

WuXi AppTec scRNA Sequencing Service



WuXi AppTec Research Service Division, Oncology & Immunology Unit



2021.01

OncoWuXi Newsletter

■ Introduction of single cell RNA-sequencing (scRNA-seq)

- Why scRNA-seq?
- Droplet-based scRNA-seq

■ Preparing for scRNA-seq

- Workflow of scRNA-seq
- Optimization of sample processing for reliable data
- Required number of cells and sequencing depth

■ Quality control and data analysis

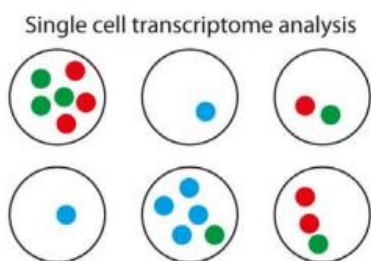
- Quality control metrics
- Data analysis for scRNA-seq

■ Case Study

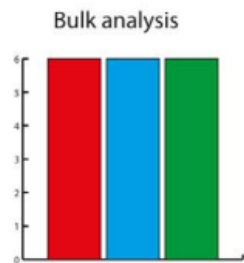
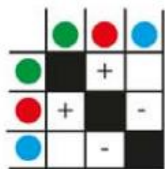
Why single cell RNA -sequencing?

- Understanding heterogeneous tissues and environment
- Identifying and analysis of rare cell types
- Finding gene profile changes in cellular composition
- Dissection of temporal changes

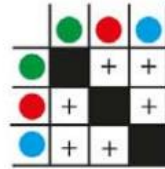
Single cell vs Bulk Analysis



Coexpression Matrix
(single cell)

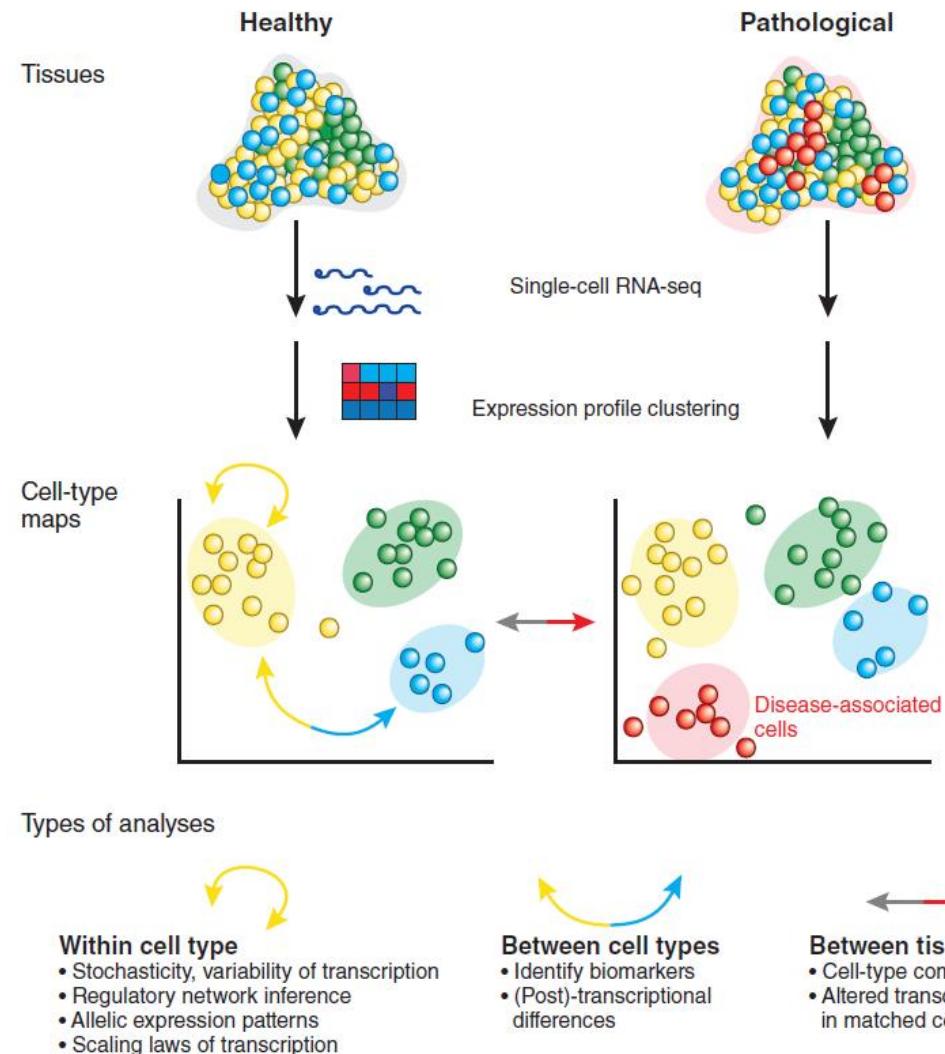


Coexpression Matrix
(bulk analysis)



Macaulay and Voet, PLOS Genetics, 2014

Multi-dimensional Comparison



Sandberg, Nature Methods 2014

Highlight of single cell gene expression

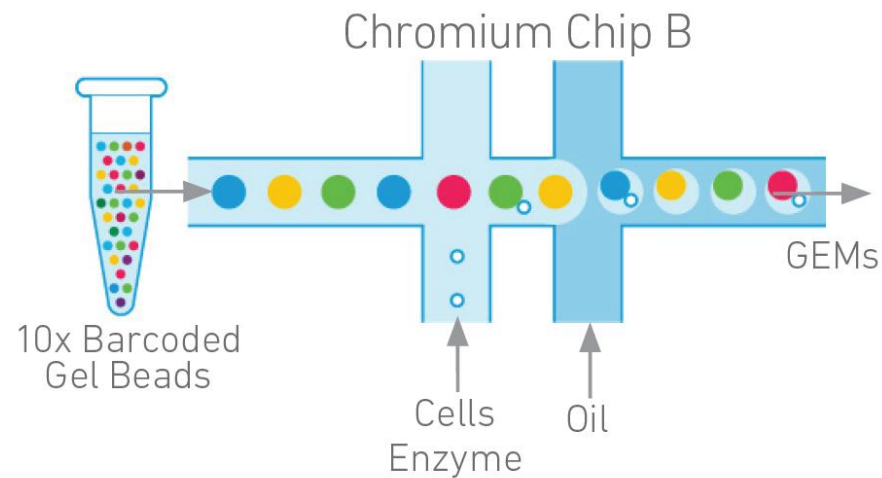
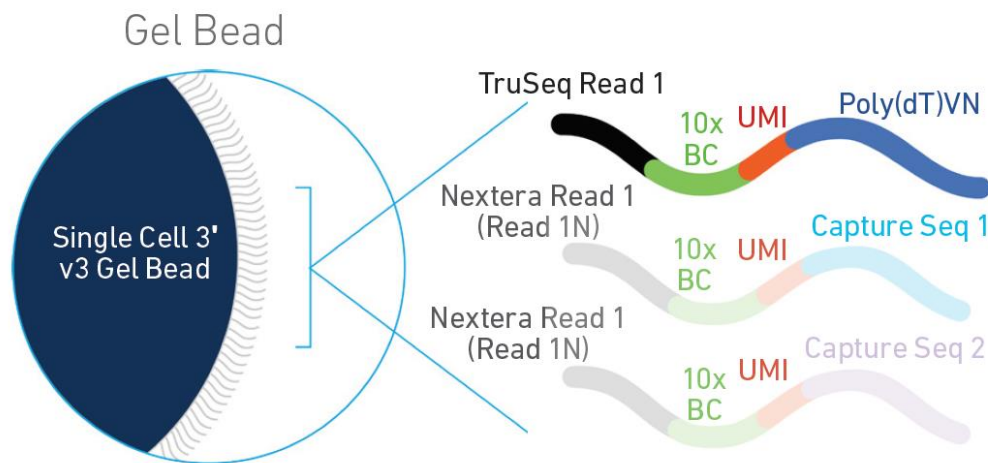
■ RNA-Seq

