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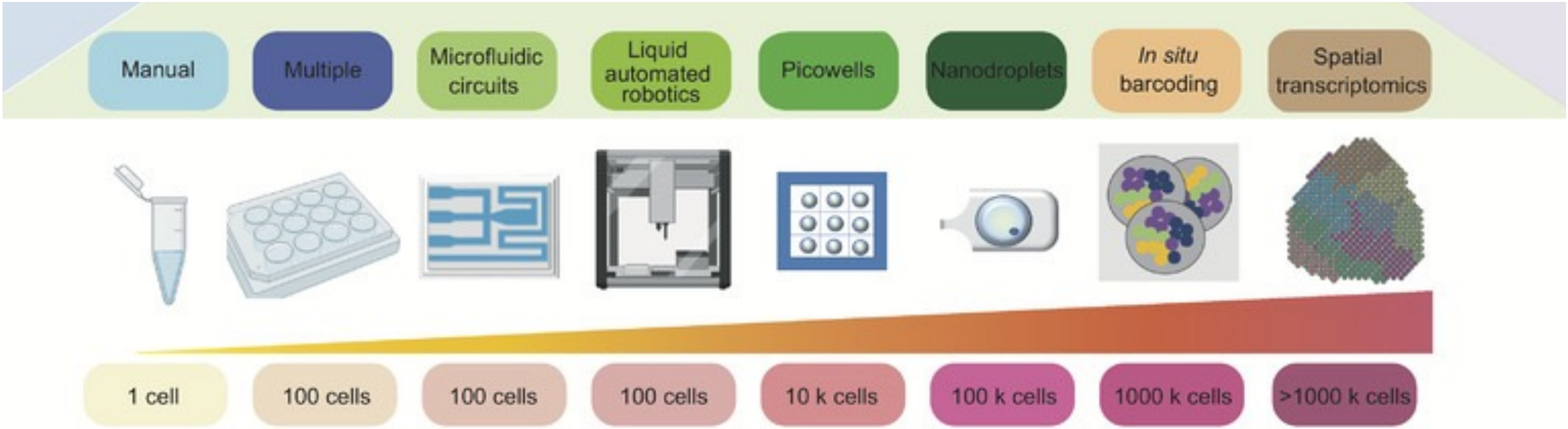
Enhancing Single-Cell Data and Cell Recovery Rates for Complex Tissue Samples

Agne Antanaviciute, PhD

14th April 2023

MRC Weatherall Institute of Molecular Medicine

Single-Cell RNA Sequencing



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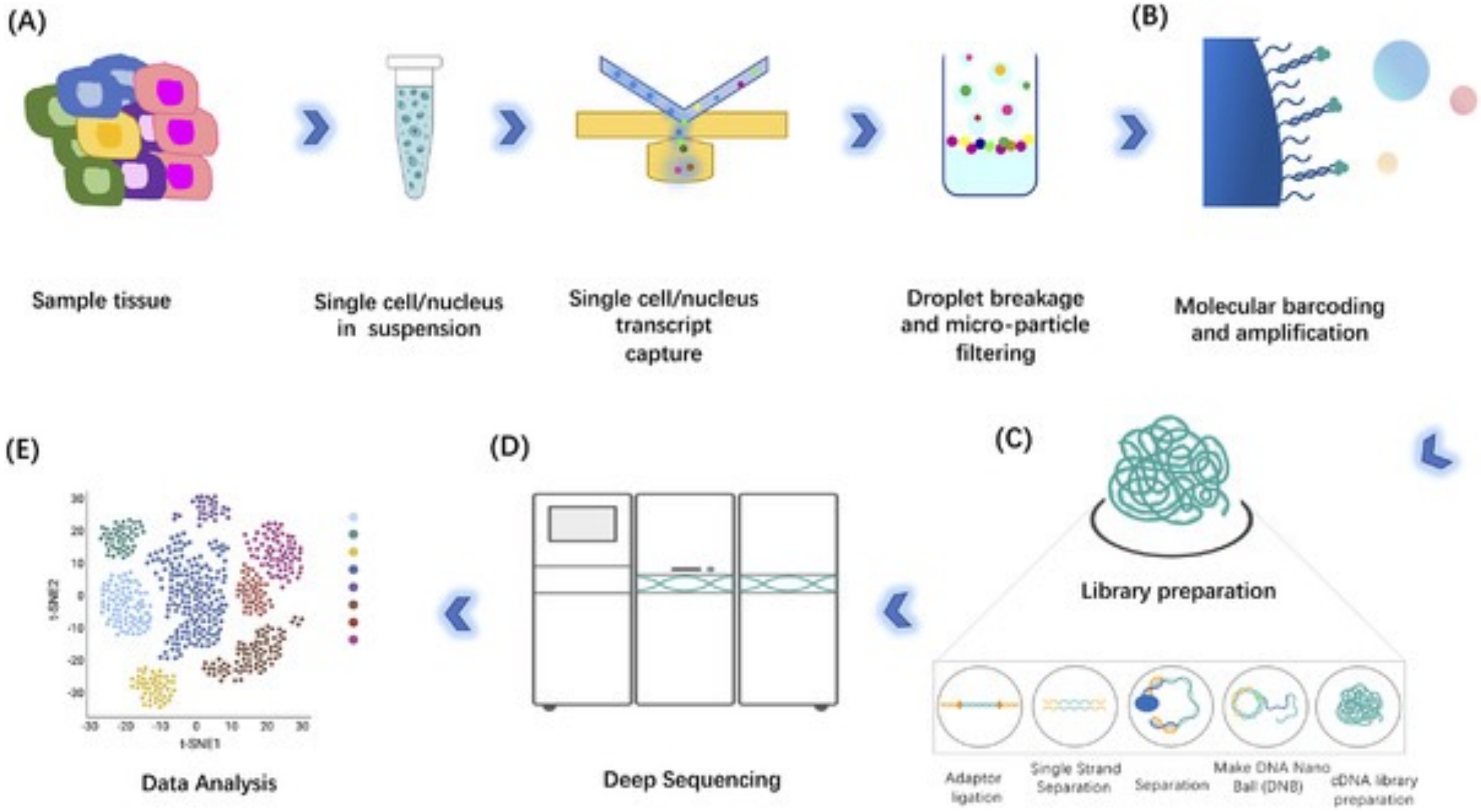
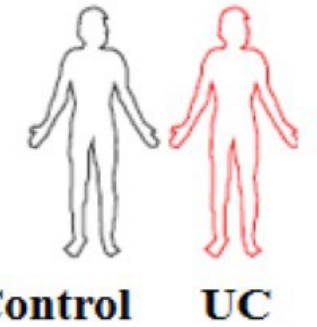
Jovic *et al*, 2022

Single-Cell RNA Sequencing

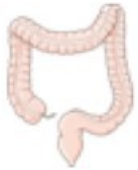


Jovic *et al*, 2022

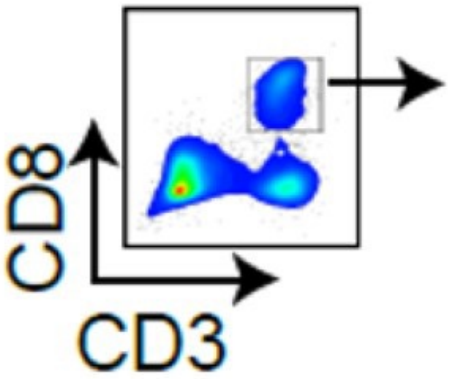
Multi-modal Single-Cell RNA Sequencing of CD8+ T-cells



