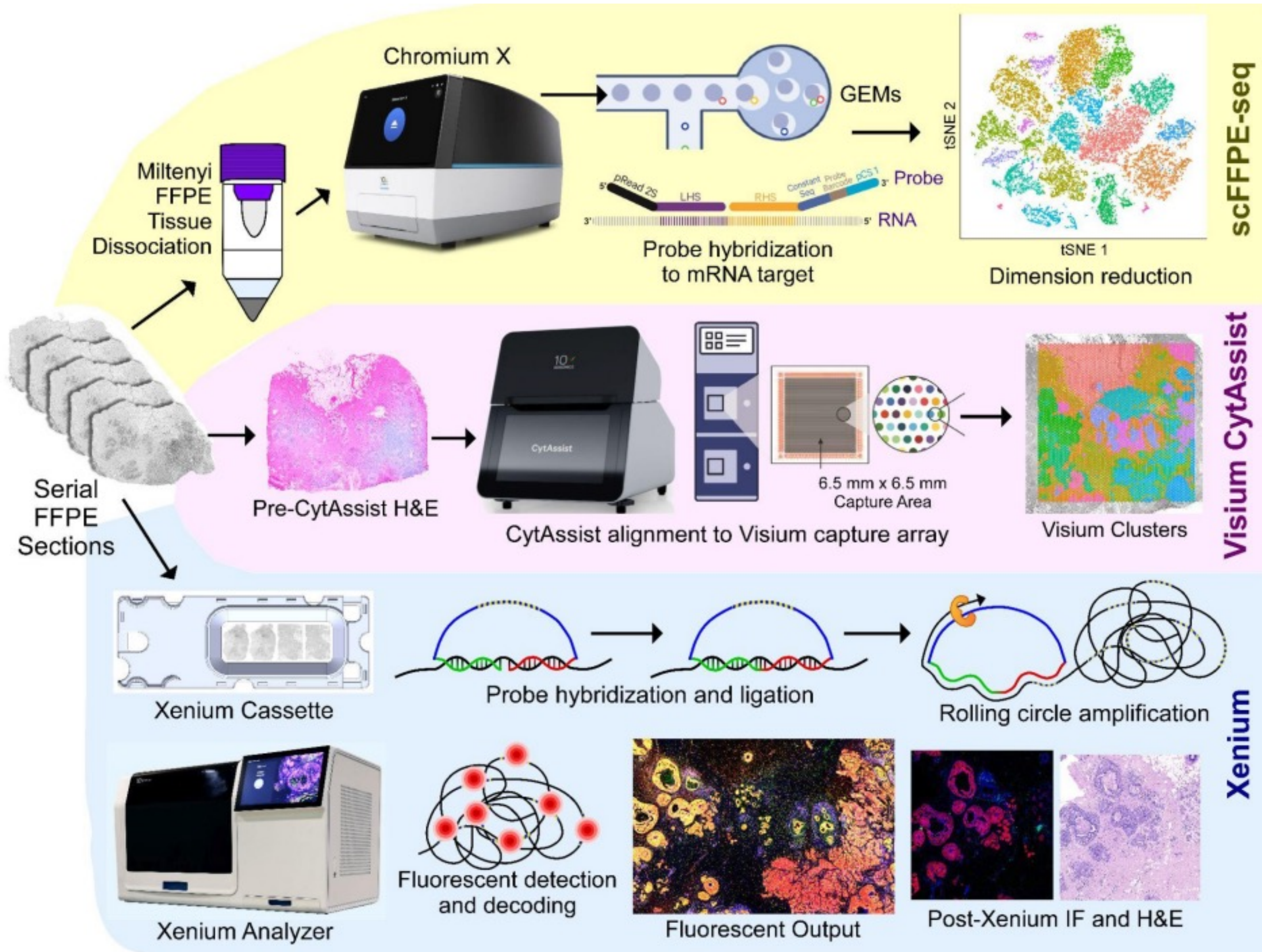




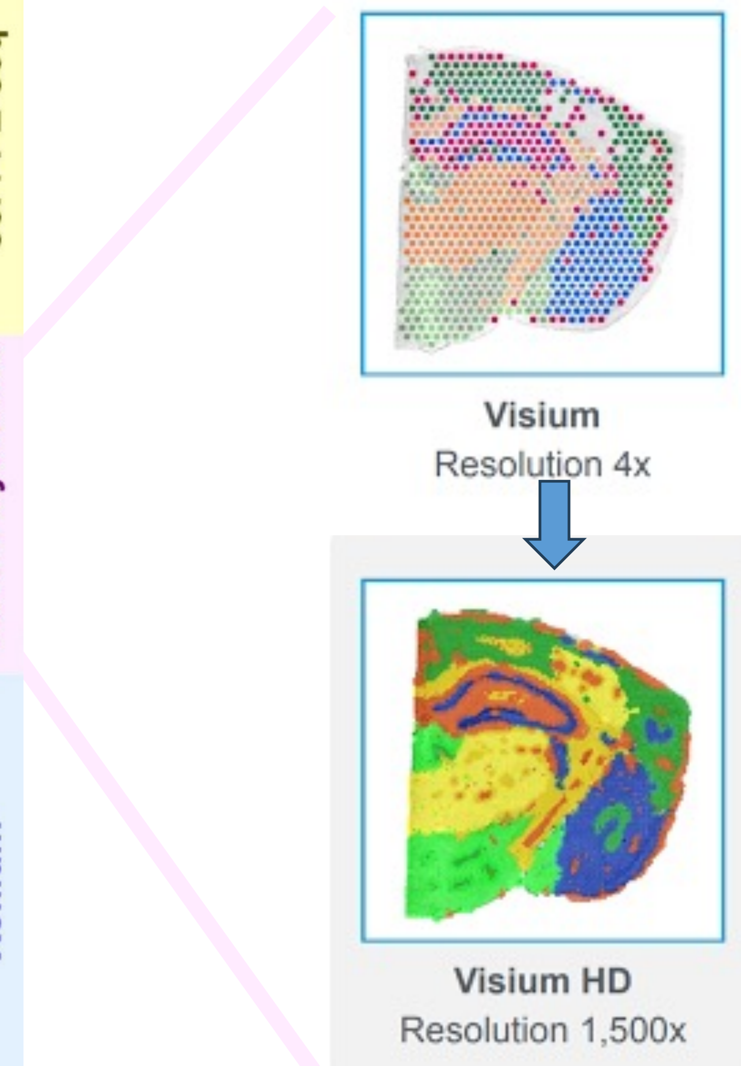
https://www.10xgenomics.com/in-situ-technology

Stuart and Satija. *Nature Reviews Genetics.* 20:257-272. May 2019

Spatial technologies are currently panel-based, but moving rapidly to whole transcriptome + protein at subcellular resolution

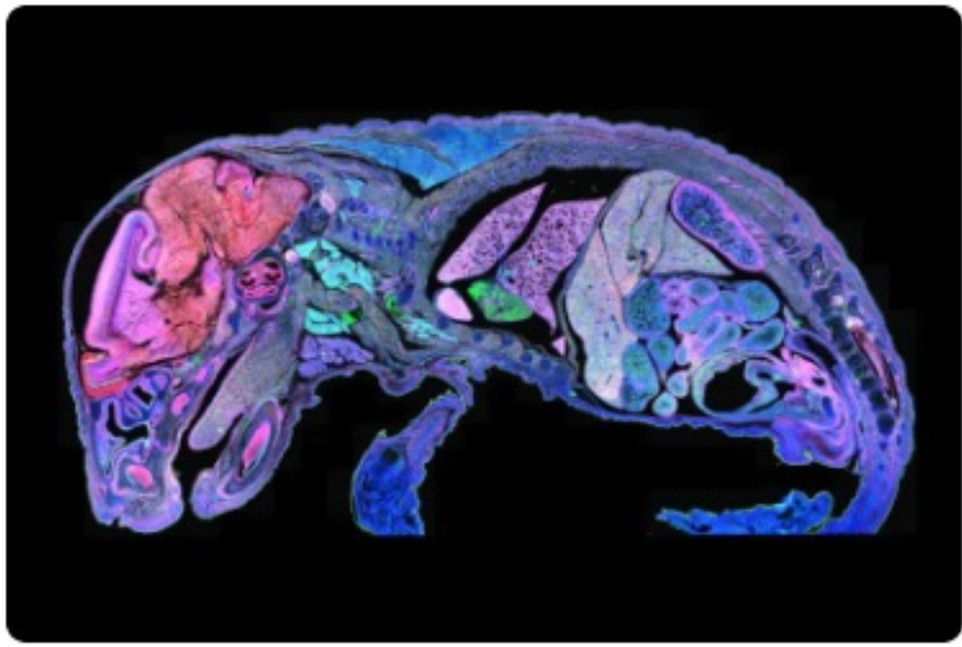


Janesick et al. bioRxiv 2022.10.06.510405 doi: https://doi.org/10.1101/2022.10.06.510405



https://omicsomics.blogspot.com/2021/02/more-details-on-10xs-sample-profiling.html

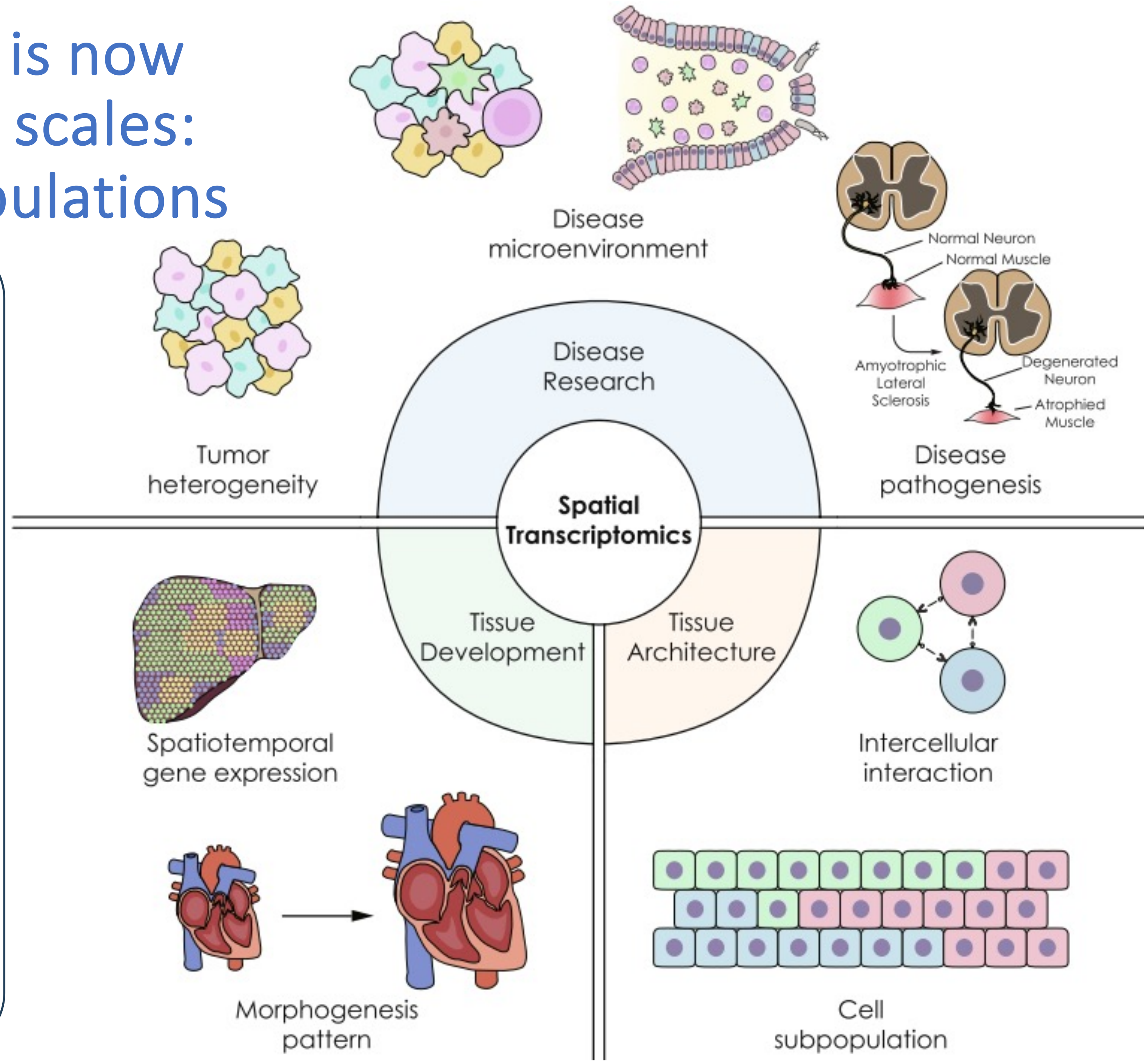
Cell organization & cell state is now measureable at all biological scales: cells, tissues, organisms, populations



Mouse Tissue Atlassing (379 genes)

Includes gene markers for bladder, bone marrow, brain, heart, kidney, limb muscle, lung, pancreas, thymus, etc.

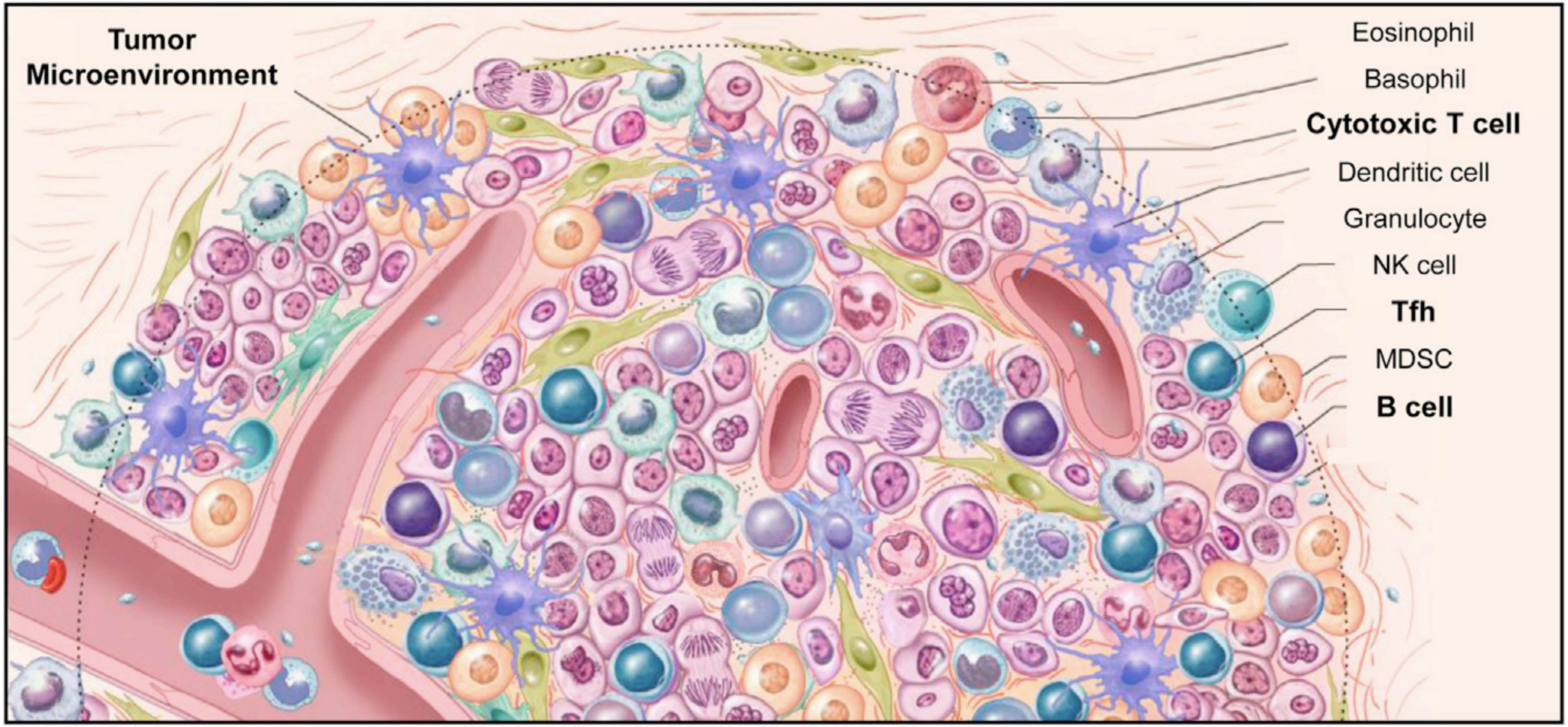
https://www.10xgenomics.com/products/xenium-panels



Du, Yang et al. *J Transl Med.* 2023; 21: 330.

