

# Single-cell RNA Sequencing Service



WuXi AppTec, WuXi Biology, Oncology & Immunology Unit



2023.05

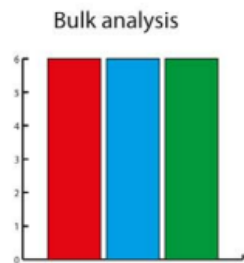
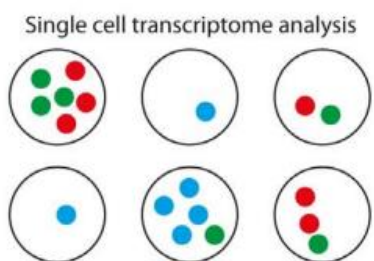
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# Why single cell RNA-sequencing?

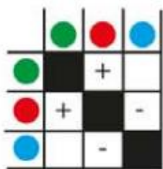
## Advantages of scRNA-seq technology compared with bulk-RNAseq:

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

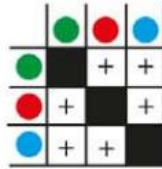
### Single cell vs Bulk Analysis



Coexpression Matrix (single cell)

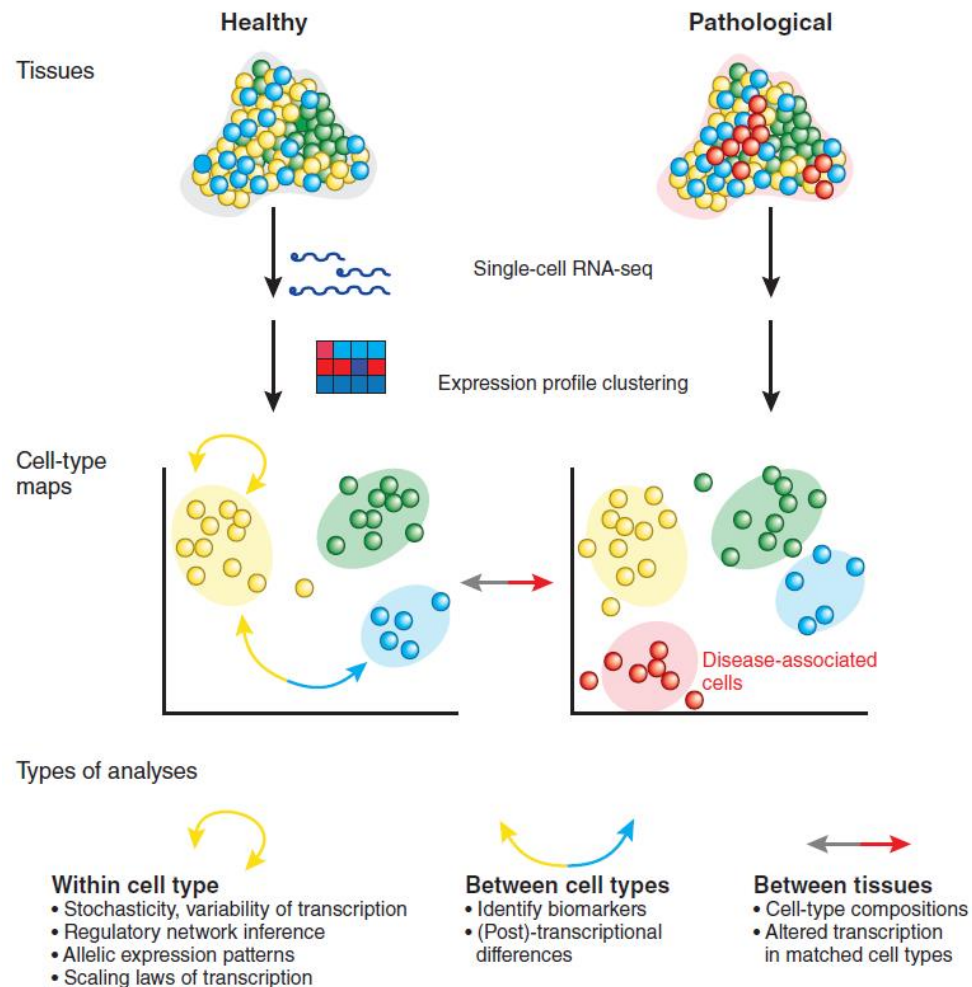


Coexpression Matrix (bulk analysis)



Macaulay and Voet, PLOS Genetics, 2014

## Multi-dimensional Comparison



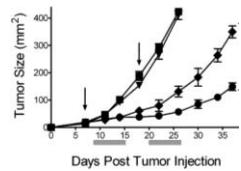
Sandberg, Nature Methods 2014

# Schematic workflow of scRNA sequencing

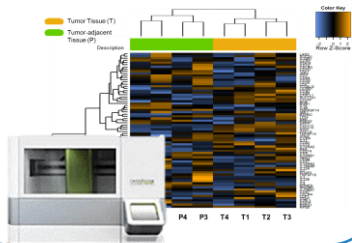
- How to decide the dosage for a scRNA-seq study?
- How to select the time points?