- Omics analysis to reveal the gene expression profile
- Identification of novel targets and biomarkers
- Global changes by intervention
- Good for homogenous tissue or cells

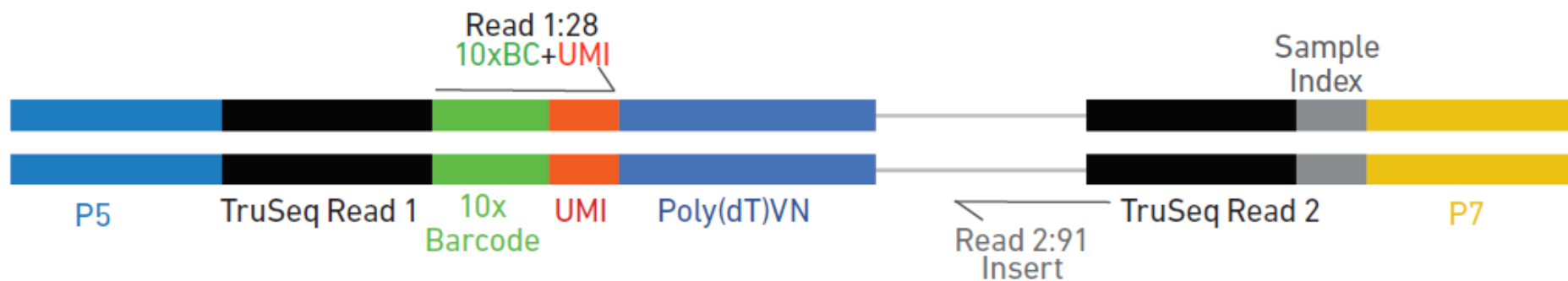
■ scRNA-Seq

- Identify and characterize **rare cell types**
- Analyze and understand **cellular heterogeneity** and how this contributes to your biological system
- Perform cellular phenotyping with single cell RNA-Seq **to identify novel targets, biomarkers, and cell types** and states without the need for pre-selected targets
- Evaluate mRNA and cell surface protein **expression profiles** within the same cell
- Perform **high-throughput and high-resolution** functional genetic screens in tens of thousands of cells simultaneously

Droplet-based single cell sequencing



Chromium Single Cell 3' Gene Expression Library



Schematic workflow of scRNA sequencing

- How to decide the dosage for a scRNA-seq study?
- How to select the time points?

Pilot Study

In Vivo Treatment

- Fit-for-purpose design and processing optimization

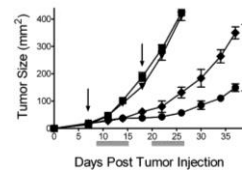
Sample Collection & Single Cell Prep

Library Prep & Sequencing

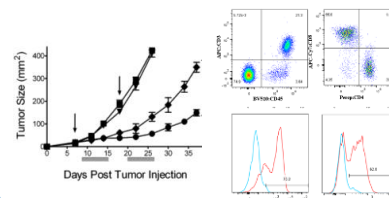
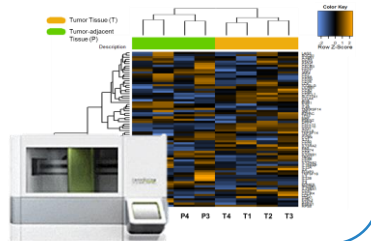
- What cell types are affected by the treatment?
- What's the MOA?

Bioinformatics Analysis

Dose Selection by Efficacy Study



Time Points Selection



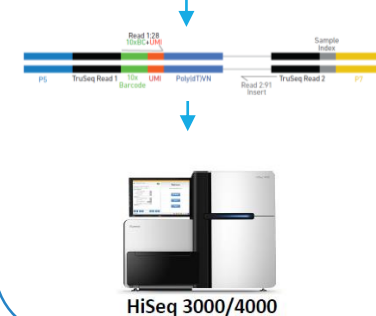
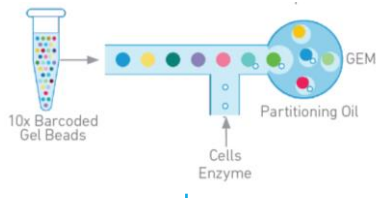
Complex tissue



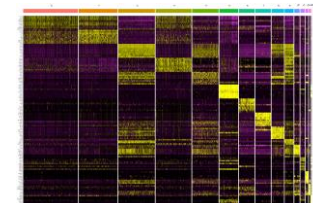
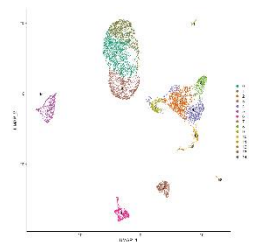
Cell isolation



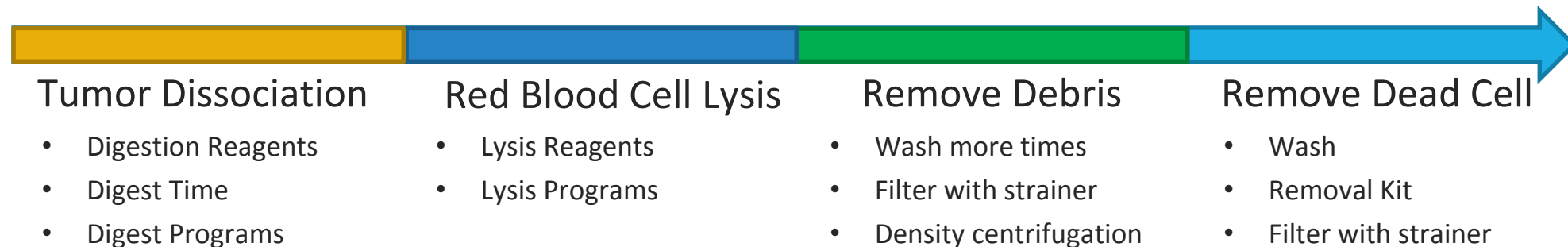
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HiSeq 3000/4000



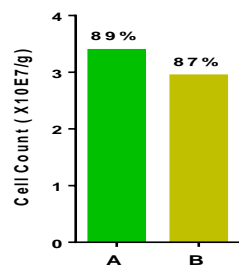
Fit-for-purpose sample processing optimization



