

Integration of single-cell transcriptome in retinoblastoma

Jing Liu

PhD student

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Variations in single-cell transcriptome

❑ Technical variations (batch effects) :

- Technology platforms (Smart-seq, 10X Chromium)
- Labs (equipments, handling personnel, reagents)
- Sequencing (depth, read length, lanes, flow cells)
- Sampling time

❑ Biological variations:

- Species
- Tissues
- Spatial locations
- Time points
- Inter-individual variations



**How to remove batch effects
while preserving biological
variations?**

Batch-effect correction in single-cell transcriptome

❑ 97 Integration methods (2020.09.24)



- Limma/Combat
- MNN
- Dimention Reduction (Seurat 2 CCA, LIGER NMF)
- Dimention Reduction + MNN (Seurat 3 CCA + MNN, HARMONY PCA + MNN)
- Neural network (scVI, scGen)

❑ Benchmarking studies

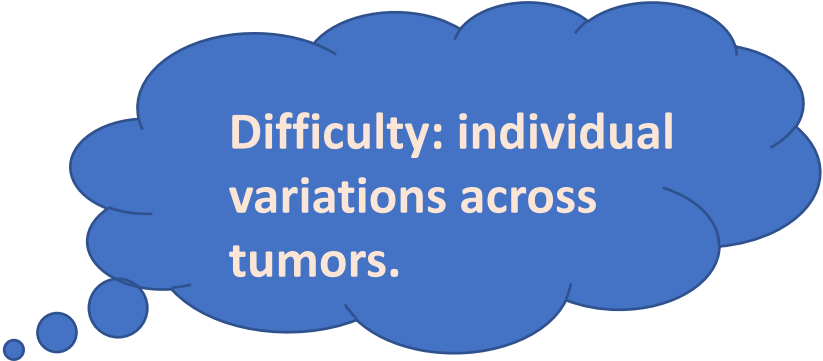
Büttner, Miao et al, 2019 Nature Methods (7 methods)

Tran, Ang, Chevrier, Zhang et al, 2020 Genome Biology (14 methods)

Luecken et al, 2020 bioRxiv (10 methods)

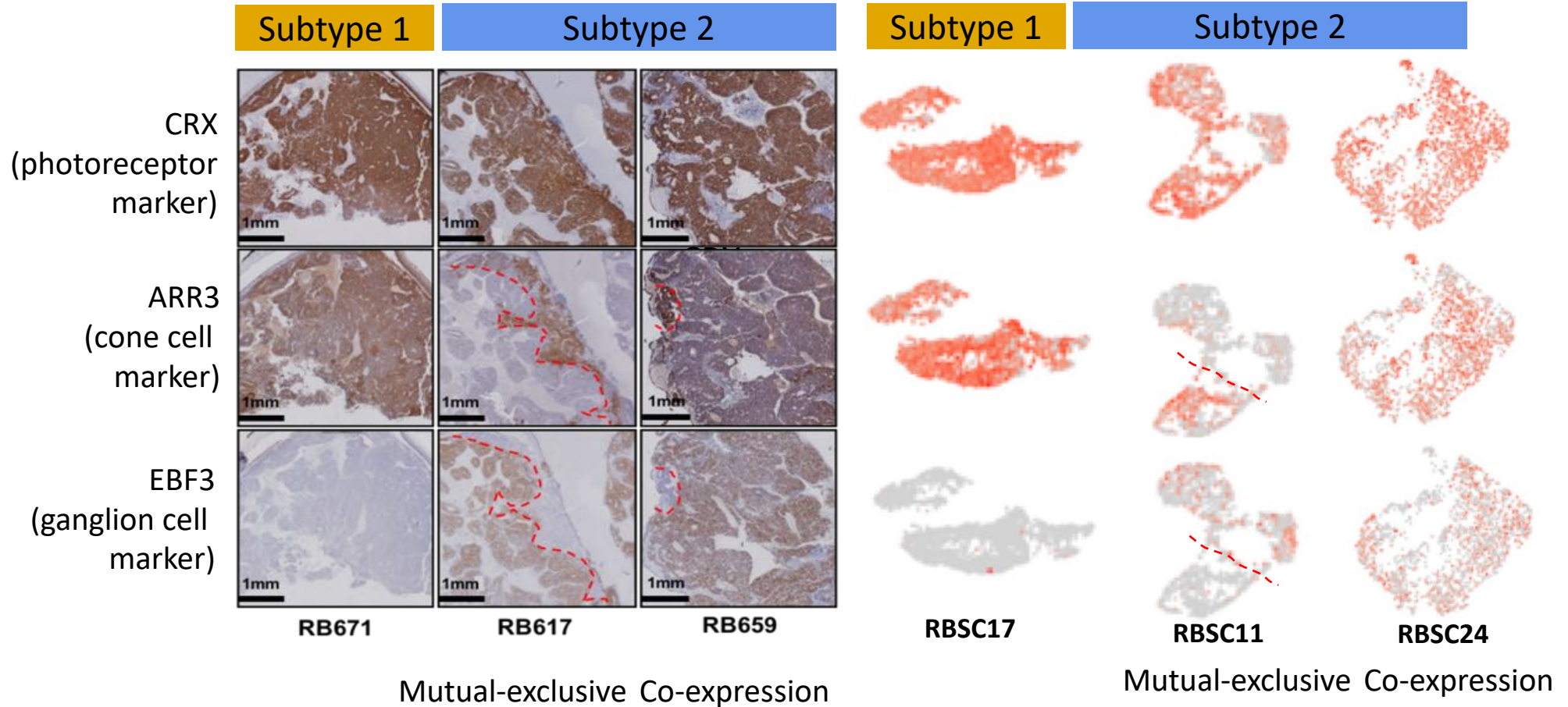
- Different scenarios:
 - Different technology
 - Different labs
 - Species
 - Tissues
 - Individual variations
 - Unbalanced batches