Pilot Study

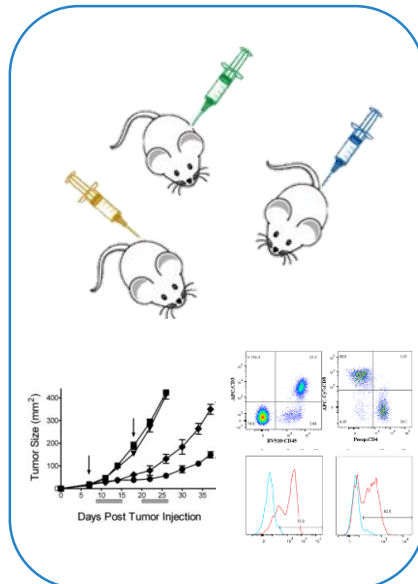
Dose Selection by Efficacy Study



Time Points Selection



*In Vivo* Treatment



- Fit-for-purpose design and processing optimization

Sample Collection & Single Cell Prep

Complex tissue

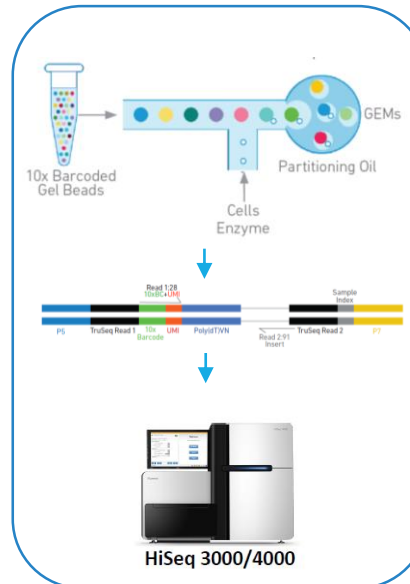


Cell isolation



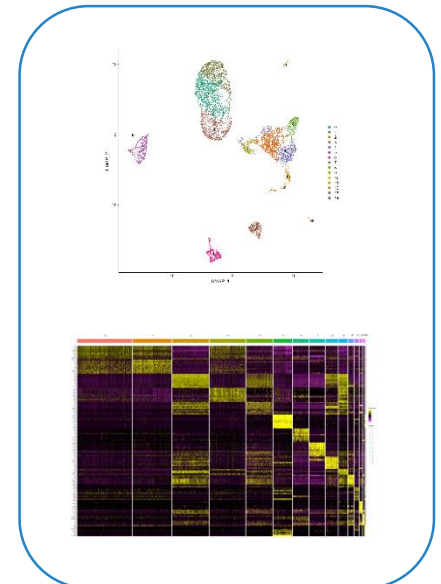
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Library Prep & Sequencing



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

Bioinformatics Analysis



# 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

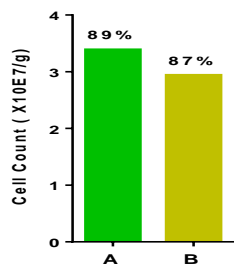
## Remove Dead Cell

- Wash
- Removal Kit
- Filter with strainer

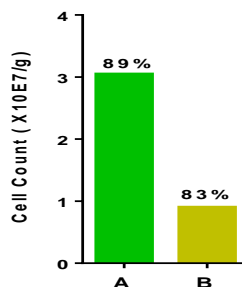
### Tumor Dissociation Optimization

Cell Yield & Viability

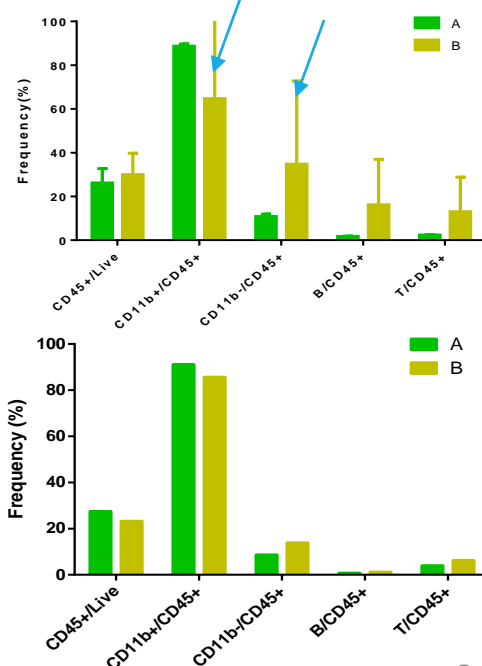
Model 1



Model 2



Immune Profiling



### 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	Green	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

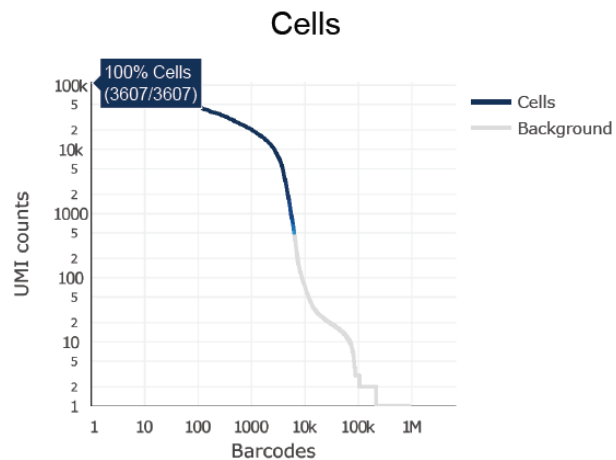
## Sample Preparation

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

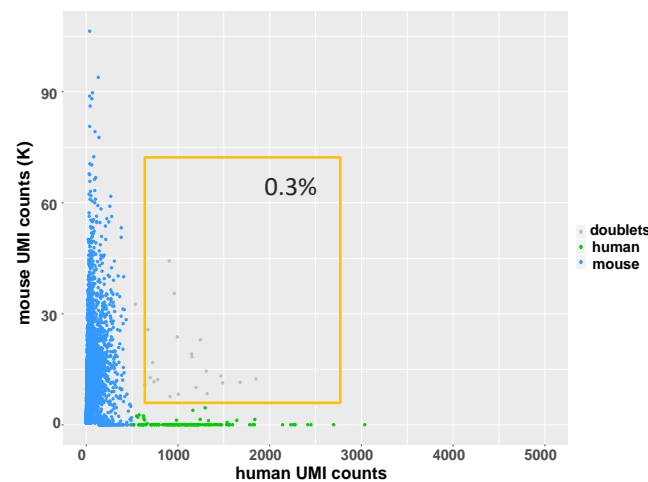
Step	RNA Conc ng/ul	Fragmentation (nt)
cDNA	$15 \pm 5$ ng/ul	-
Library	$25 \pm 5$ ng/ul	$450 \pm 50$ bp

## Cell Count QC- Cell Ranger



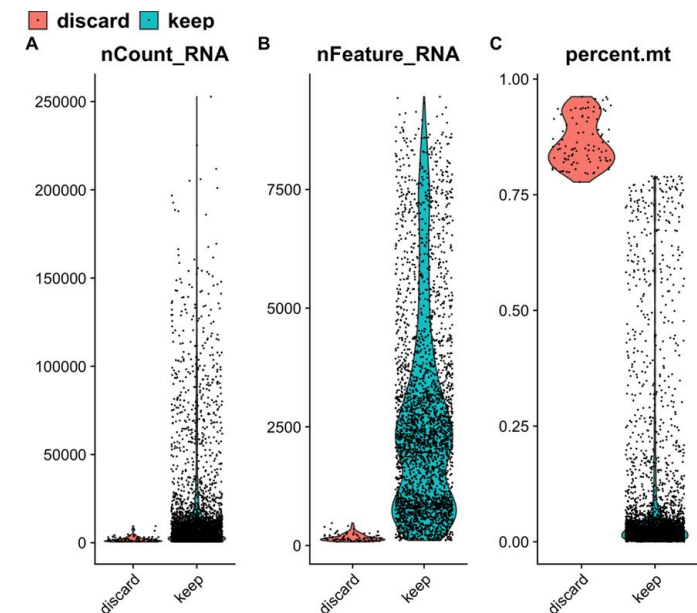
Estimated Number of Cells	5,969
Fraction Reads in Cells	95.6%
Mean Reads per Cell	27,451
Median Genes per Cell	2,632
Total Genes Detected	19,346
Median UMI Counts per Cell	8,349