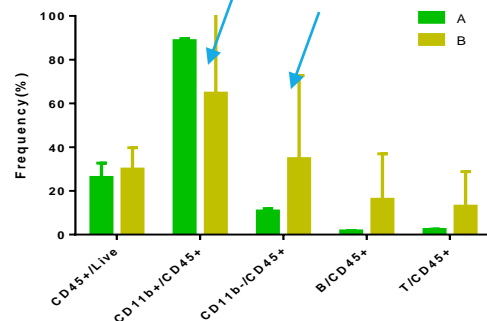
Tumor Dissociation Optimization

Cell Yield & Viability

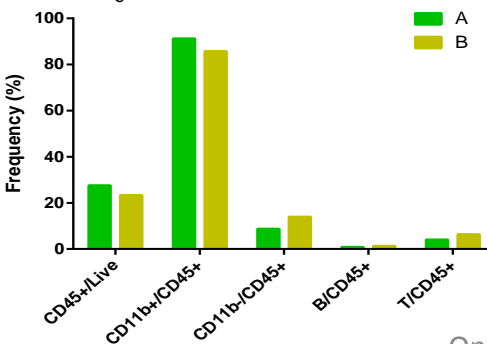
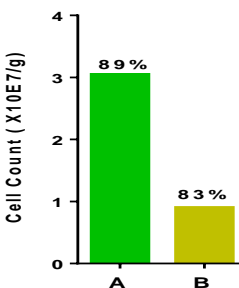
Model 1



Immune Profiling



Model 2



RBC Lysis Optimization

Model 1

Method	A	B	C	D	E	F
Cell Yield	Green	Green	Green	Green	Green	Green
RBC Residue	Green	Yellow	Green	Green	Green	Green
Cell Viability	Green	Green	Yellow	Green	Green	Yellow
Cell Type Bias	Green	Green	Green	Green	Green	Green
Time Cost	Yellow	Green	Green	Green	Green	Green

Model 2

Method	A	B	C	D	E	F
Cell Yield	Yellow	Yellow	Green	Yellow	Green	Green
RBC Residue	Green	Green	Green	Green	Yellow	Yellow
Cell Viability	Green	Yellow	Green	Green	Green	Green
Cell Type Bias	Green	Green	Green	Green	Green	Green
Time Cost	Yellow	Green	Green	Green	Green	Green

Example of quality control

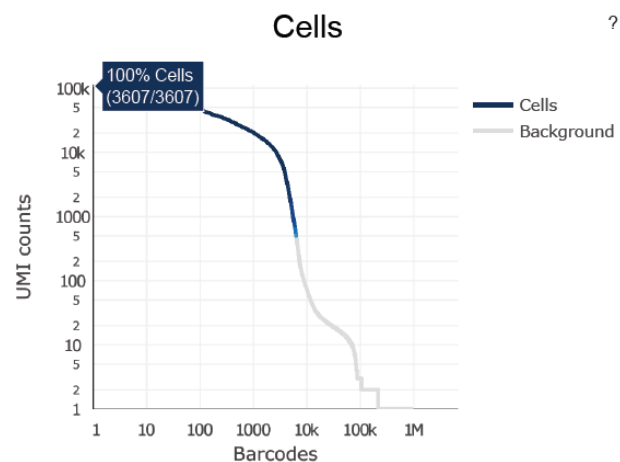
Sample Preparation

Model ID	Live Cells (Counts) /g	Viability (Mean \pm SD)
Model 1	$(6.15 \pm 4.24)E+07$	$87.96 \pm 1.92\%$
Model 2	$(2.8 \pm 0.54)E+07$	$86.40 \pm 4.13\%$

Library Preparation

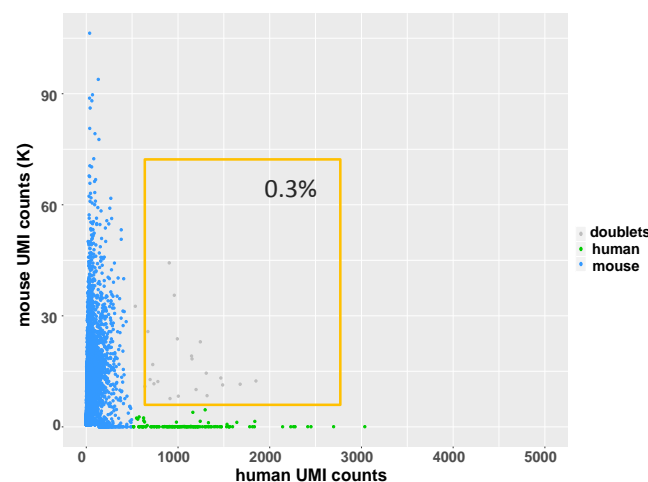
Step	RNA Conc ng/ul	Fragmentation (nt)
cDNA	15 \pm 5 ng/ul	-
Library	25 \pm 5 ng/ul	450 \pm 50 bp

Cell Count QC- Cell Ranger



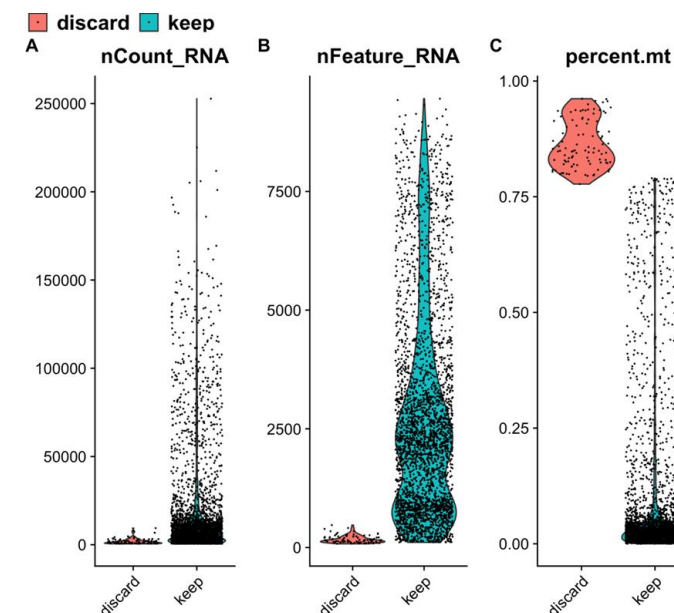
Estimated Number of Cells	5,969
Fraction Reads in Cells	95.6%
Mean Reads per Cell	27,451
Median Genes per Cell	2,632
Total Genes Detected	19,346
Median UMI Counts per Cell	8,349

