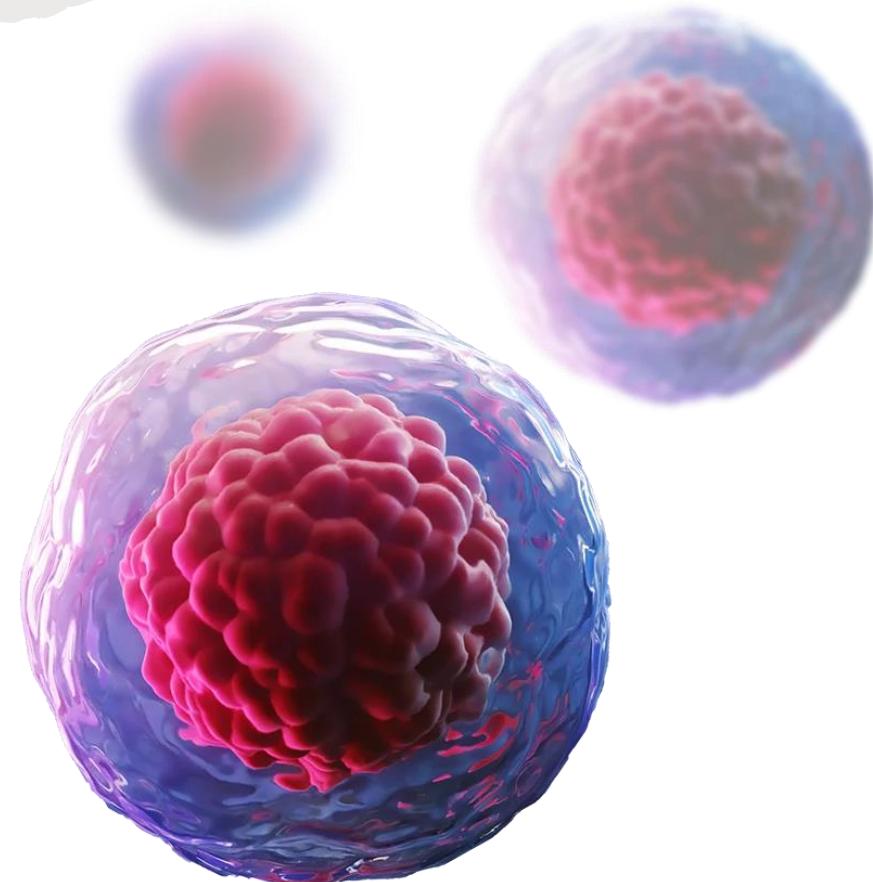
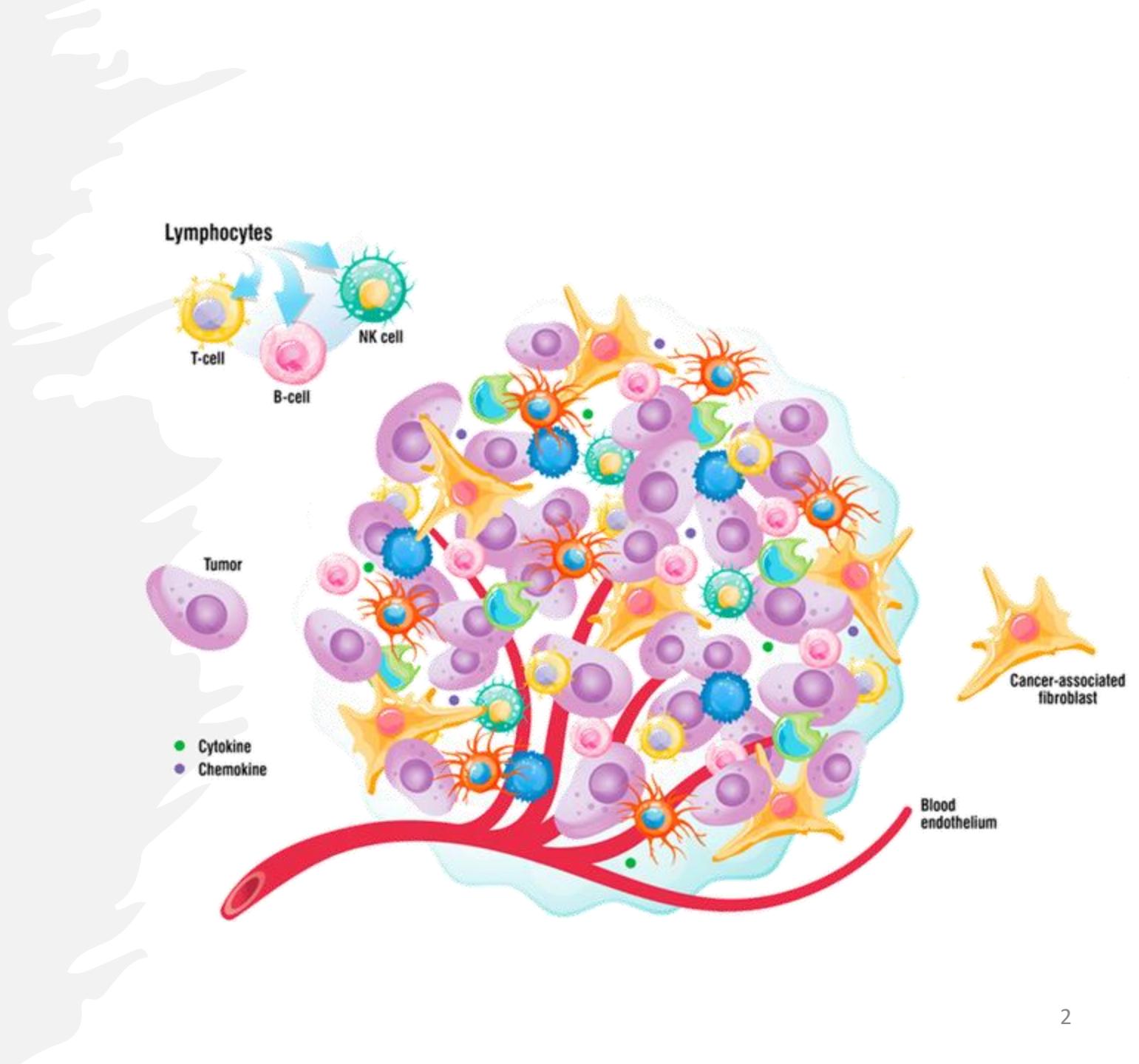


Single-cell transcriptomics in unravelling