## Doublets Rates Analysis



## Data Processing

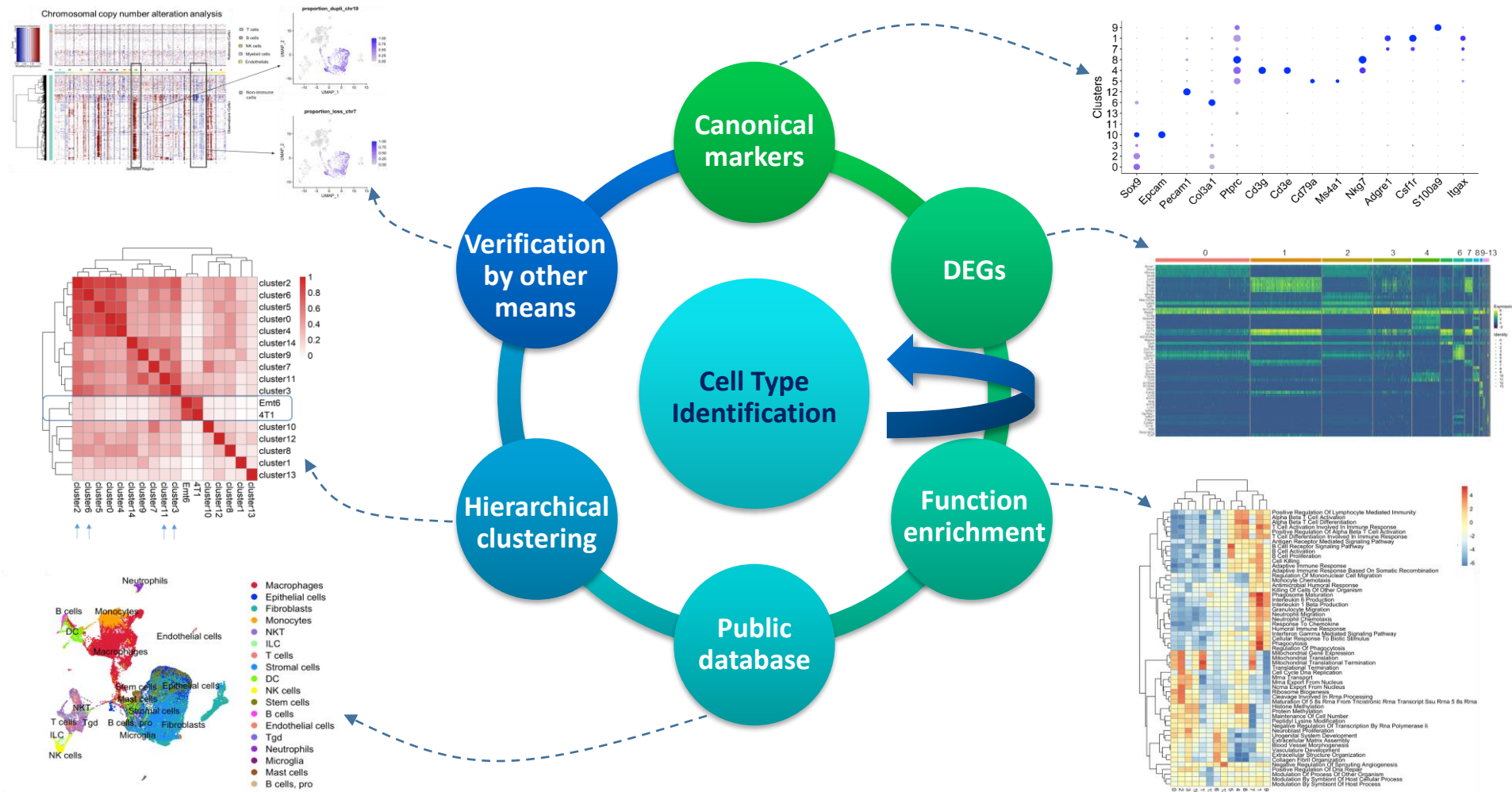
### QC metrics



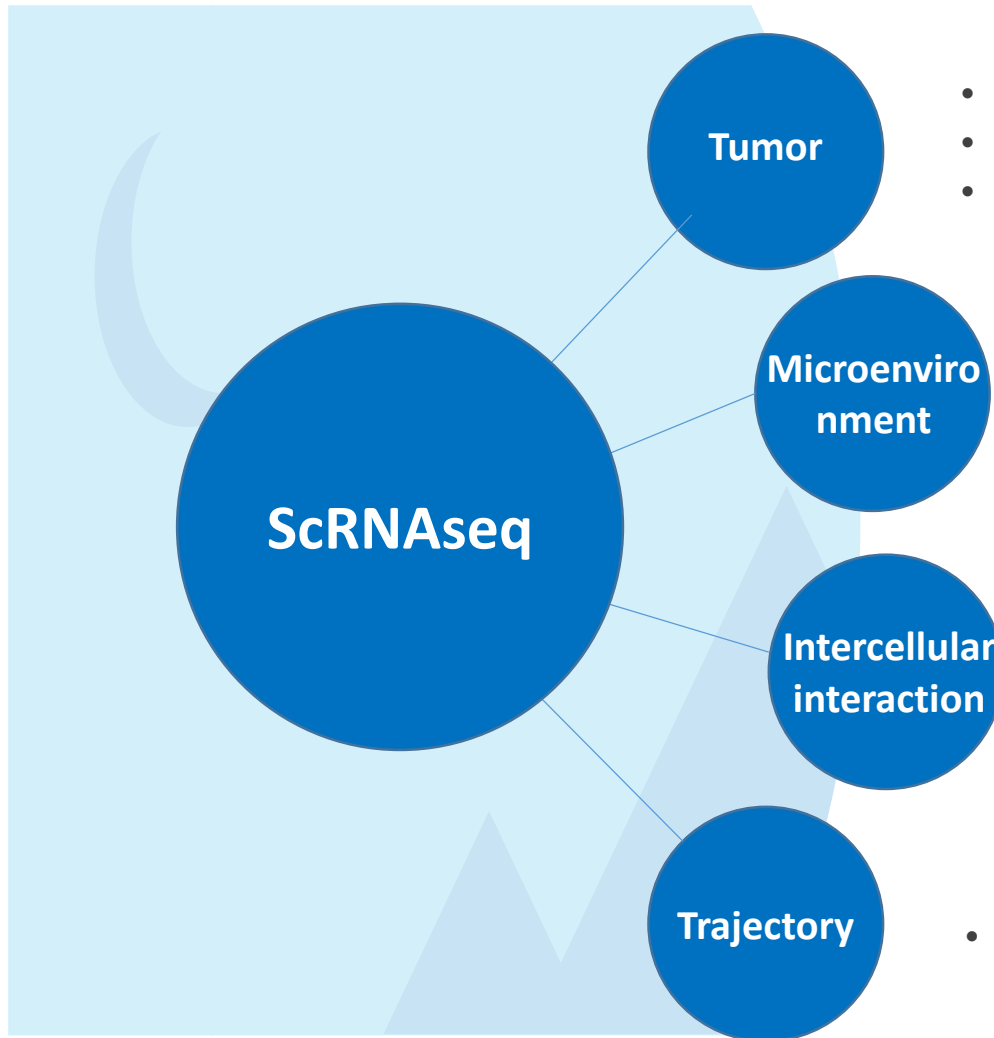


# Cell type annotation by multiple approaches

- 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



# Single-cell sequencing in immuno-oncology research

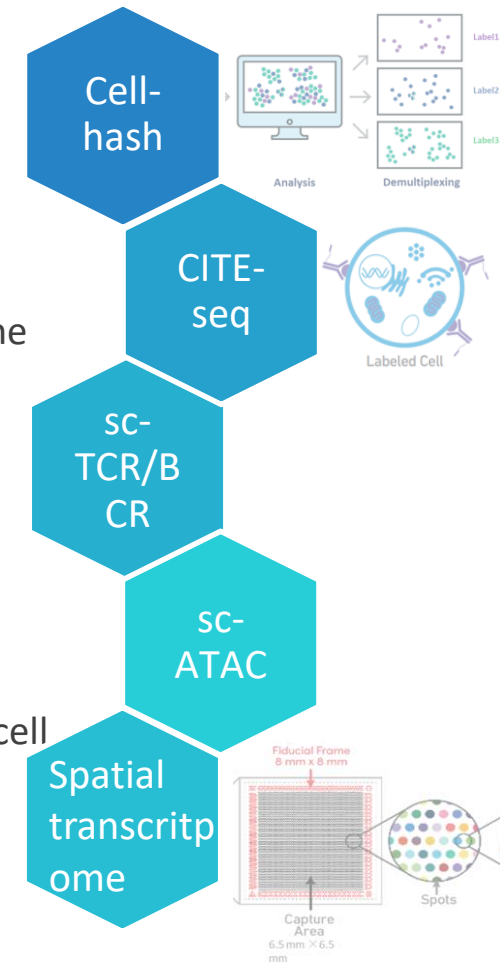


- Tumor cell heterogeneity
- MOA
- Development of drug resistance