Doublets Rates Analysis



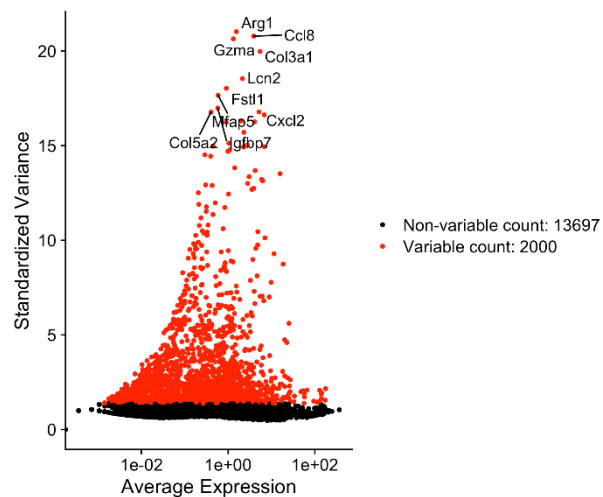
Data Processing

QC metrics

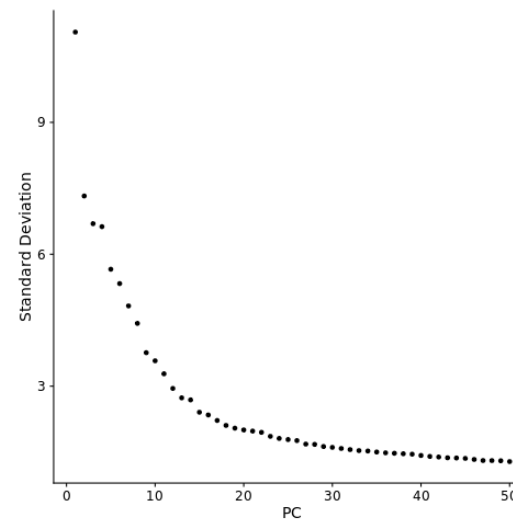


Feature selection, dimension reduction and clustering

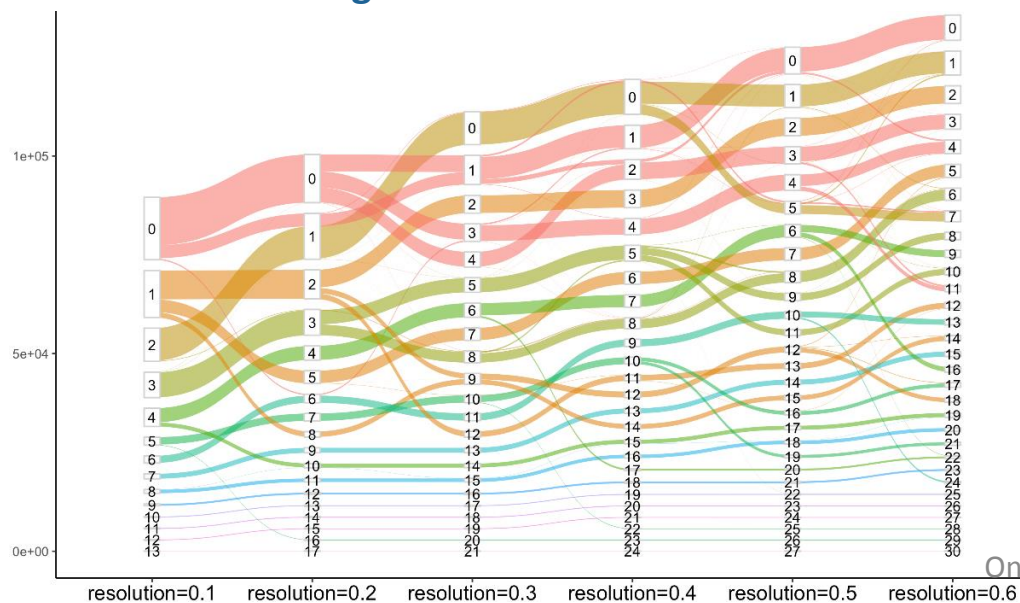
Feature Selection



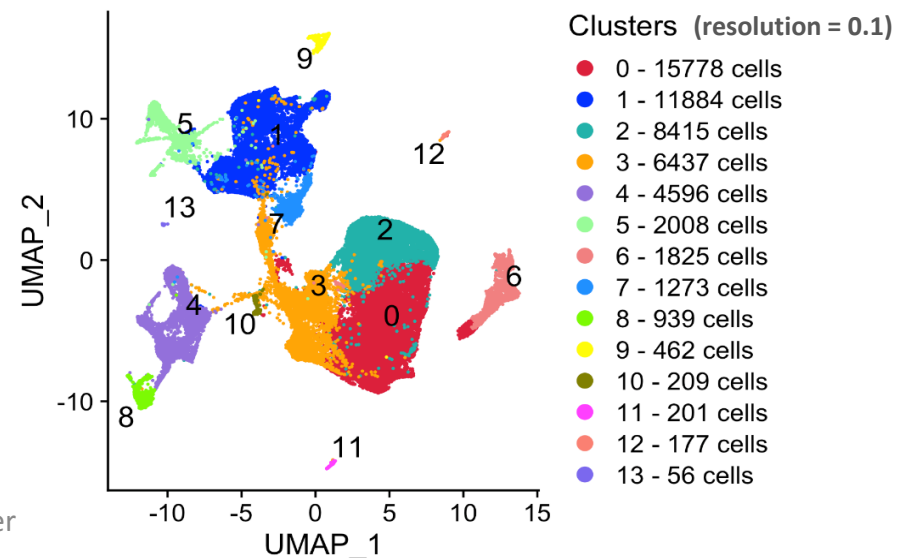
PCs selection by Elbow Plot



Clustering with a series of resolution



