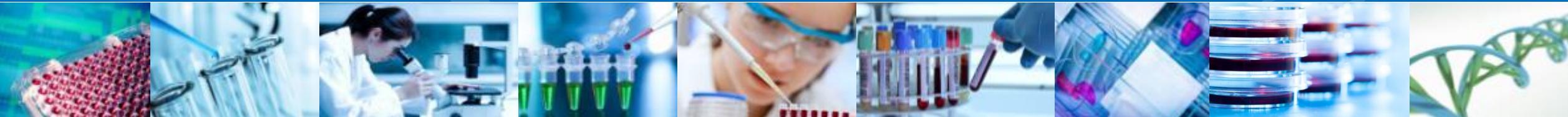


# WuXi AppTec scRNA Sequencing Service



WuXi AppTec Research Service Division, Oncology & Immunology Unit



2021.01

OncowuXi Newsletter

# Outline

## ■ 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

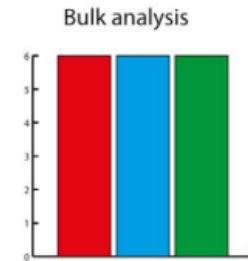
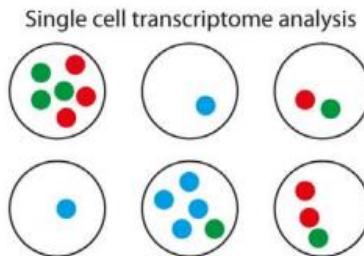
- Quality control metrics
- Data analysis for scRNA-seq

## ■ Case Study

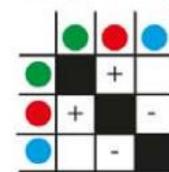
# Why single cell RNA -sequencing?

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

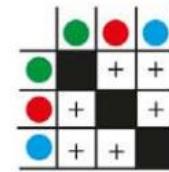
## Single cell vs Bulk Analysis



Coexpression Matrix  
(single cell)

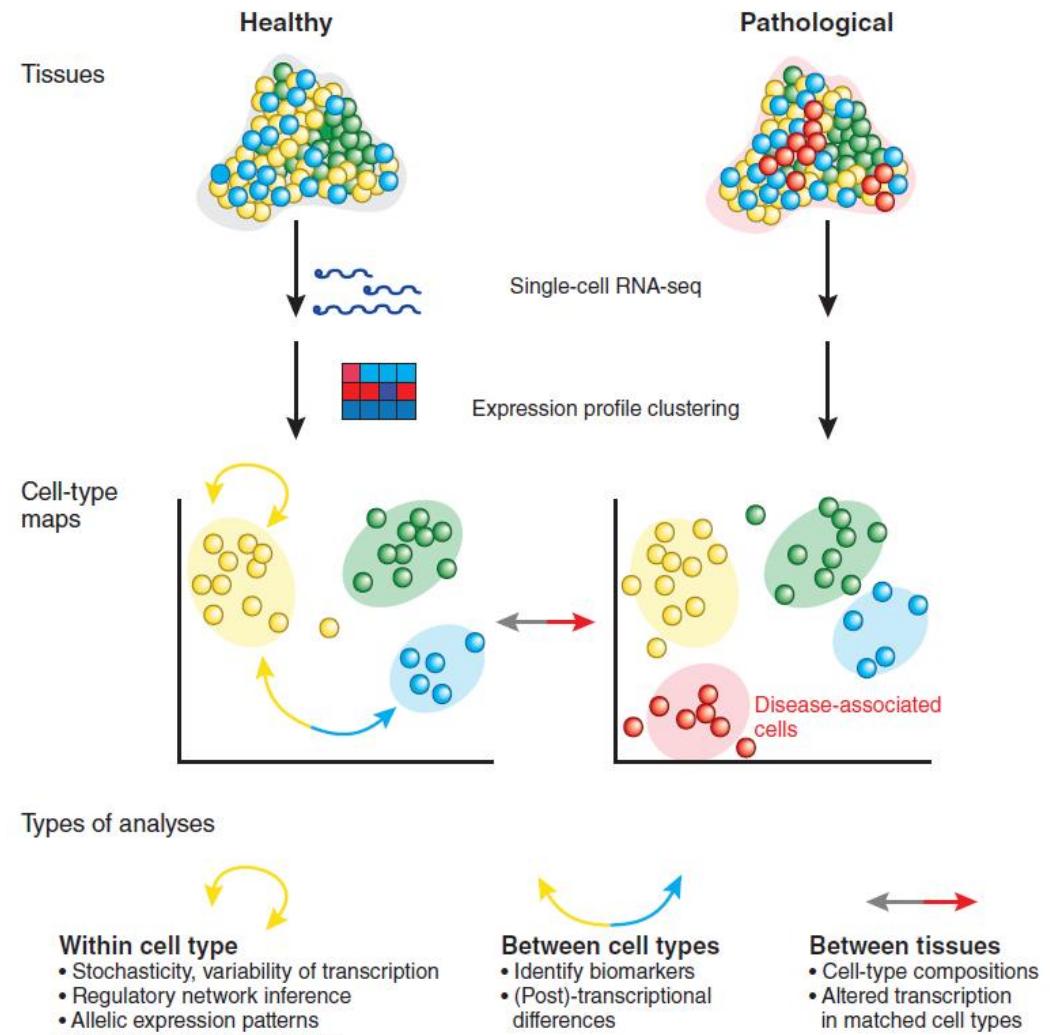


Coexpression Matrix  
(bulk analysis)



Macaulay and Voet, PLOS Genetics, 2014

## Multi-dimensional Comparison



# Highlight of single cell gene expression

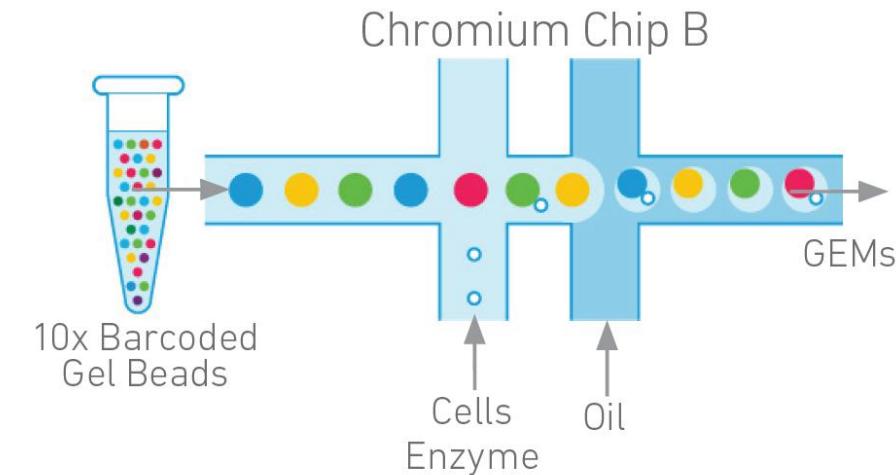
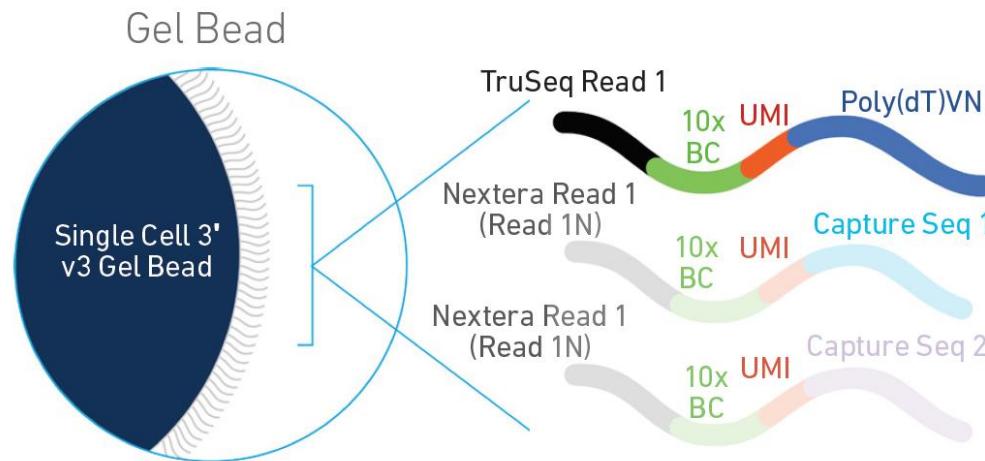
## ■ RNA-Seq