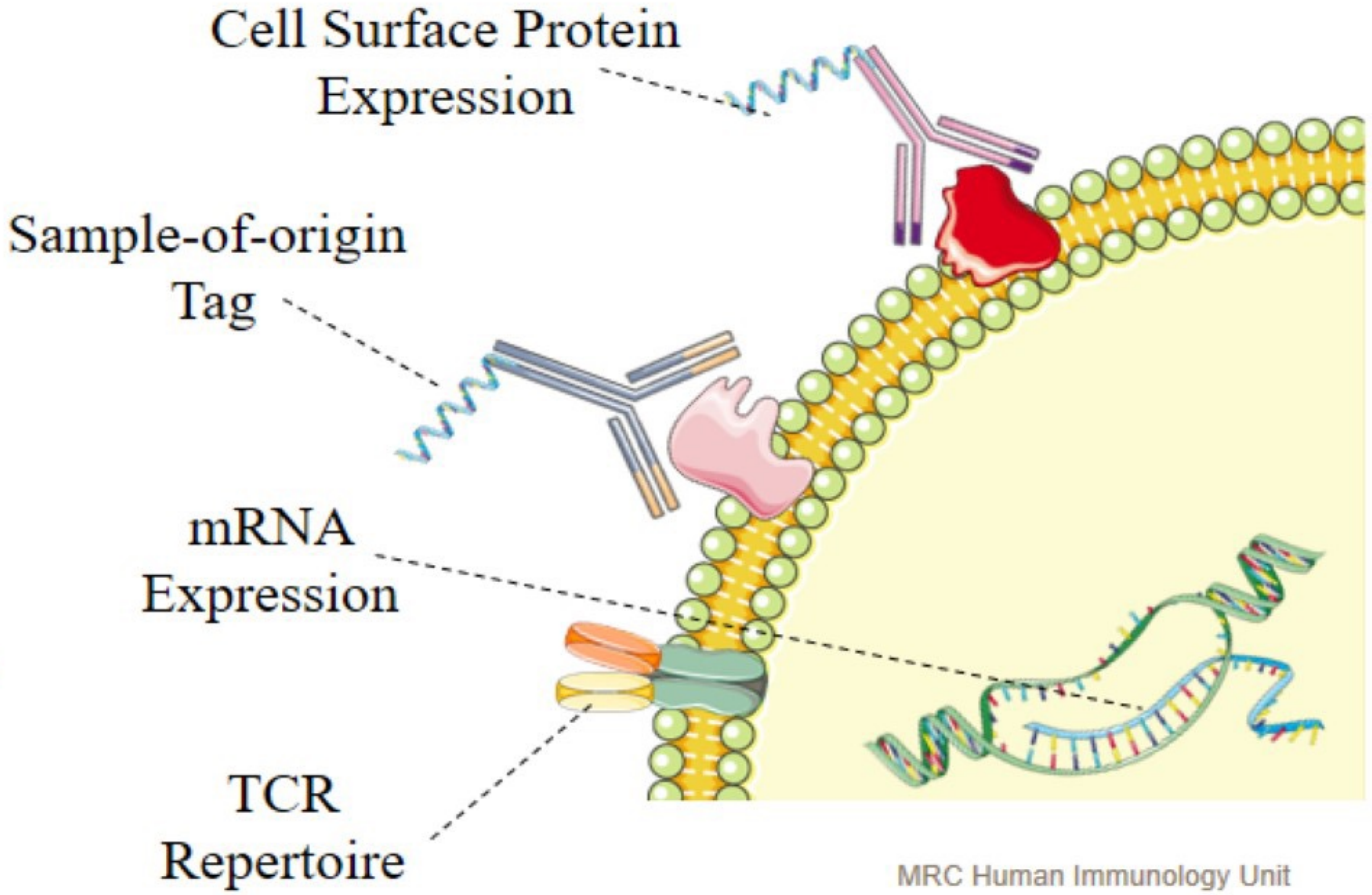
Control UC



Colon Biopsies



5' scRNA-Seq
+
VDJ Sequencing
+
Feature Barcoding
+
Cell Hashing



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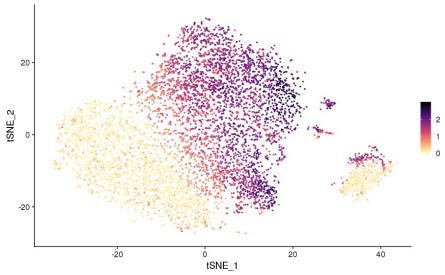
*Corridoni, Antanaviciute & Gupta *et al*, 2020

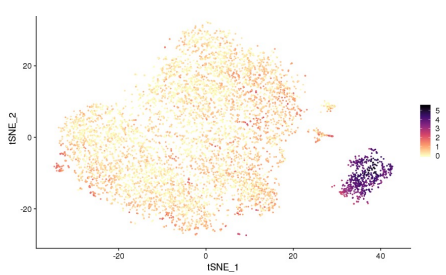
Harsh cell isolation protocols impact single-cell recovery and RNA quality

tSNE embedding of 14 Antibody CITE-seq panel

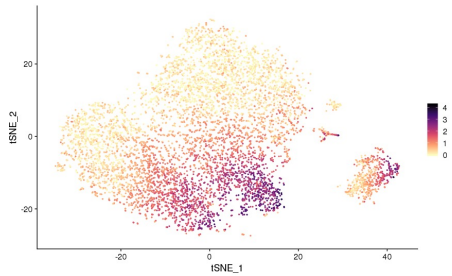
CD103



CD4



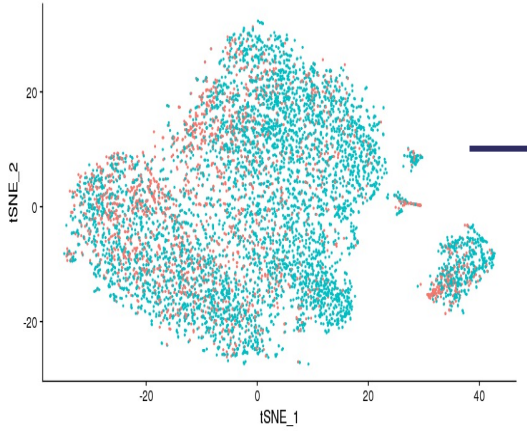
PD1



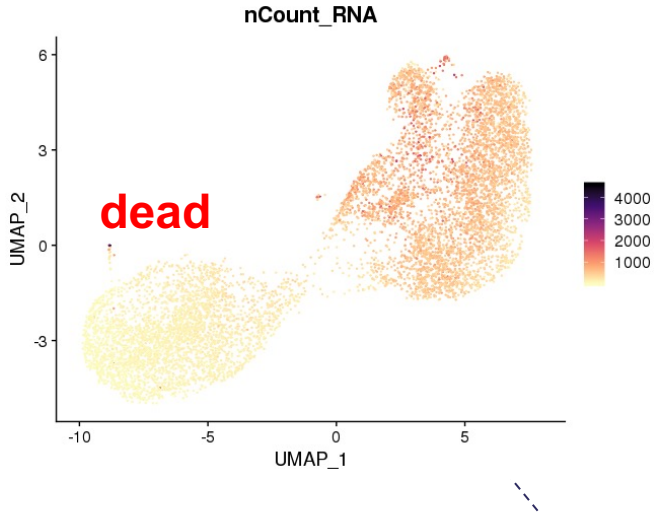
Sample 1 – 34% low quality by RNA
Sample 2 – 25% low quality by RNA
Sample 3 – 23% low quality by RNA
Sample 4 – 36% low quality by RNA