the therapeutic potential of myeloid cells in breast cancer

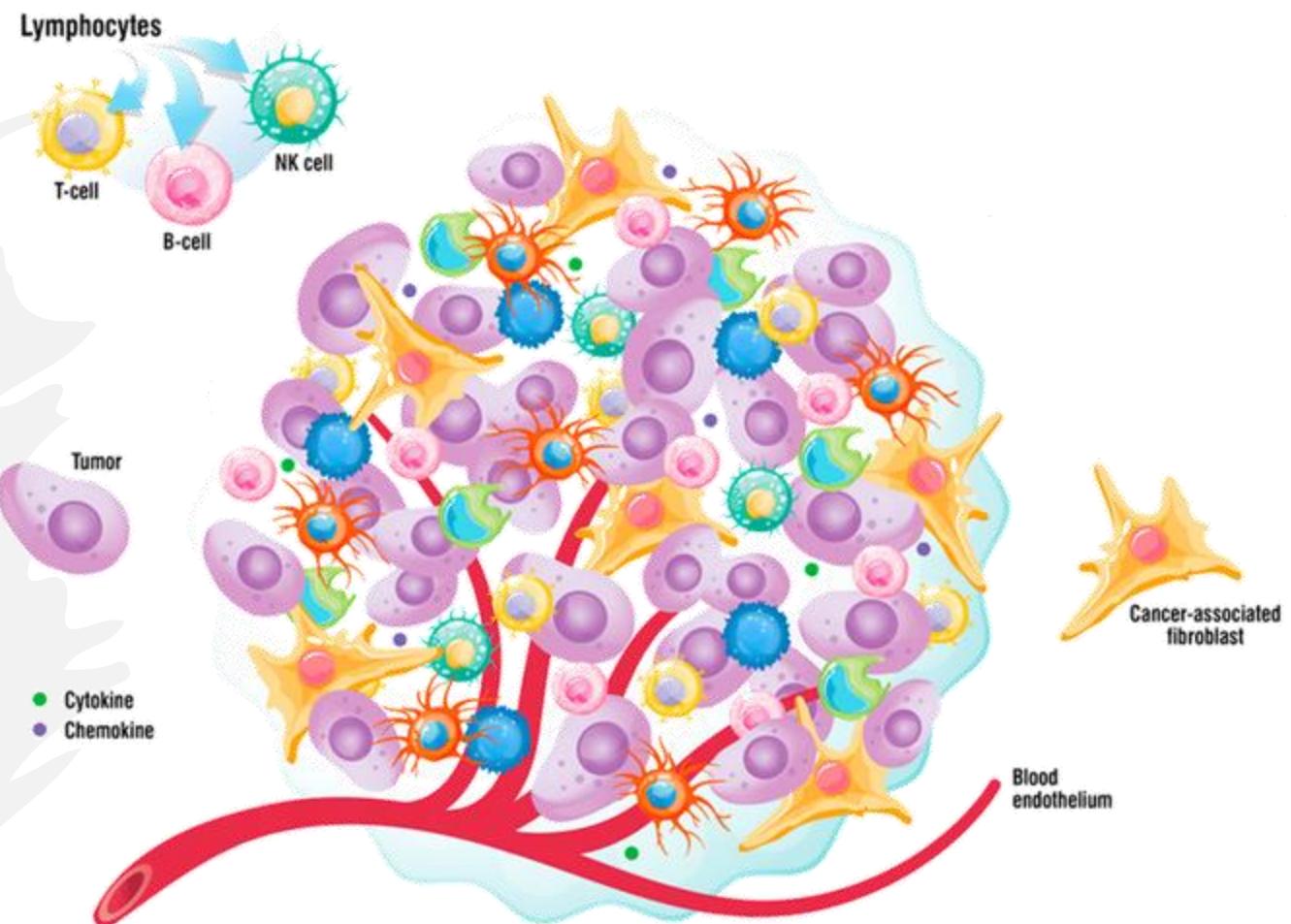