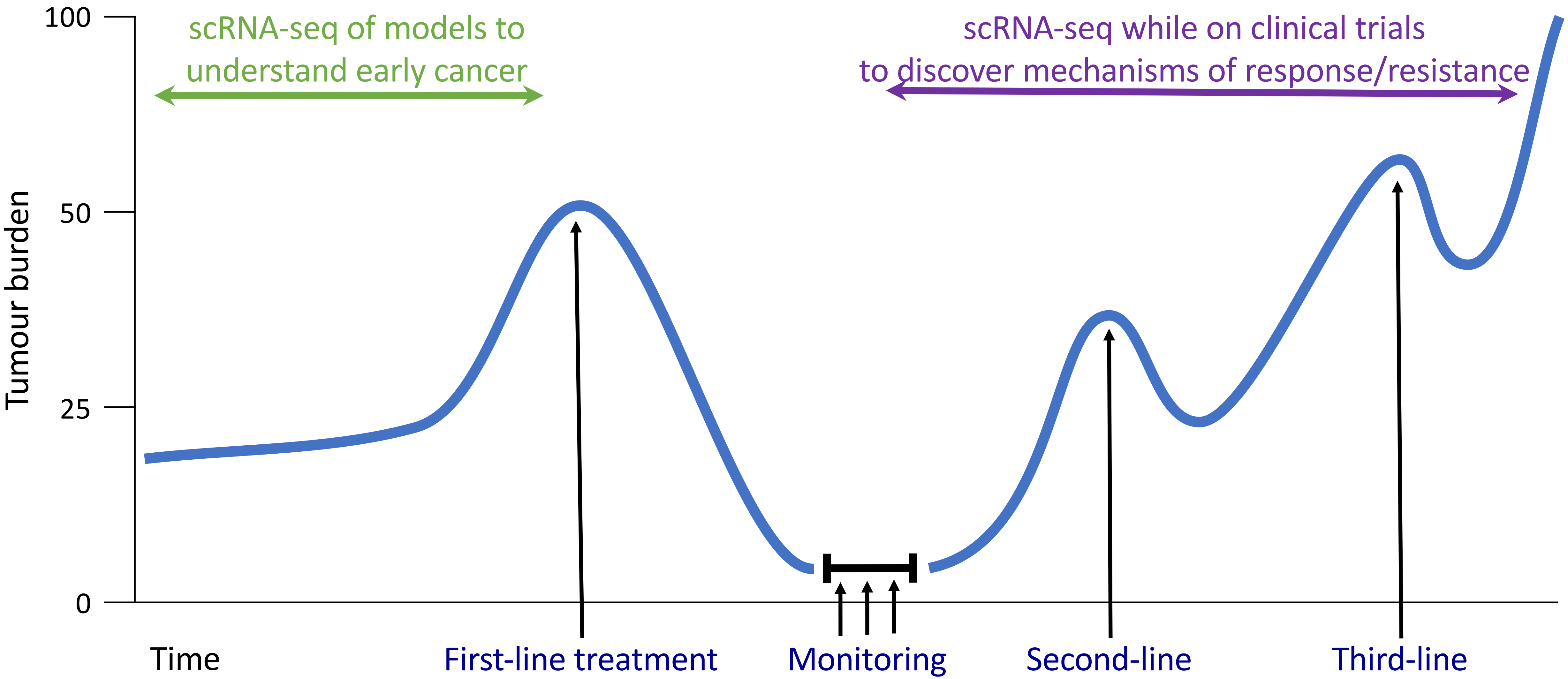
Cancer as an example

Tumours are dynamic populations of cancer, immune, and other cells that change in frequency and function over the course of treatment



Restifo NP. *Immunity*. 2013 Oct 17;39(4):631-2.

scRNA-seq in practice: How do cancer and immune systems change over time? Is there clinical relevance?

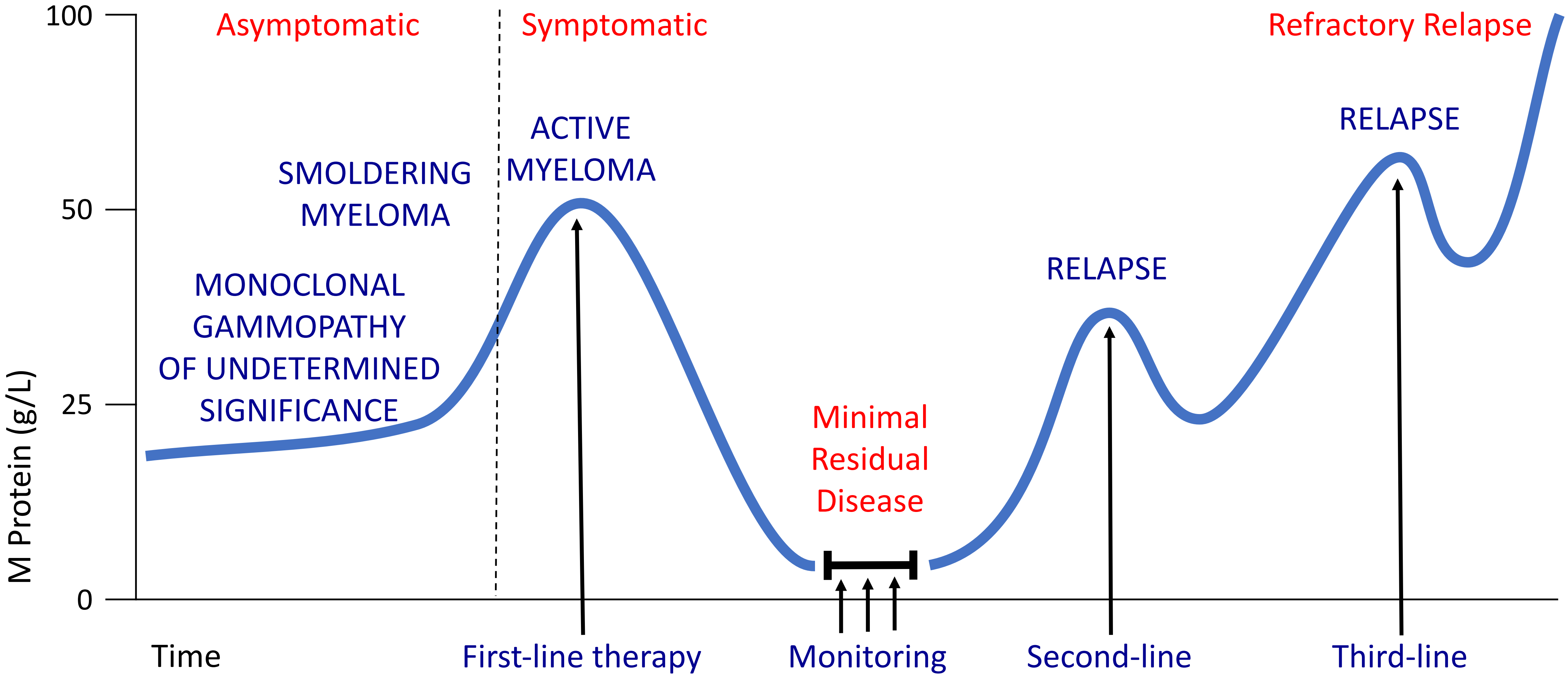


Watching immune systems evolve at the single cell level as cancer develops

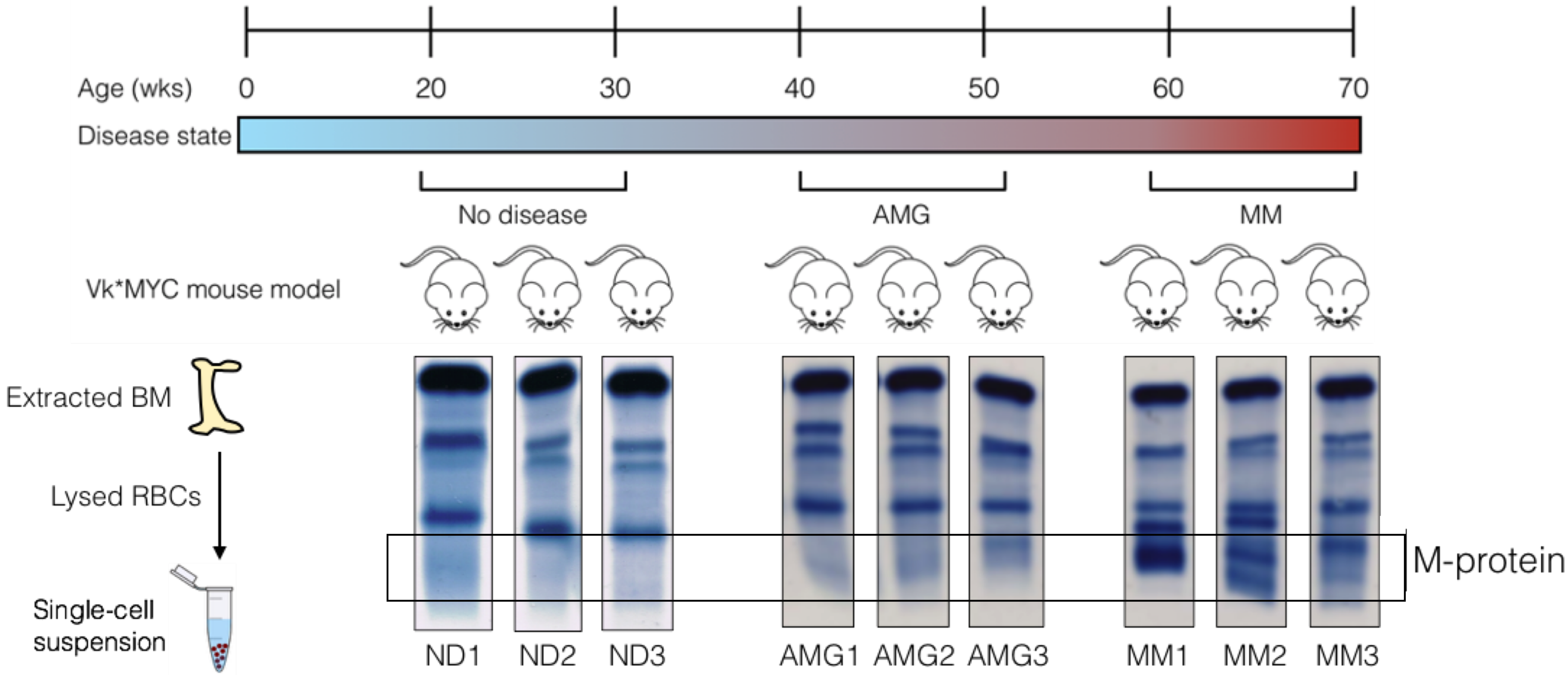
Croucher et al. *bioRxiv.* 2021 Jan. 10.22.464971
Single-cell transcriptional analysis of the immune tumour microenvironment during myeloma disease evolution.

Croucher et al. *Nature Communications.* 2021 Nov; 12(6322).
Longitudinal single-cell analysis of a myeloma mouse model identifies subclonal molecular programs associated with progression.

Myeloma begins as a benign condition that progresses to incurable malignancy that tides during treatment and can be tolerated as MRD

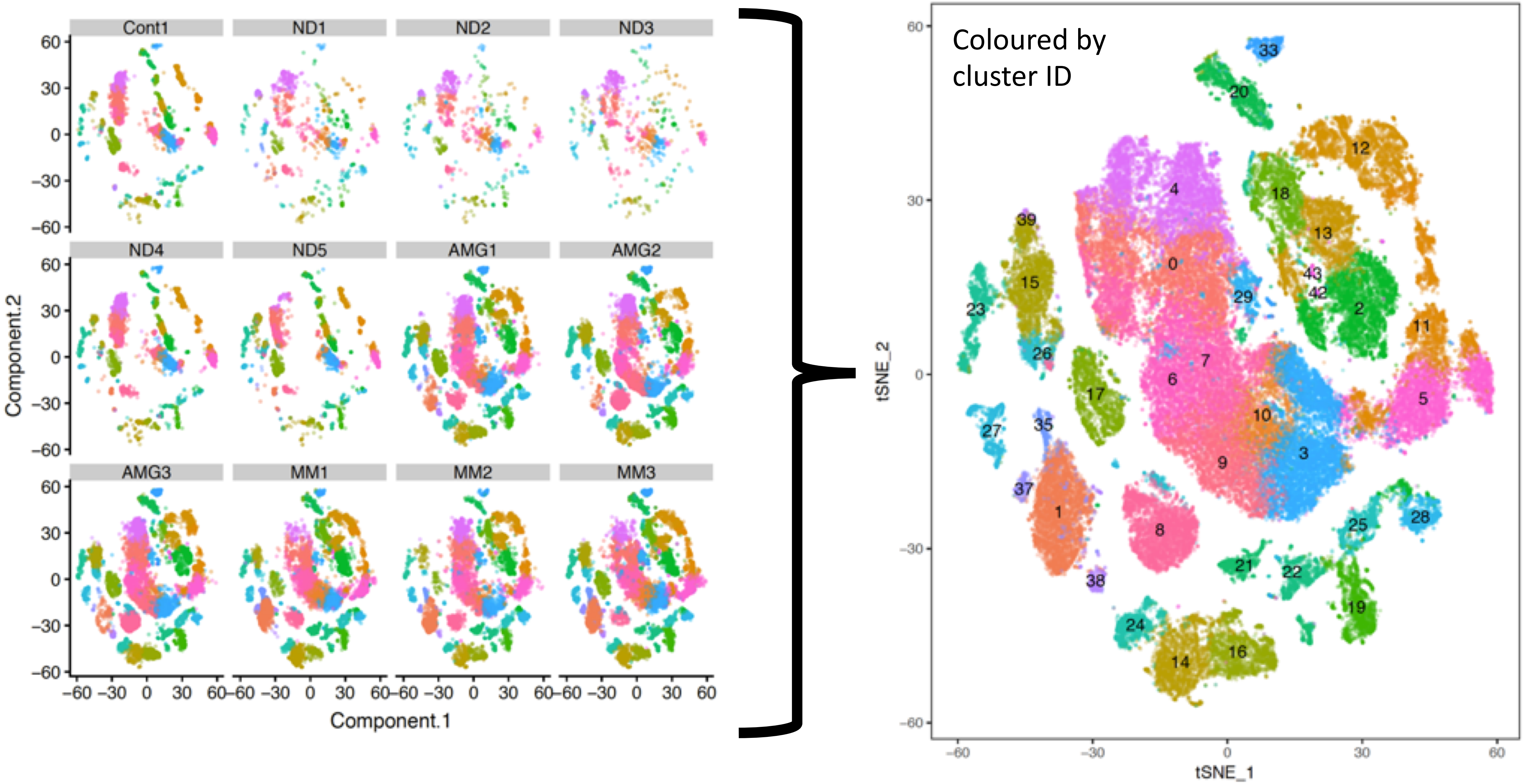


Vκ*MYC mouse model enables serial dissection of bone marrow microenvironments during transition from MGUS to MM

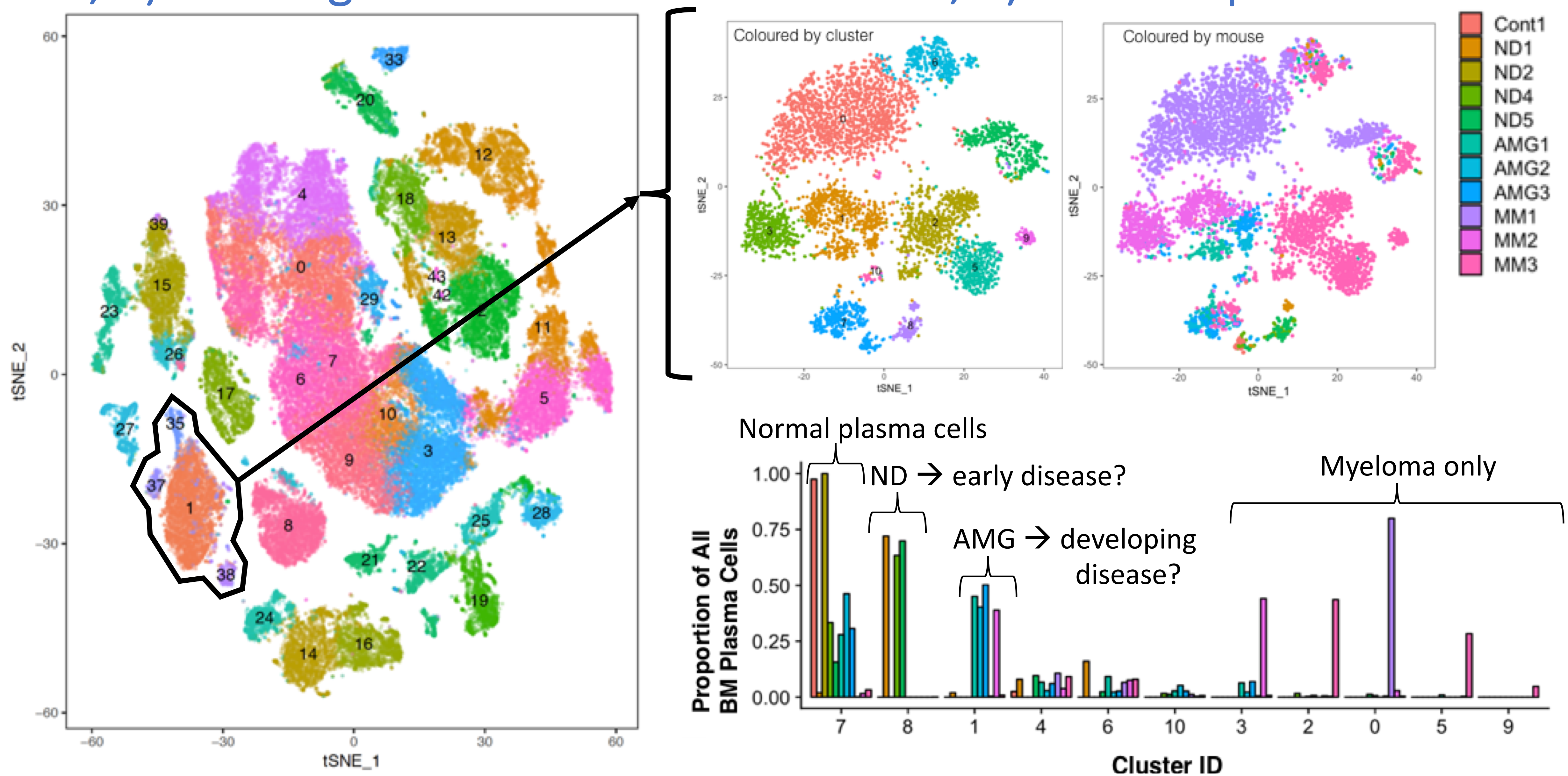


Collaboration with Michael Sebag (McGill) and Leif Bergsagel (Mayo)
Mouse model published by Chesi et al. Cancer Cell. 2008 Feb; 13(2): 167–180.