- Evaluation:
 - Removal of batch effects
 - Conservation of biological variances

A blue thought bubble with a white outline, containing the text 'Difficulty: individual variations across tumors.' in white, bold, sans-serif font. The bubble has three smaller circles leading to it from the bottom left.

**Difficulty: individual
variations across
tumors.**

scRNA-seq captured retinoblastoma heterogeneity



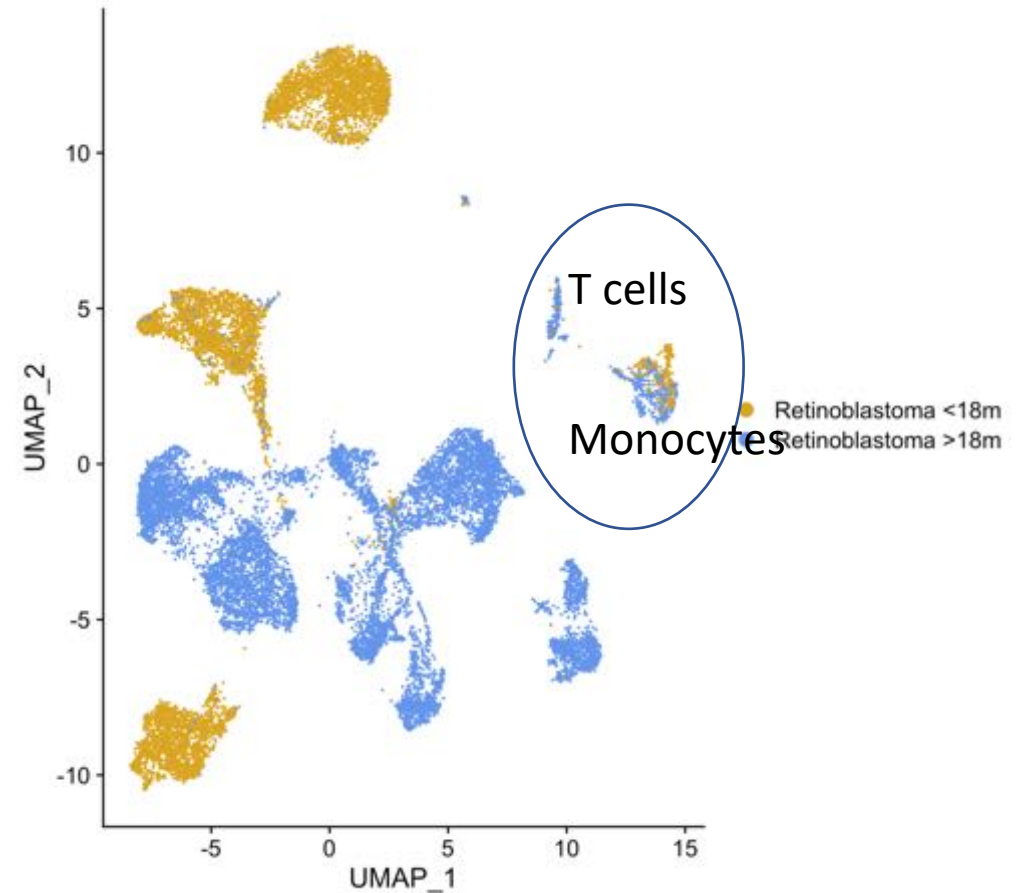
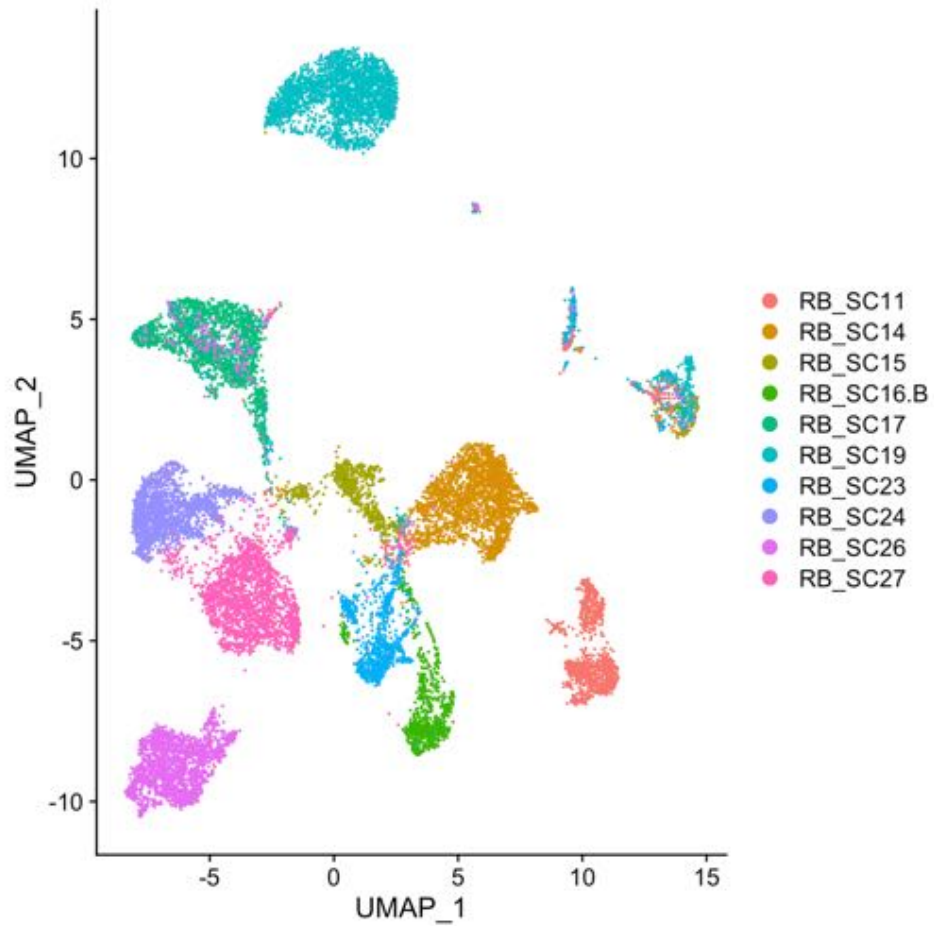
Question: Comparisons between ARR3+ cells in Subtype 1 vs ARR3+ cells in Subtype 2
EBF3+ARR3- cells vs EBF3+ARR3+ cells in subtype 2