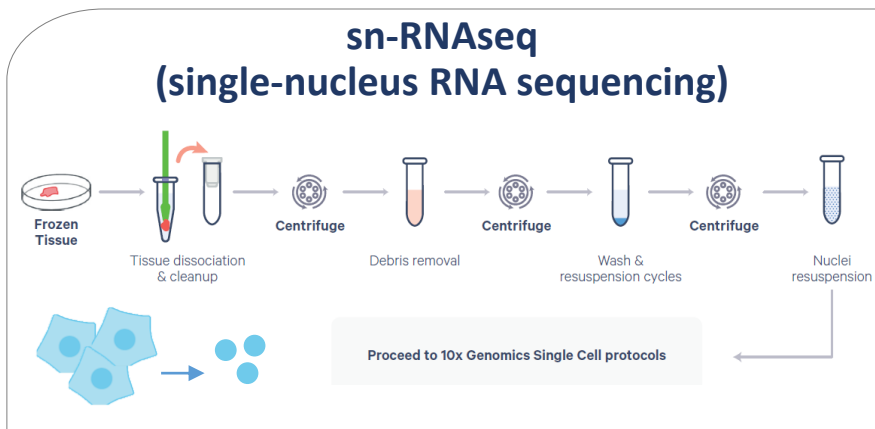
- Immunophenotyping
- Function changes of immune cell populations
- Changes in the position of immune cells

- Interacting groups of cells
- Interacting receptor ligand pairs
- Changes in the position of cell pairs

- Trajectory analysis

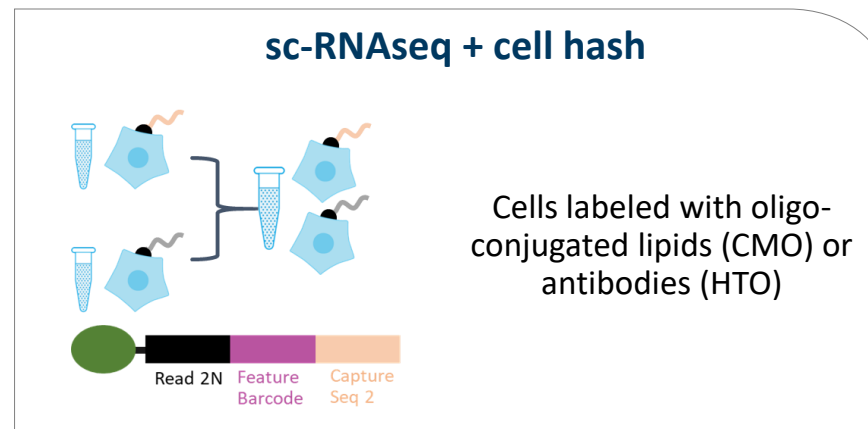


# Case study—Single cell multi-omics capability



- Freeze sample in time and preserve the biological state
- Minimize batch effects