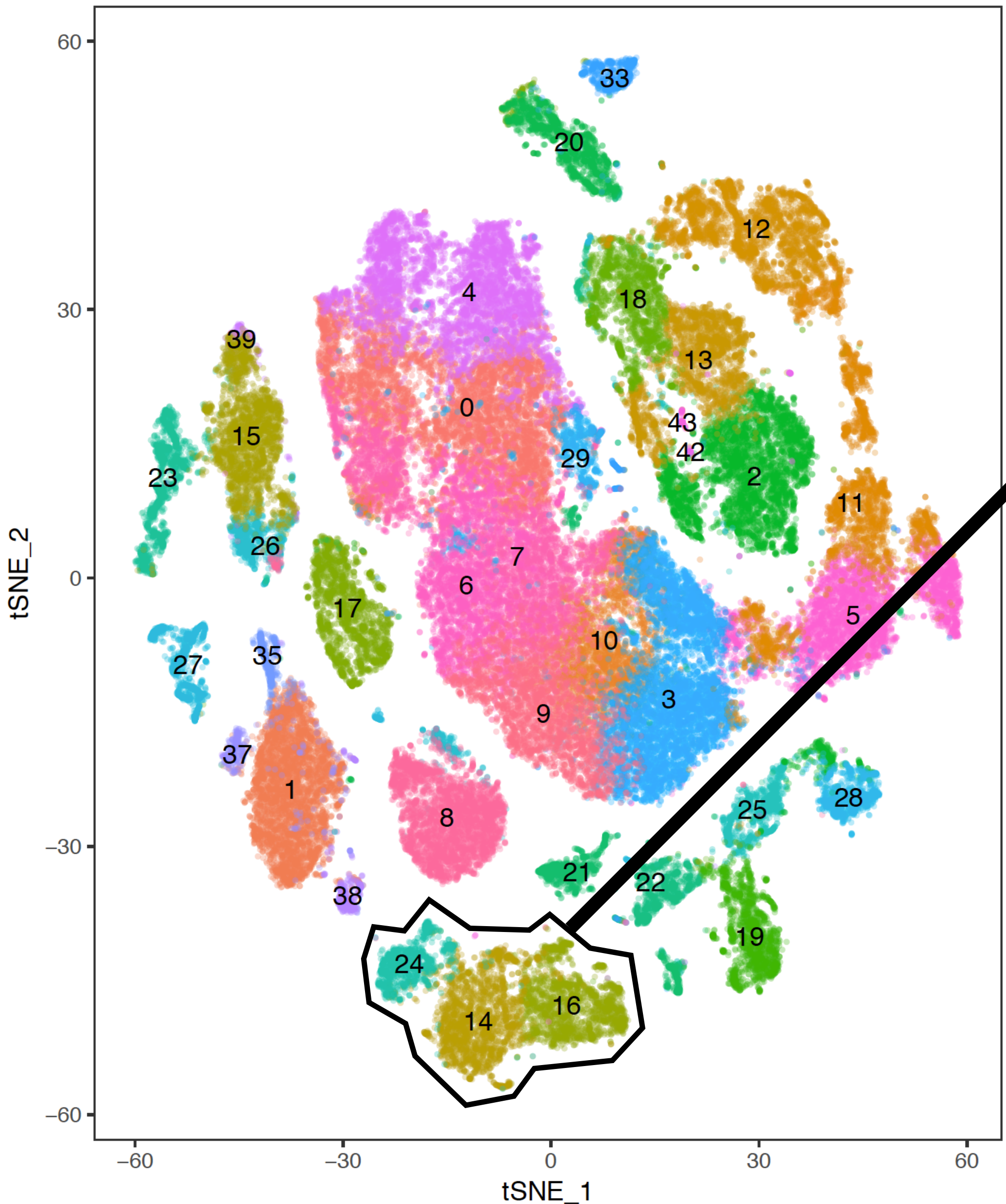
Integrated data from >90k cells from 12 mice during disease evolution

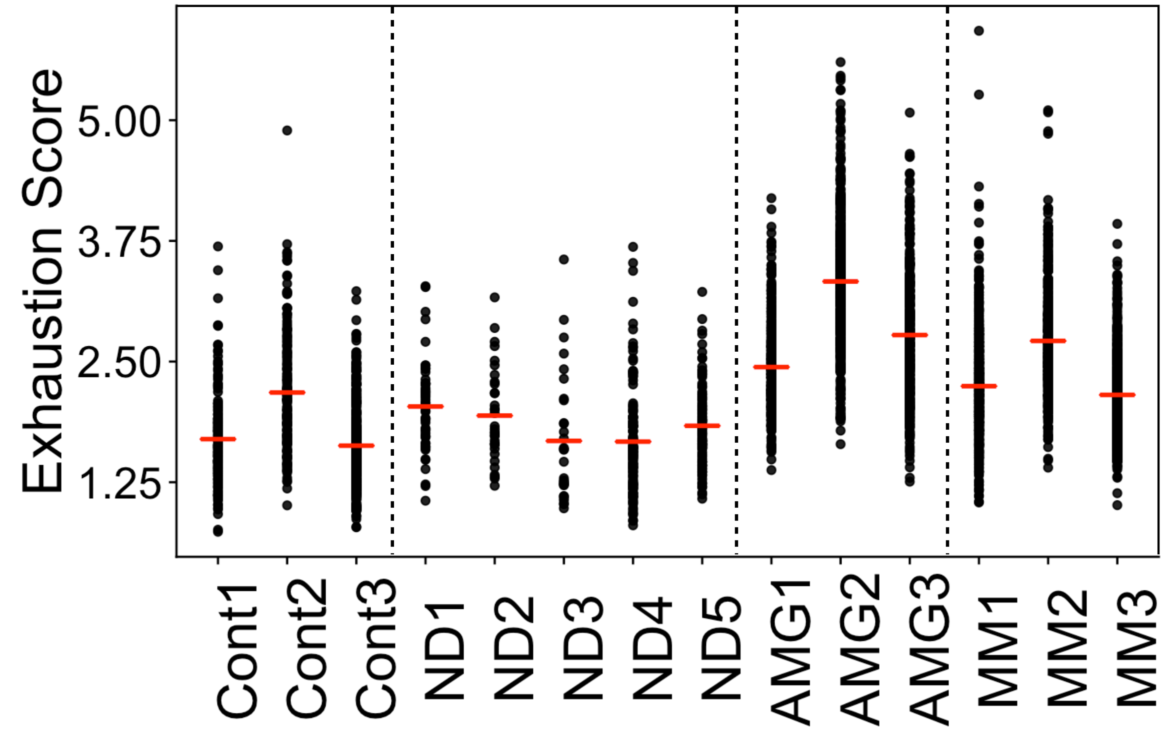


Focused re-analysis of plasma cells found A) normal plasma cells in all mice, B) evolving disease in ND & AMG mice, C) cells unique to each MM

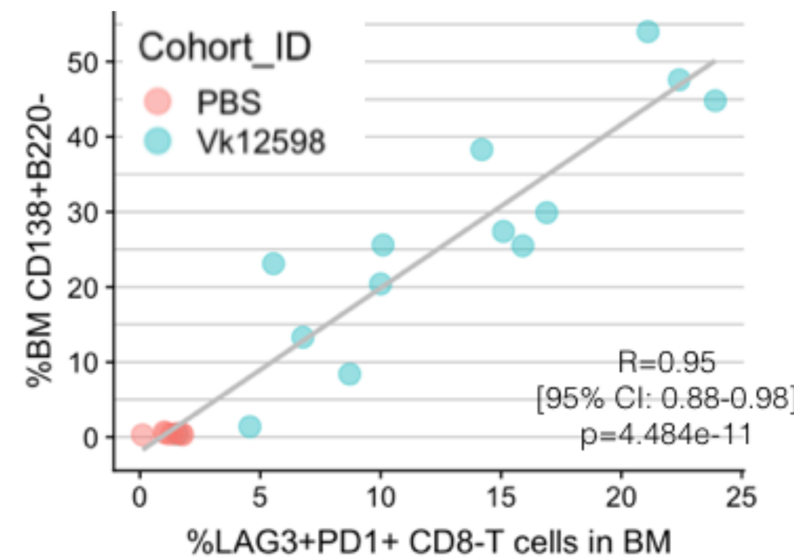


T-cells display increased exhaustion signatures as myeloma develops

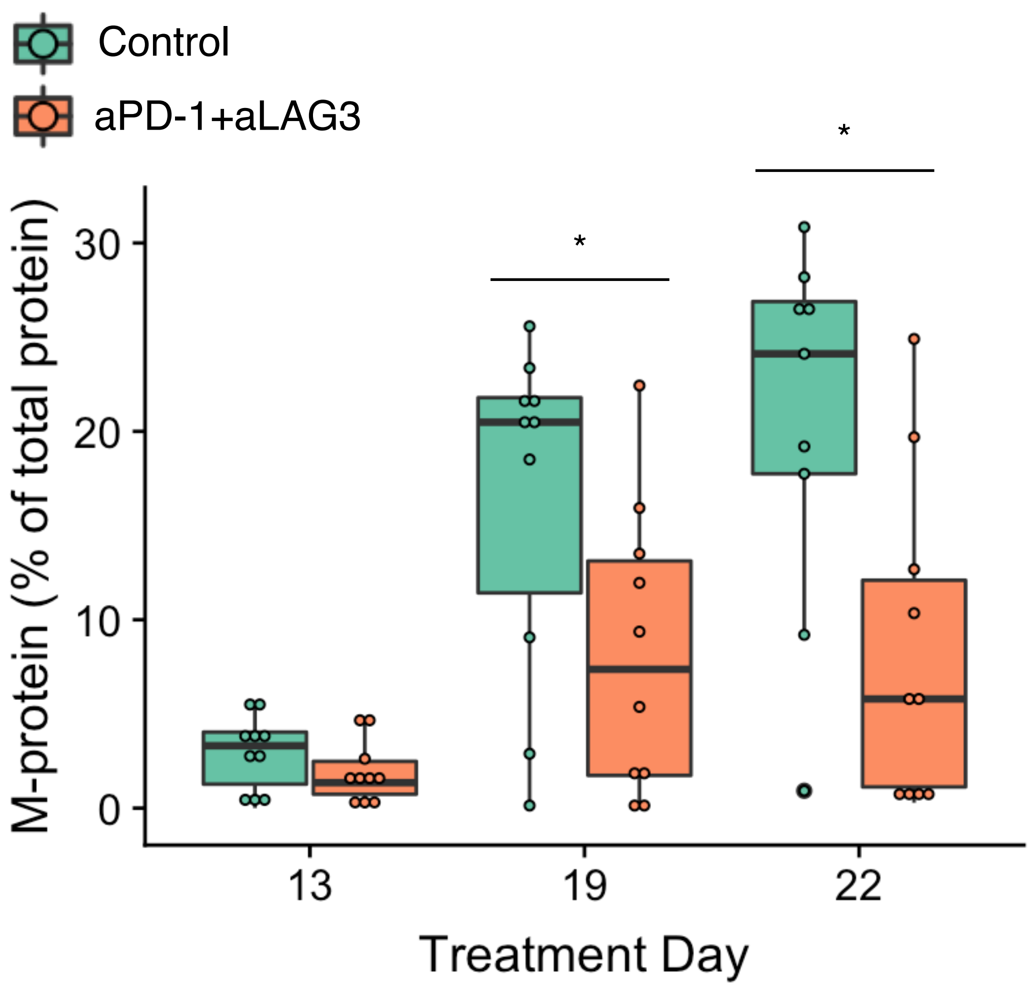


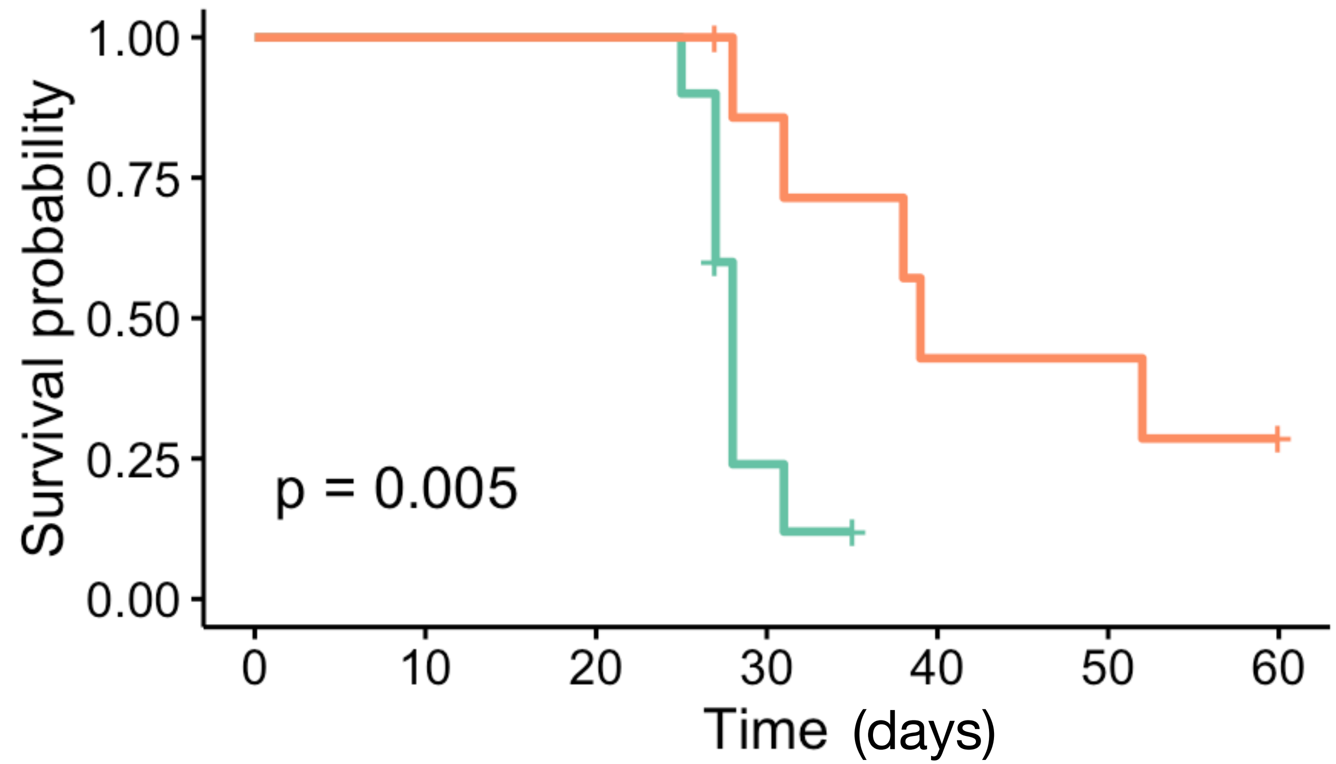


Validation by flow cytometry in transplantable mouse model (Vk12598)



Combinatorial treatment with anti-LAG3 + anti-PD-1 antibodies delays myeloma progression in transplantable mouse model