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

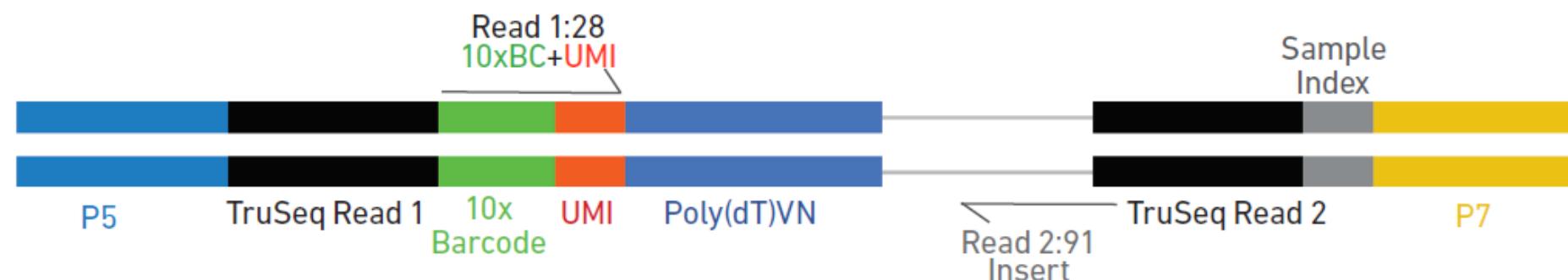
## ■ scRNA-Seq

- 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?

- Fit-for-purpose design and processing optimization

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

## Pilot Study

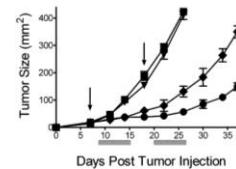
## *In Vivo* Treatment

## Sample Collection & Single Cell Prep

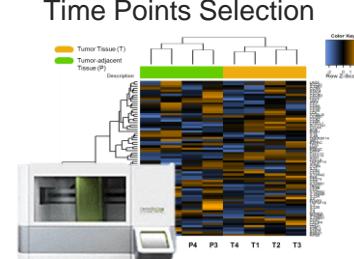
## Library Prep & Sequencing

## Bioinformatics Analysis

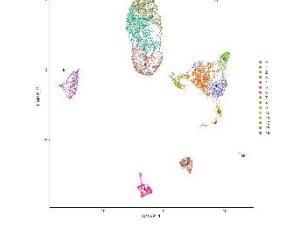
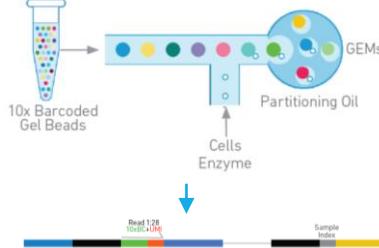
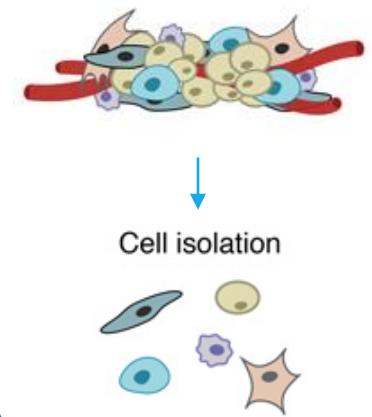
### Dose Selection by Efficacy Study



### Time Points Selection



### Complex tissue



# Fit-for-purpose sample processing optimization



## Tumor Dissociation

- Digestion Reagents
- Digest Time
- Digest Programs

## Red Blood Cell Lysis

- Lysis Reagents
- Lysis Programs

## Remove Debris

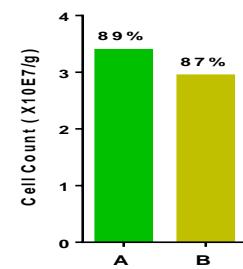
- Wash more times
- Filter with strainer
- Density centrifugation

## Remove Dead Cell

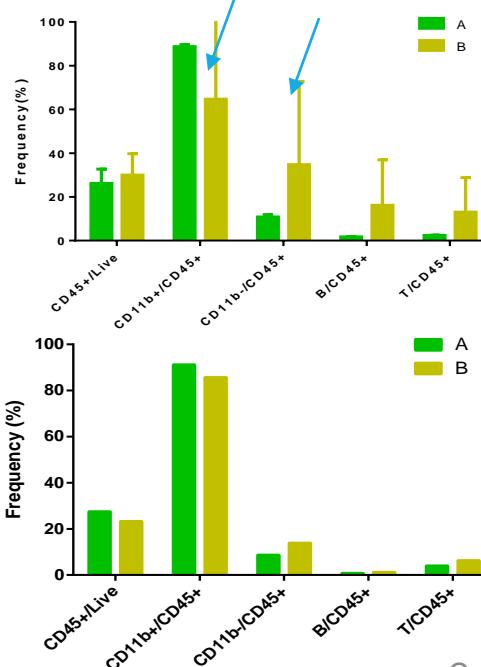
- Wash
- Removal Kit
- Filter with strainer

### Tumor Dissociation Optimization

#### Cell Yield & Viability



#### Immune Profiling



Model 1

Model 2

### RBC Lysis Optimization

Method	A	B	C	D	E	F
Cell Yield	High	Medium	High	Very High	Medium	High
RBC Residue	Medium	Very High	Medium	Very Low	Medium	Medium
Cell Viability	Medium	Medium	Very Low	Very Low	Medium	Medium
Cell Type Bias	Medium	Medium	Medium	Medium	Medium	Medium
Time Cost	Very High	Medium	Medium	Very Low	Medium	Medium

Model 1

Model 2

Method	A	B	C	D	E	F
Cell Yield	Very High	Very High	Very Low	Very Low	Medium	Medium
RBC Residue	Very Low	Medium	Medium	Very Low	Very High	Very High
Cell Viability	Medium	Very Low	Very Low	Very Low	Medium	Medium
Cell Type Bias	Medium	Medium	Medium	Medium	Medium	Medium
Time Cost	Very High	Medium	Medium	Very Low	Medium	Medium

# Example of quality control

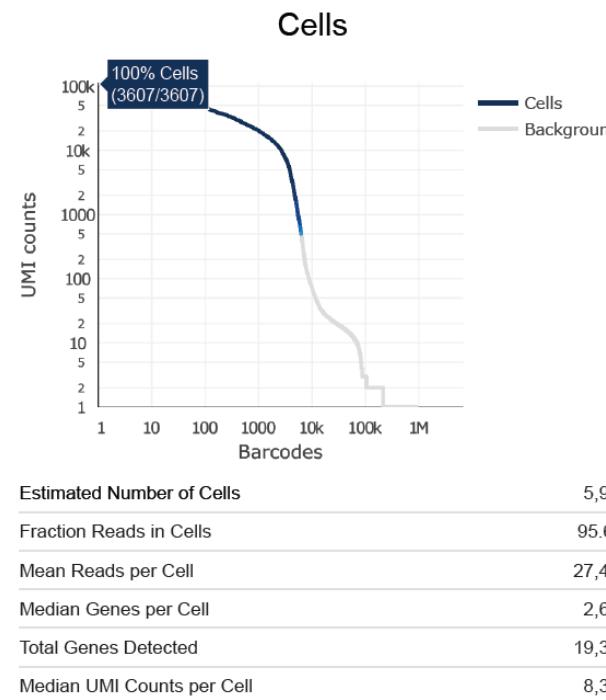
## Sample Preparation

Model ID	Live Cells (Counts) /g	Viability (Mean $\pm$ SD)
Model 1	$(6.15 \pm 4.24) \times 10^7$	$87.96 \pm 1.92\%$
Model 2	$(2.8 \pm 0.54) \times 10^7$	$86.40 \pm 4.13\%$