An example of integration of 10 retinoblastoma samples

Integration method	Gene selection	Cell selection
No batch effect correction	Most variable 2000 genes	All cells/Tumor cells
Seurat 3 (CCA + MNN)	Most variable 2000 genes	All cells/Tumor cells
	Differentially expressed genes between Subtype 1 and Subtype 2 retinoblastoma	Tumor cells
	177 retinal markers	Tumor cells
Harmony (PCA + MNN)	Most variable 2000 genes / 3000 genes	Tumor cells

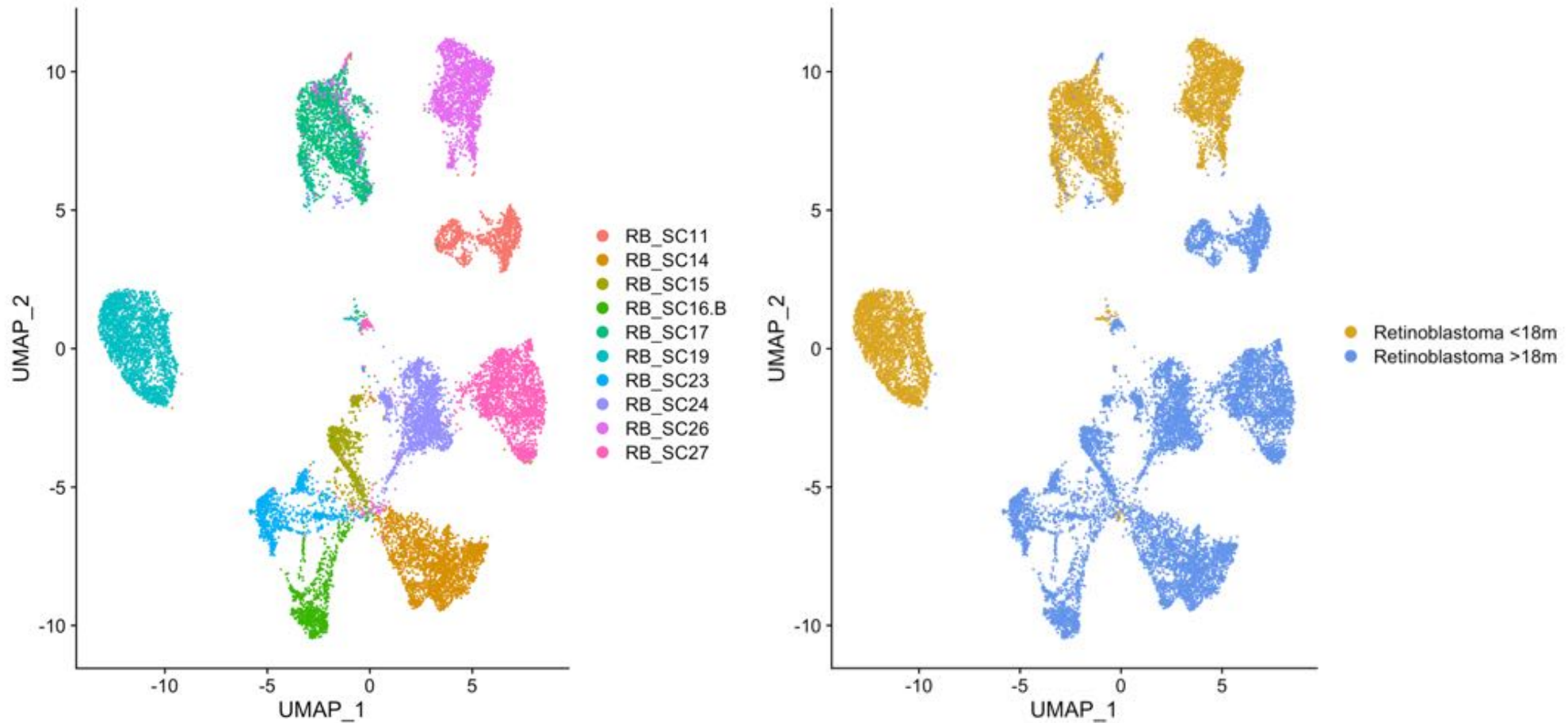
An example of integration of 10 retinoblastoma samples