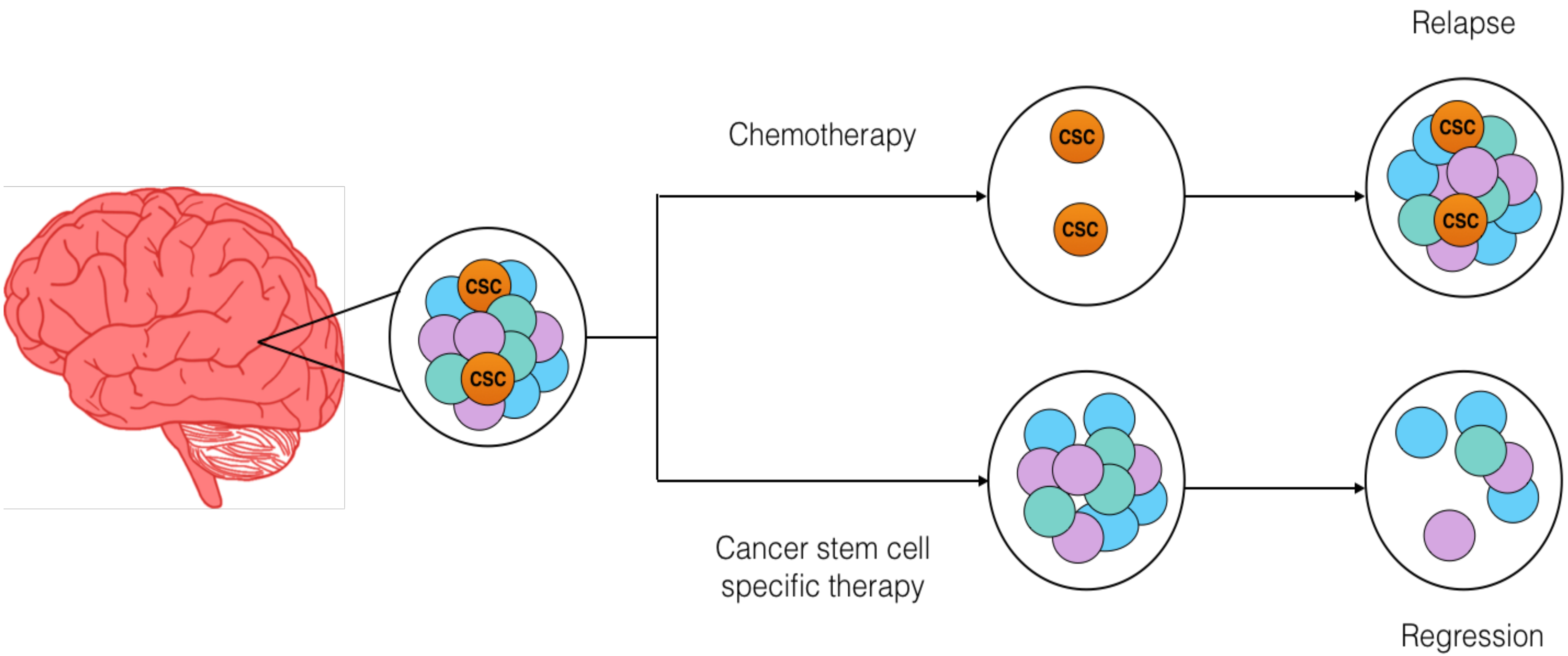




Cancer stem cells & subclones inform tumour development & treatment outcome

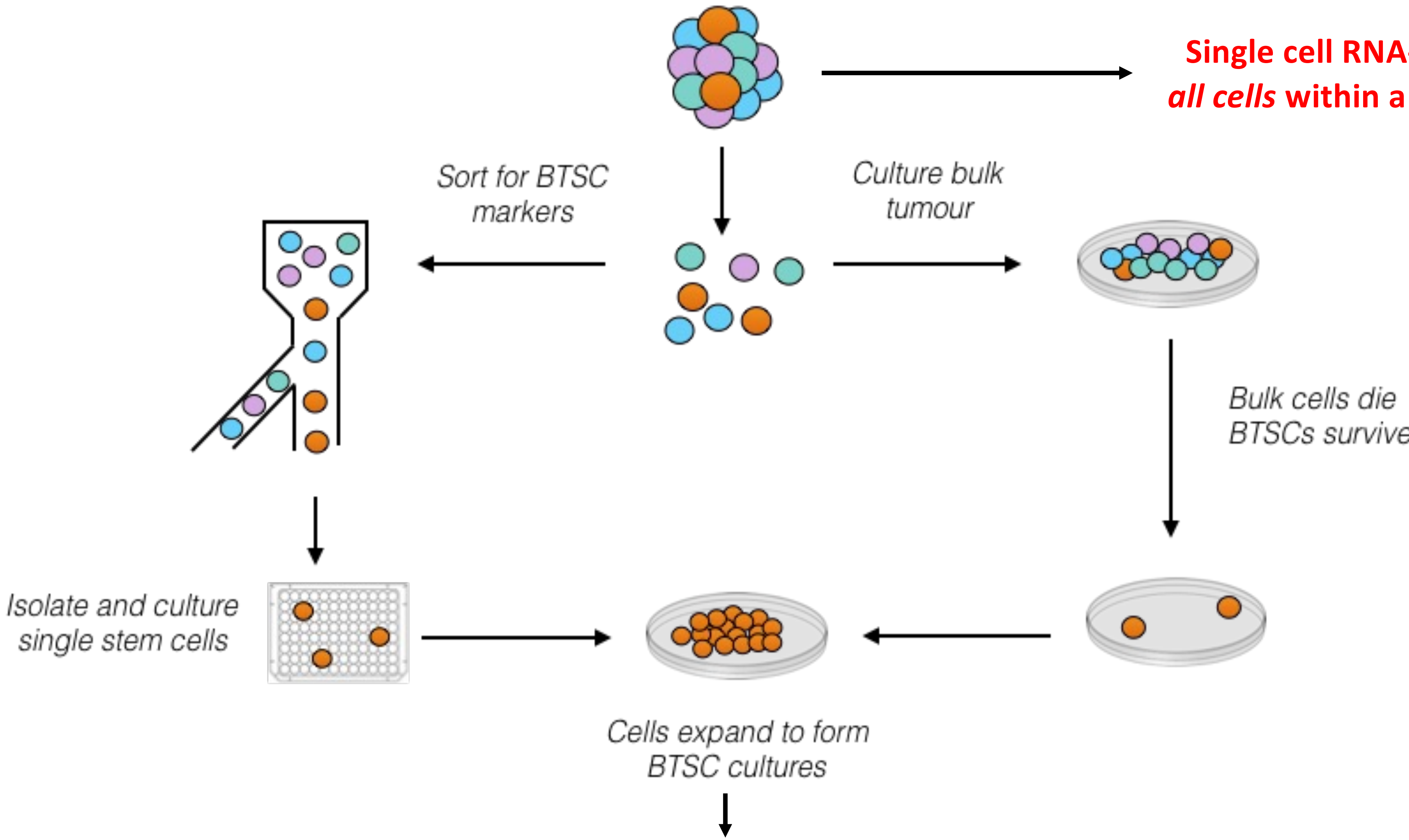
Richards, Whitley et al. *Nature Cancer*. 2021 Feb.
Gradient of developmental and injury-response transcriptional states defines functional vulnerabilities underpinning glioblastoma heterogeneity

Glioblastomas contain self-renewing cancer stem cells that contribute to tumour initiation and therapeutic resistance



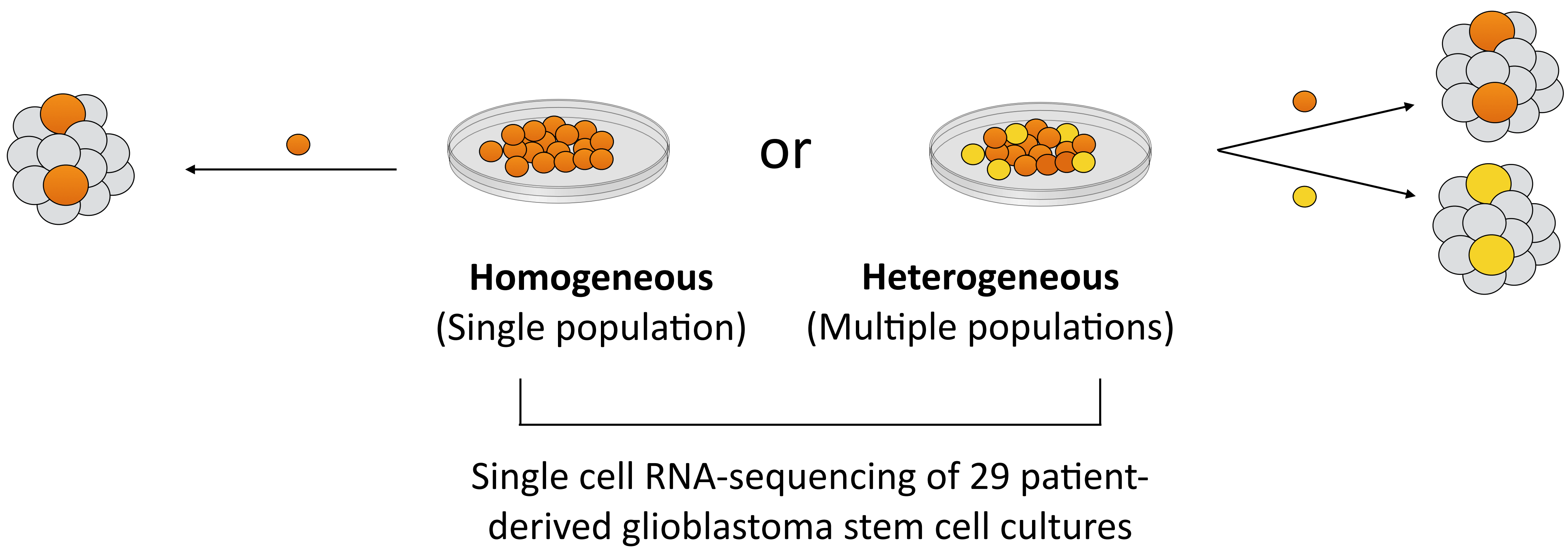
Brain tumour stem cell cultures derived from primary GBMs

Single cell RNA-seq of *all cells* within a tumour

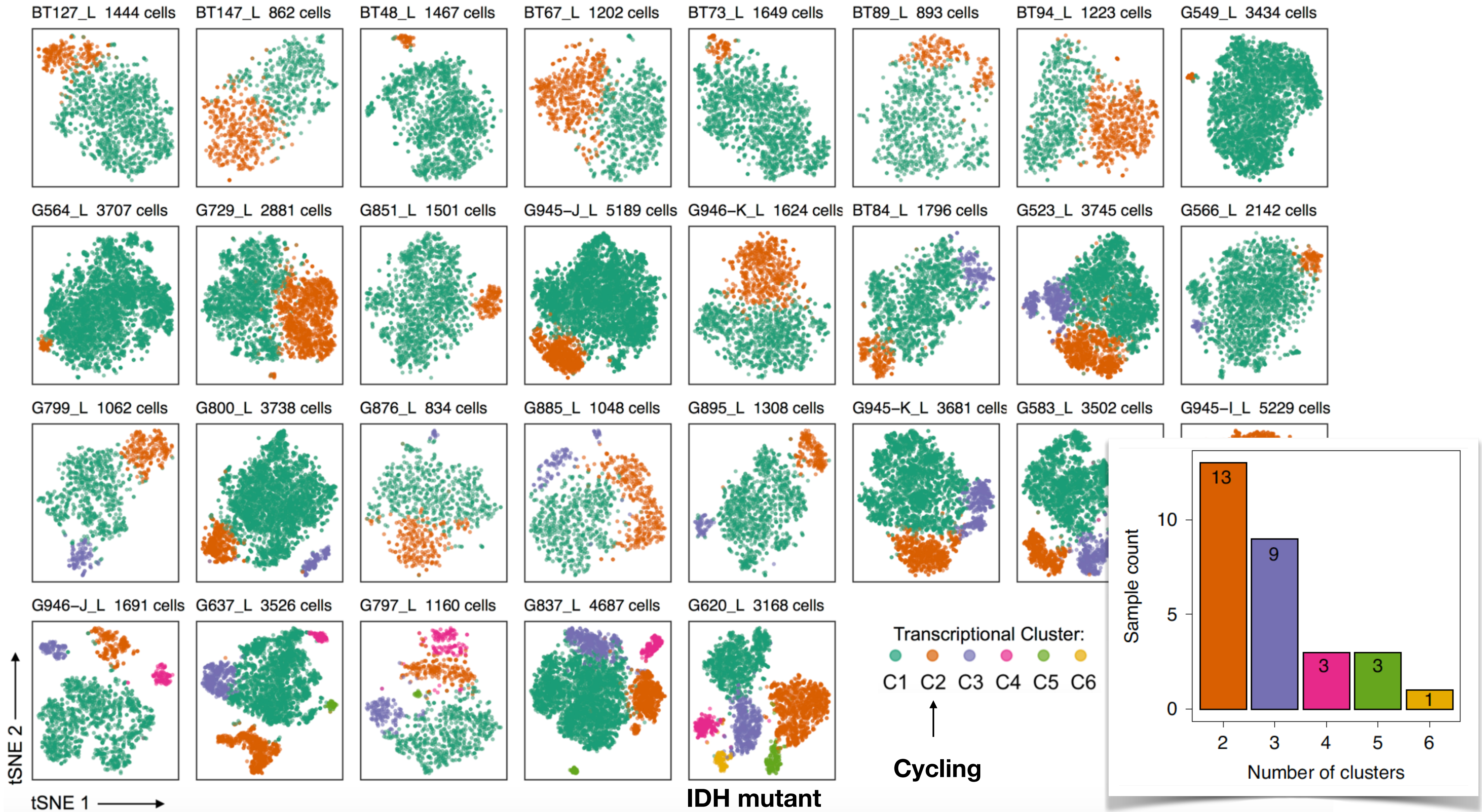


Single cell RNA-seq of only the brain tumour stem cells

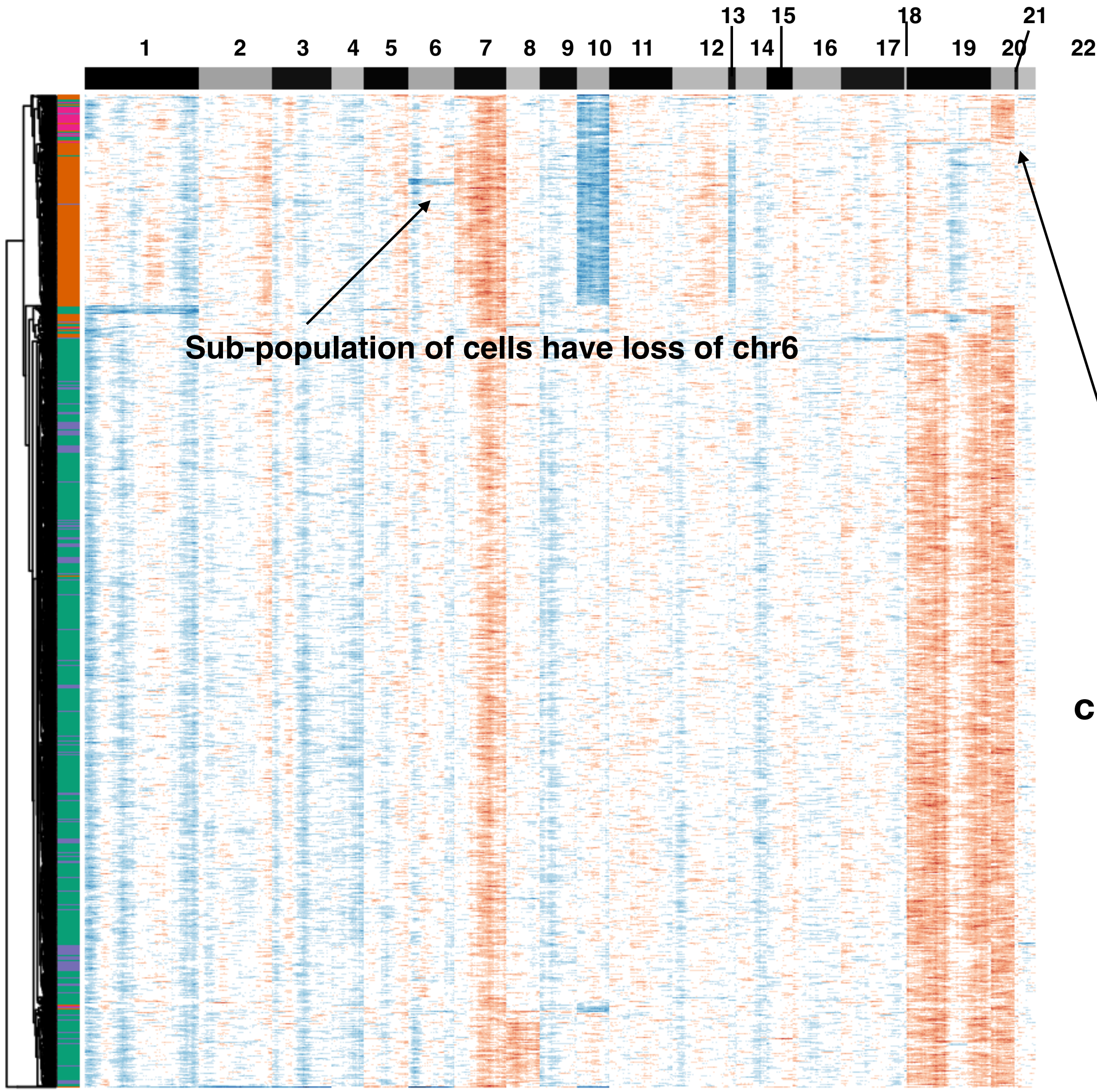
Are Brain Tumour Stem Cells comprised of genetic and transcriptional subpopulations?