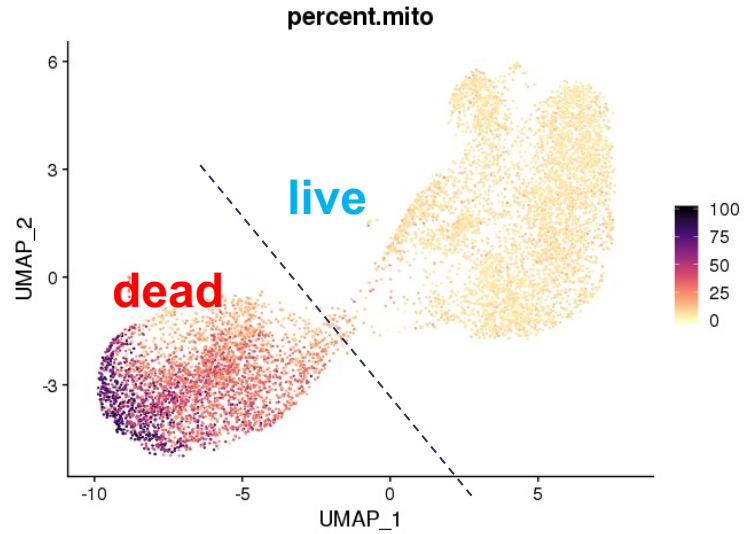
Protein-based embedding

live-dead by mRNA



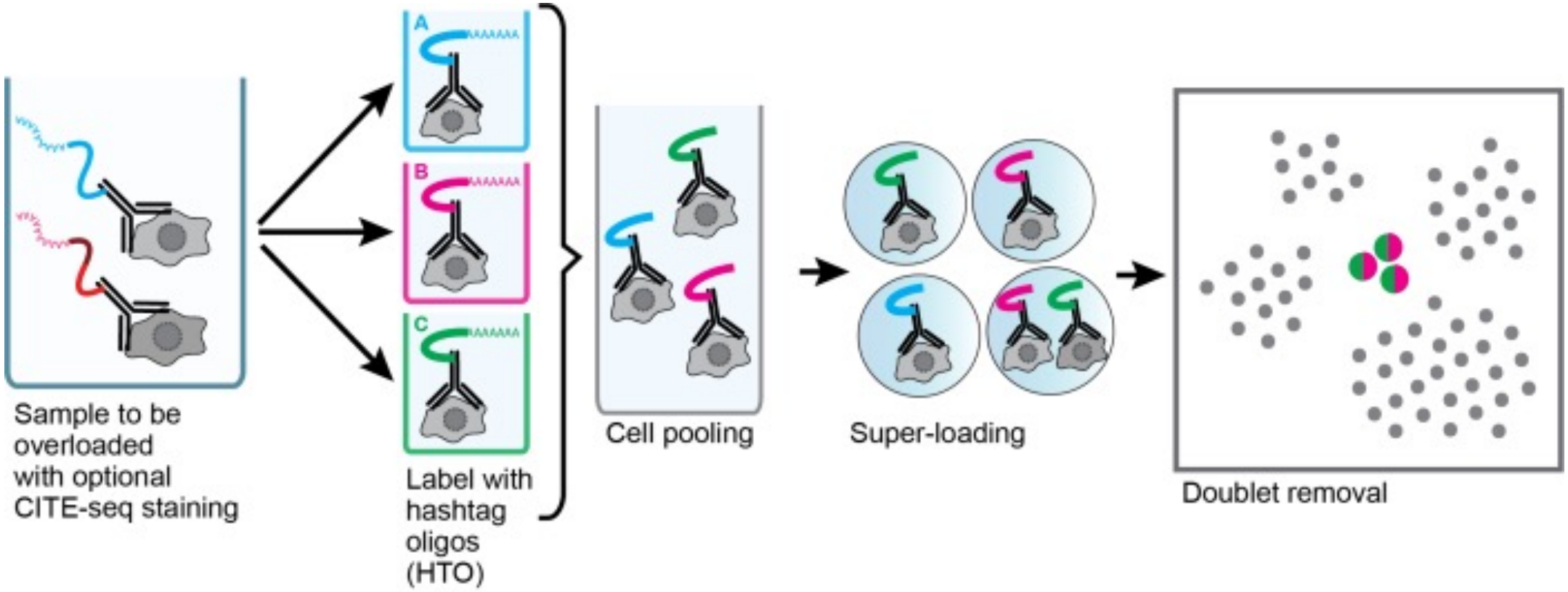
mRNA-based embedding





*Data from Corridoni, Antanaviciute & Gupta *et al*, 2020

Sample Multiplexing for Single Cell RNA Sequencing



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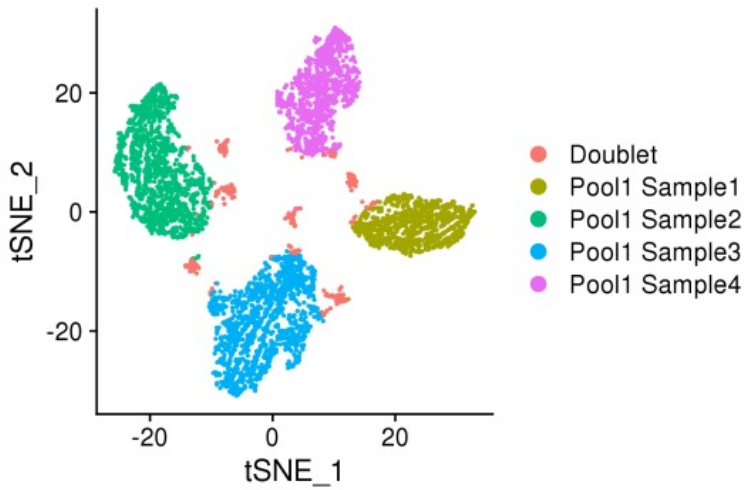
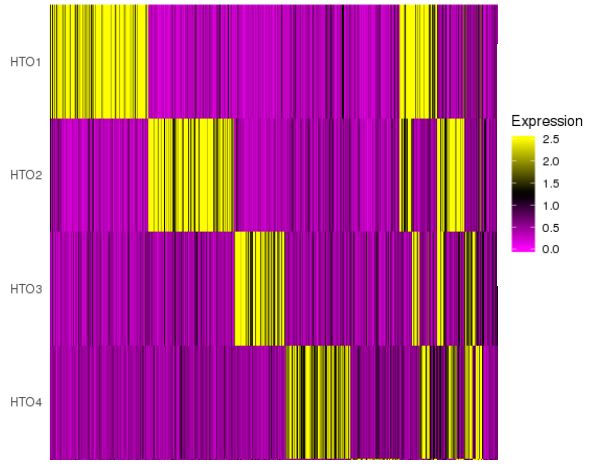
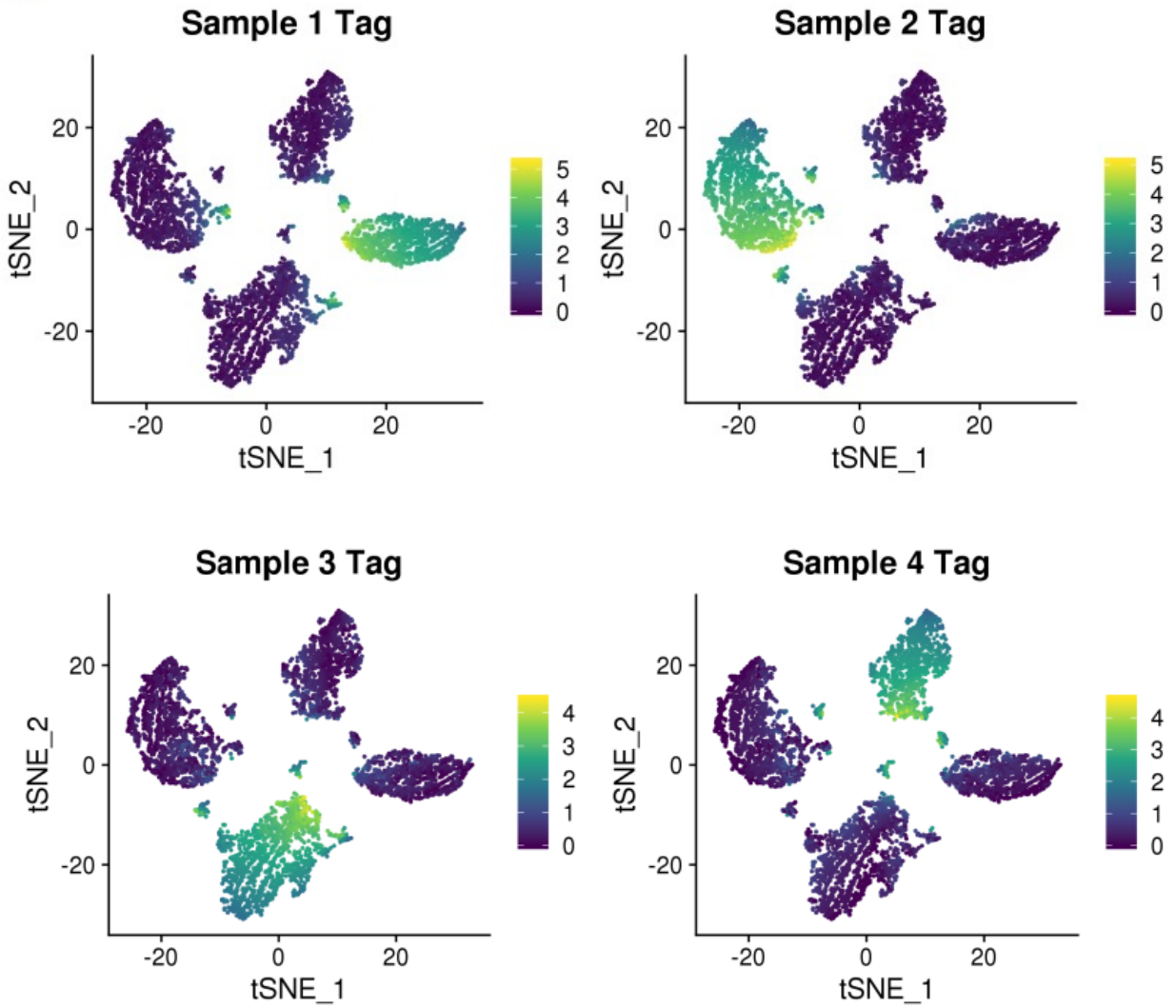
From Stoeckius *et al*, 2018