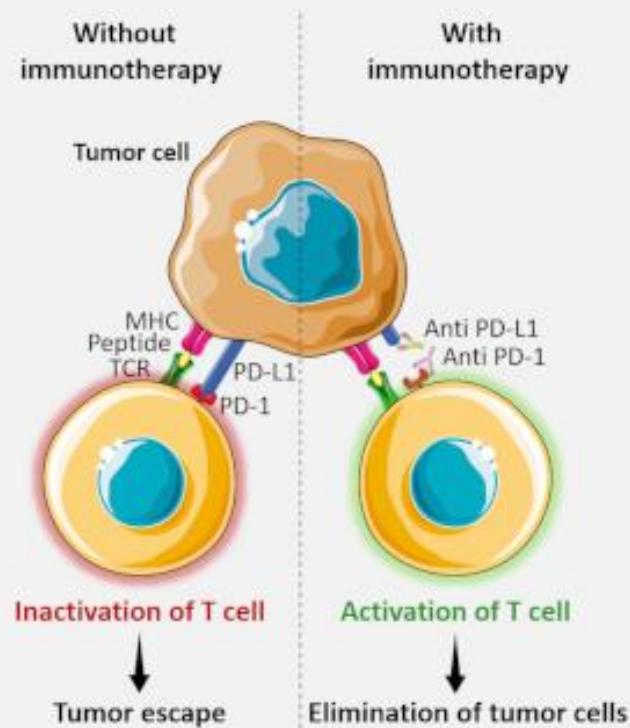
Marta Bica
Instituto de Medicina Molecular
João Lobo Antunes
28th May 2024