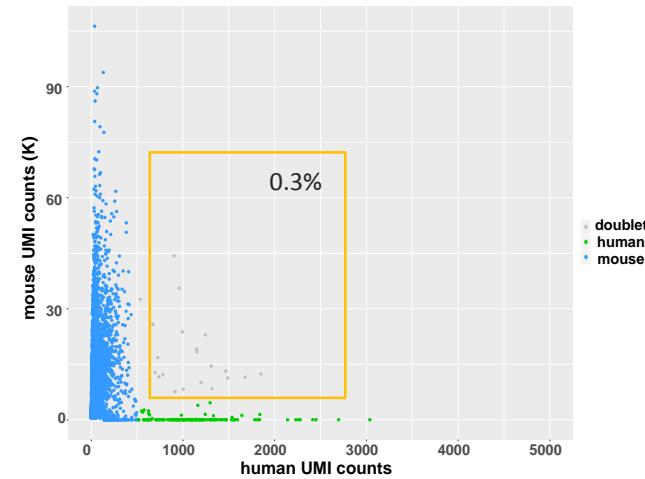
## Library Preparation

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

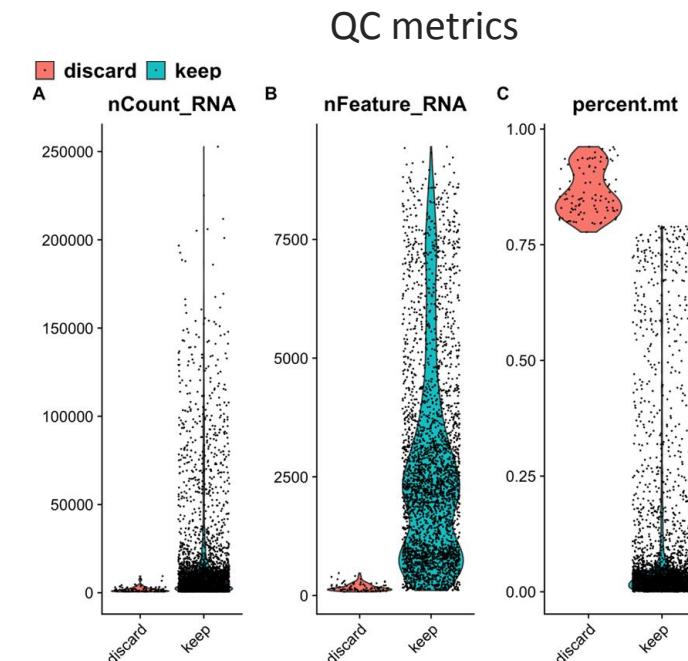
## Cell Count QC- Cell Ranger



## Doublets Rates Analysis

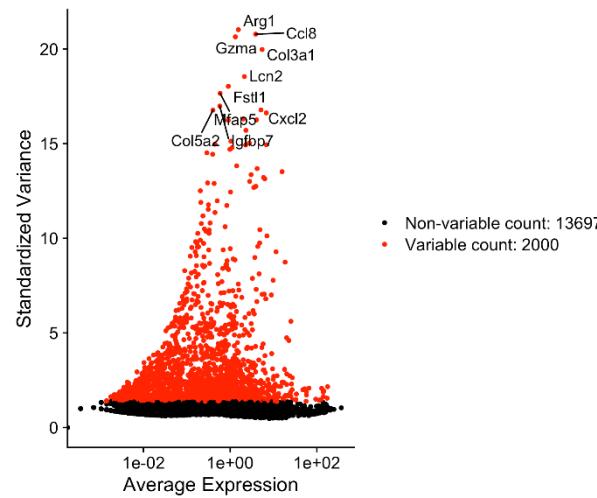


## Data Processing

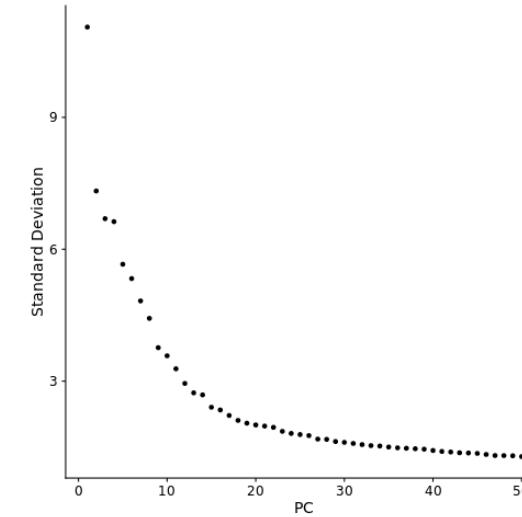


# Feature selection, dimension reduction and clustering

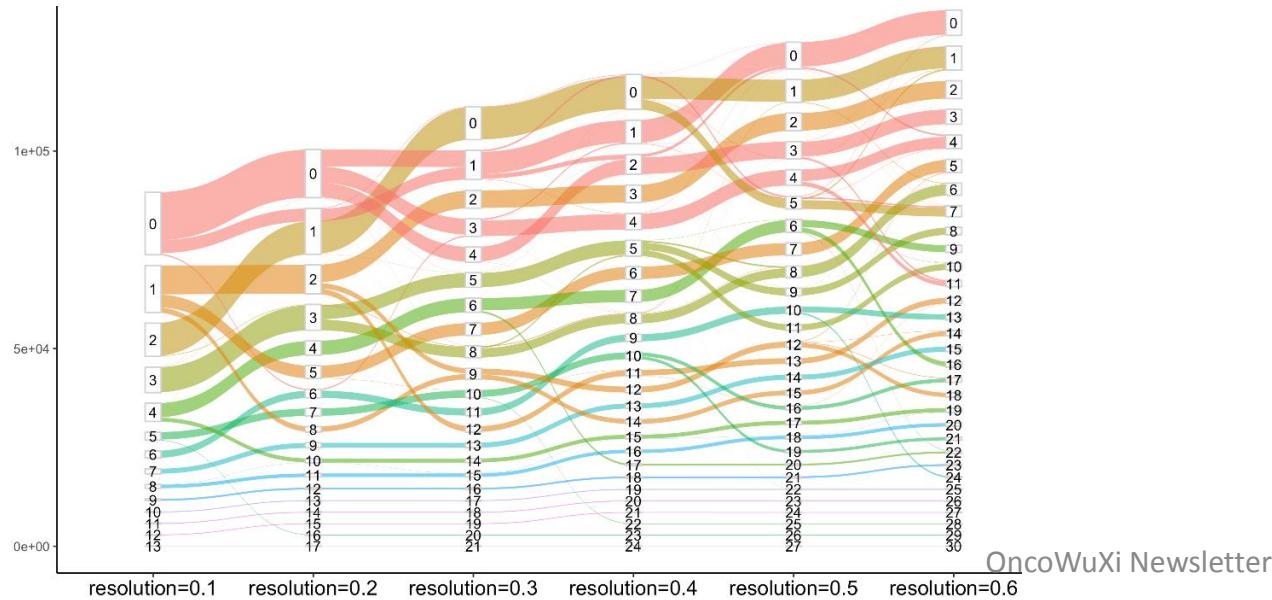