Sample Multiplexing for Single-Cell RNA Sequencing



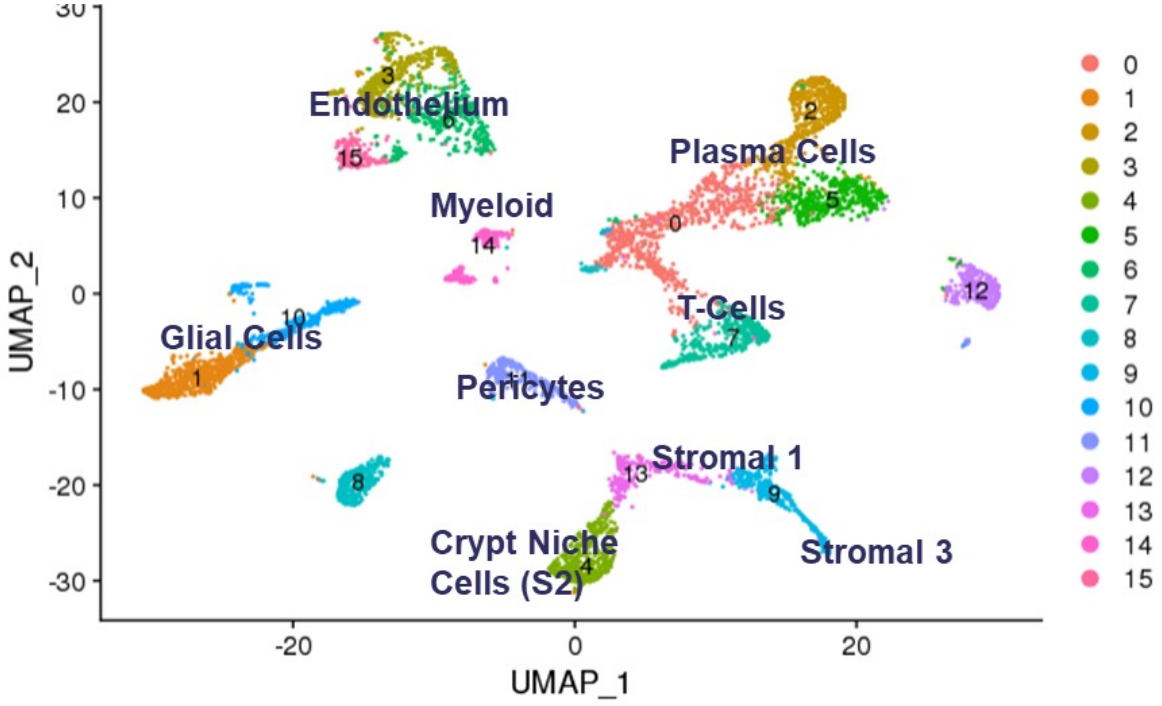




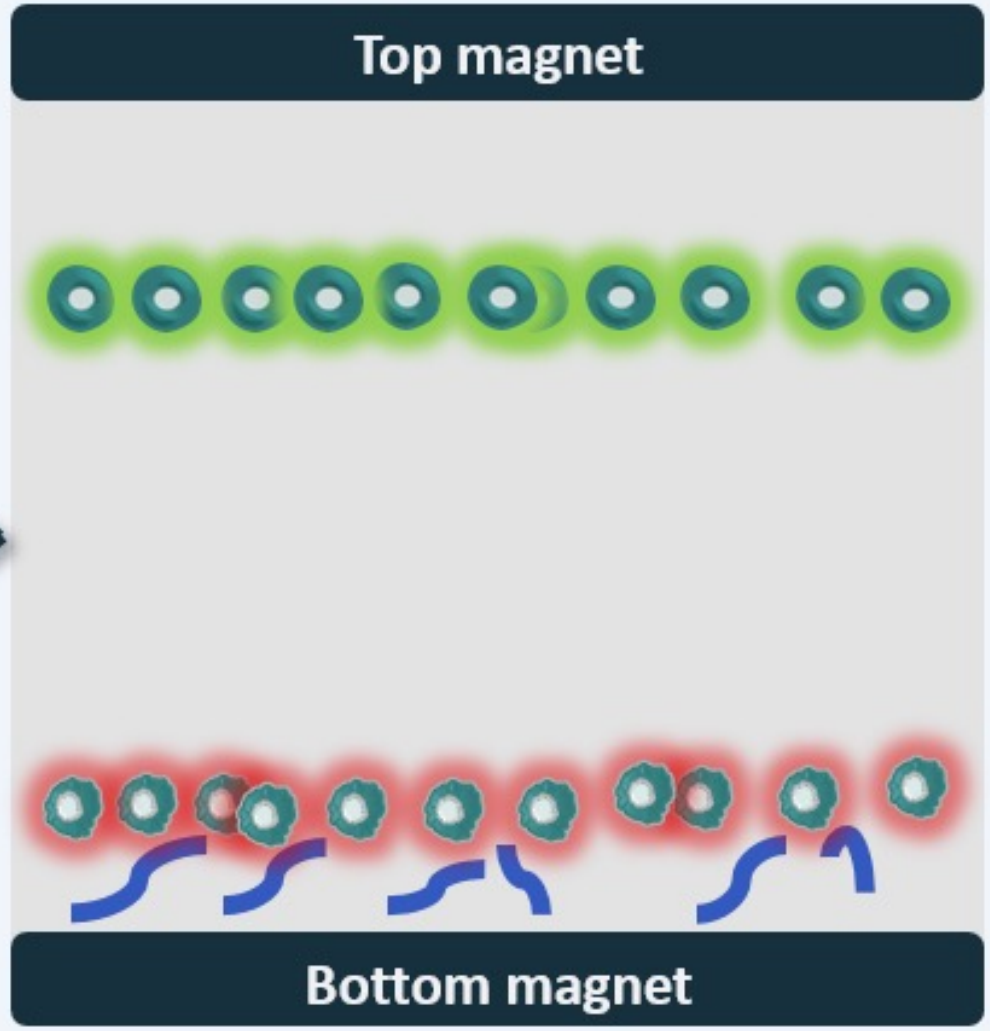
*Corridoni, Antanaviciute & Gupta *et al*, 2020

Sample Multiplexing for Single-Cell RNA Sequencing

While FACS isolation often resulted in lower-than-expected cell yields, we found that alternative, gentler protocols, such as bead isolation, did not seem to be compatible with antibody-based sample multiplexing:



LeviCell™ System for Cell Isolation



Live Cells

Intact membrane

Equilibrium levitation height

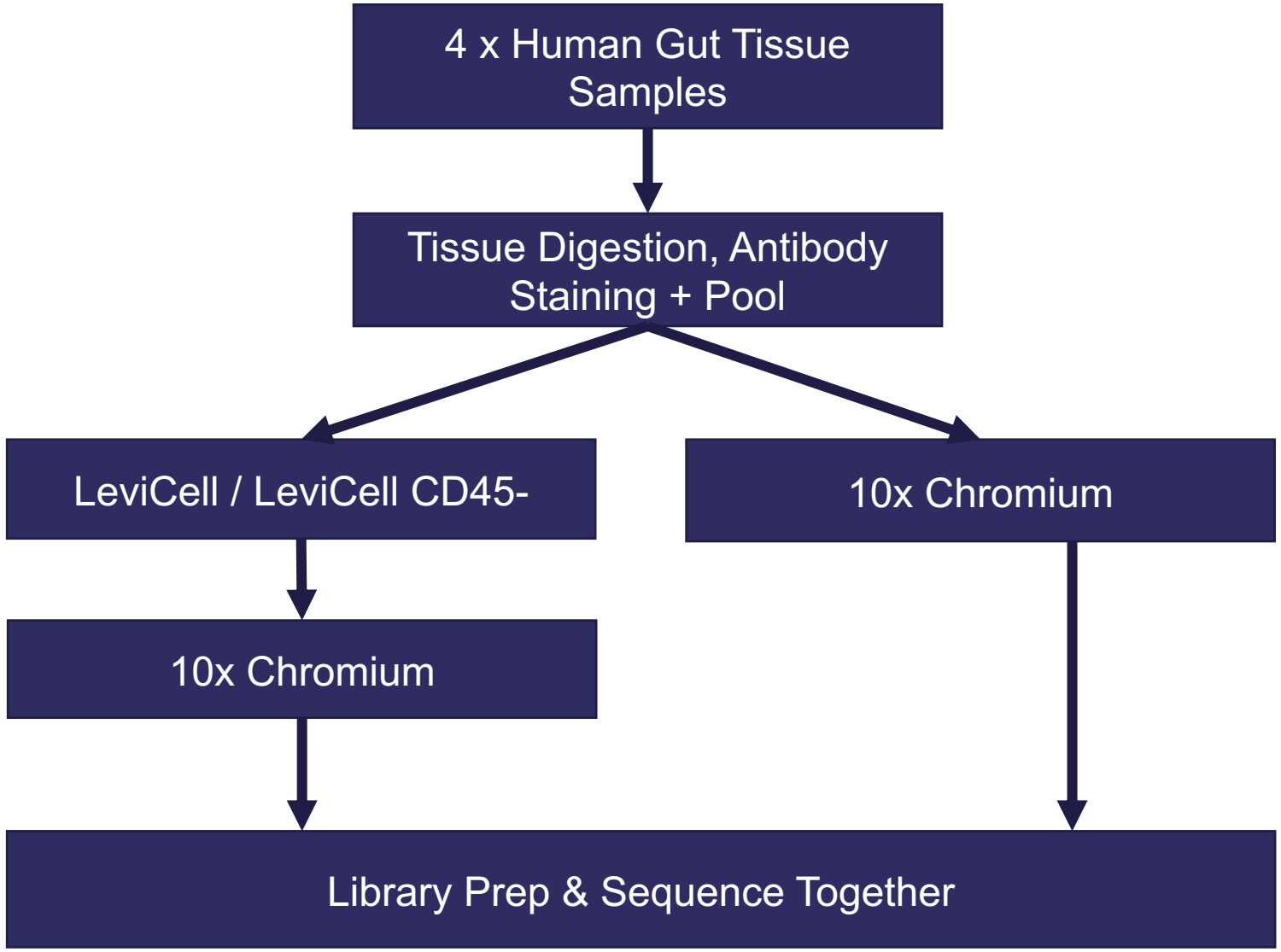
=

Dead or dying cells (Waste)