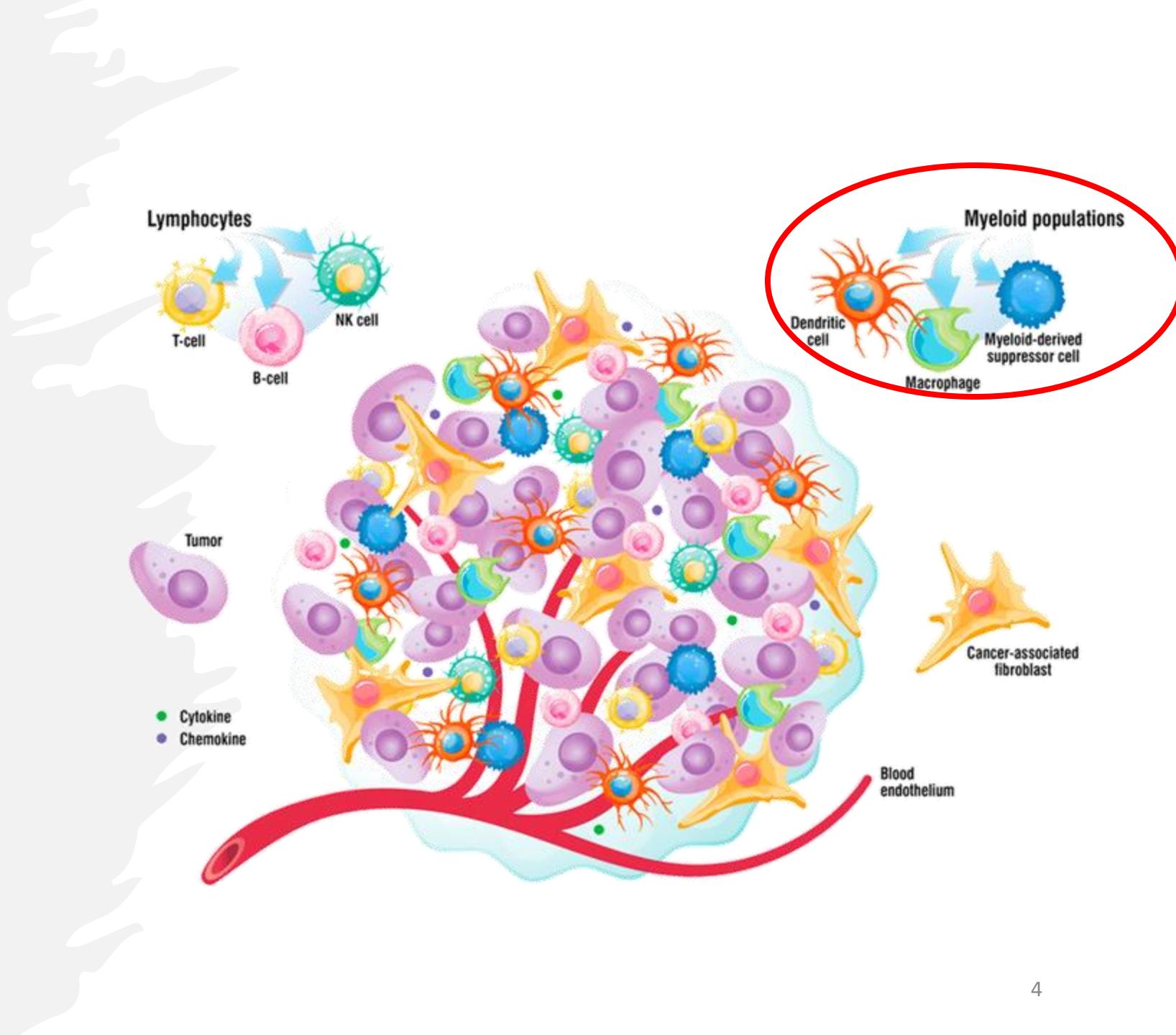
Tumour microenvironment



Tumour microenvironment



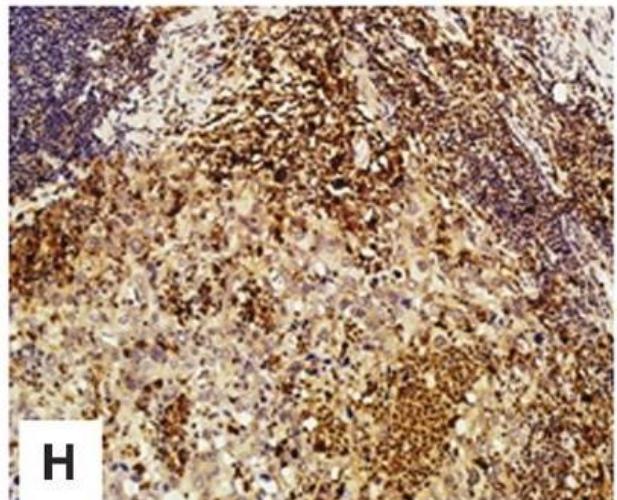