## Feature Selection



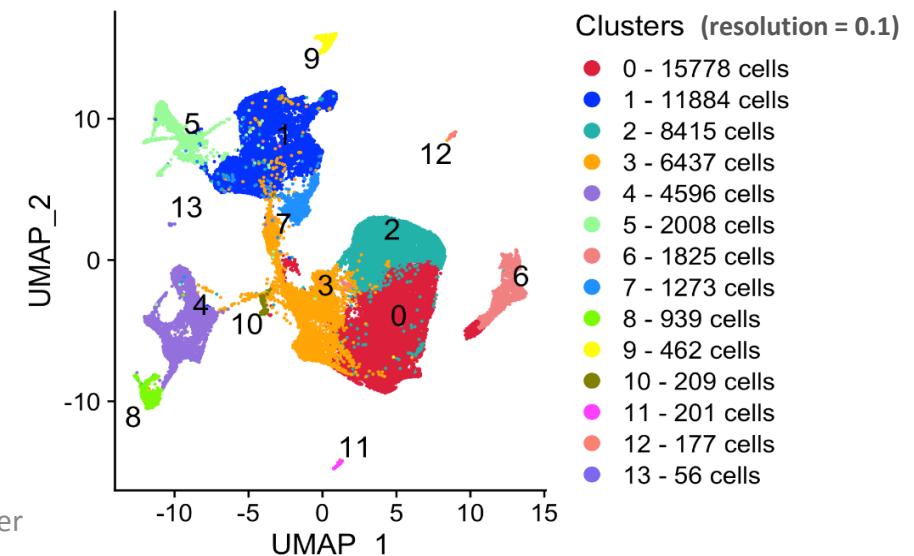
## PCs selection by Elbow Plot



## Clustering with a series of resolution



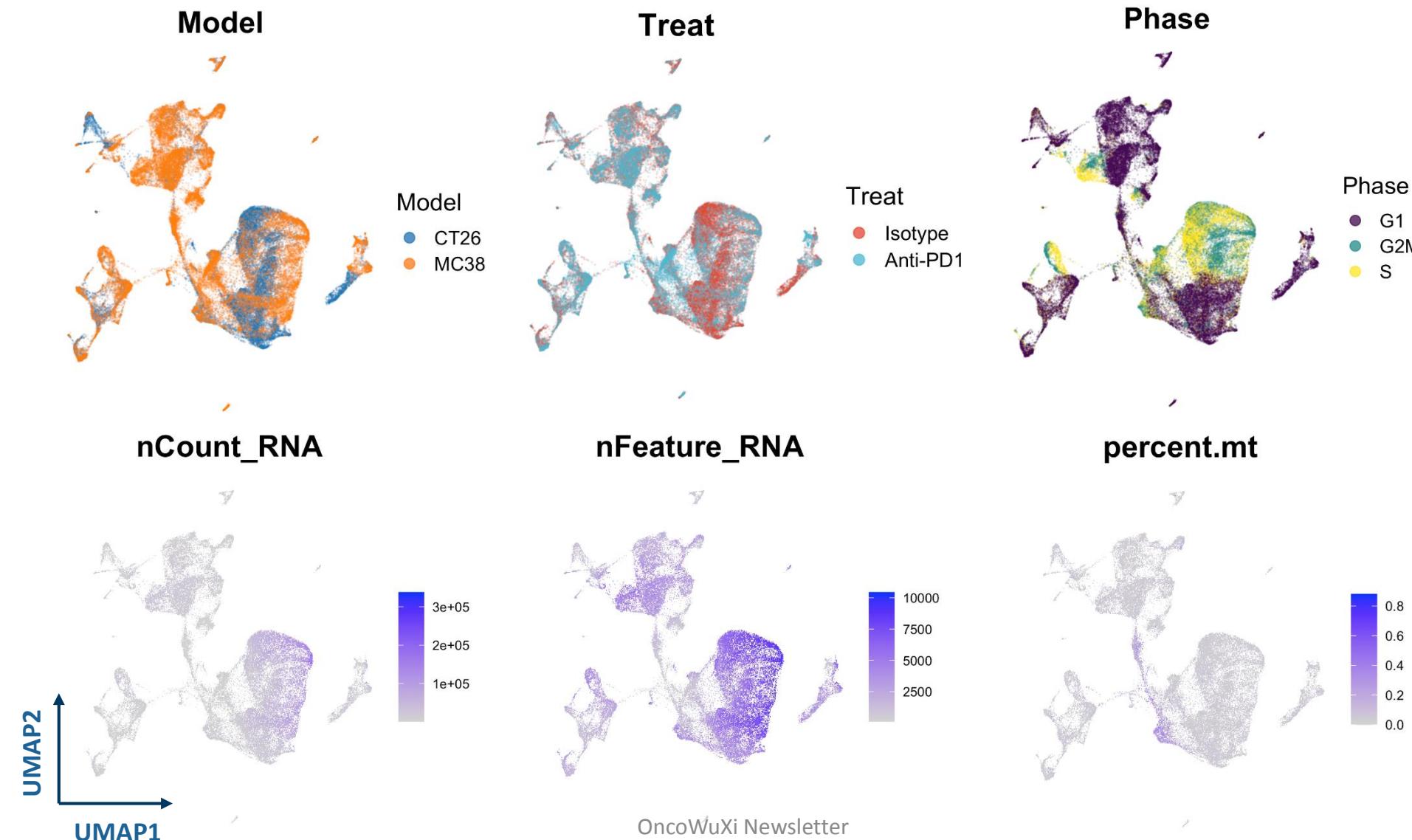
## Visualization by UMAP



# Exploration on large-scale chromosomal copy number alterations

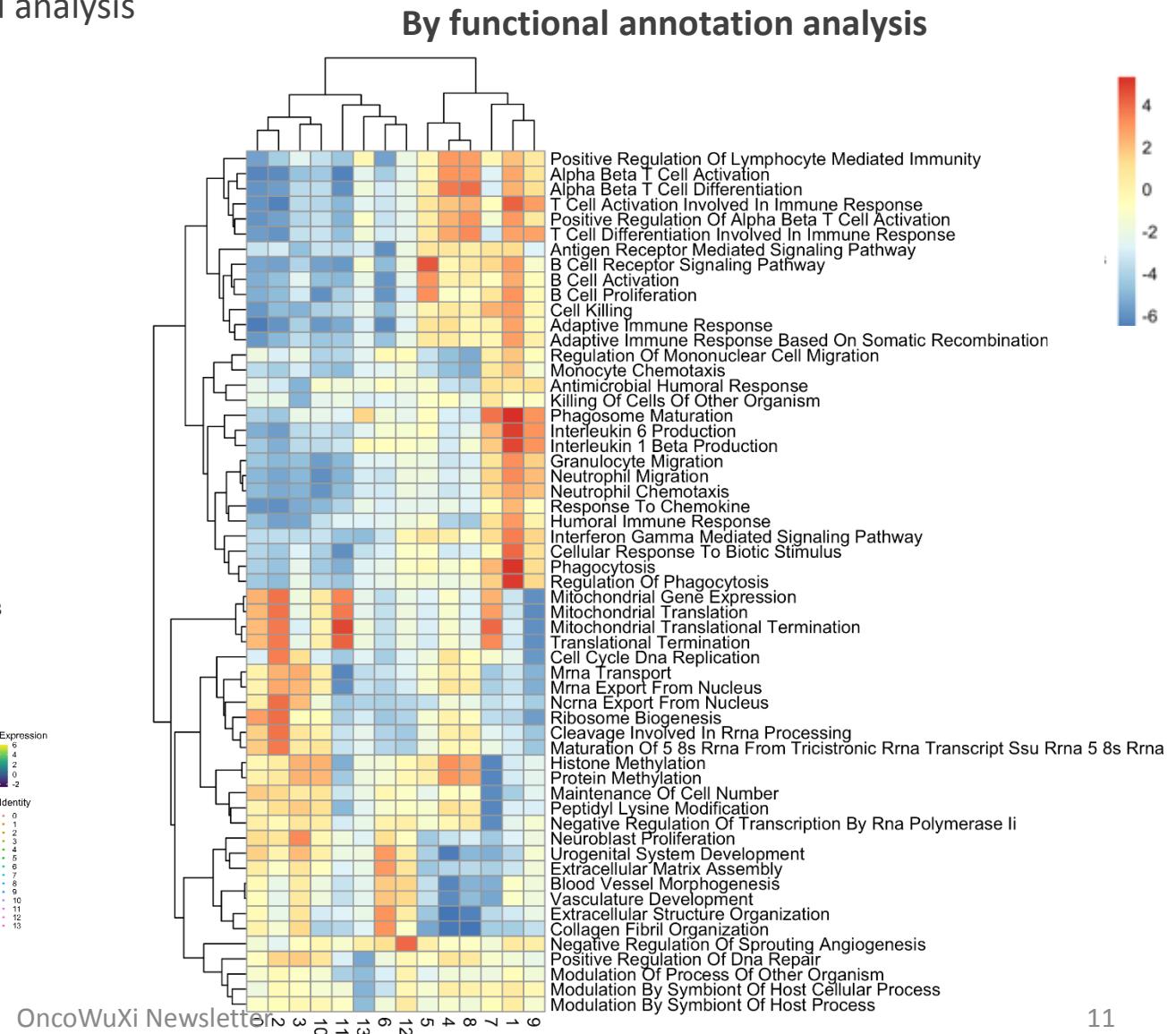
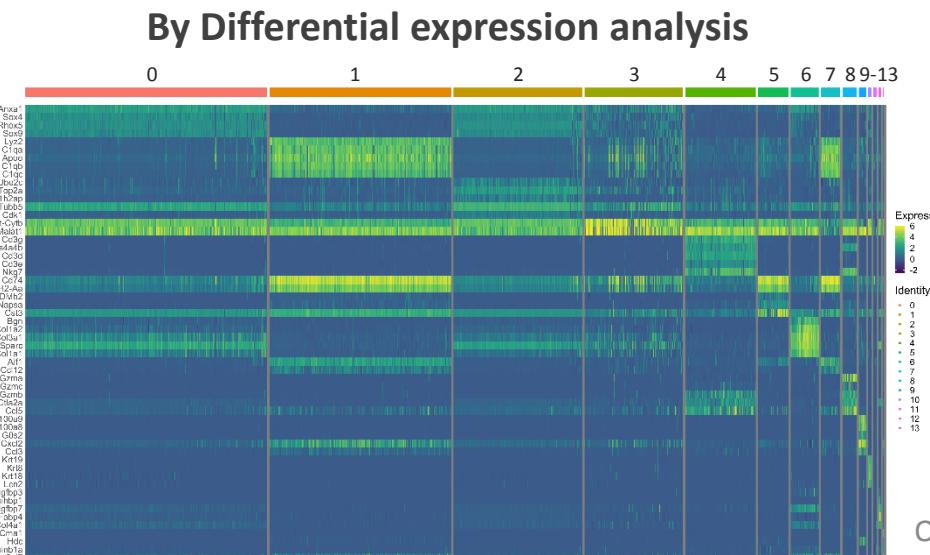
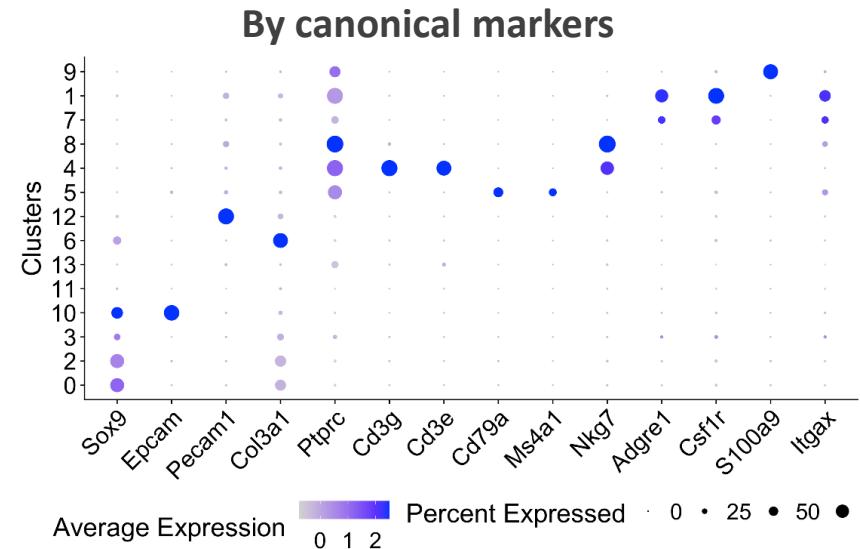
Exploration on some metrics