Merge without batch effect correction



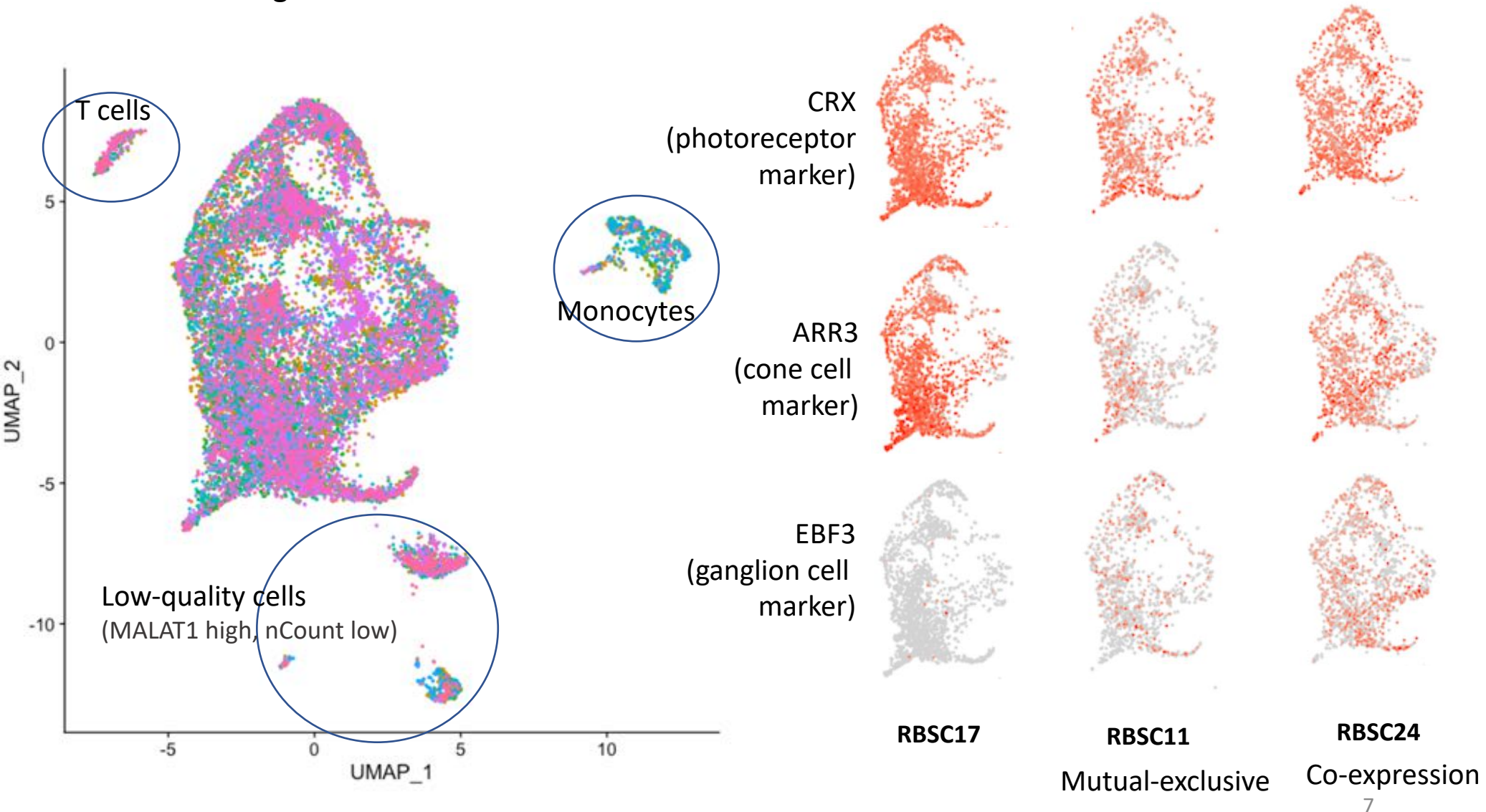
An example of integration of 10 retinoblastoma samples