Compromised membrane

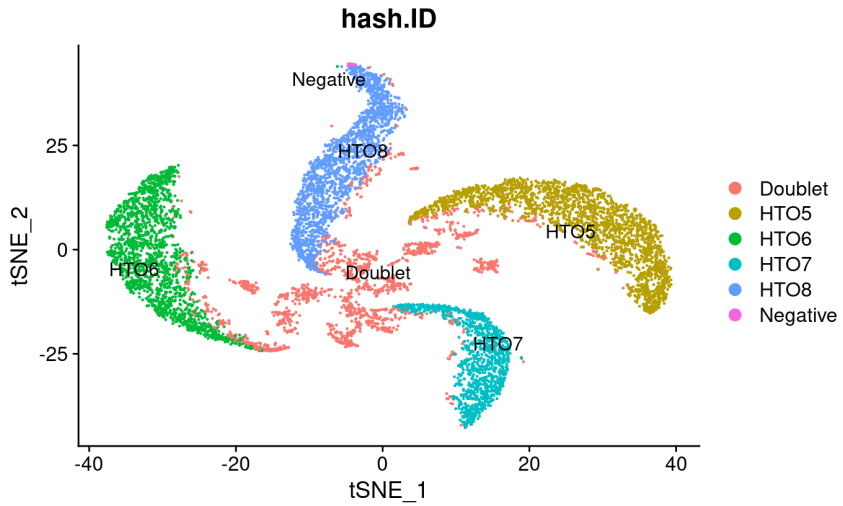
Levitates lower

Trialling the LeviCell System

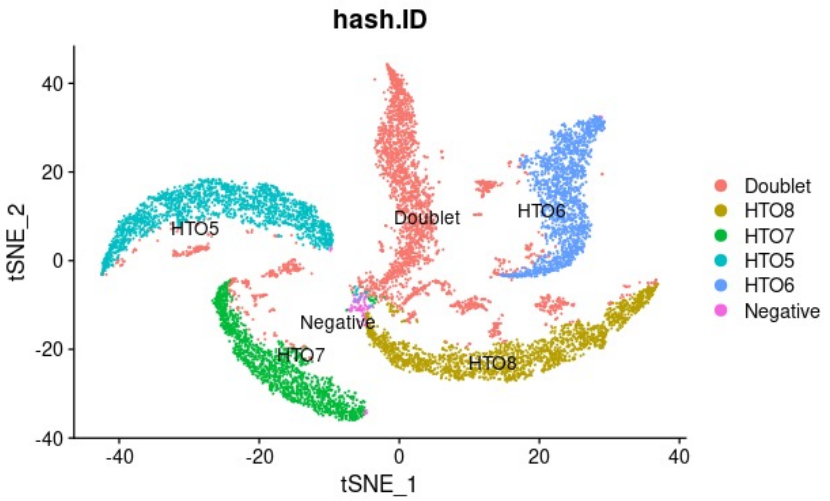


LeviCell isolation is compatible with antibody hashing

LeviCell Pool:



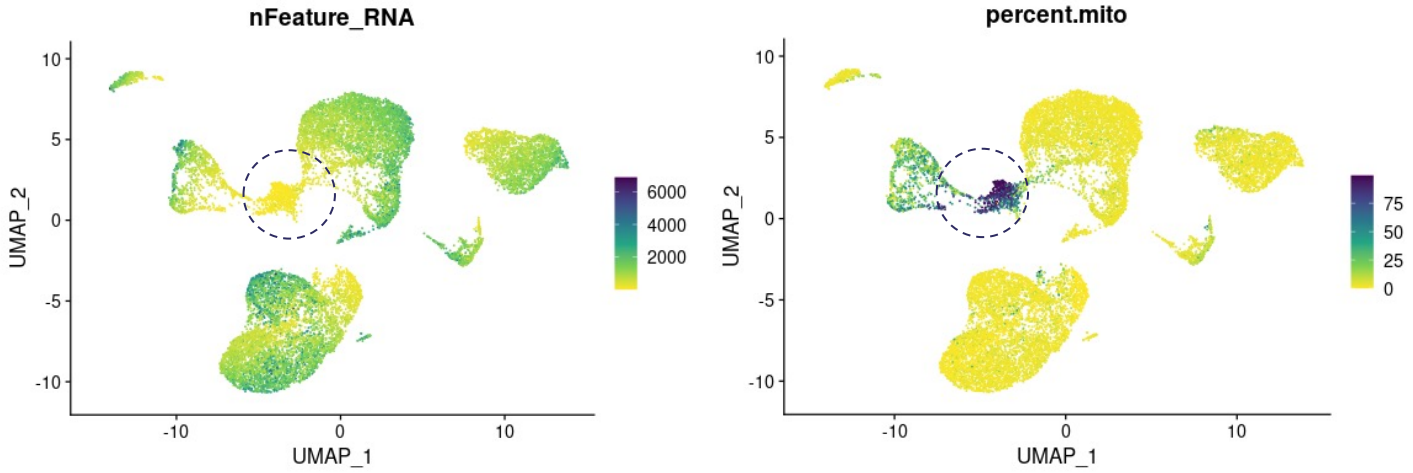
Non-LeviCell Pool:

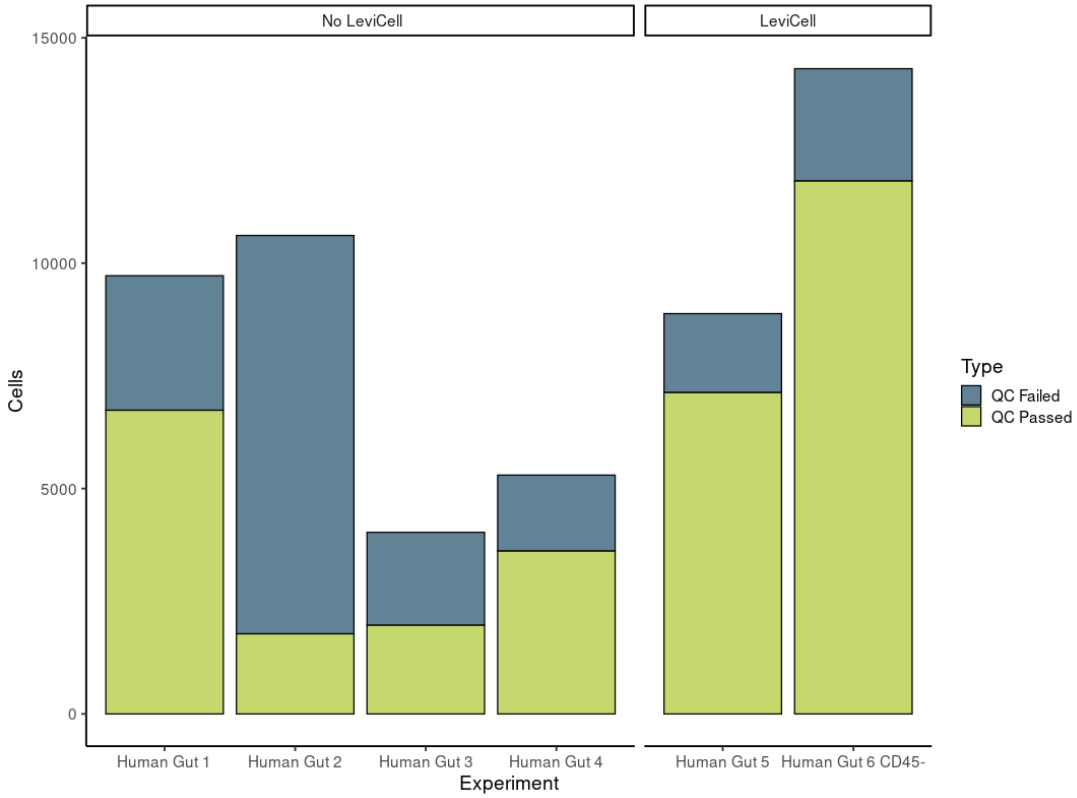


LeviCell isolation yields more QC-passing cells

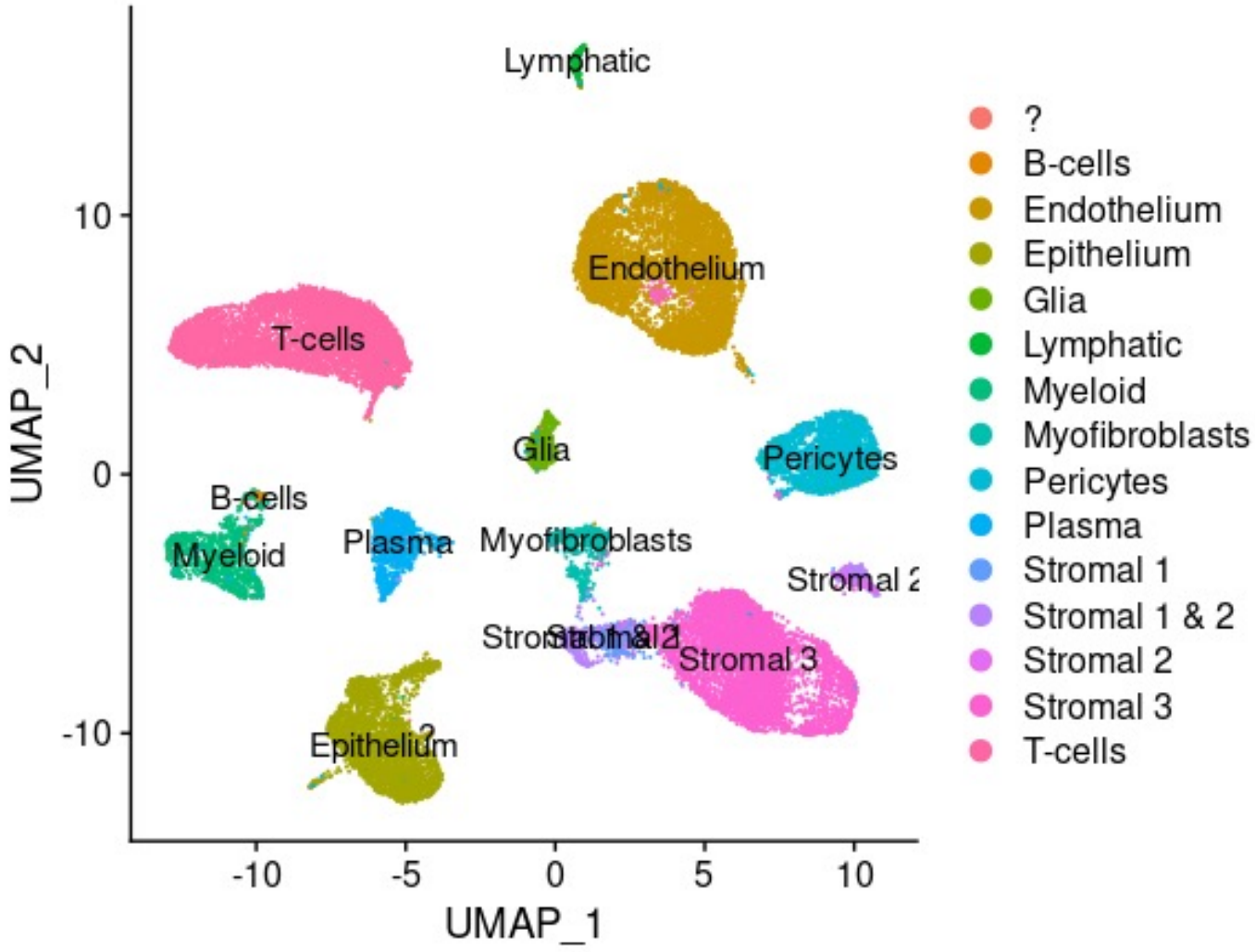
- We sequenced 6 10x reactions of cells – 2 LeviCell and 4 non-LeviCell from paired samples
- We captured more cells in LeviCell pools than in the paired samples
- We also had to filter out fewer cells due to low RNA complexity