## UMAP visualization of metrics



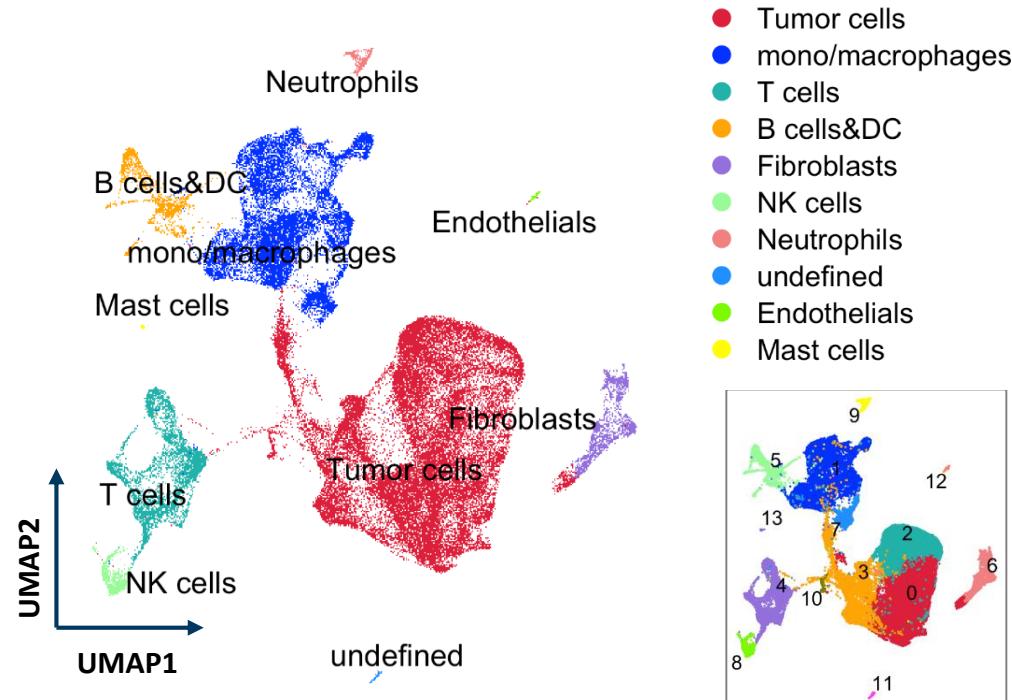
# Cell type annotation by multiple approaches

- Cell types were annotated and mutually confirmed by multiple approaches, to make sure its reliability for following differential gene analysis or other functional related analysis

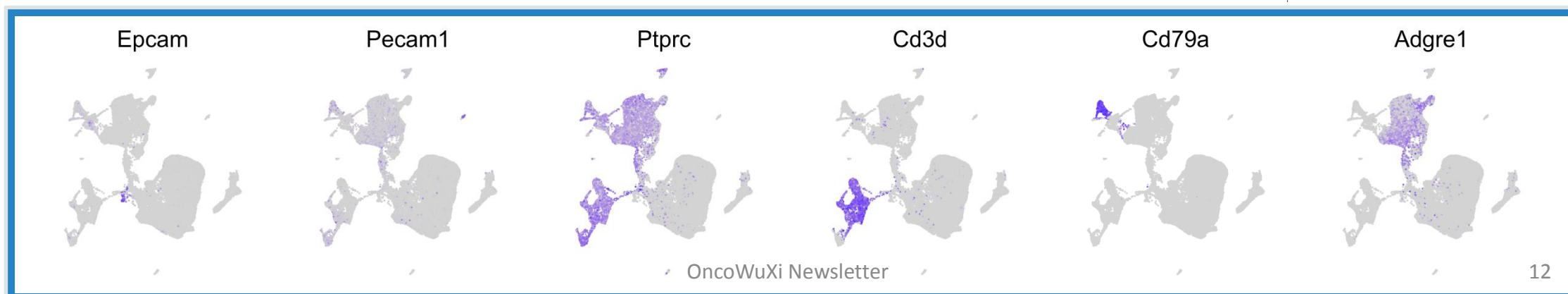
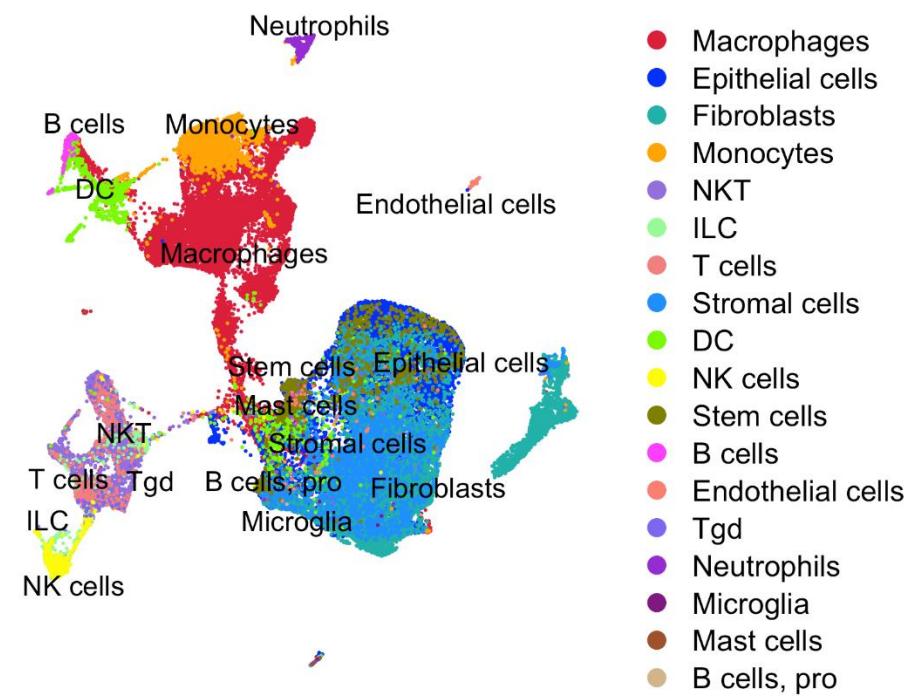