Merge, removing immune cells, without batch effect correction



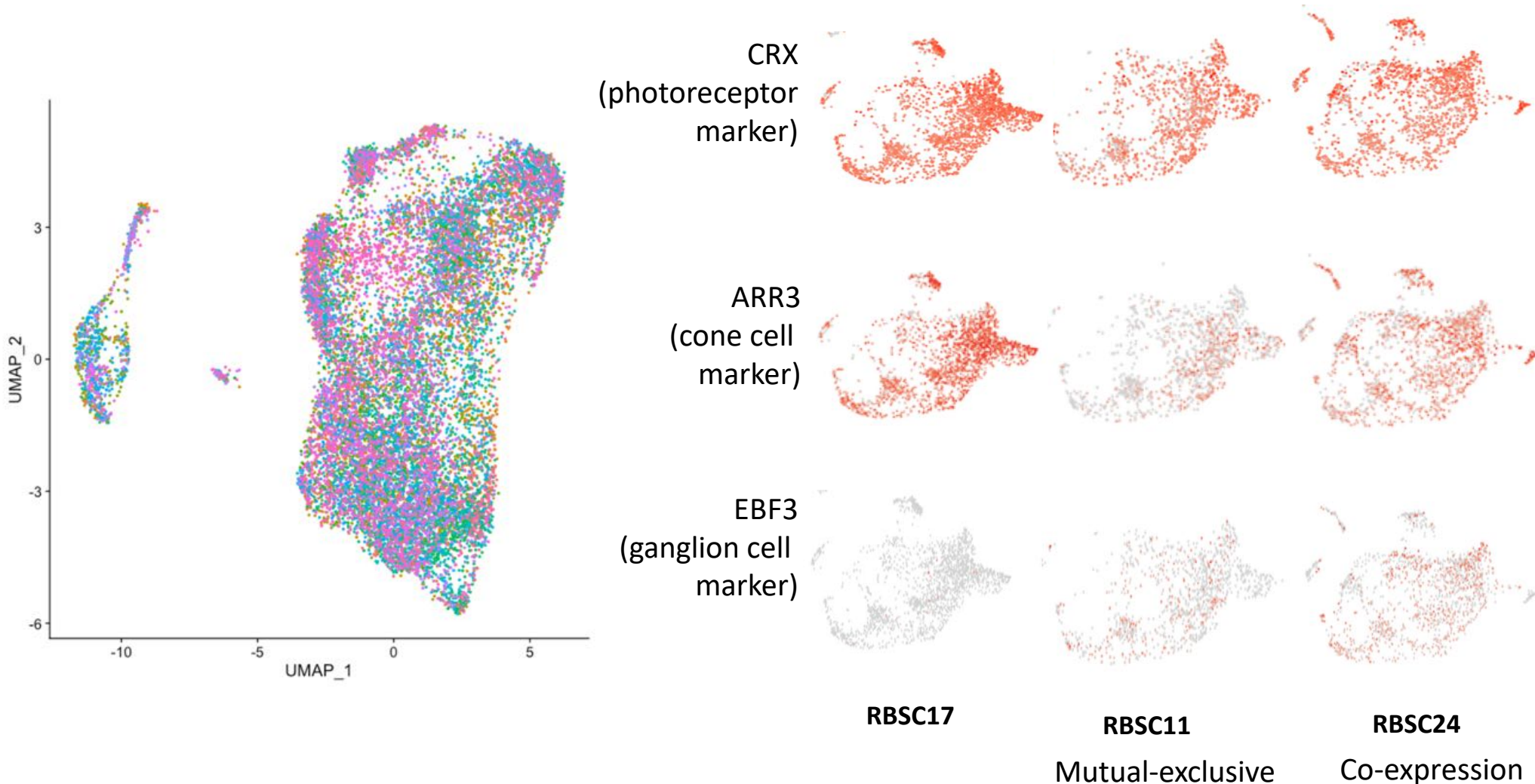
An example of integration of 10 retinoblastoma samples