- Simultaneously examine gene expression and protein abundance in the same single cell
- Accurately characterize cellular identity, states and function



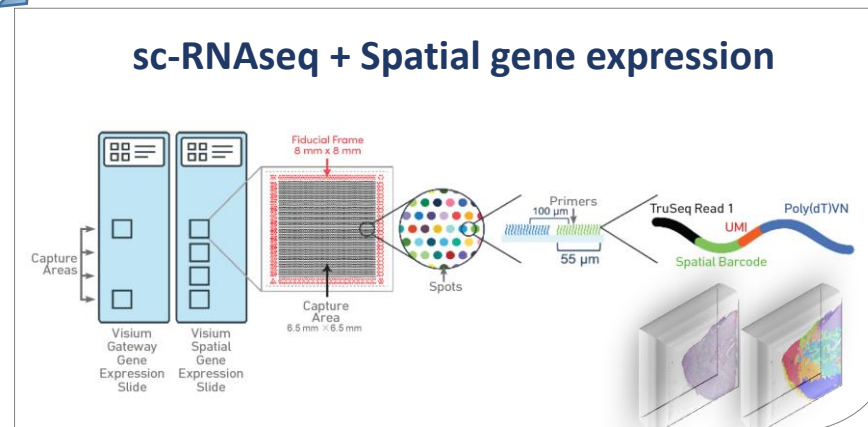
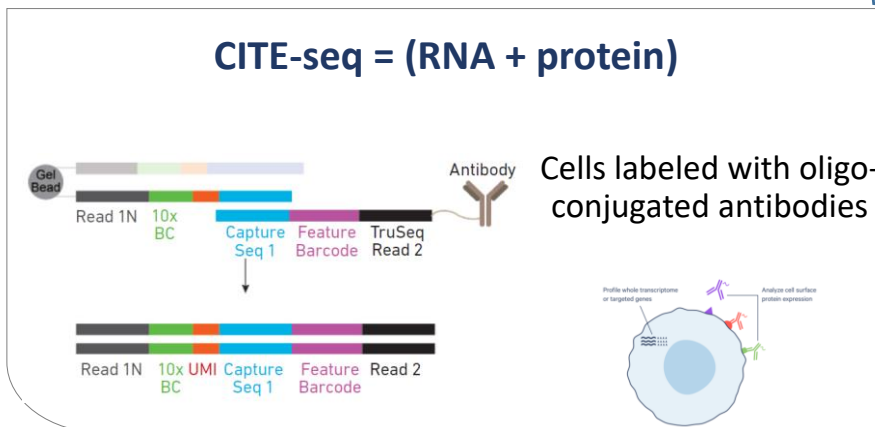
- Increase sample size and reduce batch effects
- Decrease study cost

- Deeply assess the spatial organization by integrating scRNA-seq and spatial RNA-seq

Compatibility

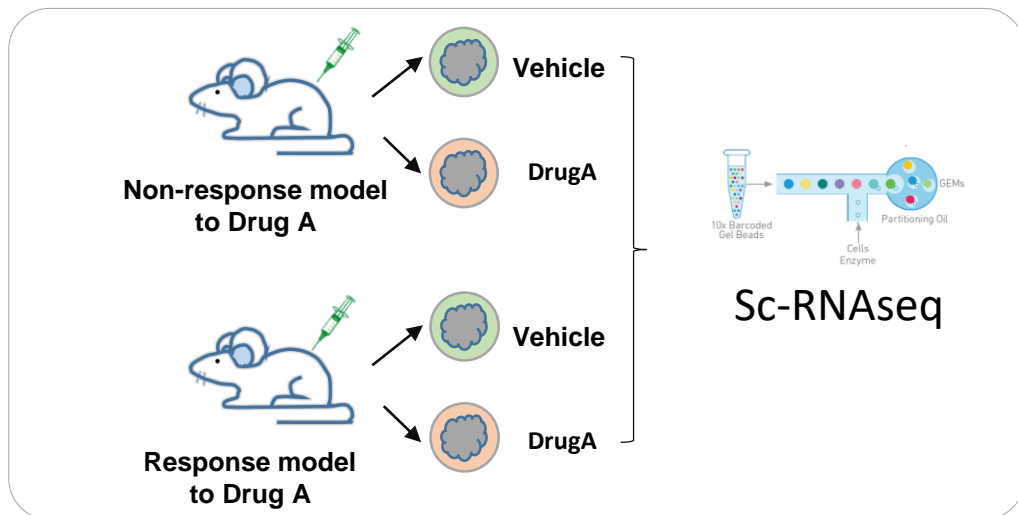
sc-RNAseq

Expansibility

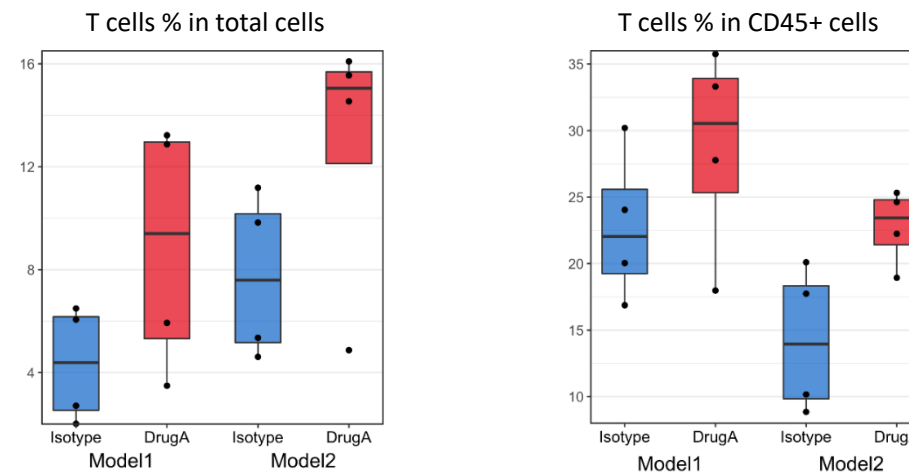




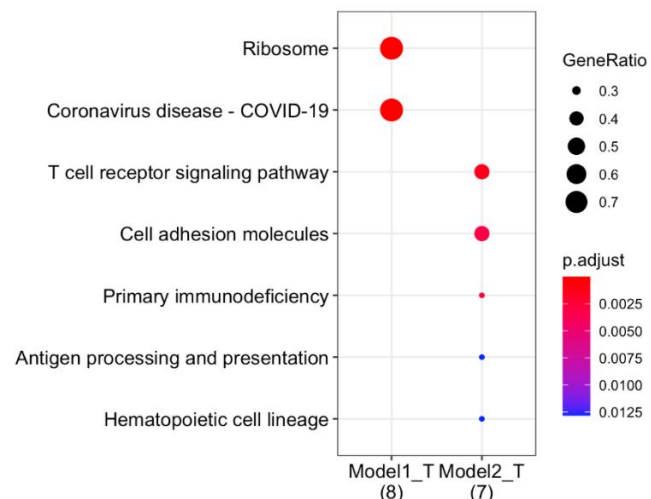
# Case study—Profiling the response to immune checkpoint inhibitors by scRNAseq