Poor quality cells:





LeviCell isolation preserves all cell types and cell type balance within samples



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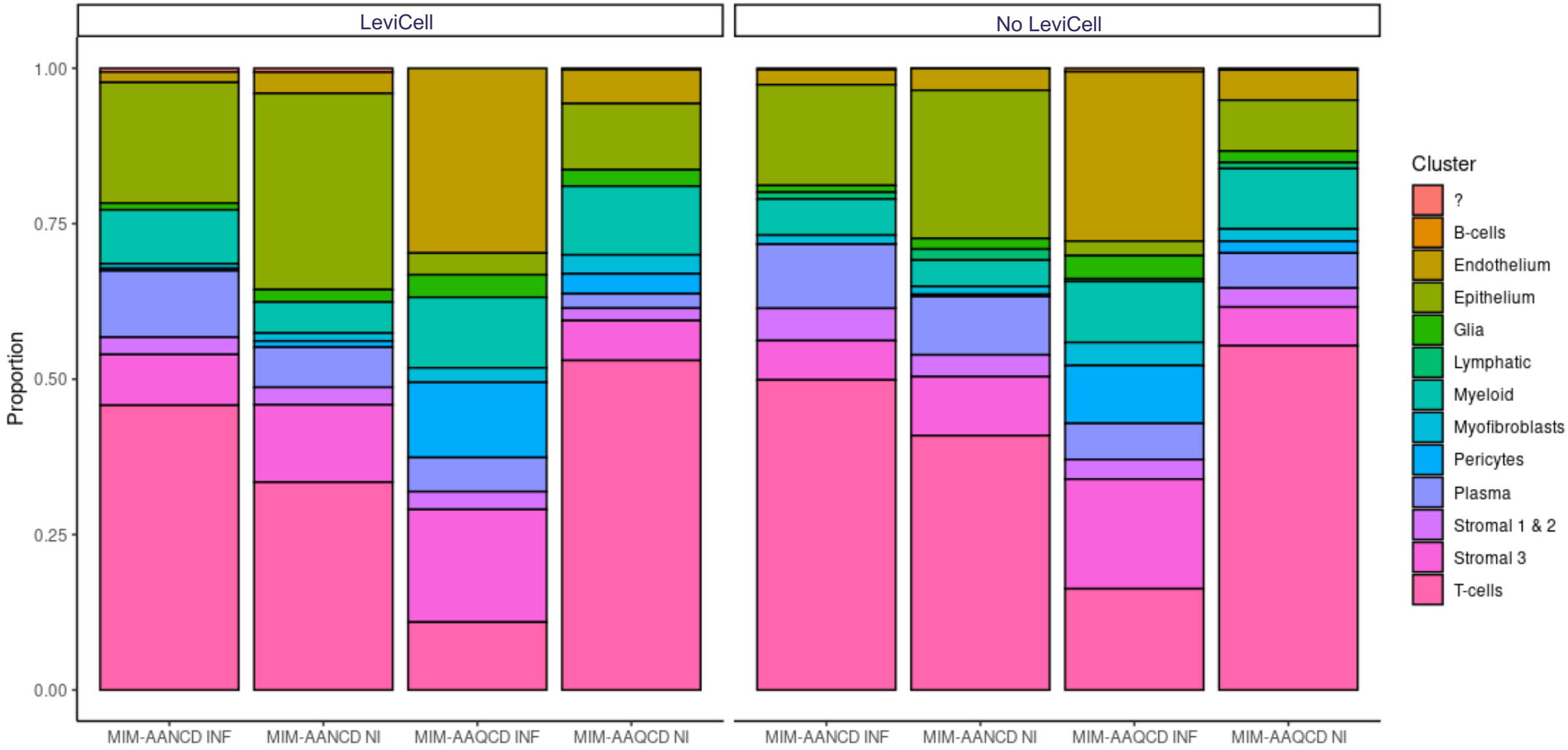
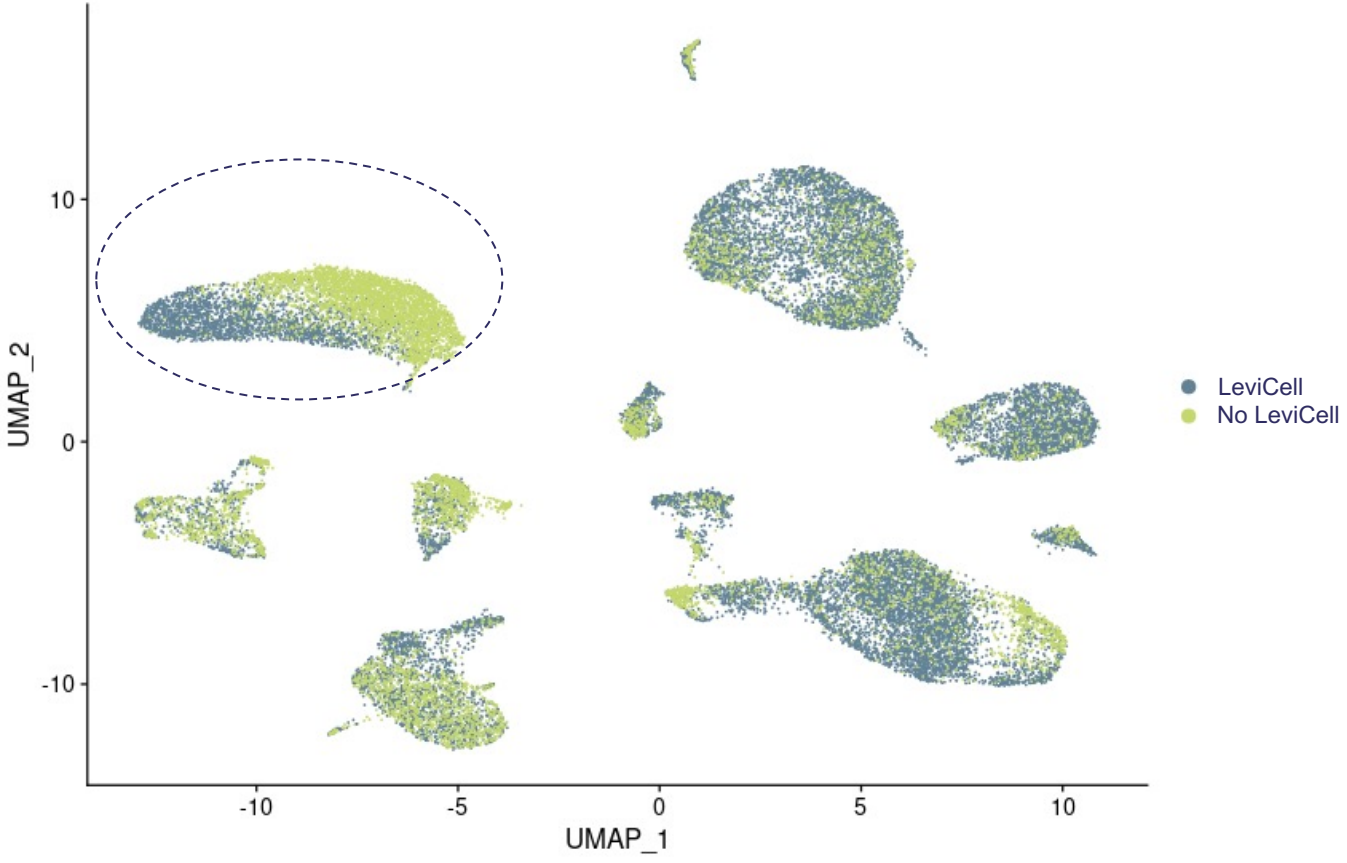
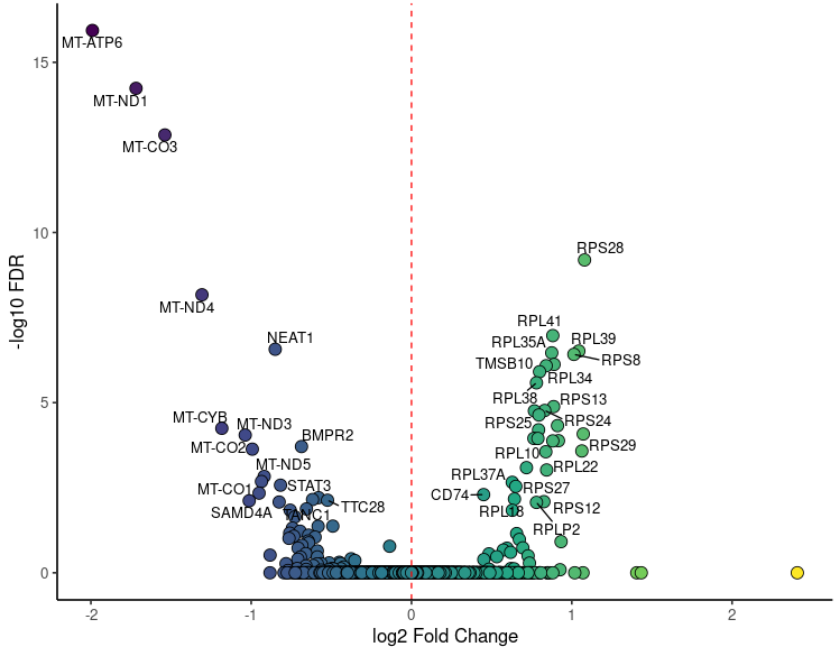
LeviCell isolation preserves all cell types and cell type balance within samples