## Visualization of cell types and markers of interest on 2D plots

## Manually by a multi-step approaches

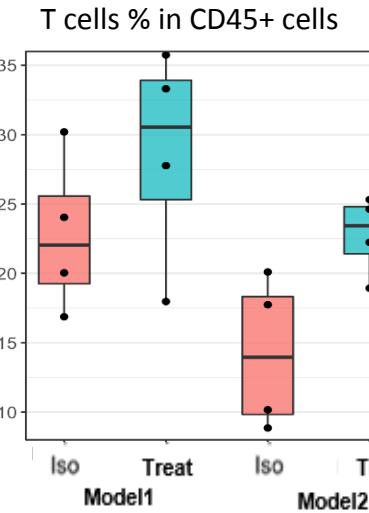
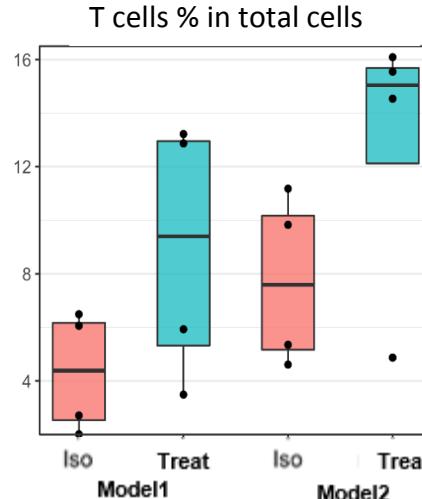


## Automatically based on database

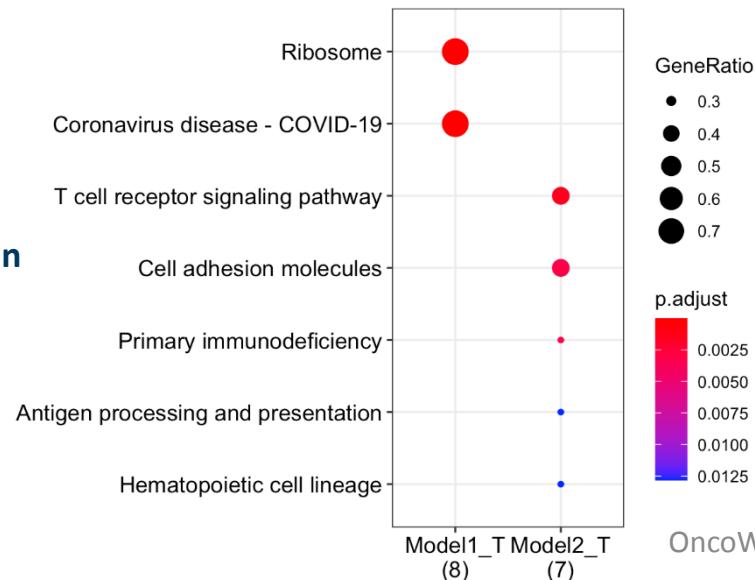


# Treatment-induced immune cell changes

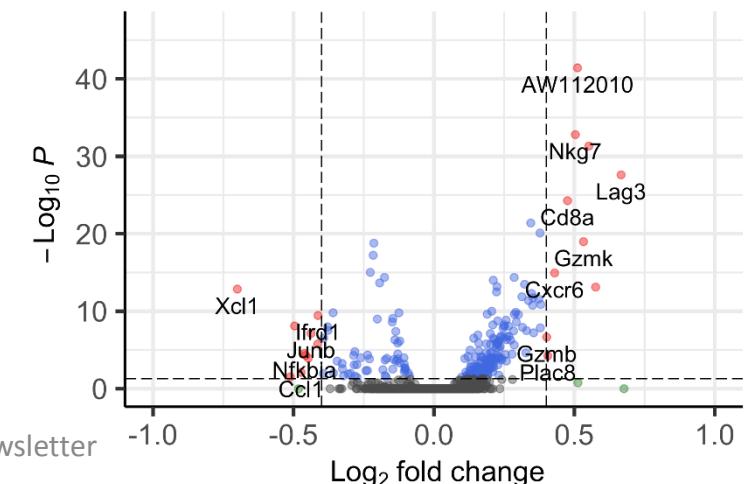
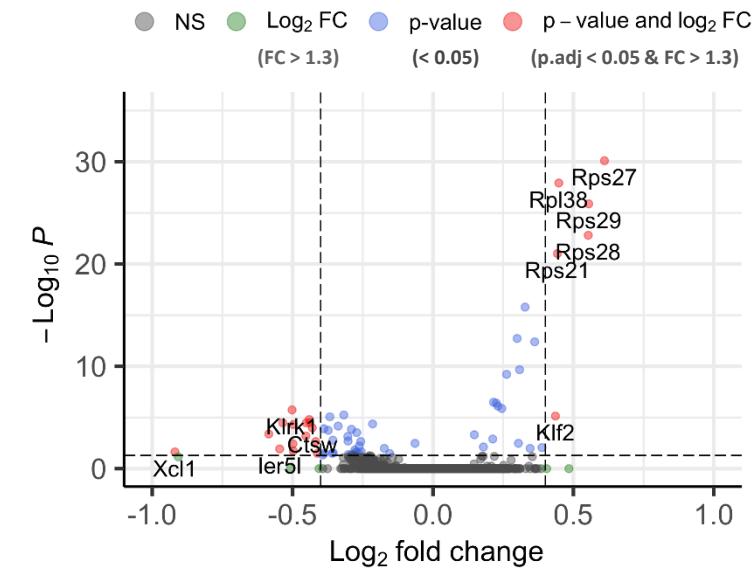
## T cell frequency changes