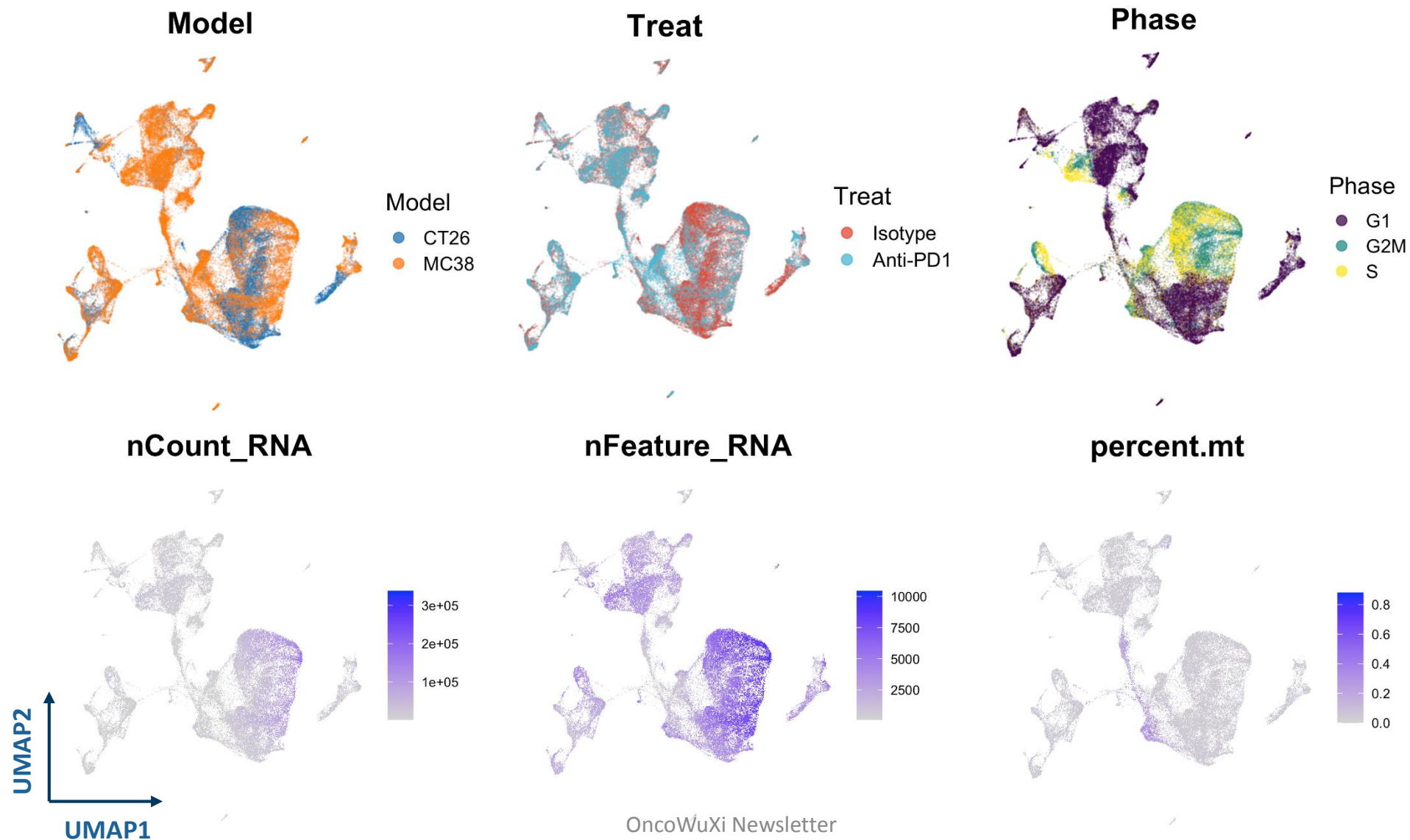
Visualization by UMAP



Exploration on large-scale chromosomal copy number alterations

Exploration on some metrics

UMAP visualization of metrics

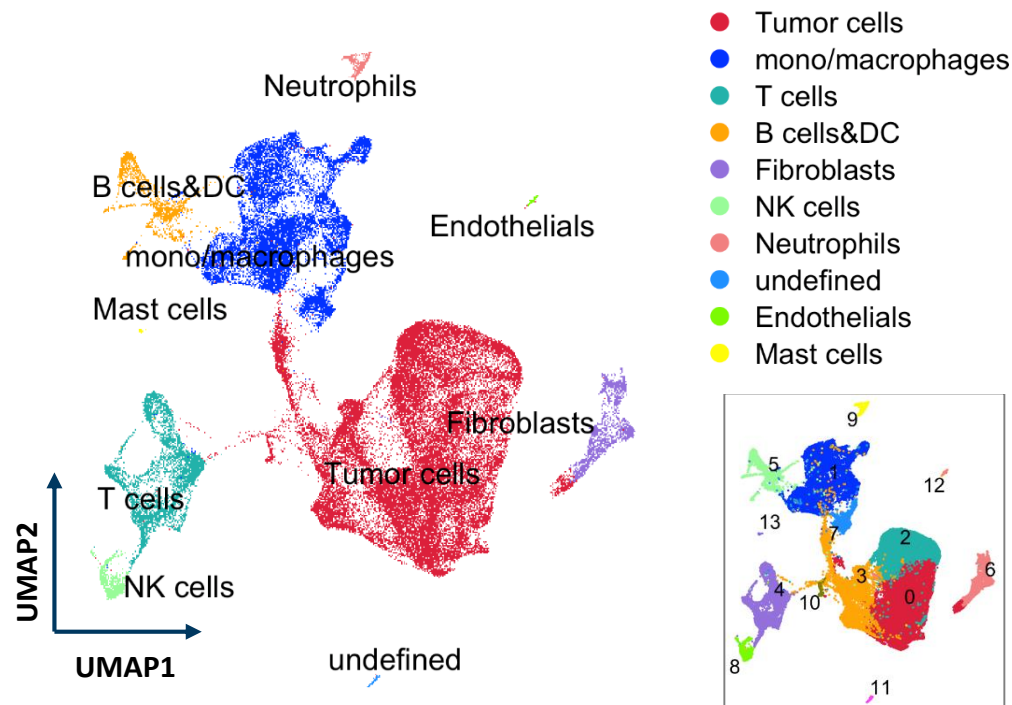


- ### By functional annotation analysis

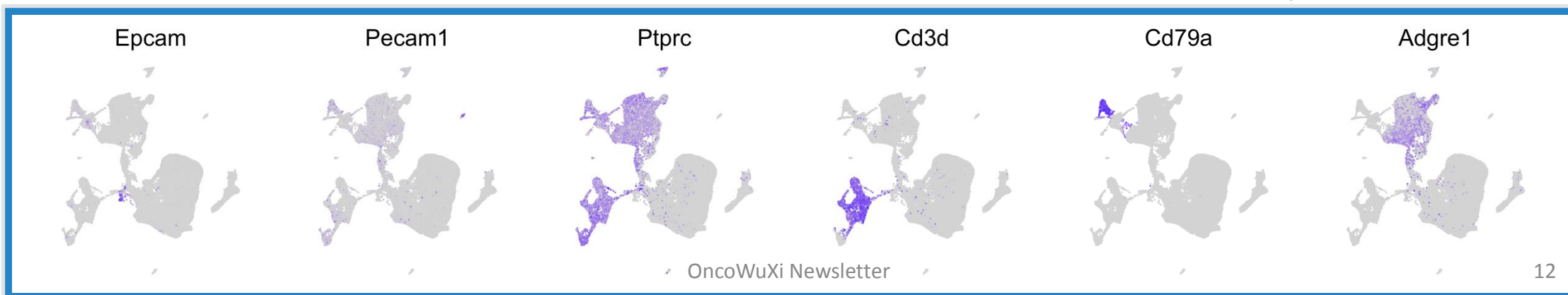
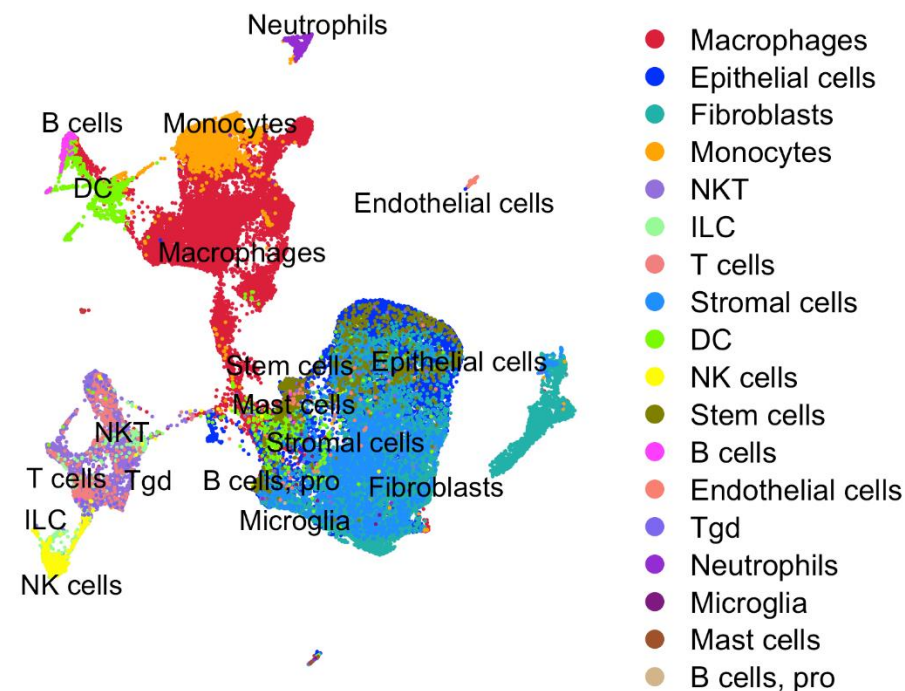