Seurat3 integration



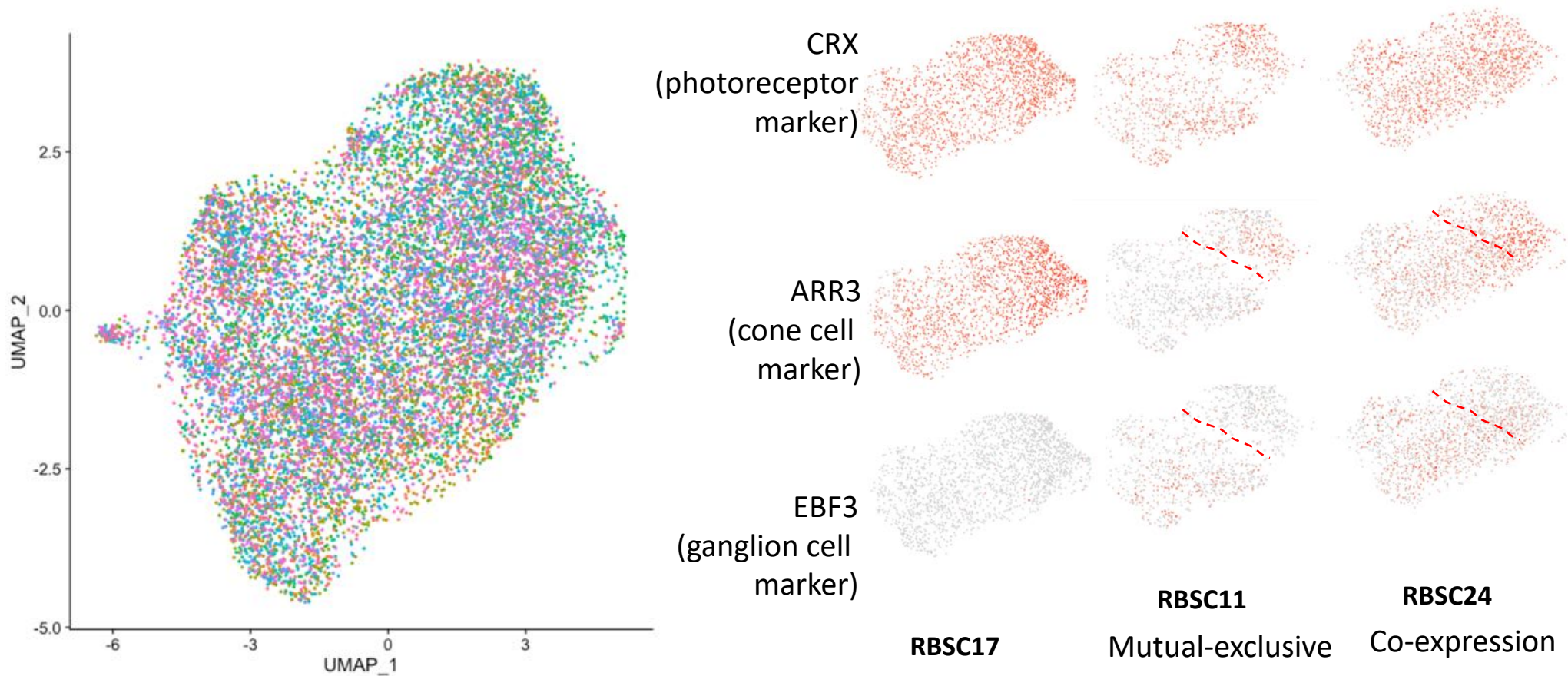
An example of integration of 10 retinoblastoma samples

Seurat3 integration, differential expressed genes between subtype 1 and subtype 2, removing immune/low-quality cells