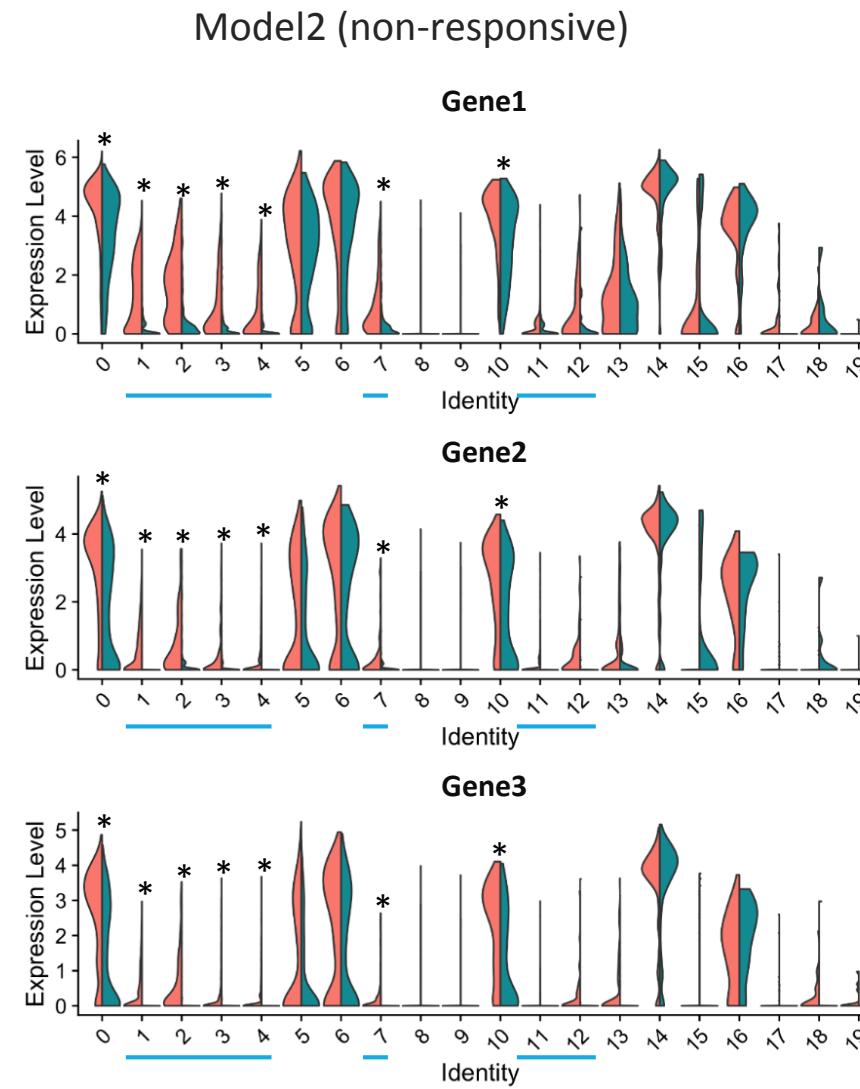
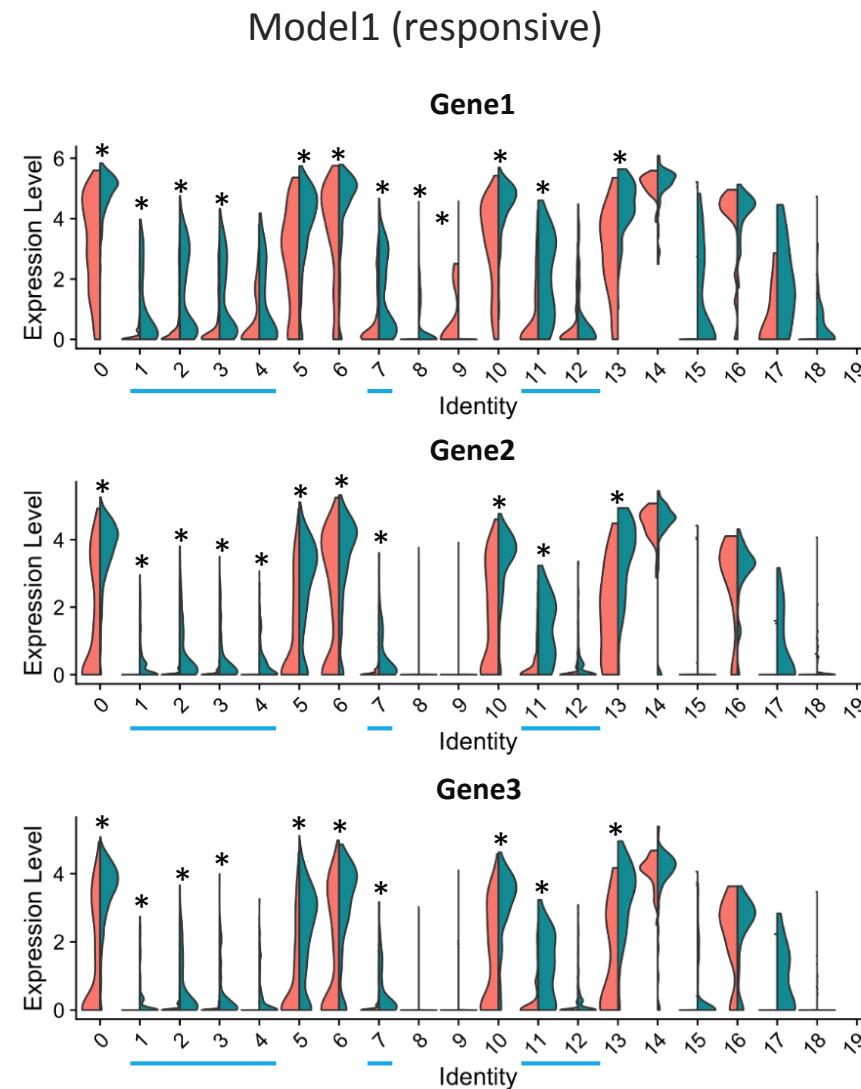
## Pathway changes in different models