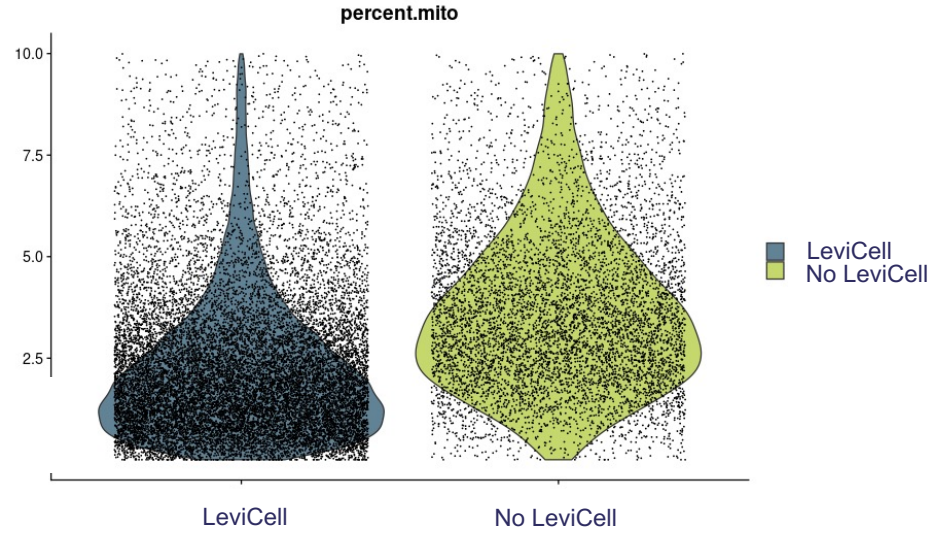


LeviCell isolation impact on mRNA profiles

There are some batch effects (uncorrected):







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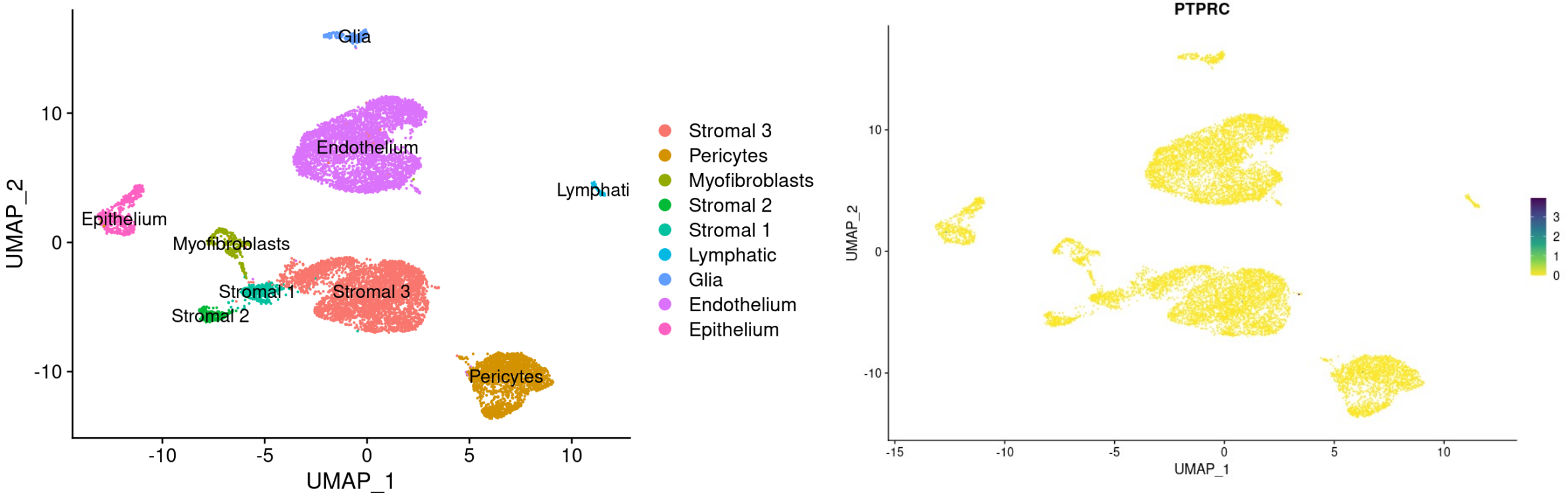
LeviCell CD45+ cell depletion successfully removes unwanted immune cells



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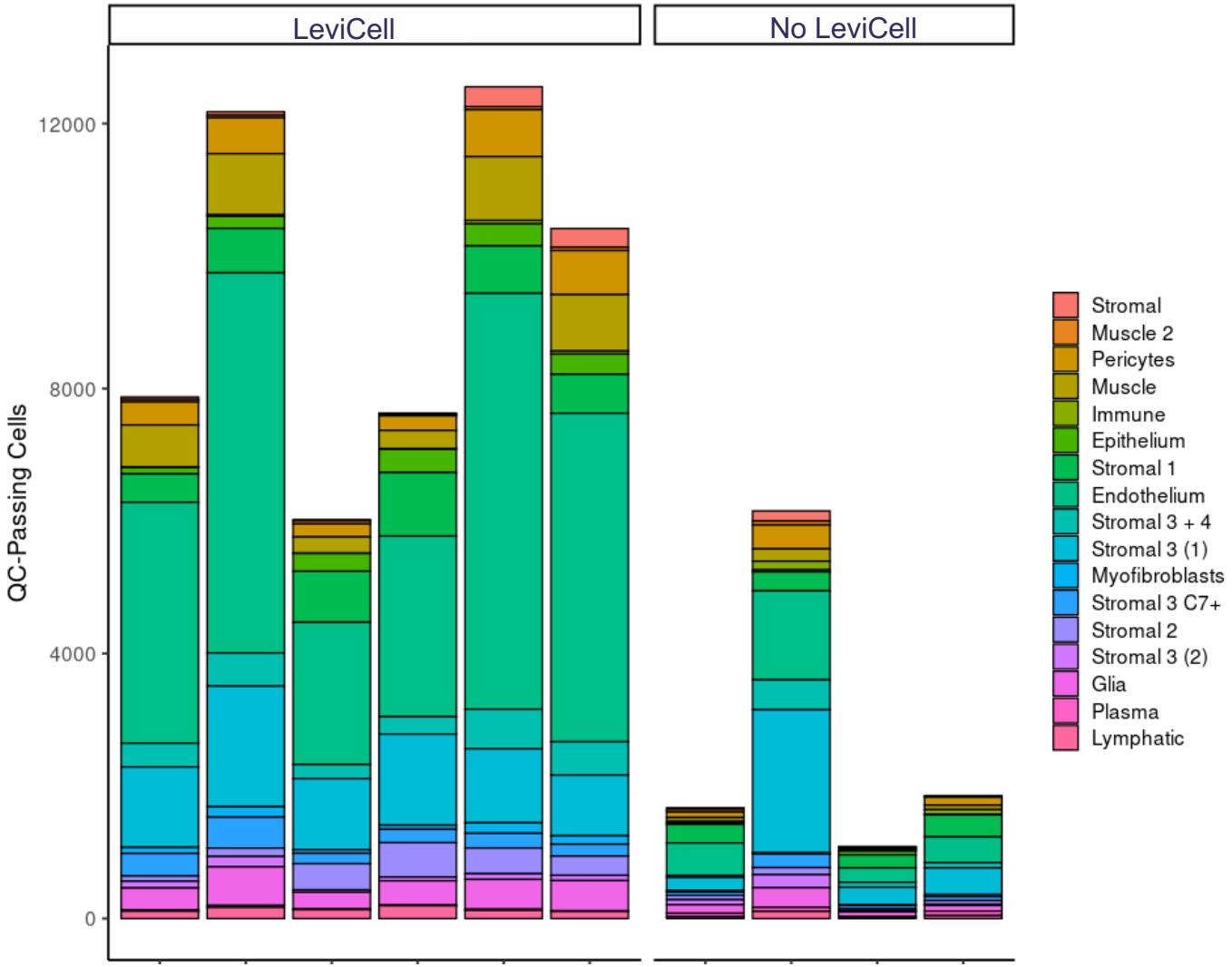
LeviCell improves the cell yield of poor-quality samples