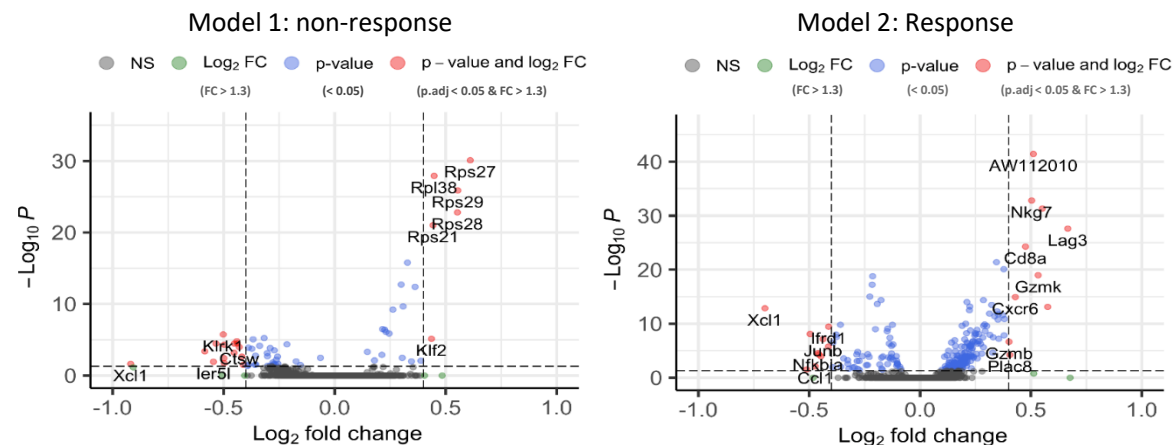
## Different changes of T cell abundance