Within each stem cell culture, we find a range of distinct subpopulations

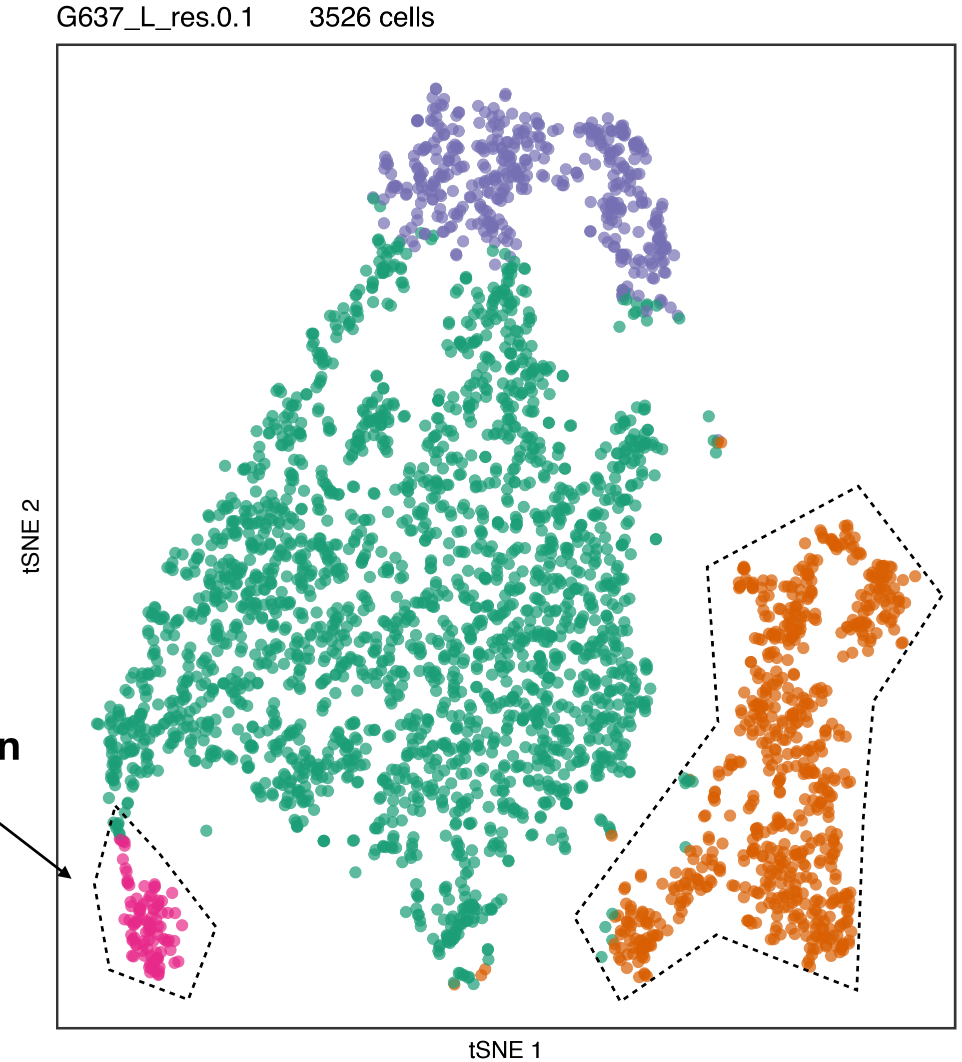


Genome-wide analysis using normal oligodendocytes as controls uncovers CNVs that *partially* distinguish clusters



Rows are individual cells coloured by transcriptional cluster

chr20 gain

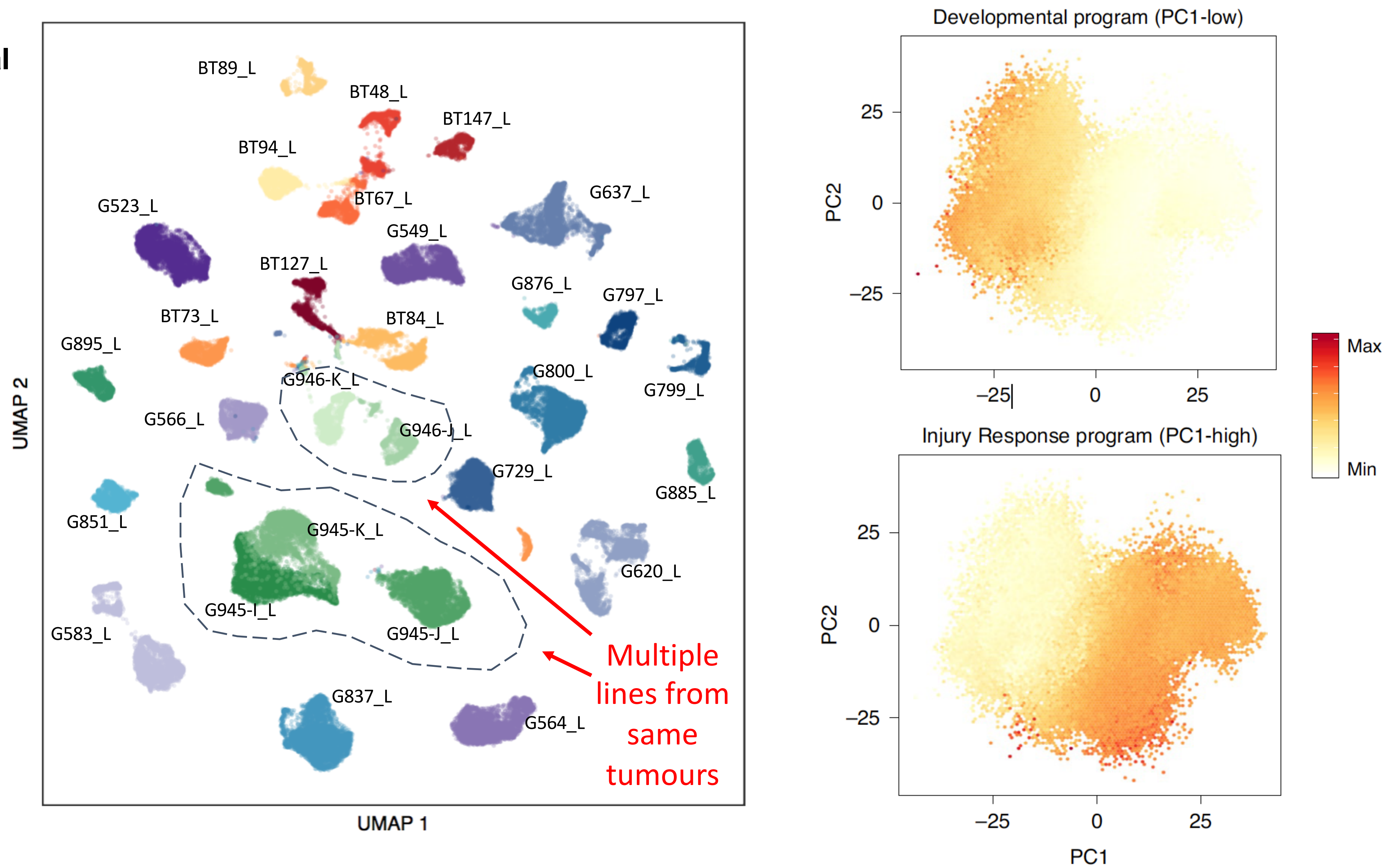


Subpopulations with loss of chr10

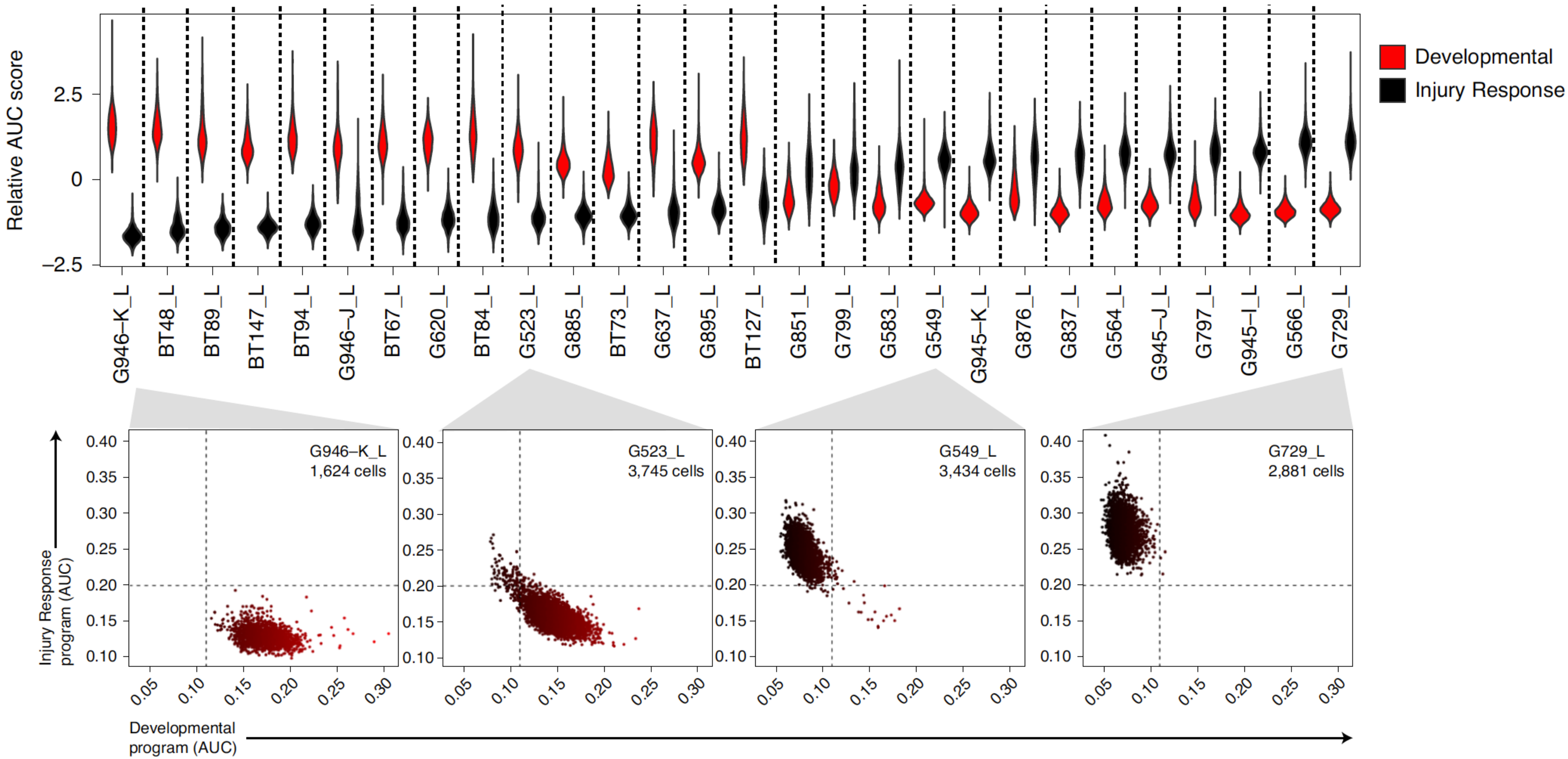
Patients' GSCs are all different....do they share any common biology?

Developmental Programs

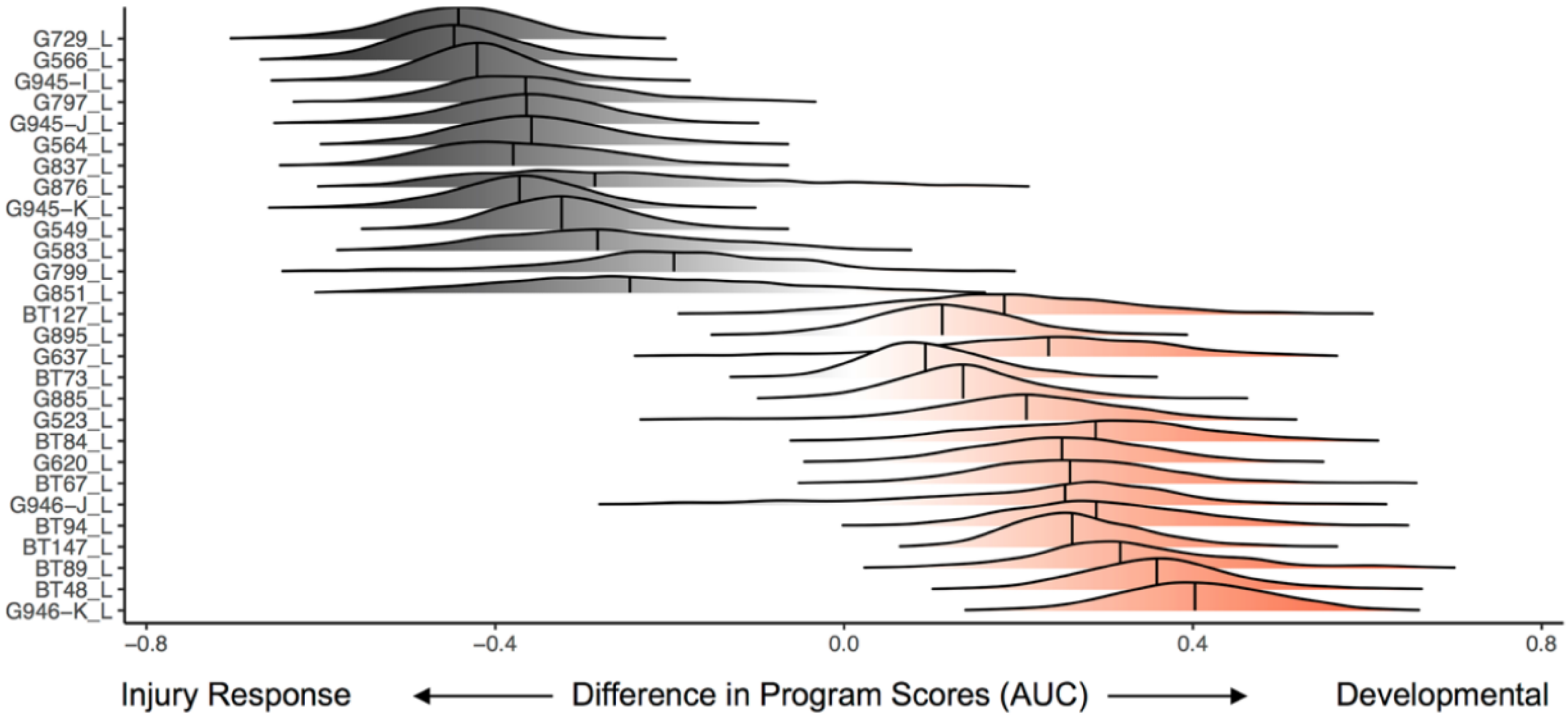
Injury-response Programs



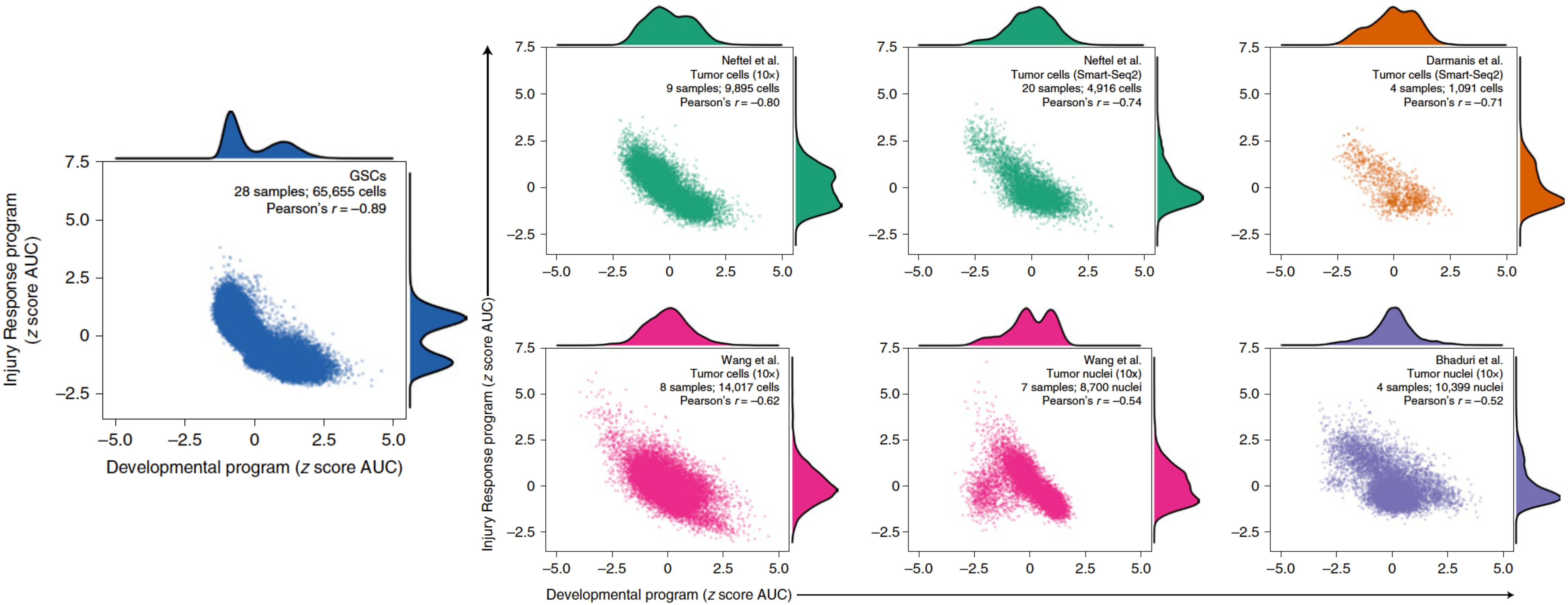
Subpopulations within brain tumour stem cells maintain relative position within the initial developmental/injury-response gradient