- We next trialled LeviCell CD45+ depletion protocol on gut tissue samples which had quite low cell viabilities
- In previous experiments, we used FACS sorting of CD45- cells, but most reactions still suffered low cell yields
- We repeated these experiments, using some of the same samples, which resulted in as much as 5-fold increase in cell recoveries



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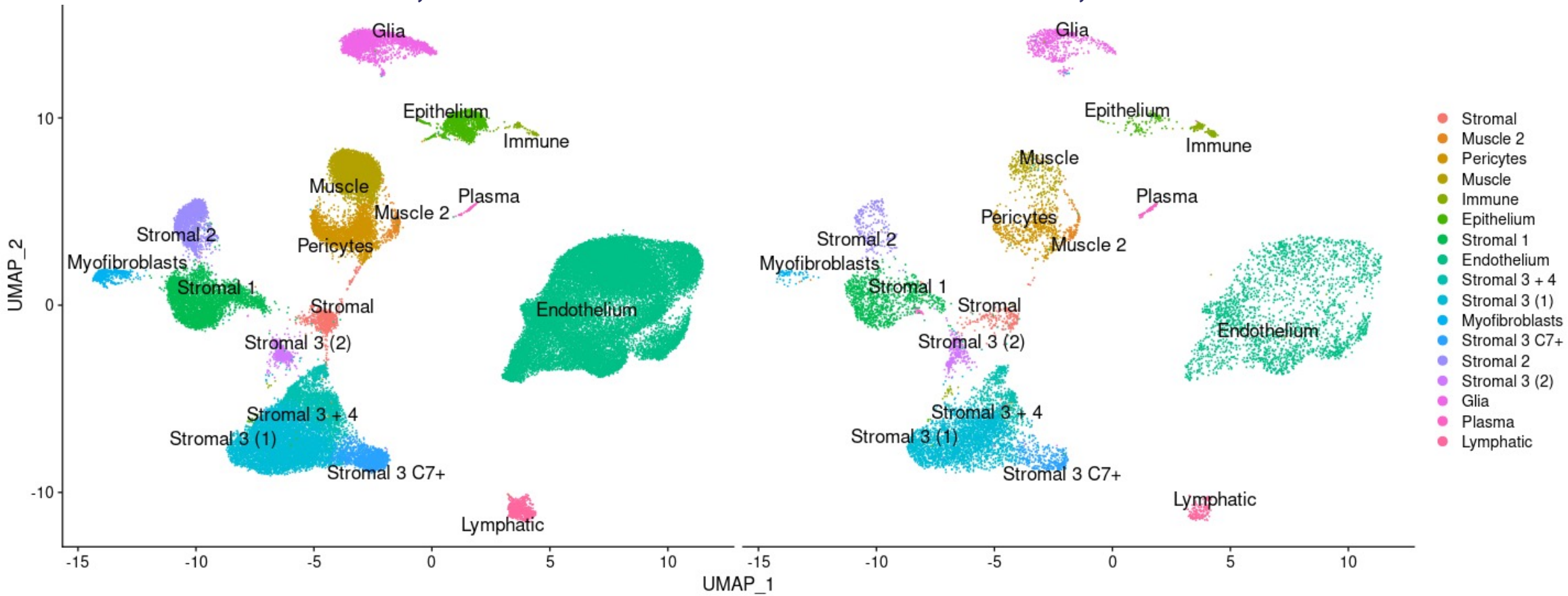
LeviCell improves the cell yield of poor-quality samples

- 6 LeviCell vs 5 FACS 10x Chromium wells of CD45- cells from tissue samples
- Data integrate well and all cell types are represented
- Some differences in “contaminating” cell populations – for example, more immune cells slip through by FACS with our current gating

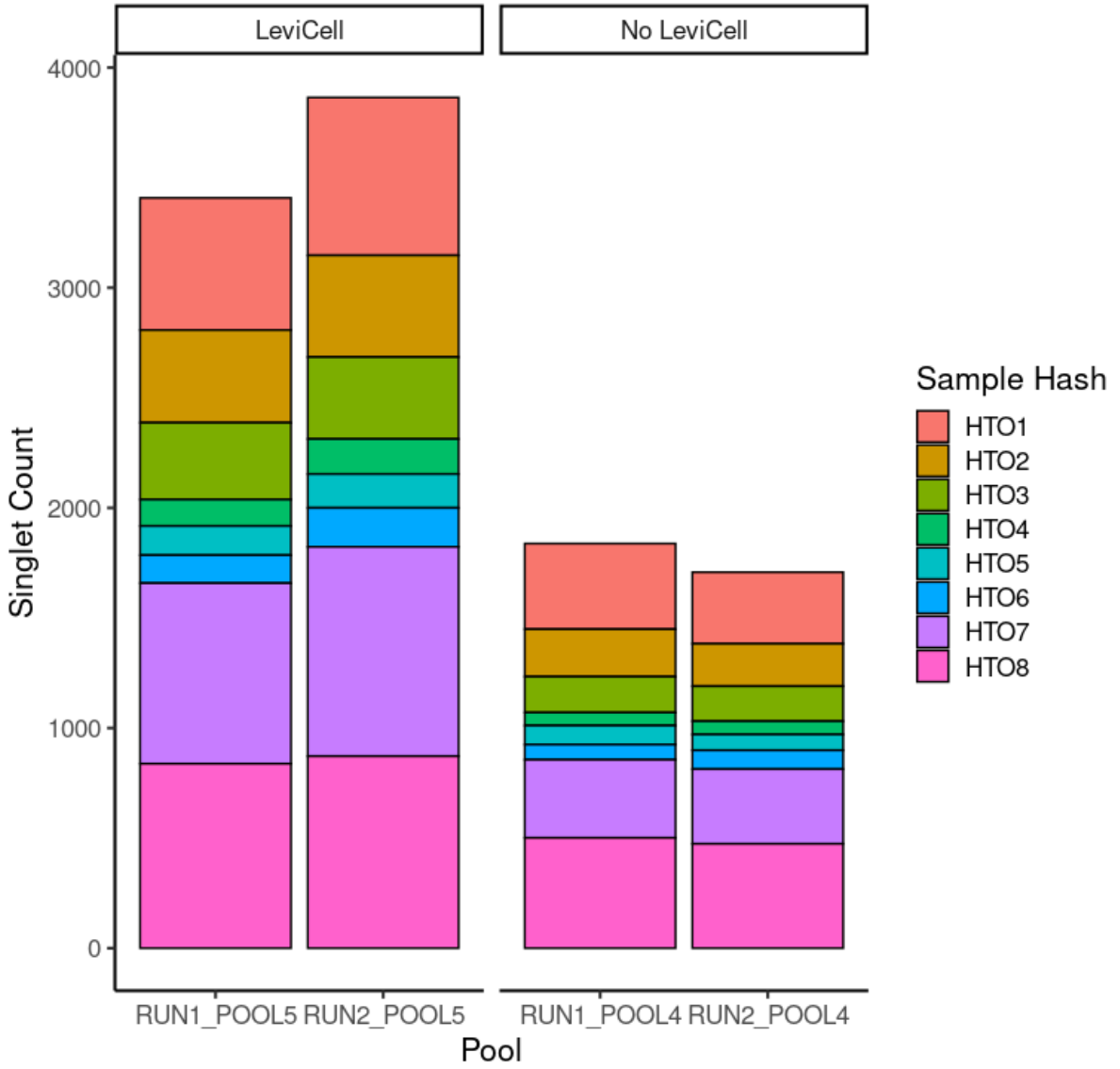
LeviCell - 56,672 cells:

FACS -10,771 cells:



LeviCell improves yield of delicate epithelial cells

- Multiplexed up to 8 samples per reaction from epithelial organoids
- Same single cell suspension was split four ways, half went through LeviCell
- Sample balance preserved
- ~ 2-fold increase in cell recovery post LeviCell selection



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Summary

- LeviCell system can greatly improve the cell yield and quality of scRNA-Seq from otherwise difficult samples without a major impact on sample cell type composition
- LeviCell system is compatible with antibody labelling, allowing multi-modal profiling such as CITE-Seq or sample multiplexing
- Sample balance is not impacted when using LeviCell system with a multiplexed sample pool – this simplifies and shortens the lab workflow, as there is no need to run each sample individually. However, for single-sample workflows, parallelisation would be important
- More limiting than FACS for specific cell type isolations – especially since kits work by target depletion rather than selection

Acknowledgments

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Tarun Gupta
Zinan Yin

Sophie Craddock
Rosana Babu

Chloe Hyun-Jung Lee
Marta Jagielowicz
David Fawkner-Corbett

WIMM Facilities
- FACS Facility
- Neil Ashley (Single Cell Facility)
- **Maria Greco (Single Cell Facility)**

TGU Biobank Team
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Patients

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University of Oxford

We are hiring!

Computational and wet lab postdocs
in single-cell and spatial biology and immunology

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agne.antanaviciute@ndm.ox.ac.uk

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