Tumour microenvironment



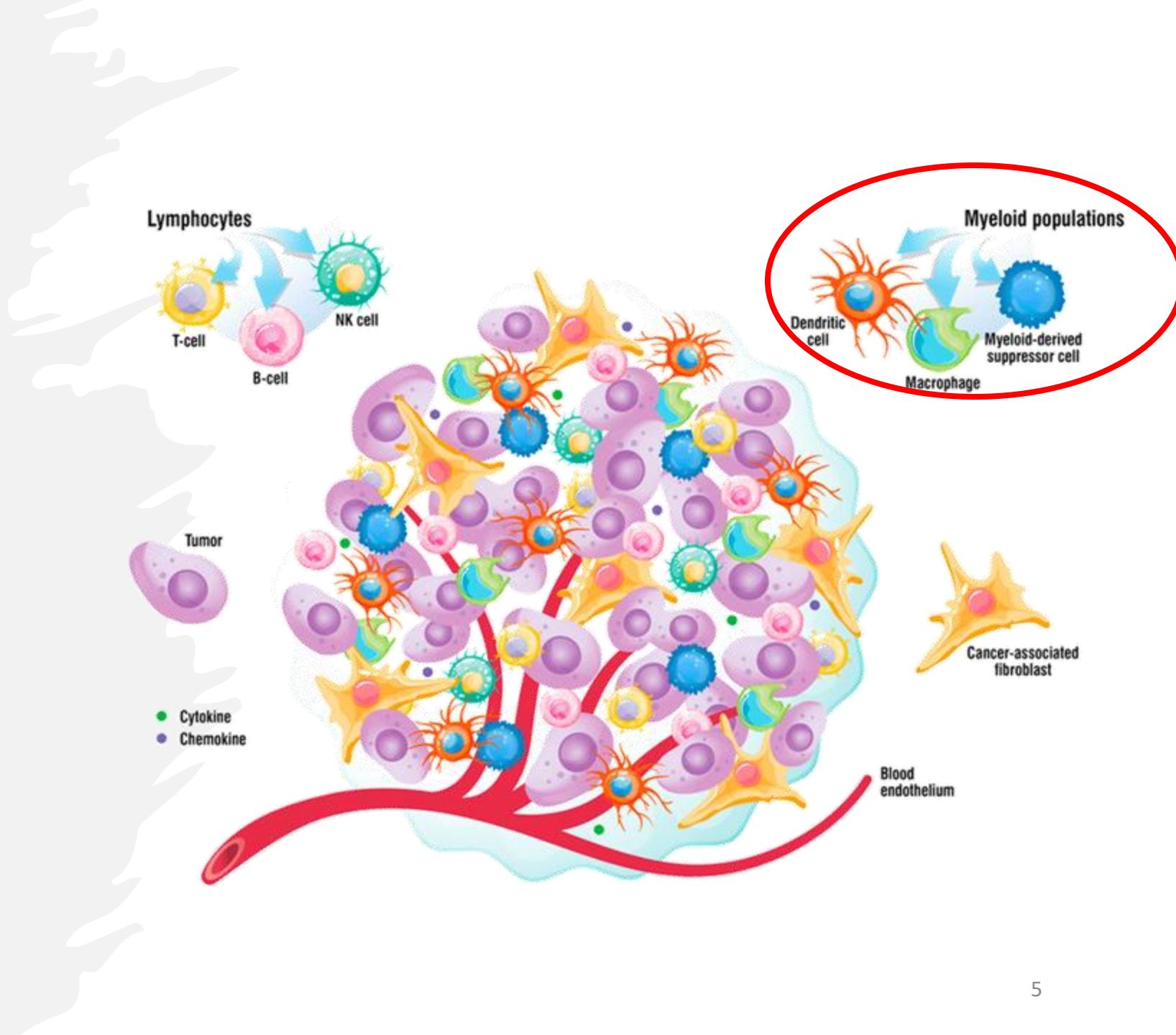
Tumour microenvironment

Up to 50% of the breast tumour mass