Visualization of cell types and markers of interest on 2D plots

Manually by a multi-step approaches

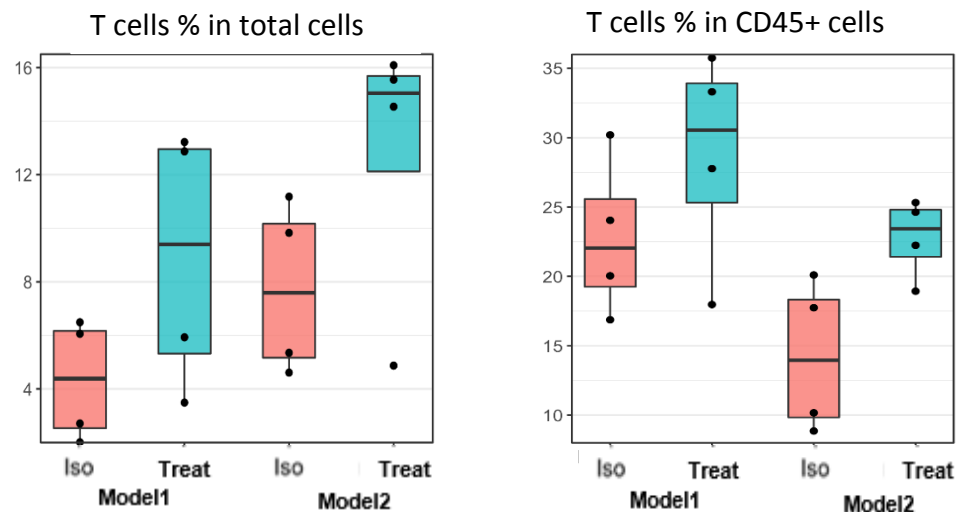


Automatically based on database

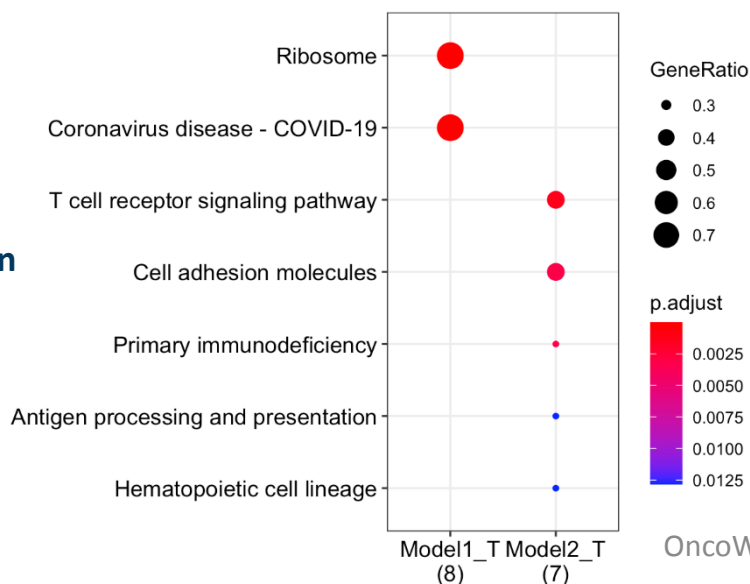


Treatment-induced immune cell changes

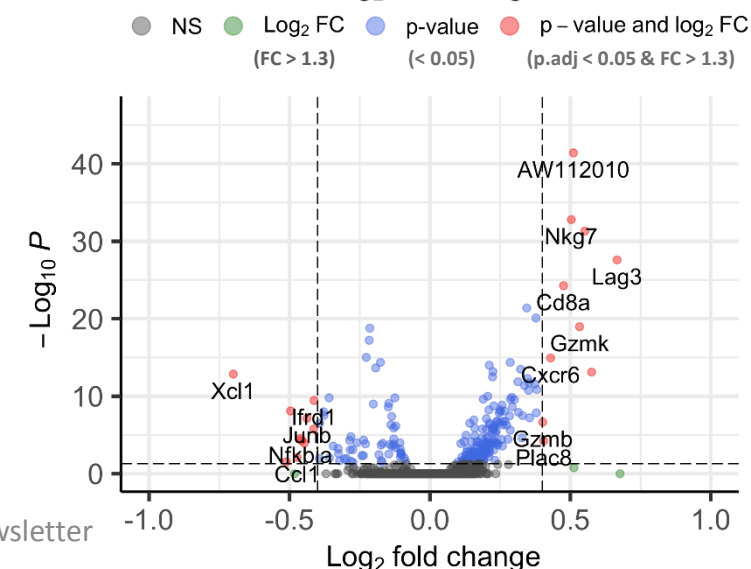
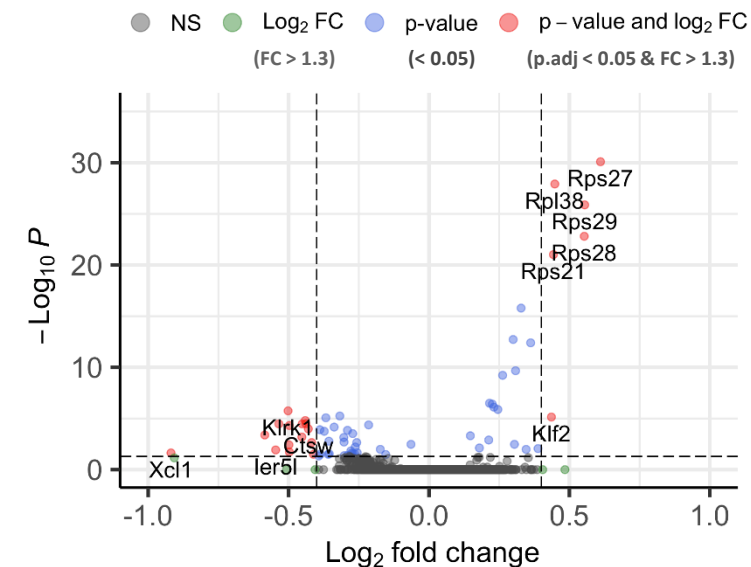
T cell frequency changes