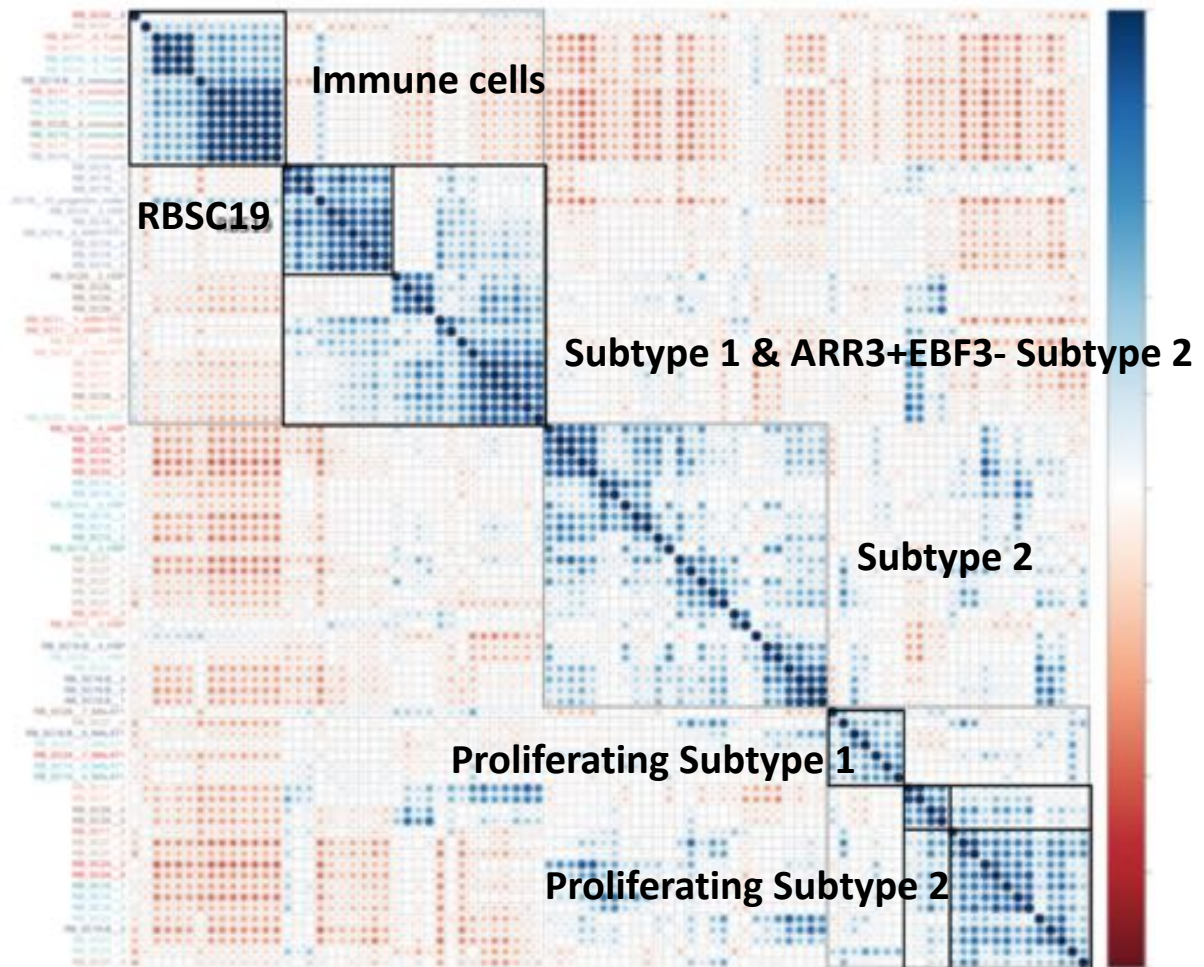
An example of integration of 10 retinoblastoma samples

Seurat3 integration, using 177 retinal cell markers, removing immune /low-quality cells



Centroid correlation of 10 retinoblastoma samples

Centroid correlation using 1084 diff genes ($> 1.5\text{FC}$), logNorm expression, Row-centered



Discussion

- Single-cell transcriptome captured cone differentiation heterogeneity in retinoblastoma in individual-sample analysis.
- Integration of single-cell transcriptome in different retinoblastoma samples without batch-effect correction identified various immune cells and separate subtype 1 tumors from subtype 2 tumors.
- Integration with batch-effect correction fails to separate Subtype 1 from Subtype 2 tumors.
- Centroid correlation of cell clusters in different retinoblastoma samples separate subtype 1 from subtype 2 retinoblastoma and showed higher similarity of subtype 2 ARR3+ cells to subtype 1 tumors
- Solutions:
 - No batch-effect correction + differential expression analysis + NMF / ICA to study common signaling programs in different samples
 - Other batch-effect correction methods