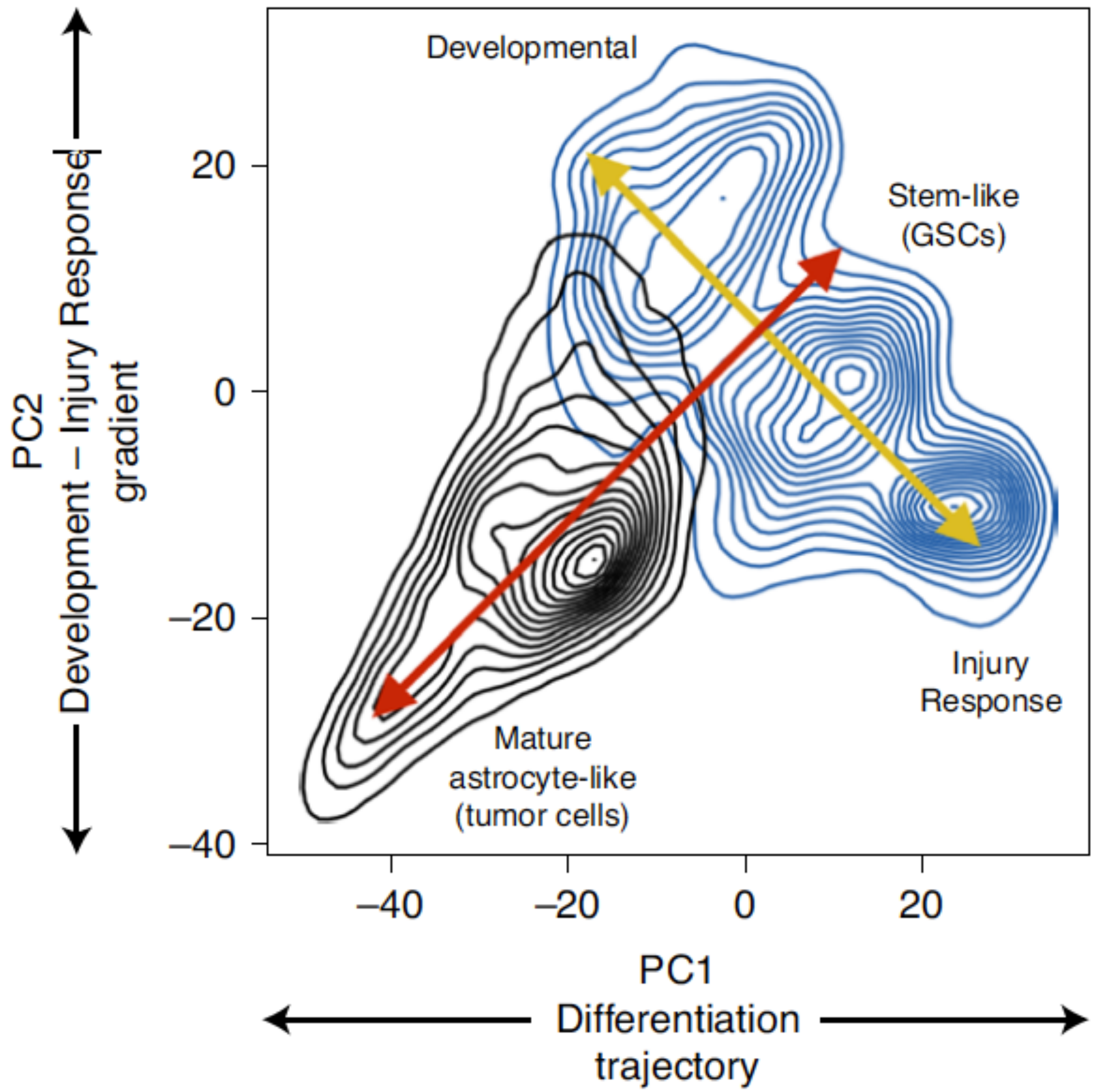
Profiling GSCs from many samples is necessary to characterize the full spectrum of possible transcriptional states giving rise to bulk GBM.

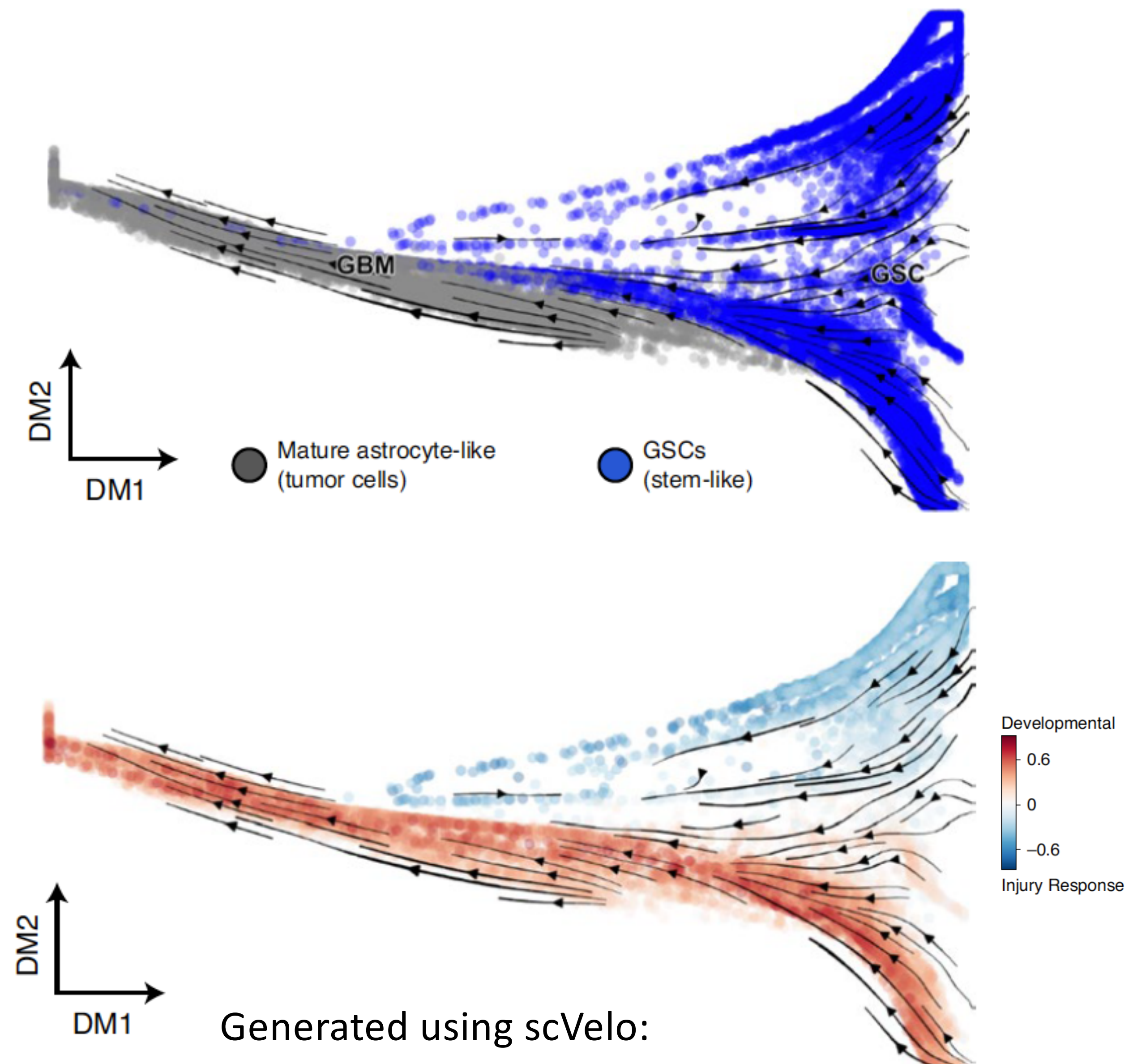


GSC gradient between Developmental and Injury Response is recapitulated in cells or nuclei from primary tumors, but bulk tumour cells can obscure



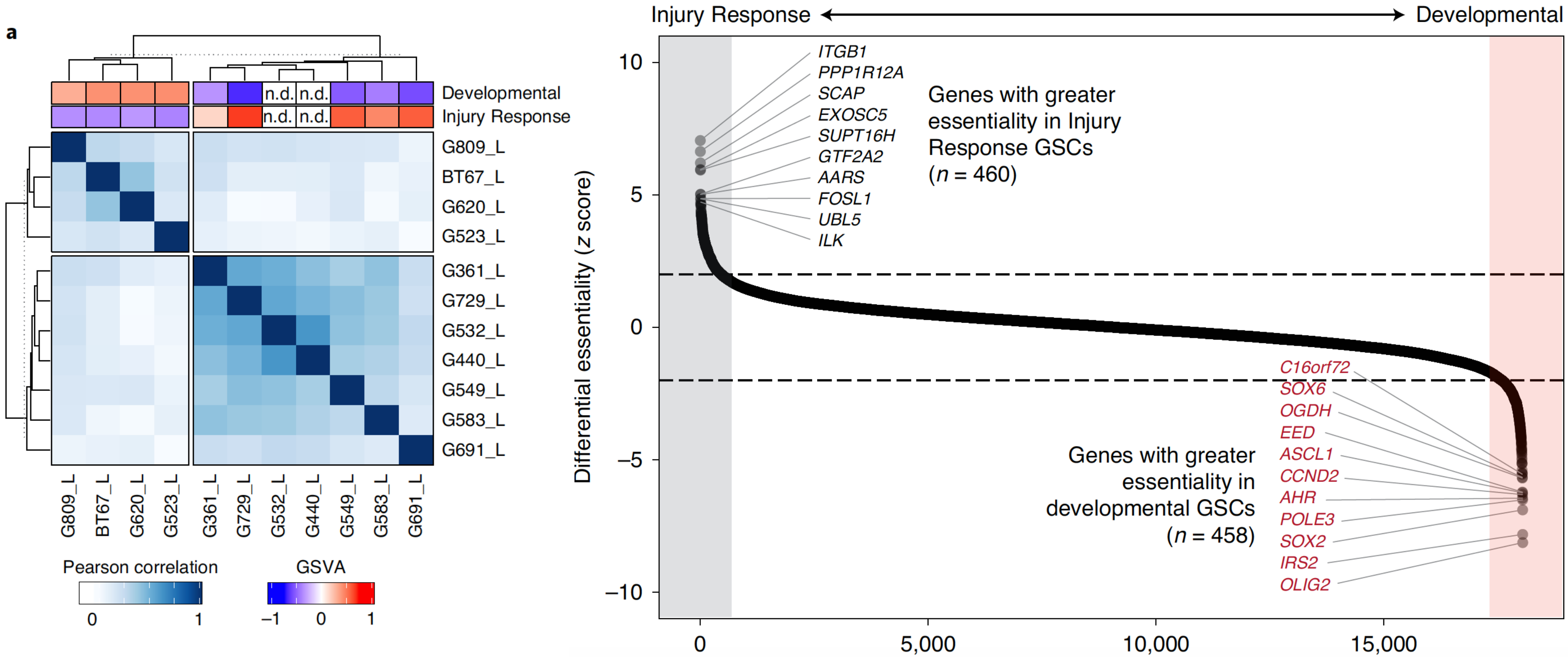
Bulk tumour cells "flow" from their progenitor GSCs' position on the Developmental/Injury Response Gradient





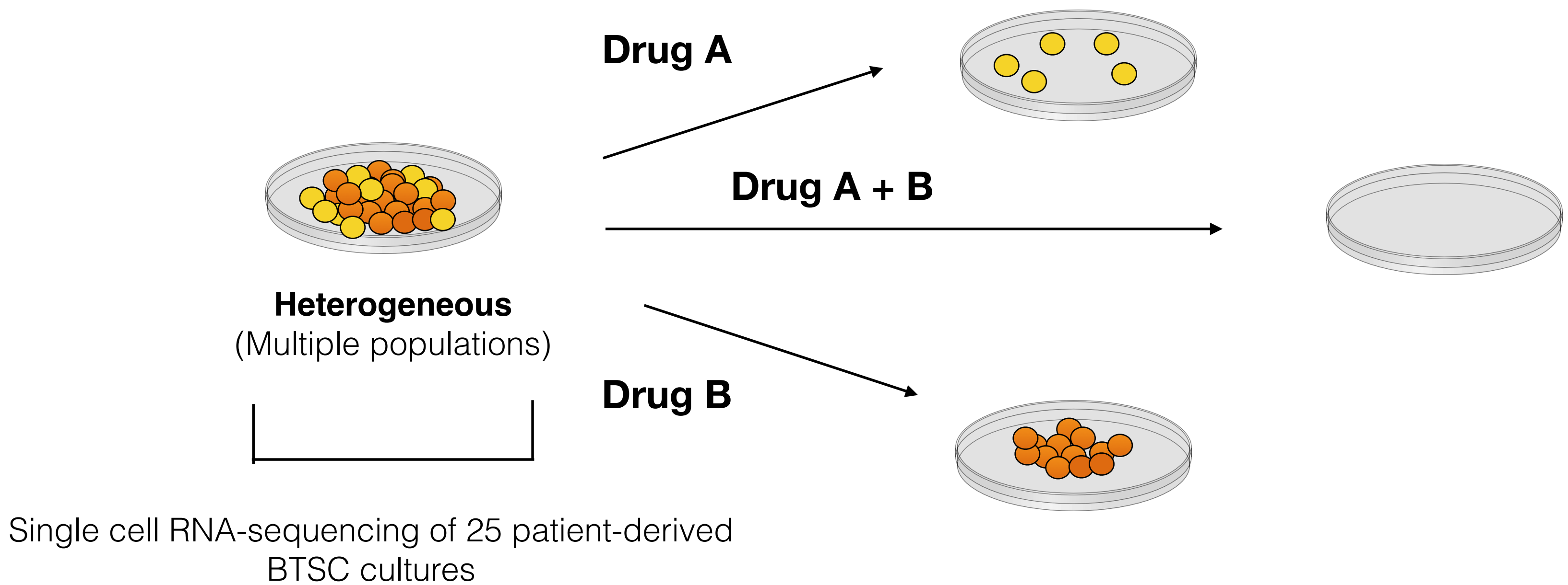
Generated using scVelo:
Bergen et al. *Nat Biotechnol* 38, 1408–1414 (2020)

Functional dependencies identified by genome-wide CRISPR screens reflect Developmental–Injury Response gradient position



TKOv3 library: 70,948 guides targeting 18,053 protein-coding genes in 11 GSCs

Gradients and clusters may be biologically interesting, but is there application for patients?



PharmacoDB aggregates gene expression, copy number, and pharmacogenomic profiles of cell lines from multiple high-throughput drug screening studies

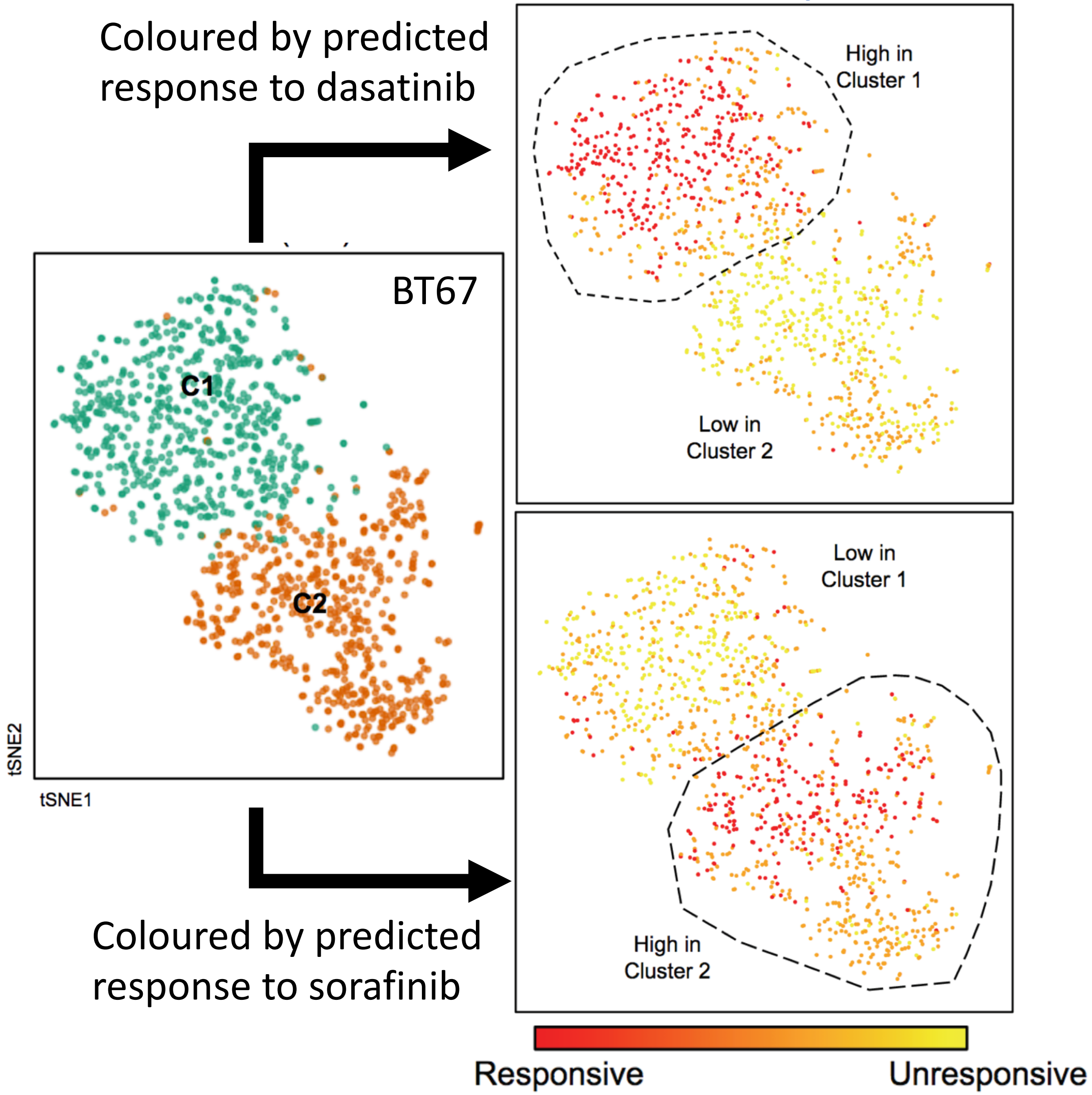
www.pharmacodb.ca

Smirnov, Petr, et al. "PharmacoDB: an integrative database for mining in vitro anticancer drug screening studies." Nucleic Acids Research (2017).