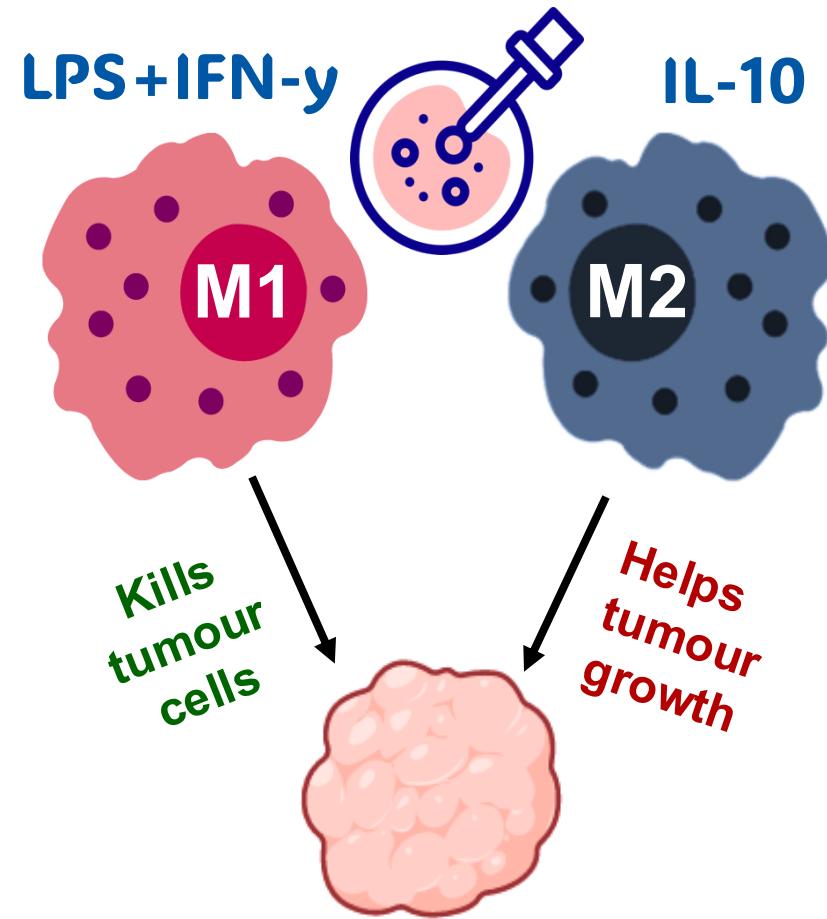
Macrophage staining



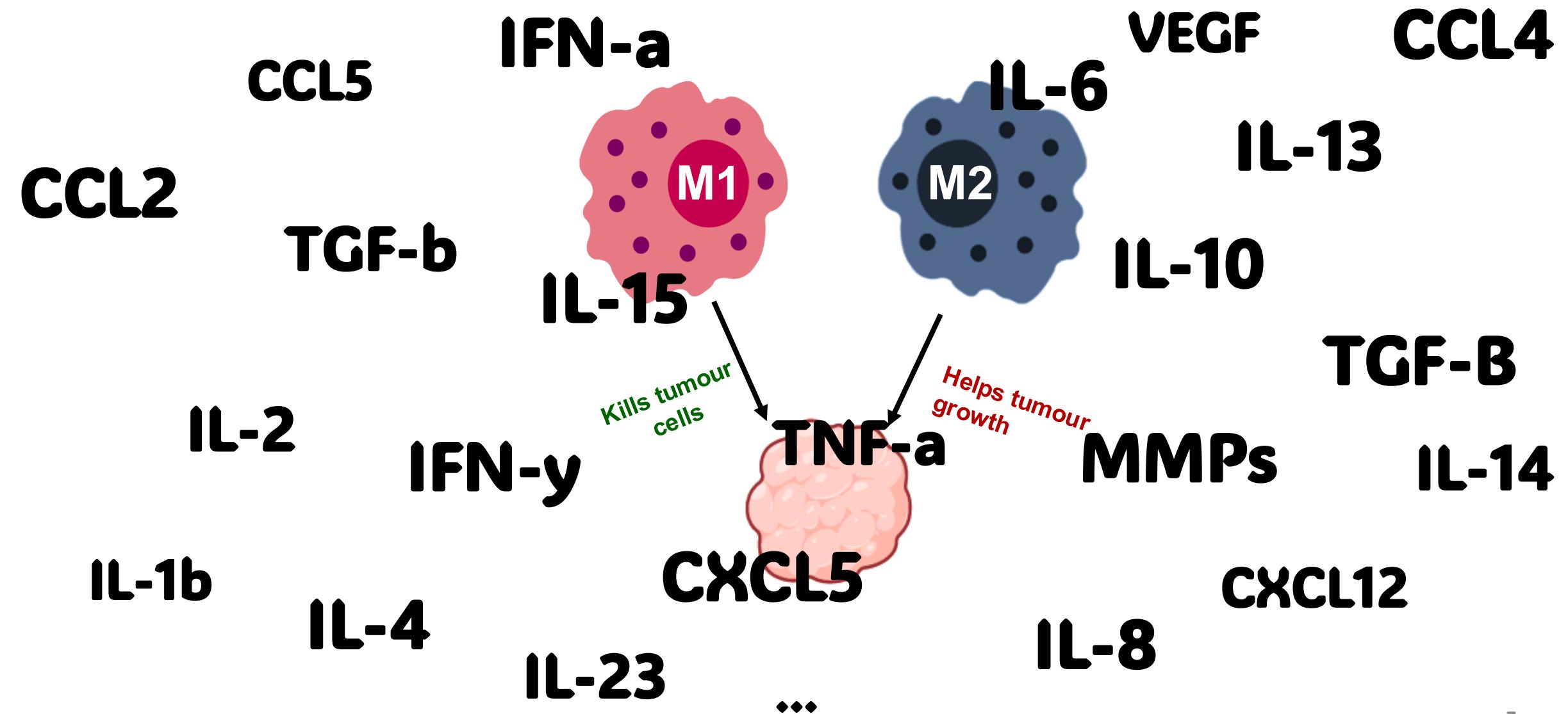
Zhang et al. 2015