Pathway changes in different models

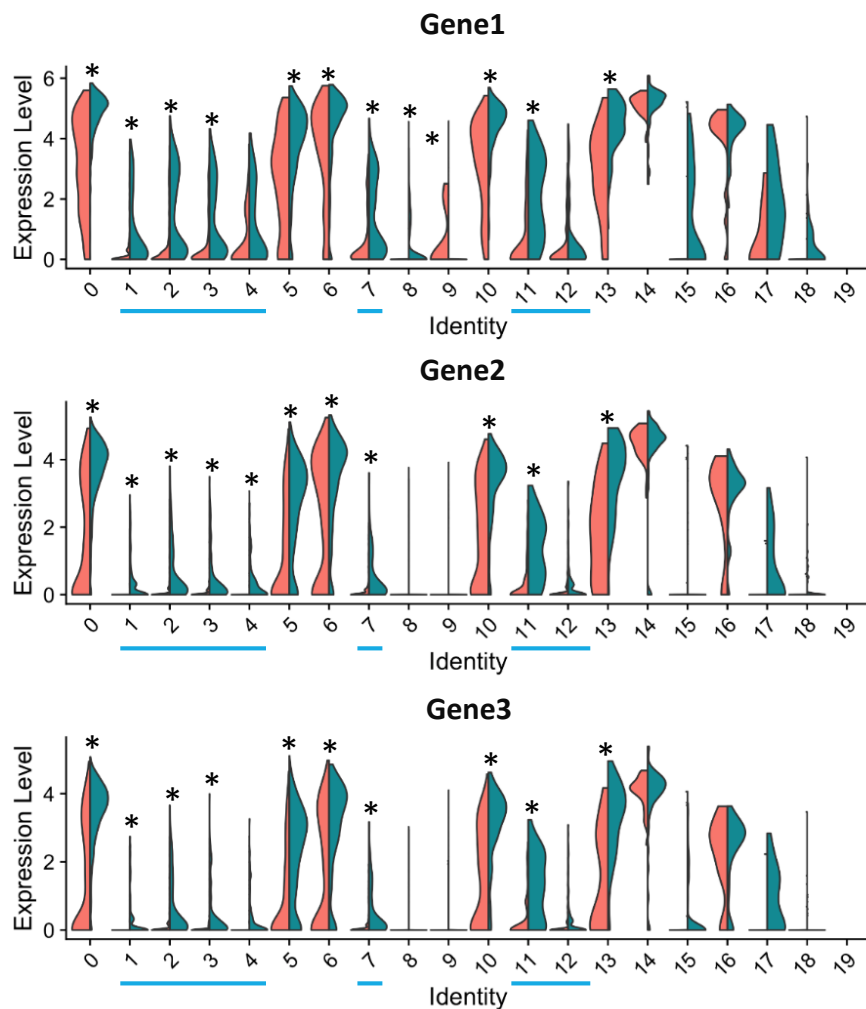


Differential gene expression across models

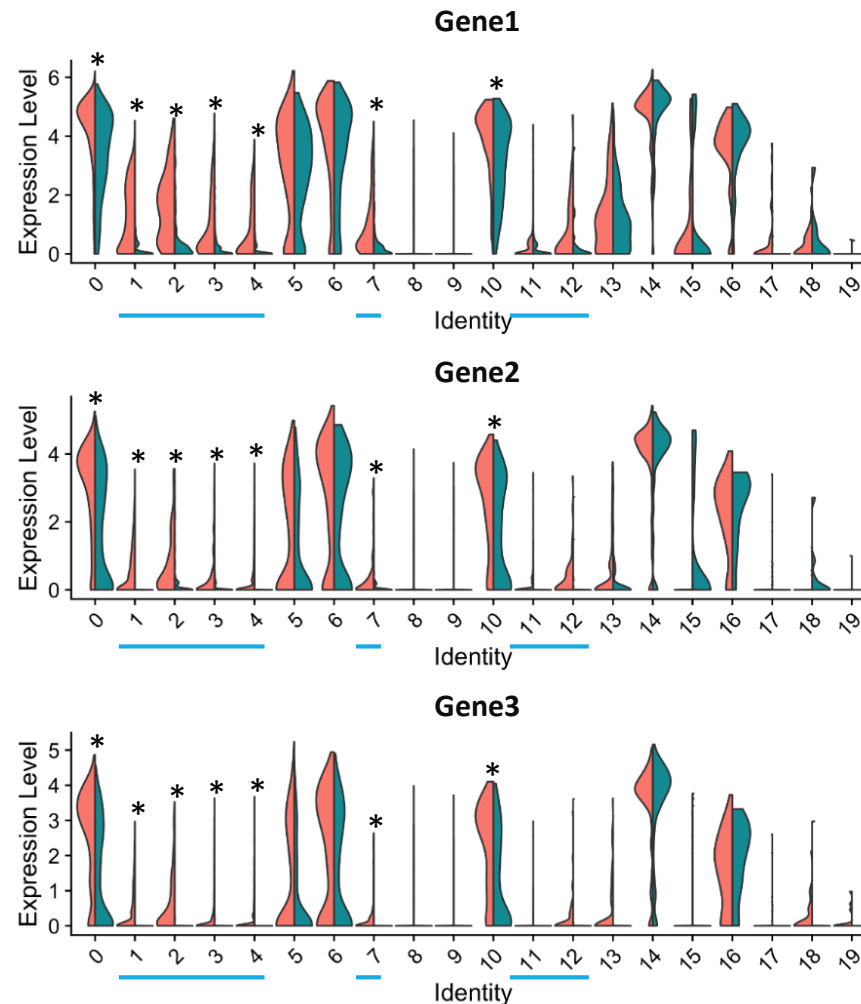


Treatment-induced gene expression alternation in tumor cells

Model1 (responsive)

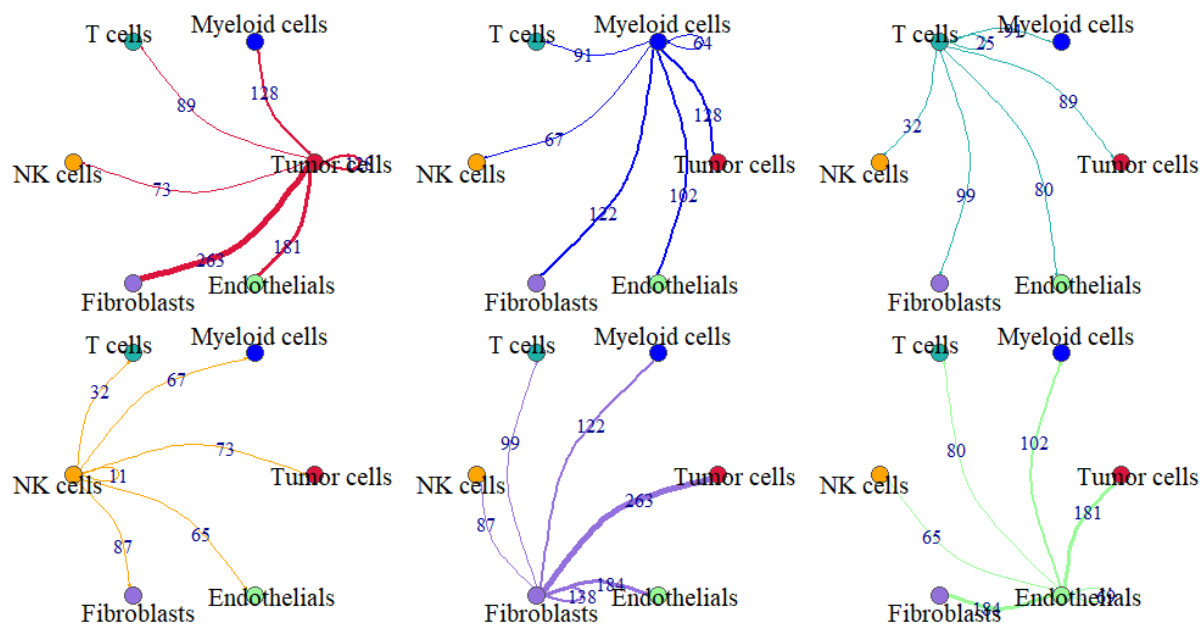


Model2 (non-responsive)