## Pathway changes in different models

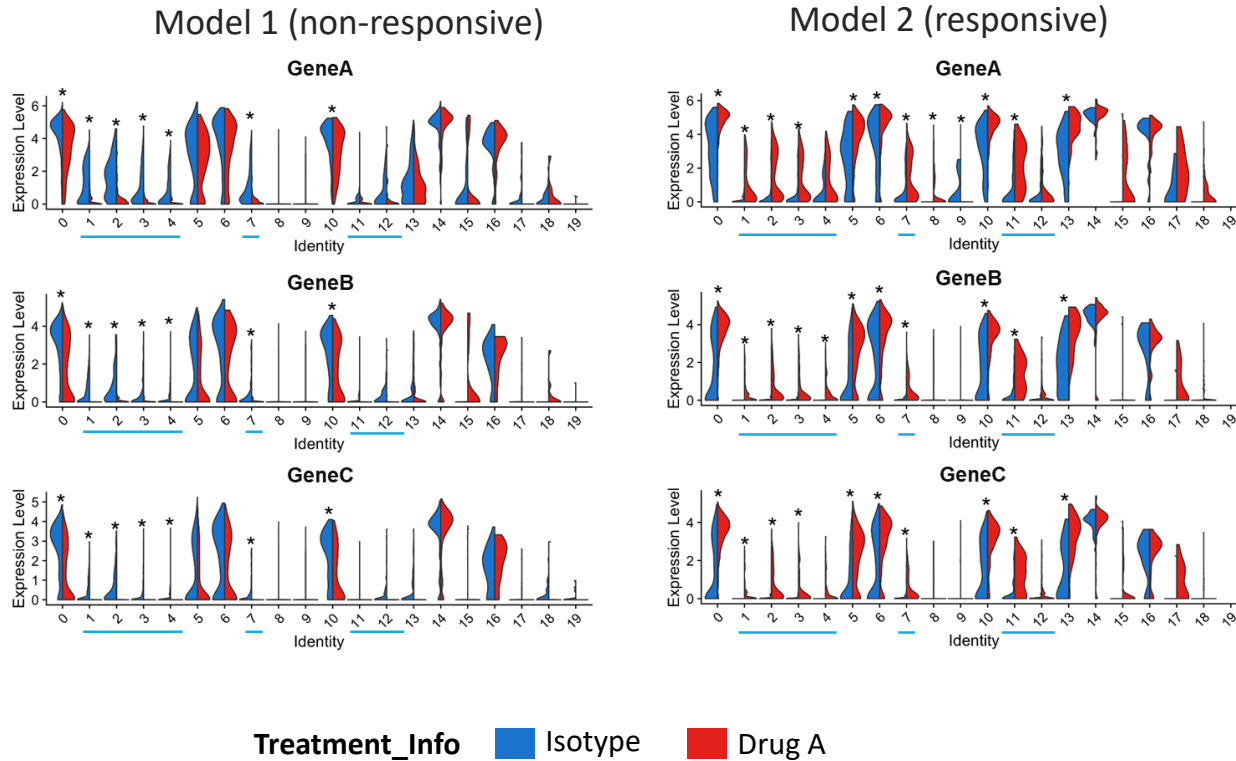


## Differential expression genes

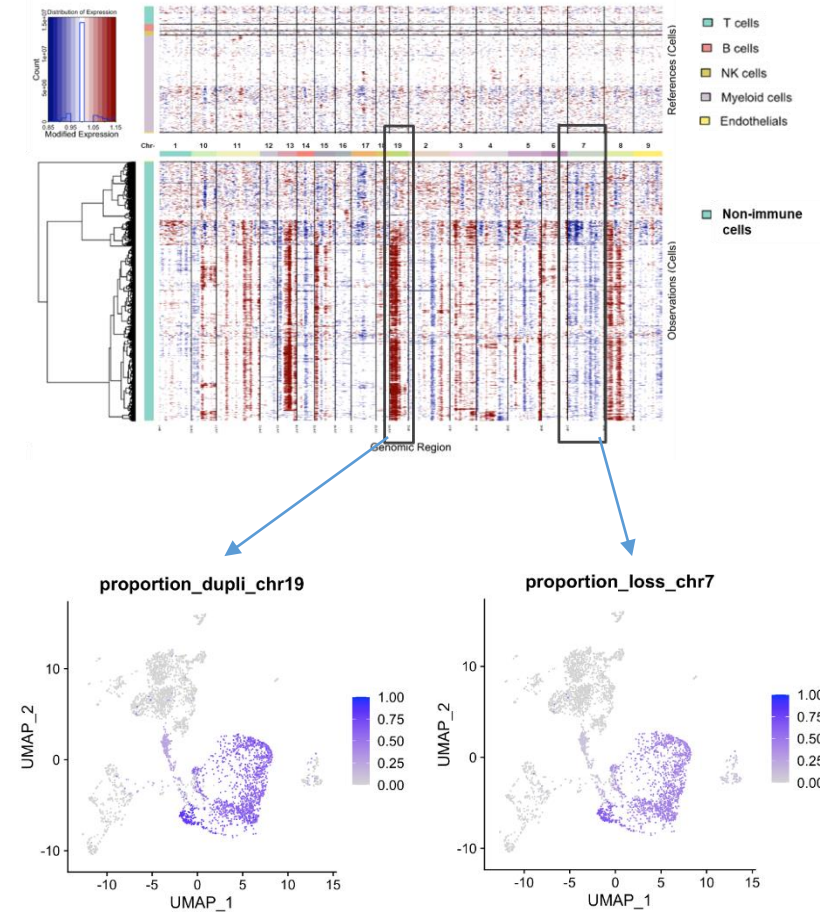


# Treatment-induced gene expression alternation in tumor cells

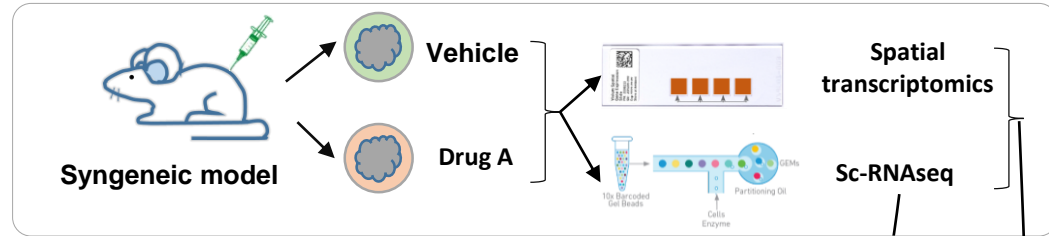
## Detecting the differential expression genes in tumor cells



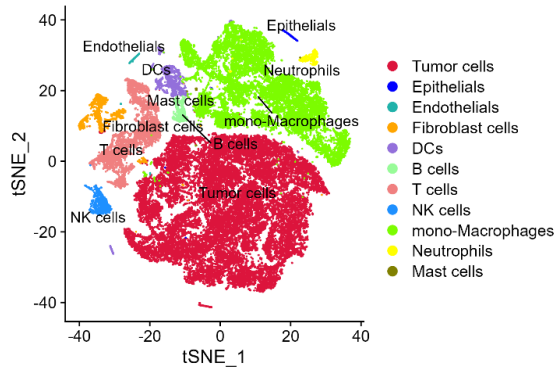
## Chromosomal copy number alteration analysis



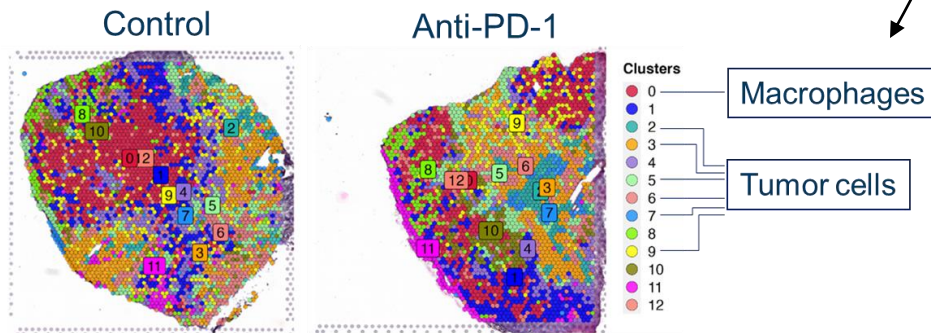
# Case study—Elucidating the crosstalk between TIL and tumor by integrating single-cell and spatial RNA-seq



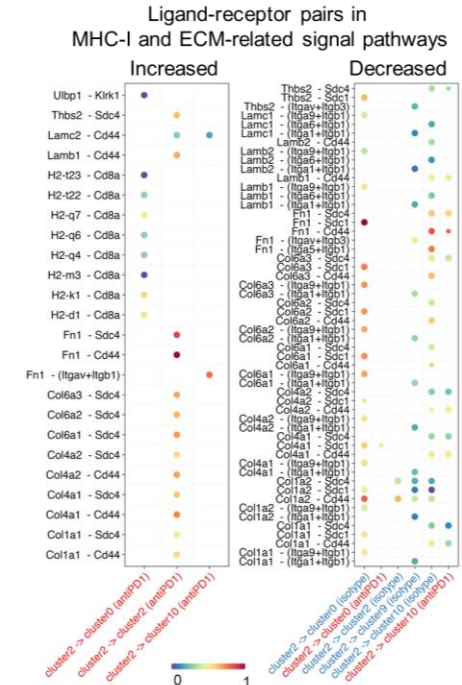
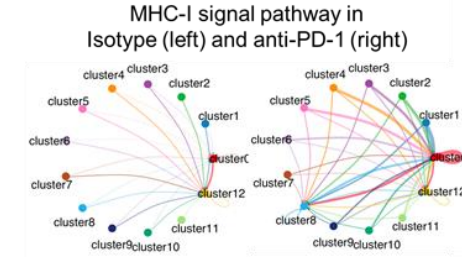
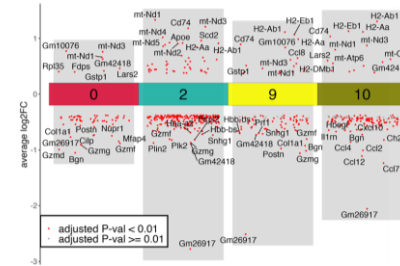
## Cell type identification using scRNA-seq data