Smirnov, Petr, et al. "PharmacoGx: an R package for analysis of large pharmacogenomic datasets." Bioinformatics 32.8 (2015): 1244-1246.

Laboratory of Benjamin Haibe-Kains

Predict drug efficacy score for each cluster, calculate variation of score across each cell, establish dose/response, synergy assay



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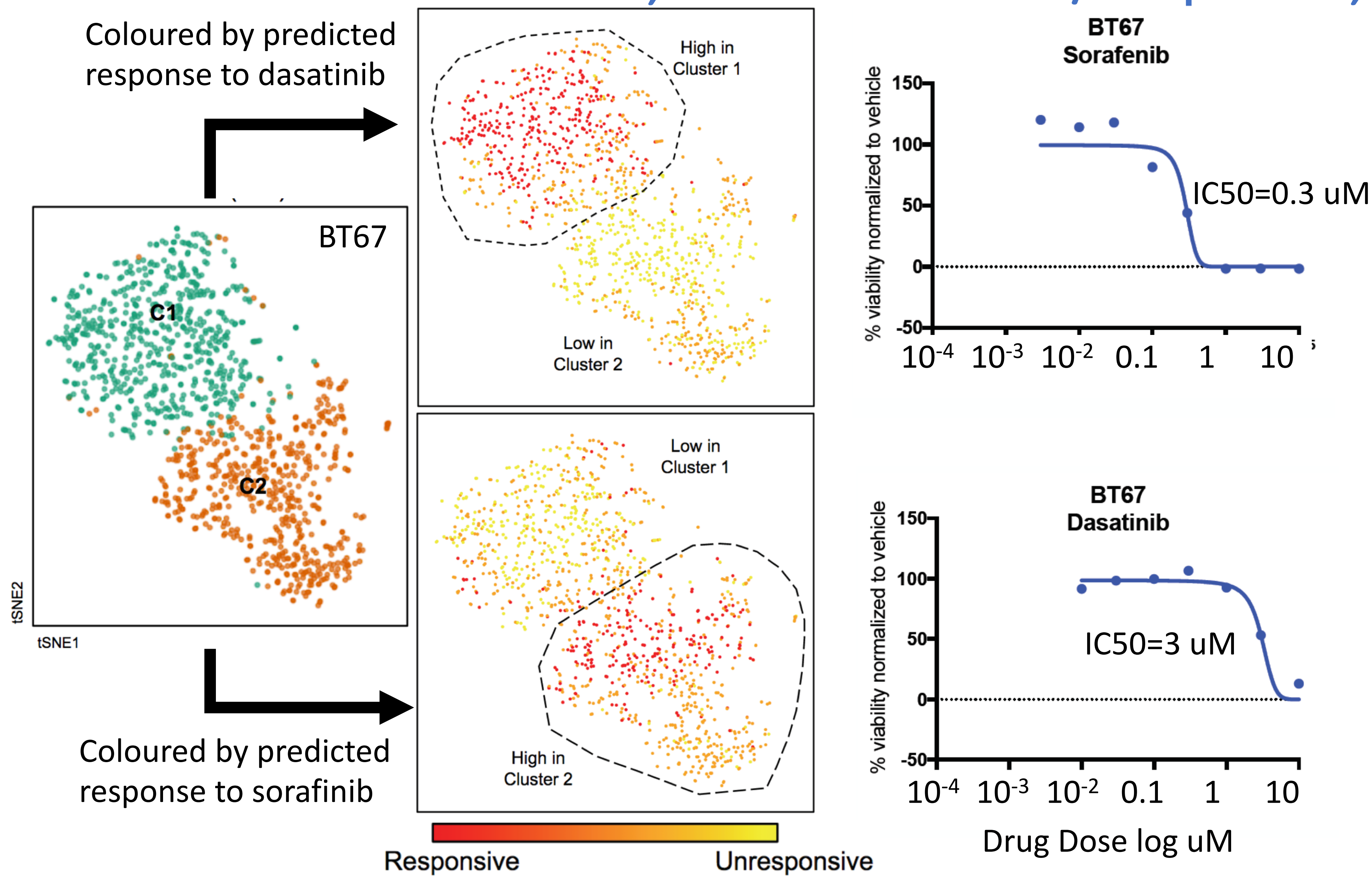

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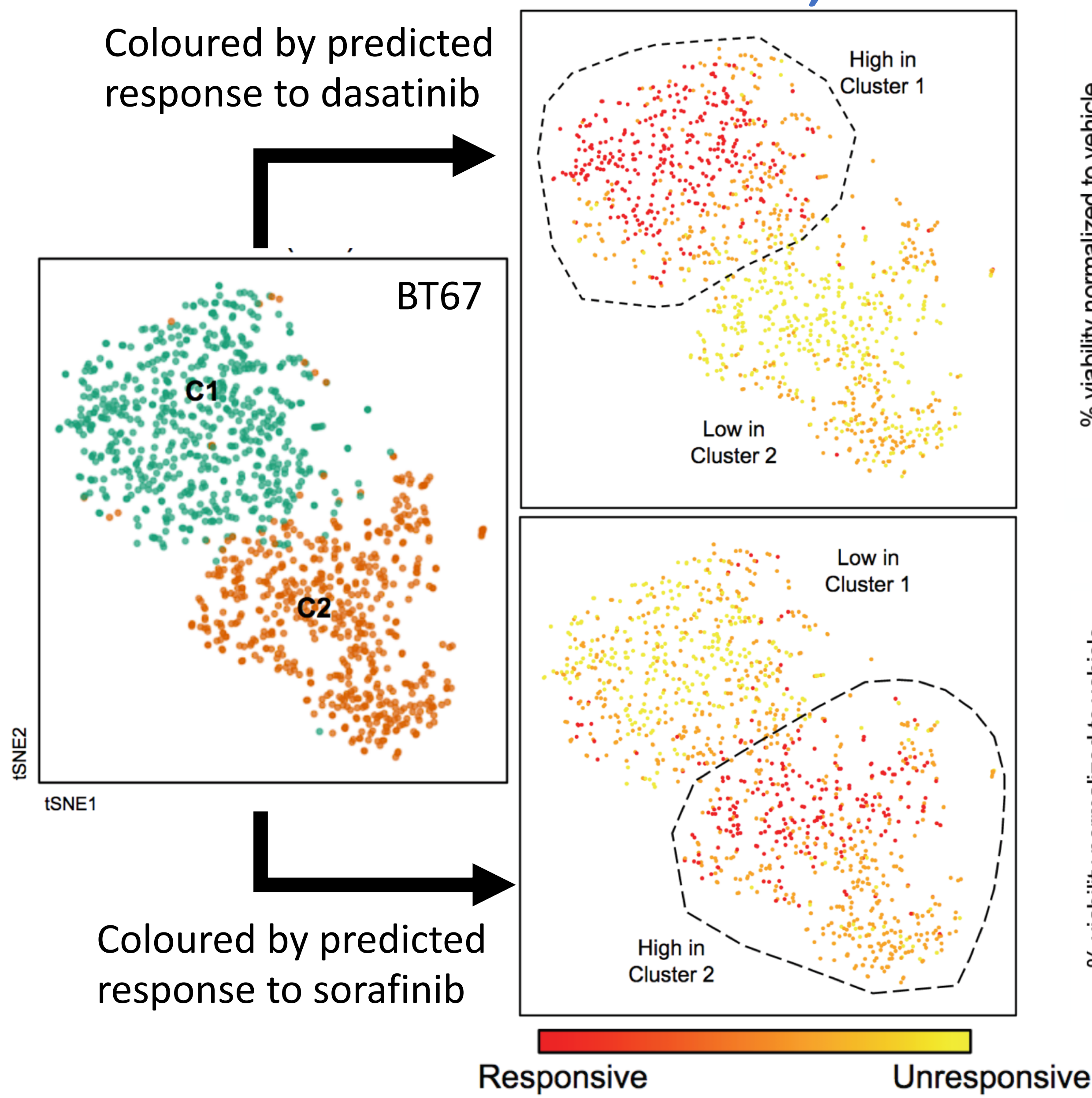
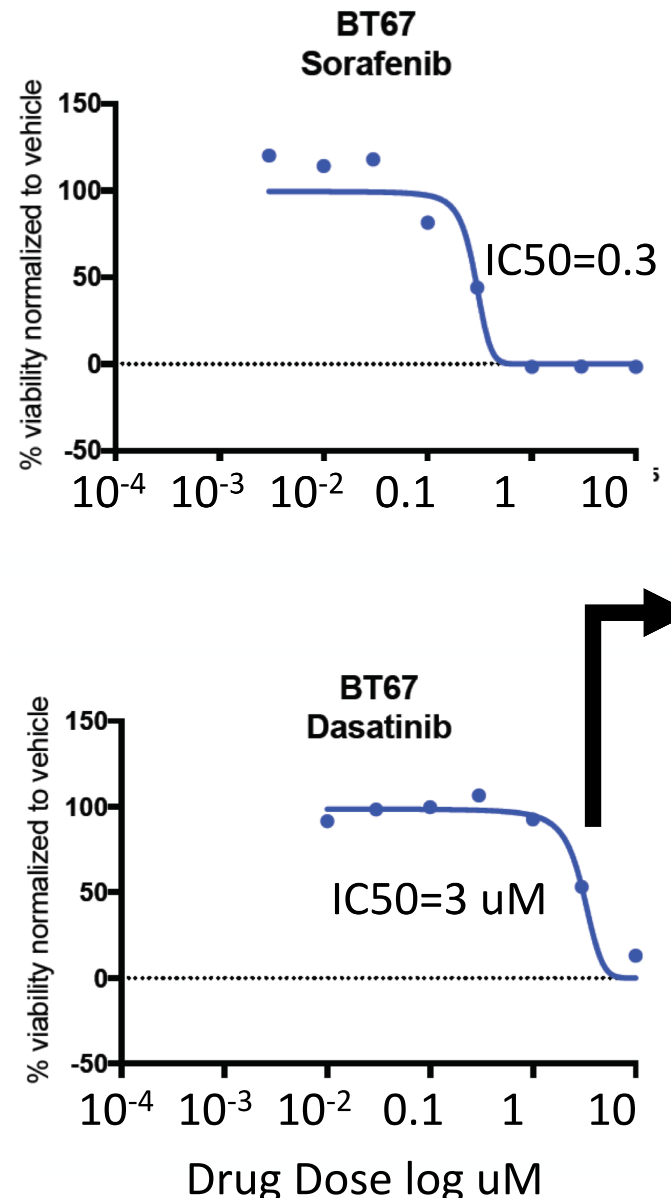
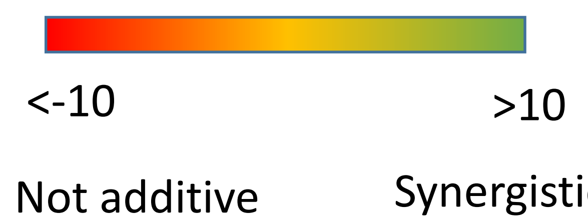


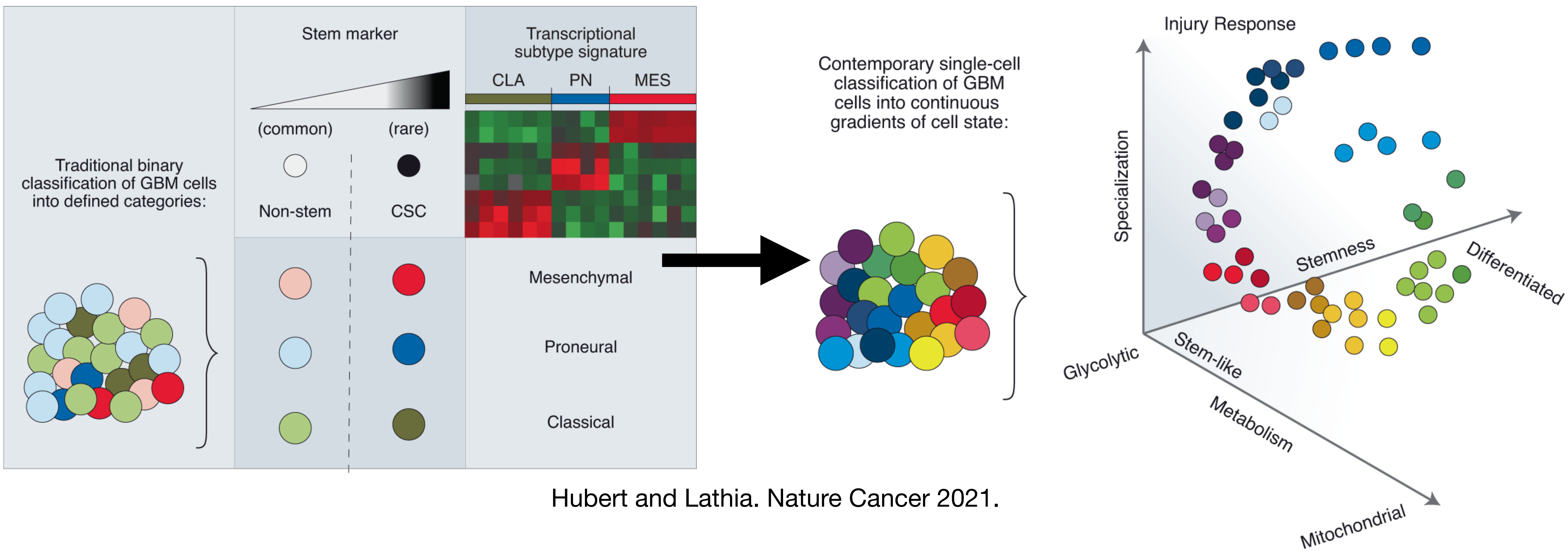


Bliss Synergy Value

uM	0.3	0.1	0.03	0.01	0.003	0.001
3	-2.65	-2.12	12.23	0.79	6.78	12.73
1	0.93	1.32	15.96	14.38	20.21	12.75
0.3	0.96	0.6	5.8	-12.31	4.08	5.48
0.1	0.1	-0.16	7.96	-1.19	0.13	-15.21
0.03	0.19	0.082	15.8	-9.59	7.63	-10.63
0.01	0.078	0.38	12.48	4.89	15.42	5.87



Gradients galore: RNA, metabolic, & proteomic profiling all identified continuous biological gradients in glioblastoma

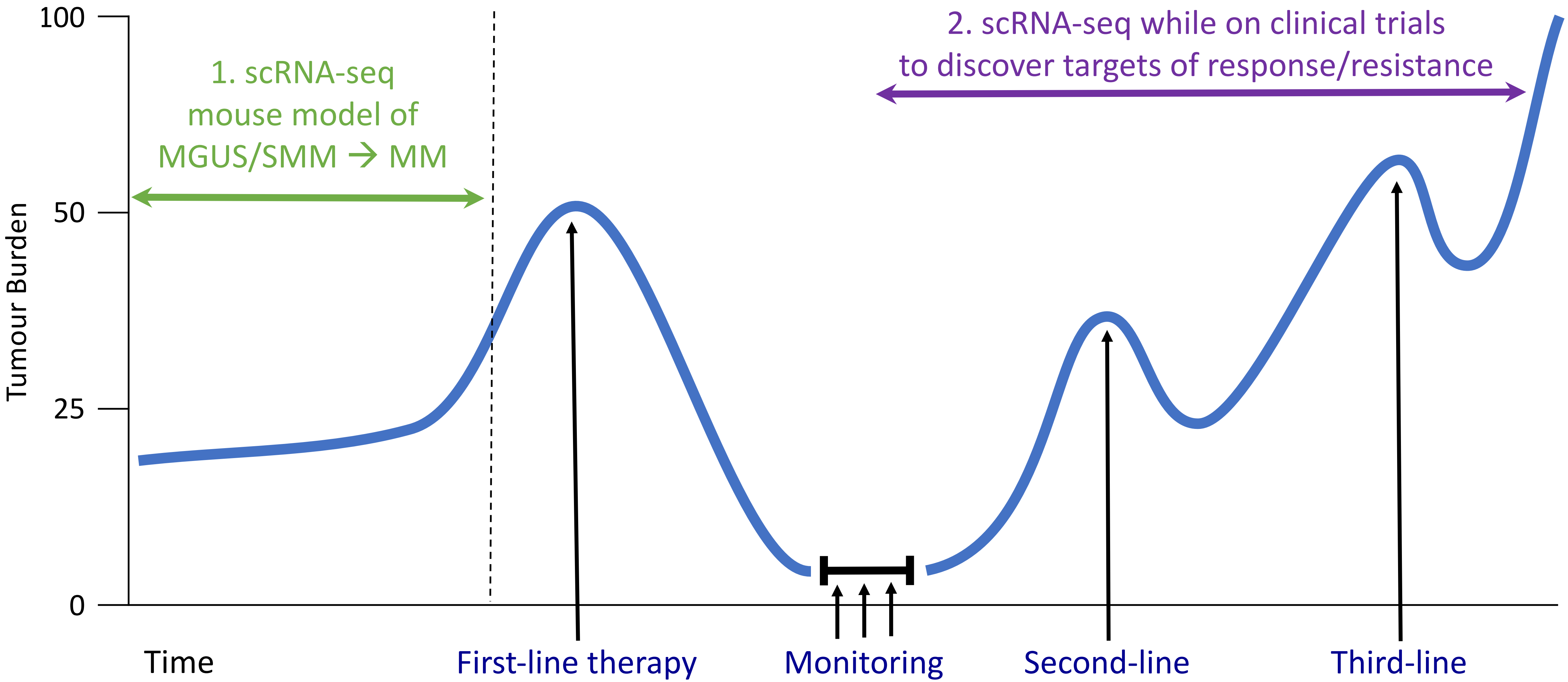


Hubert and Lathia. Nature Cancer 2021.