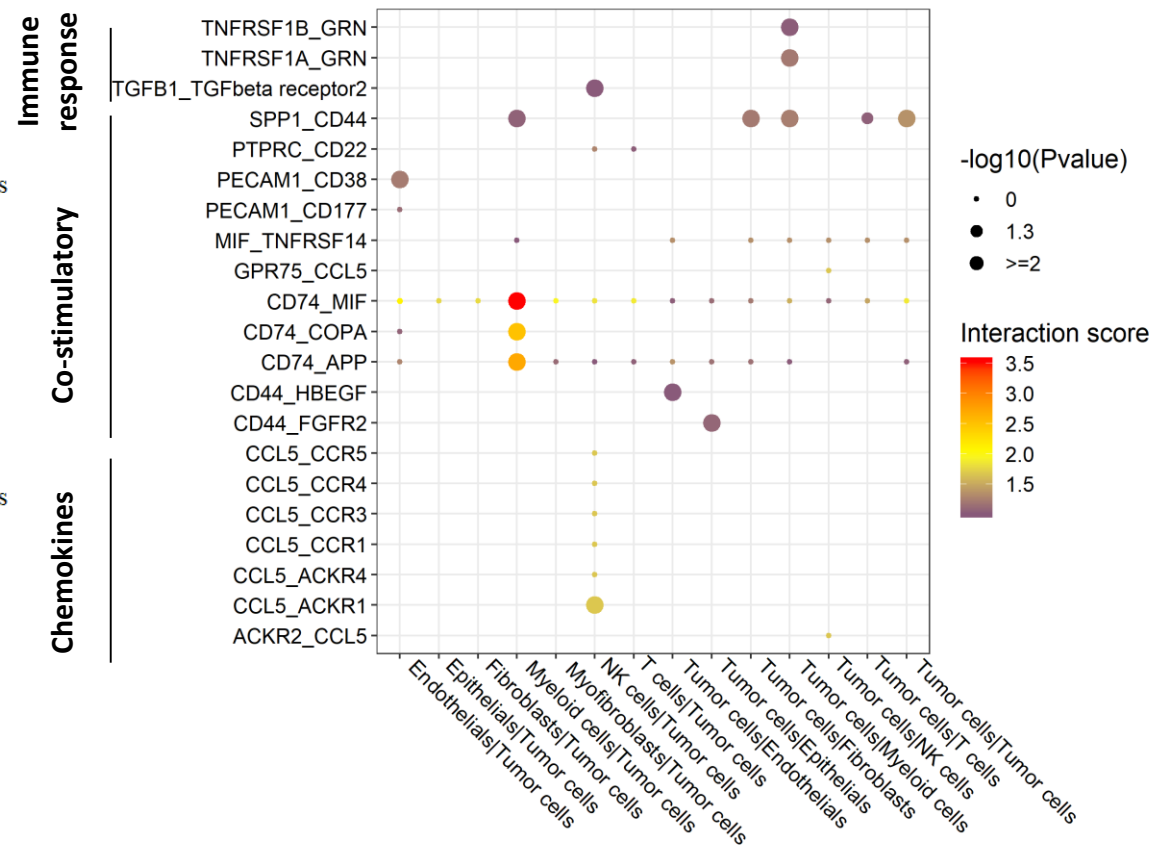


The potential key ligand-receptor pairs functioning in TME

Cell-cell communication analysis based on ligand-receptor pairs database

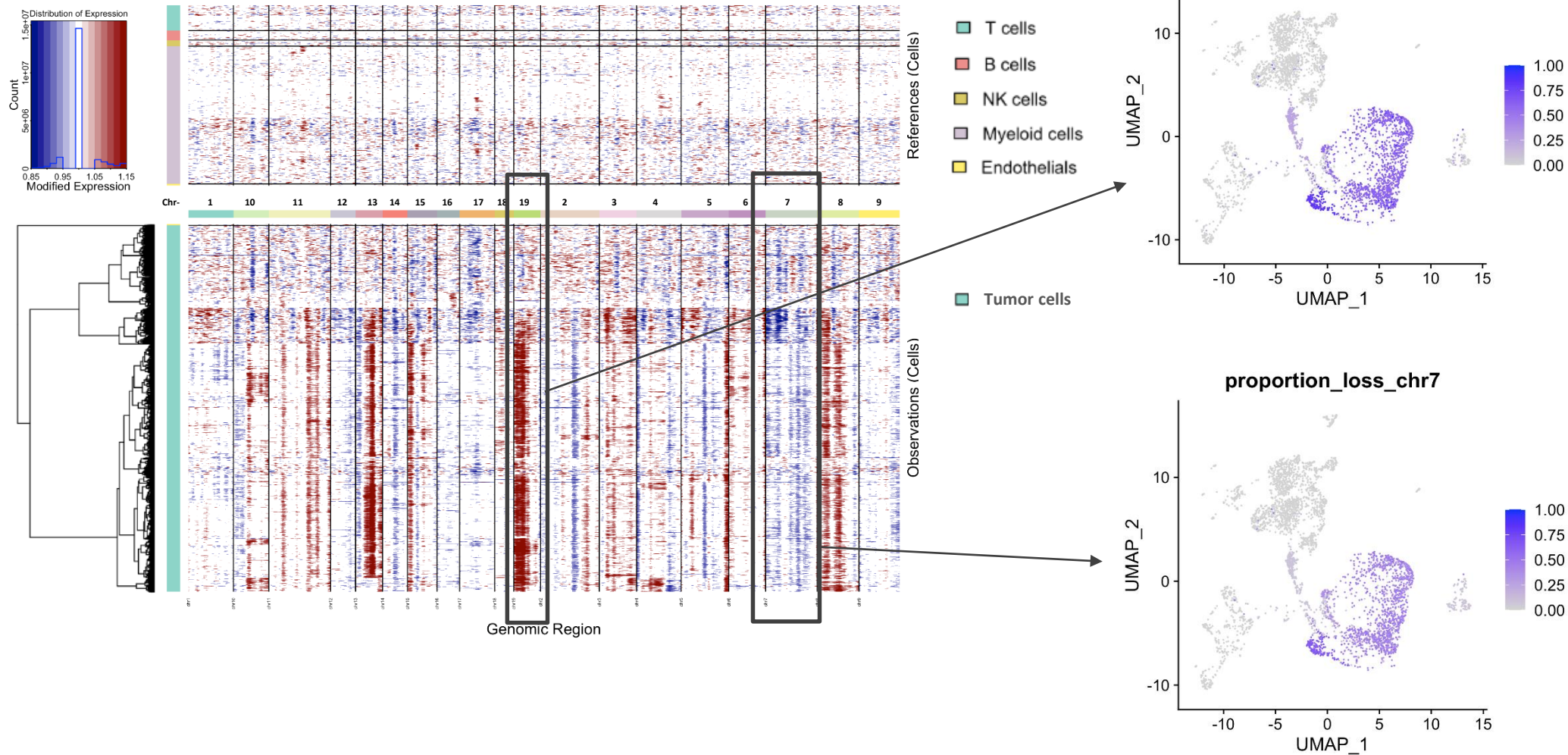


Overview of selected ligand-receptor interactions of tumor cells



Exploration on large-scale chromosomal copy number alteration

Chromosomal copy number alteration analysis





OUR COMMITMENT

Improving Health. Making a Difference.

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