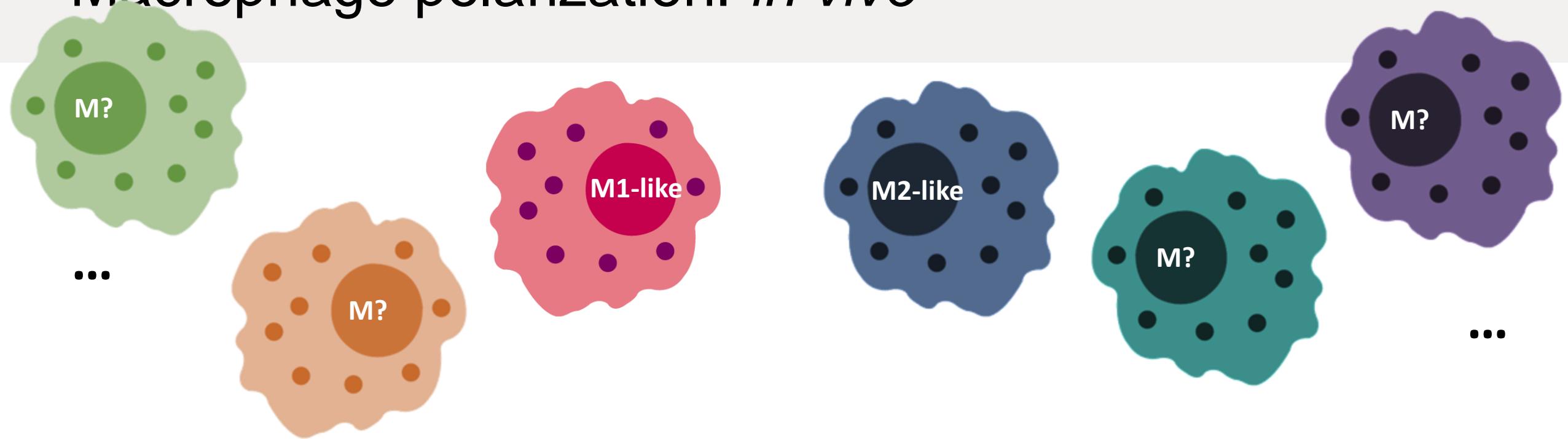
Macrophage polarization: *in vitro*



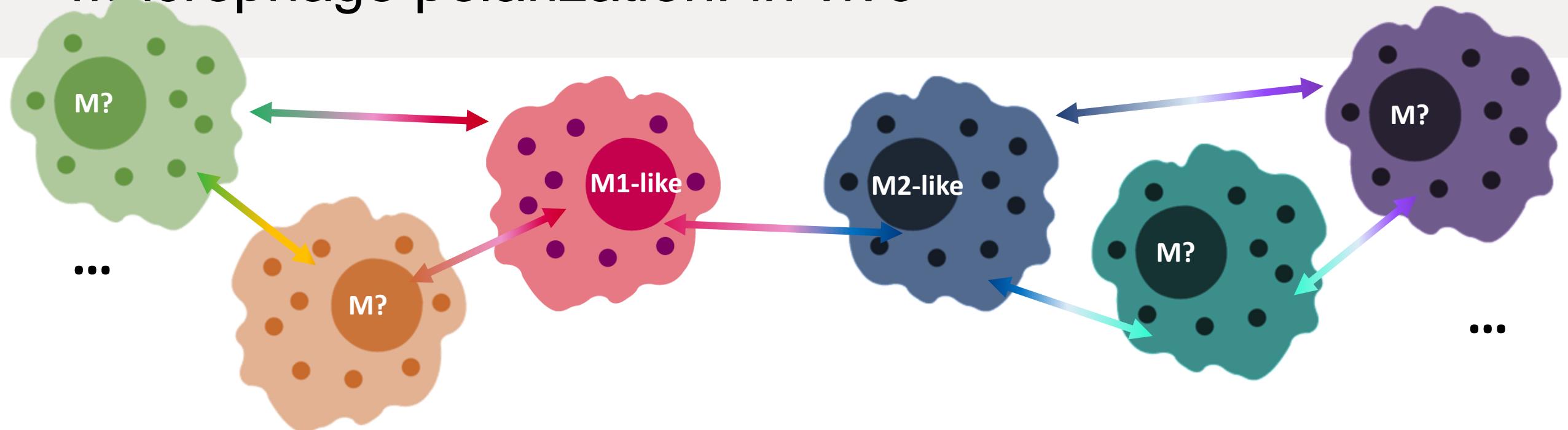