## Differential gene expression across models

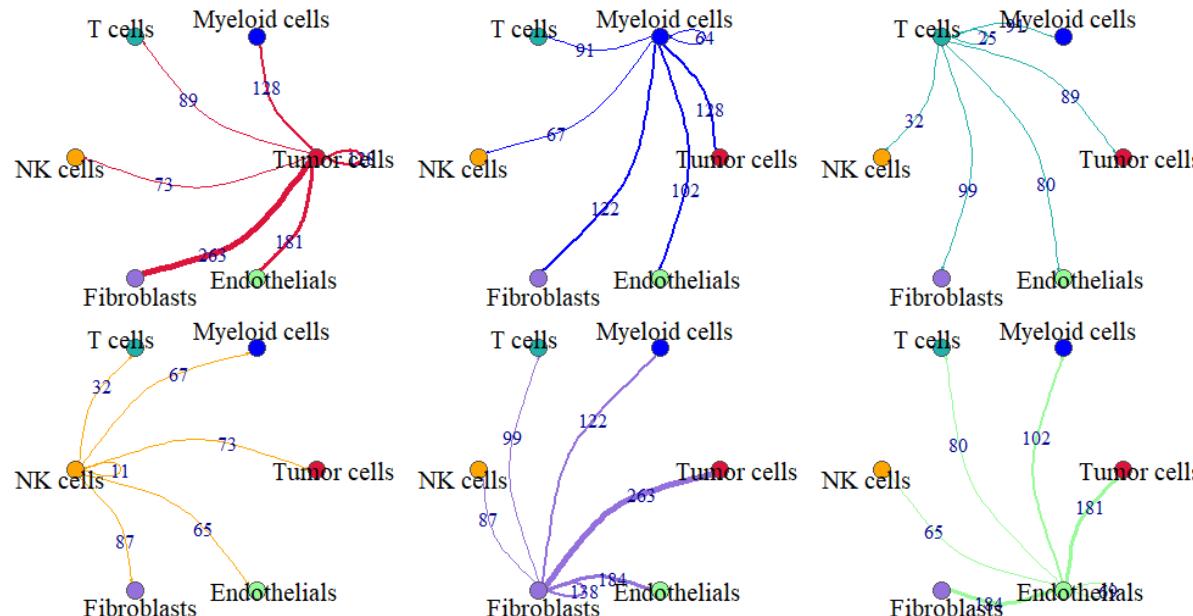


# Treatment-induced gene expression alternation in tumor cells

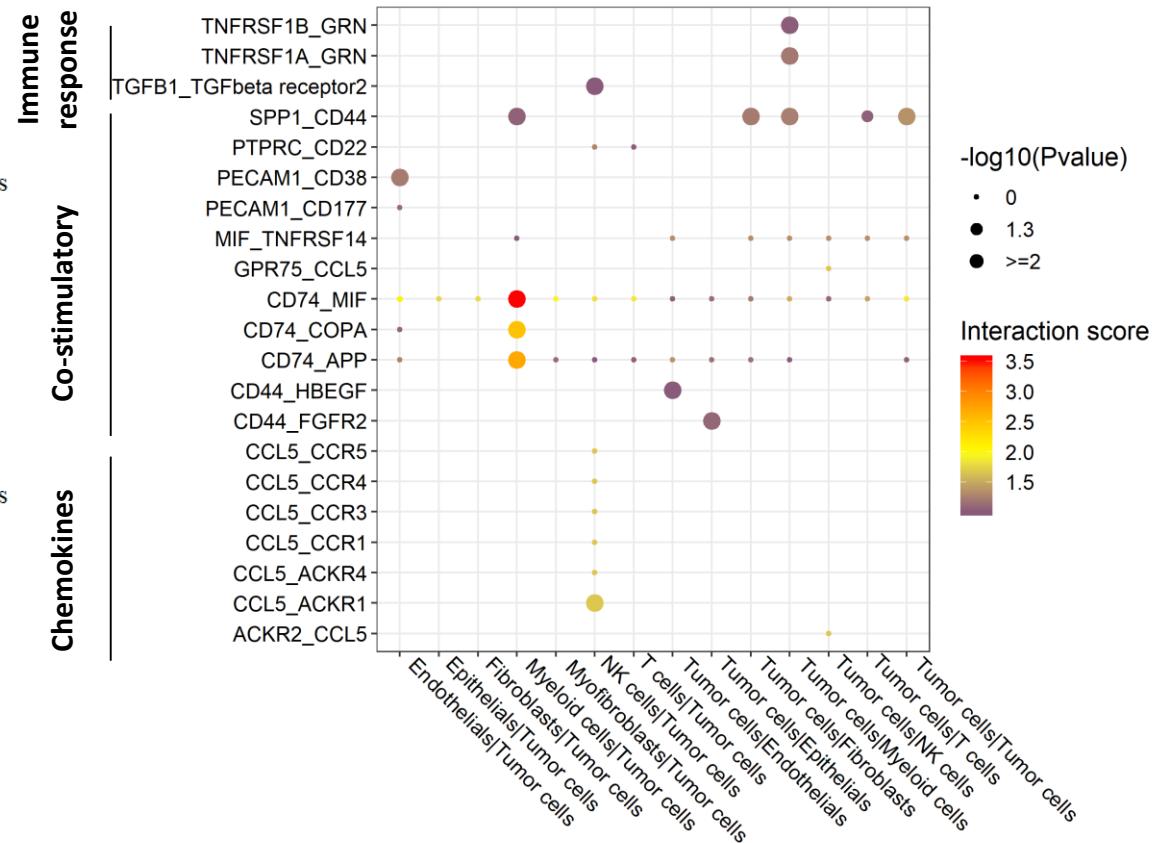


# The potential key ligand-receptor pairs functioning in TME

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

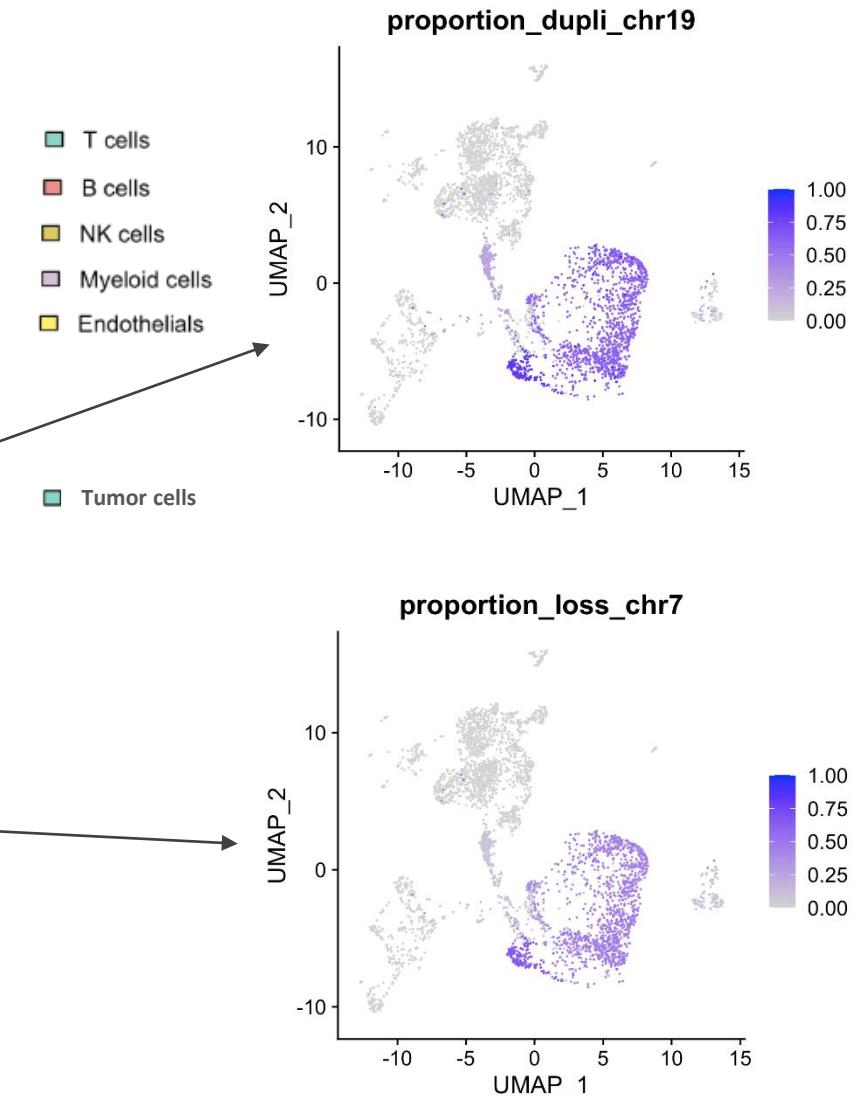
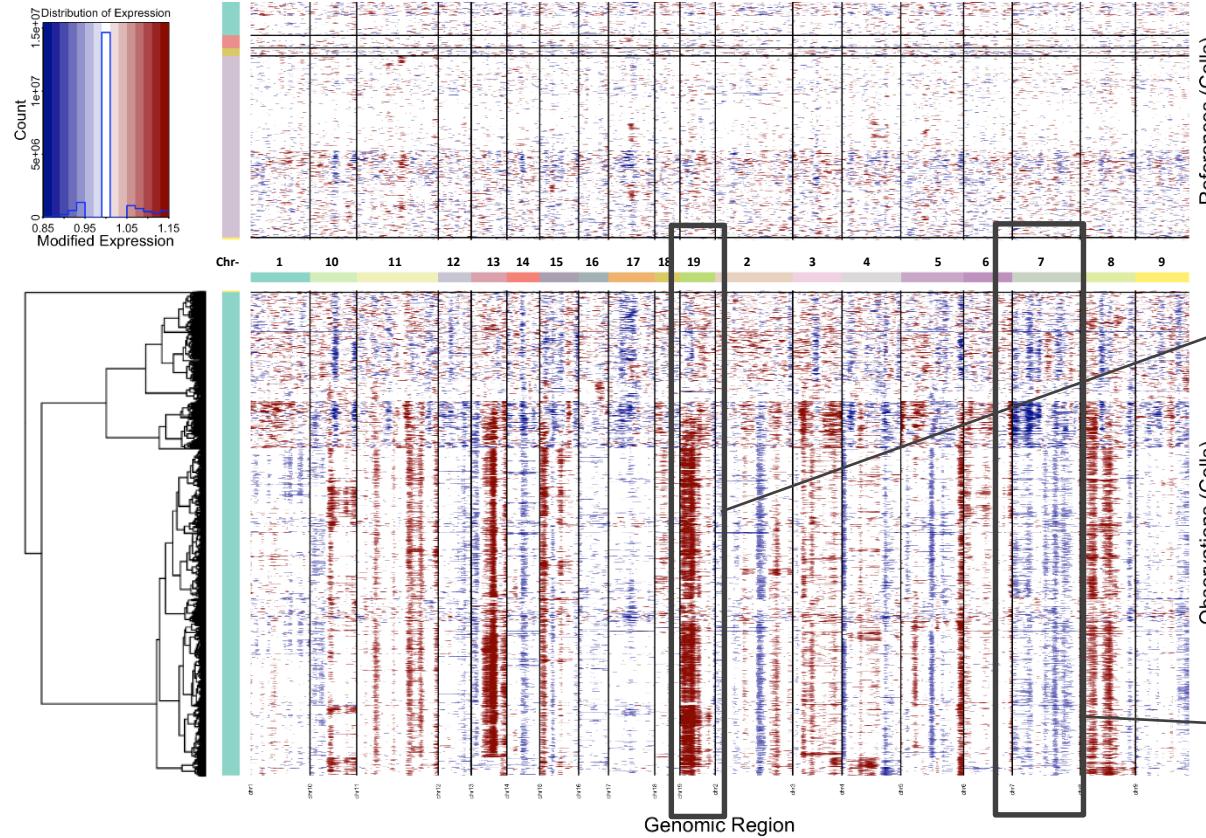


## Overview of selected ligand-receptor interactions of tumor cells



# Exploration on large-scale chromosomal copy number alteration

## Chromosomal copy number alteration analysis





# OUR COMMITMENT

## *Improving Health. Making a Difference.*

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