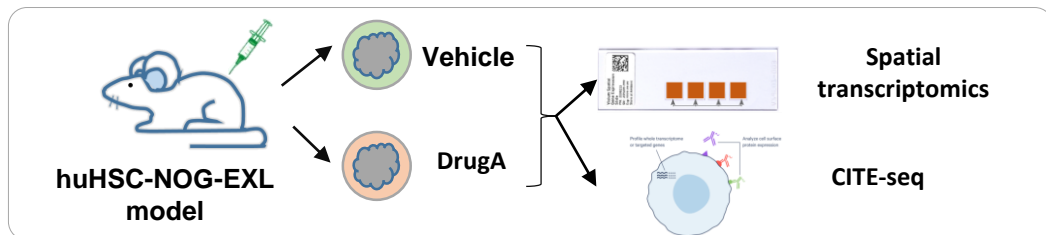
## Spatial distribution of cell types by integrating scRNA-seq and spatial RNA-seq



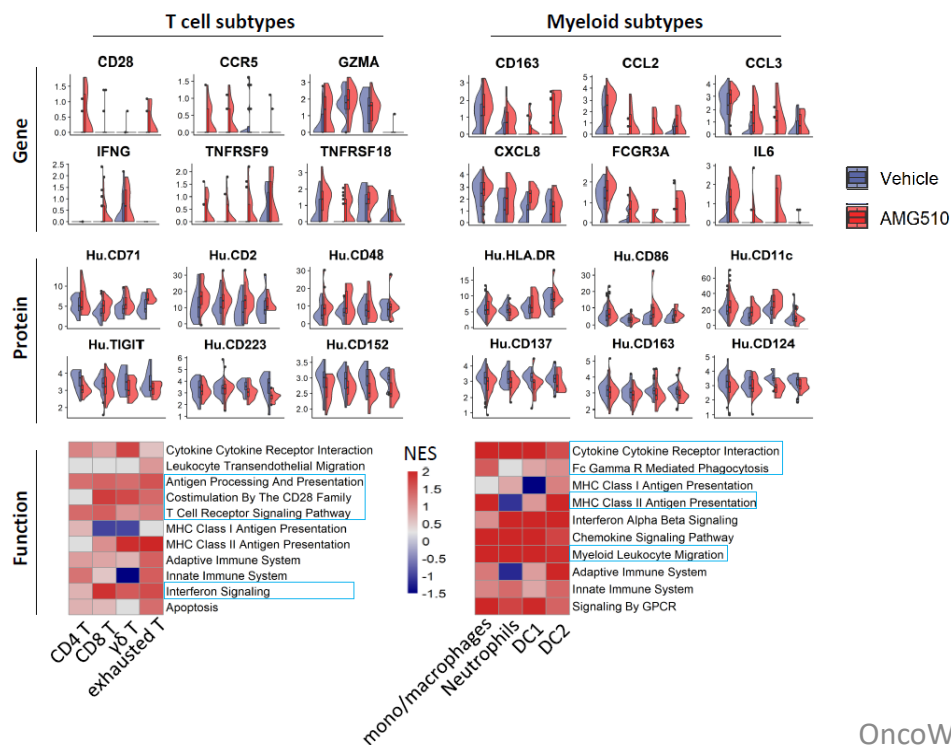
## Differential expression gene analysis and cell-cell communication analysis



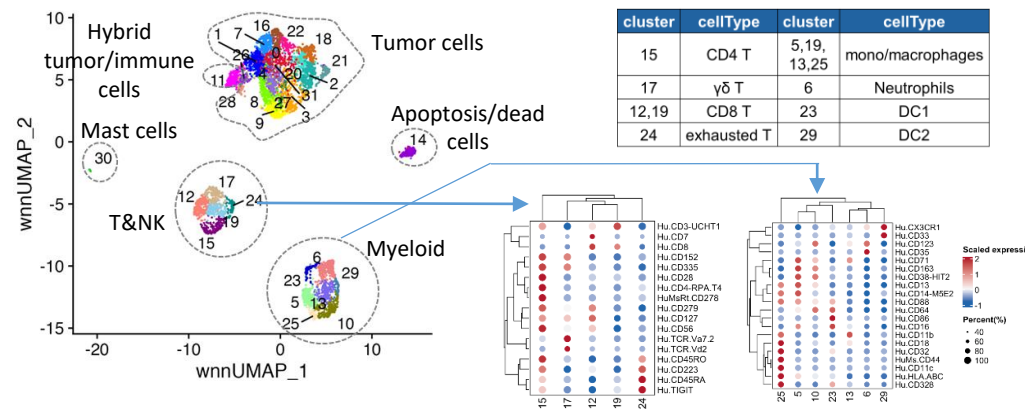
# Case study—Deeply reveal the pharmacodynamic mechanism by single cell multi-omics



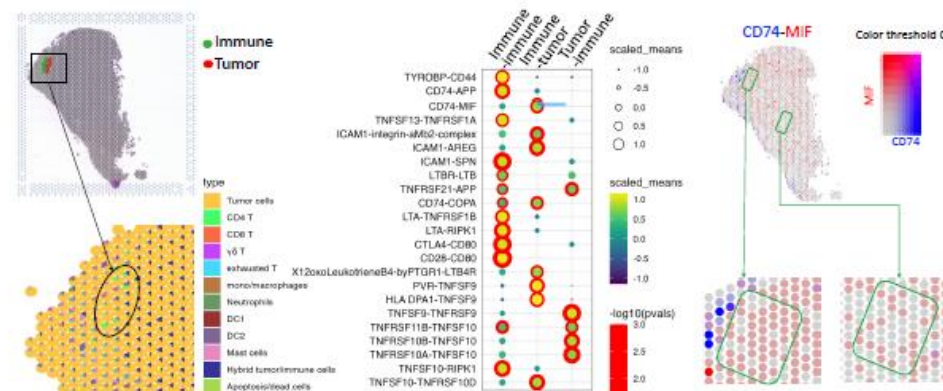
## Exploring TME by differential gene/protein expression analysis and GSEA functional analysis



## Cell type identification using CITE-seq data



## Co-localization of infiltrated immune cells and tumor cells by spatial transcriptomics







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## *Improving Health. Making a Difference.*

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