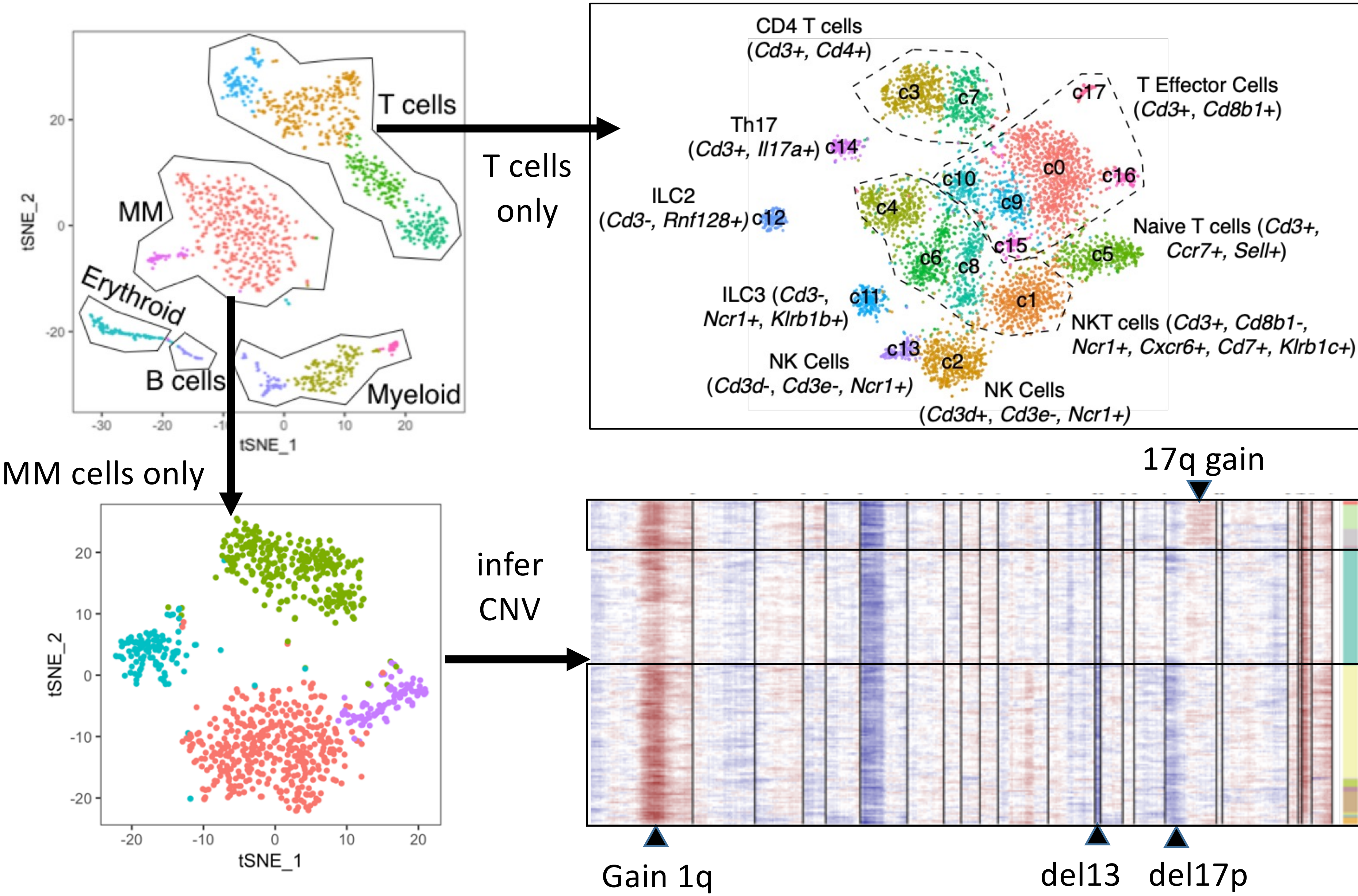
Multiple, single cell approaches can converge and cross-validate biological signals

Single cell technologies can reveal biology not apparent from bulk approaches

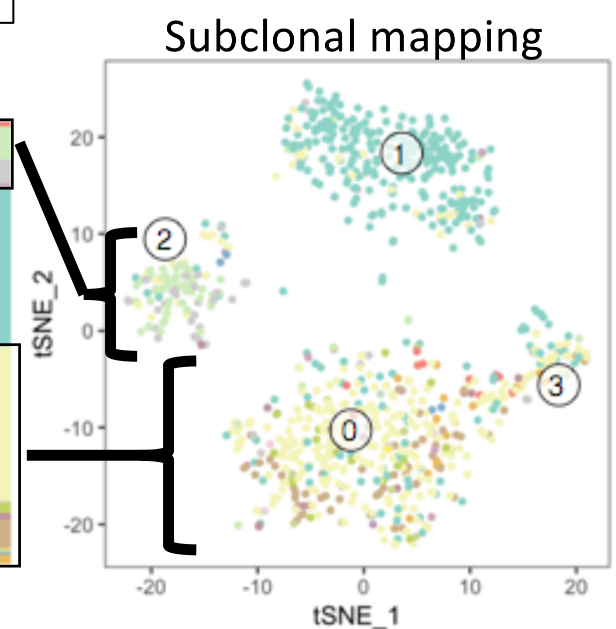
Putting it into practice: Can we prevent early cancer or observe subclonal drug responses in patients?



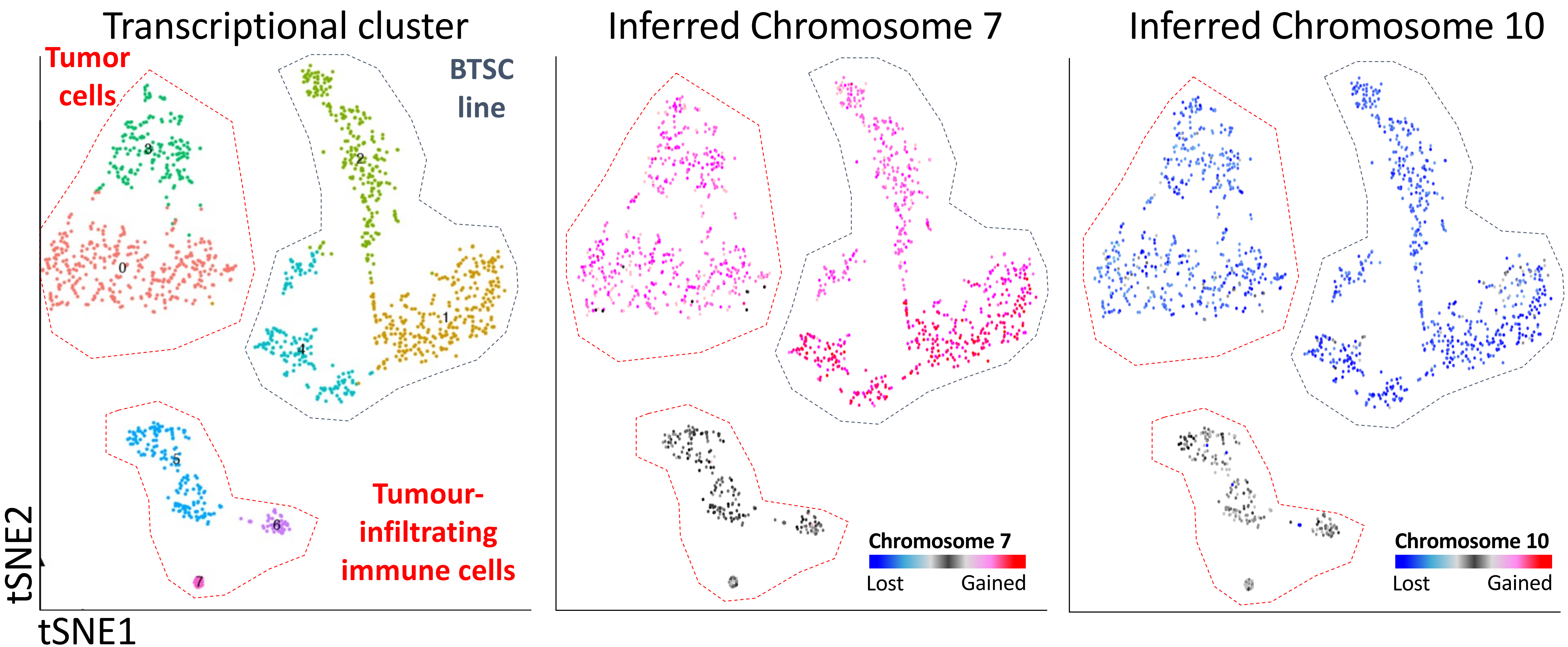
Computational dissection of cells from a patient with multiple myeloma: direct analysis of cell type, cell state, TCR/BCR, & subclonal copy number



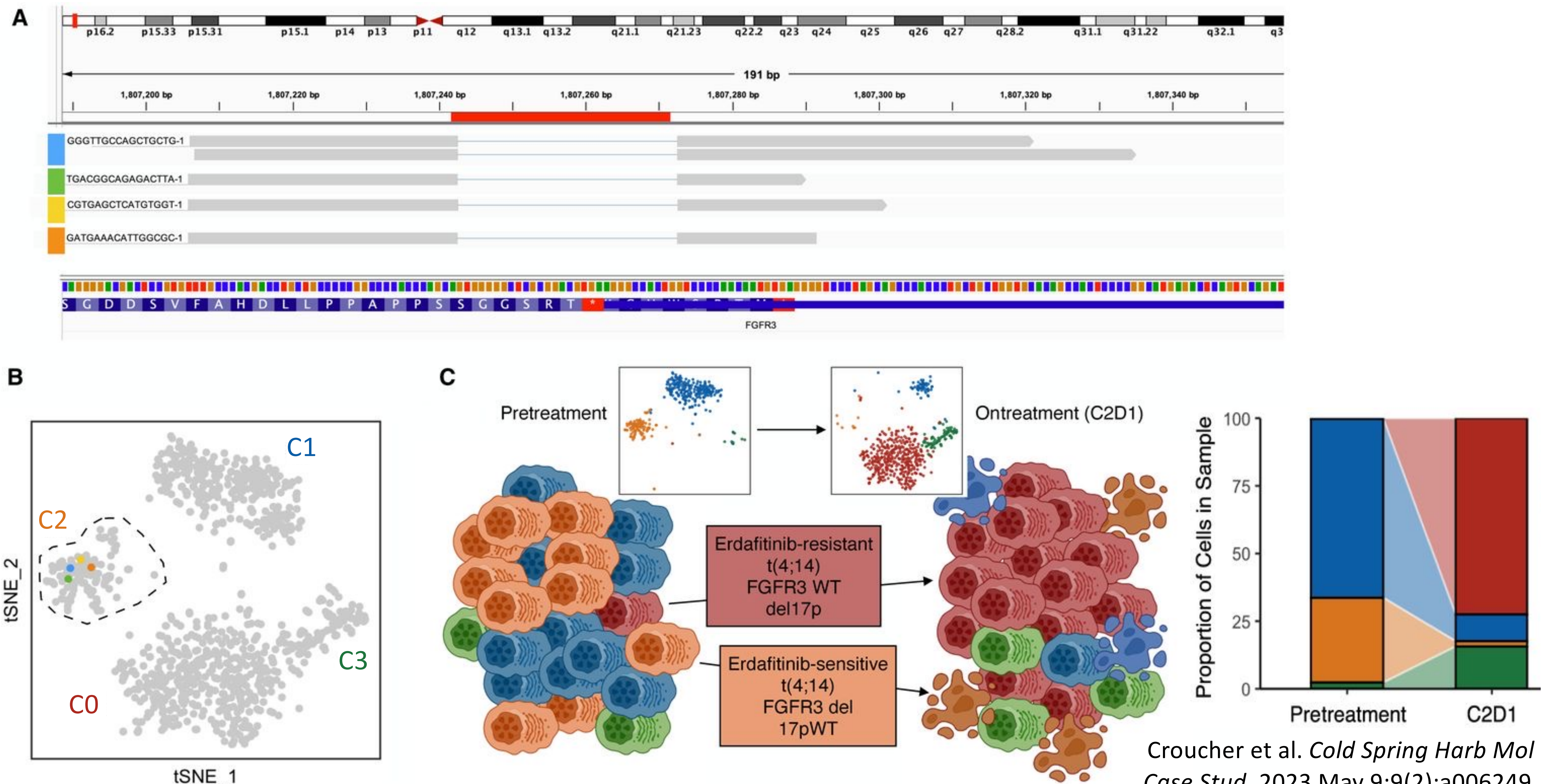
Croucher et al.
Cold Spring Harb Mol Case Stud.
2023 May 9;9(2):a006249.



Copy number variants called from 10X Genomics single cell RNA-seq using chromosomal "pathway" enrichment



Somatic mutations in scRNA-seq data explain clonal responses



Croucher et al. *Cold Spring Harb Mol Case Stud.* 2023 May 9;9(2):a006249.

Revisiting the Learning Objectives

1) A plethora of single cell technologies have opened windows into cell biology that were closed to bulk approaches that "average out" signal

2) The same biology may be measurable using multiple methods → tailor experimental approaches to specific scientific questions answerable by available samples & technologies

3) Multiple cellular components can be queried from one single cell experiment, e.g. immune & cancer cells inhabiting tumours

4) "Fact-check" data quality, integrations, & conclusions using orthogonal experiments, external data sets, & clinical outcomes

Citations - Single Cell RNA-seq (trevor.pugh@utoronto.ca)

Tumour microenvironment	Junttila & de Sauvage. Nature. 2013 Sep 19;501(7467):346-54.
Immune inference	Yoshihara et al. Nat Commun. 2013;4:2612.
Experimental design	Proserpio and Lönnberg. Immunol Cell Biol. 2016 Mar;94(3):225-9.
Cell isolation	Wang and Song. Clin Transl Med. 2017; 6: 10.
Technology scaling	Svensson, Vento-Tormo, and Teichmann. Nat Protoc. 2018 Apr;13(4):599-604.
10X vs SMART-SEQ2	Baran-Gale, Chandra, and Kirschner K. Brief Funct Genomics. 2017 Nov 8.
scRNA-seq technologies	Ziegenhain et al. Mol Cell. 2017 Feb 16;65(4):631-643.e4.
Bioinformatics	Stegle, Teichmann, and Marioni. Nat Rev Genet. 2015 Mar;16(3):133-45.
Myeloma mouse immune	Croucher et al. bioRxiv. 2021 Jan. 10.22.464971
Myeloma mouse cancer	Croucher et al. Nat Comm. Nat Comm. 2021 Nov; 12(6322).
Glioblastoma stem scRNA-seq	Richards, Whitley, et al. Nat Cancer. 2021 Feb.

Broad Single Cell Portal	portals.broadinstitute.org/single_cell/
10X Genomics TCR/Ig poster	www.10xgenomics.com/resources/posters
Human Cell Atlas	www.humancellatlas.org
Chan-Zuckerberg Initiative	www.chanzuckerberg.com
Princess Margaret Genomics	www.pmgenomics.ca

Panoramics A Vision:
Single Cell Analysis Working Group
panoramics-a-vision.slack.com
https://www.panoramics-a-vision.com