Macrophage polarization: *in vivo*



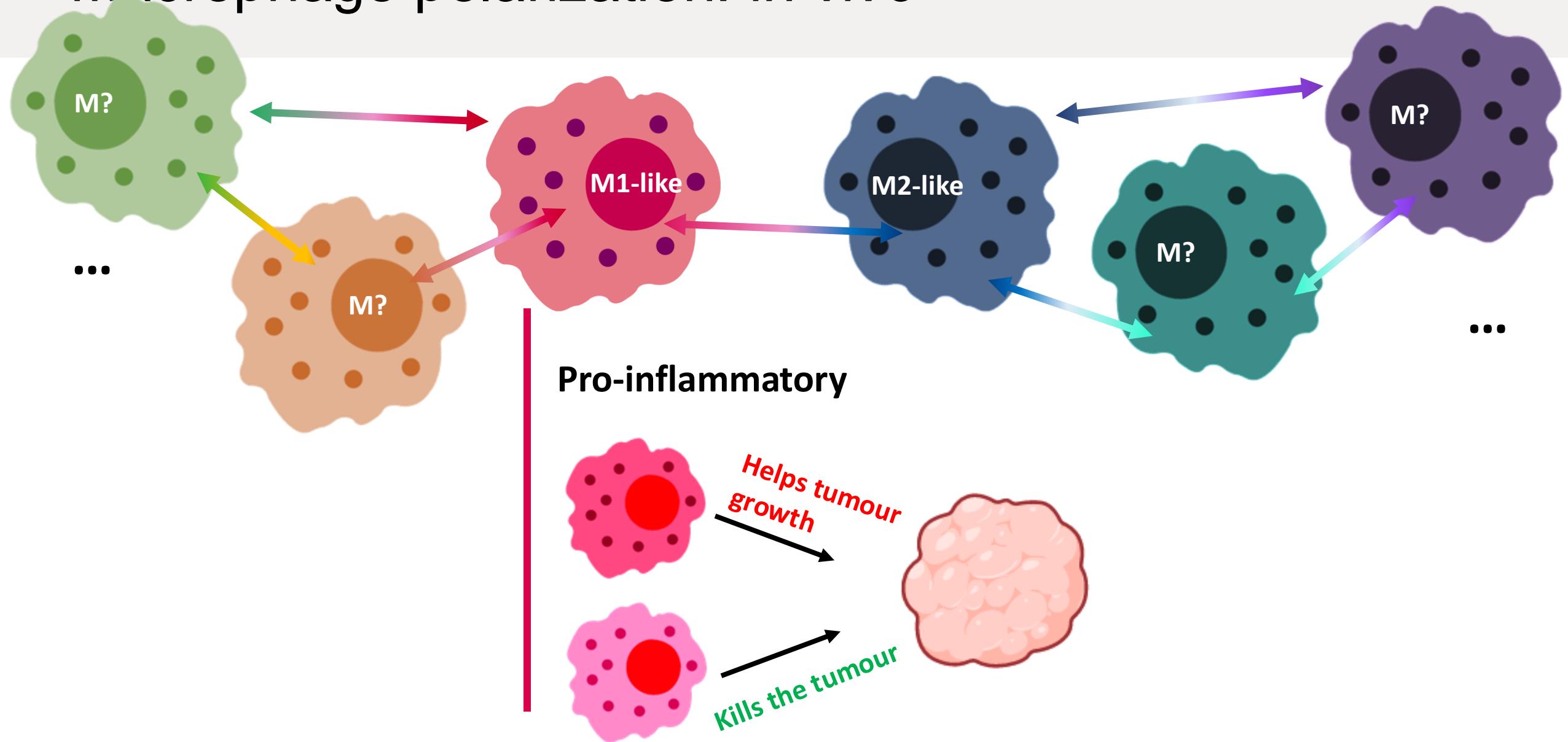
Macrophage polarization: *in vivo*



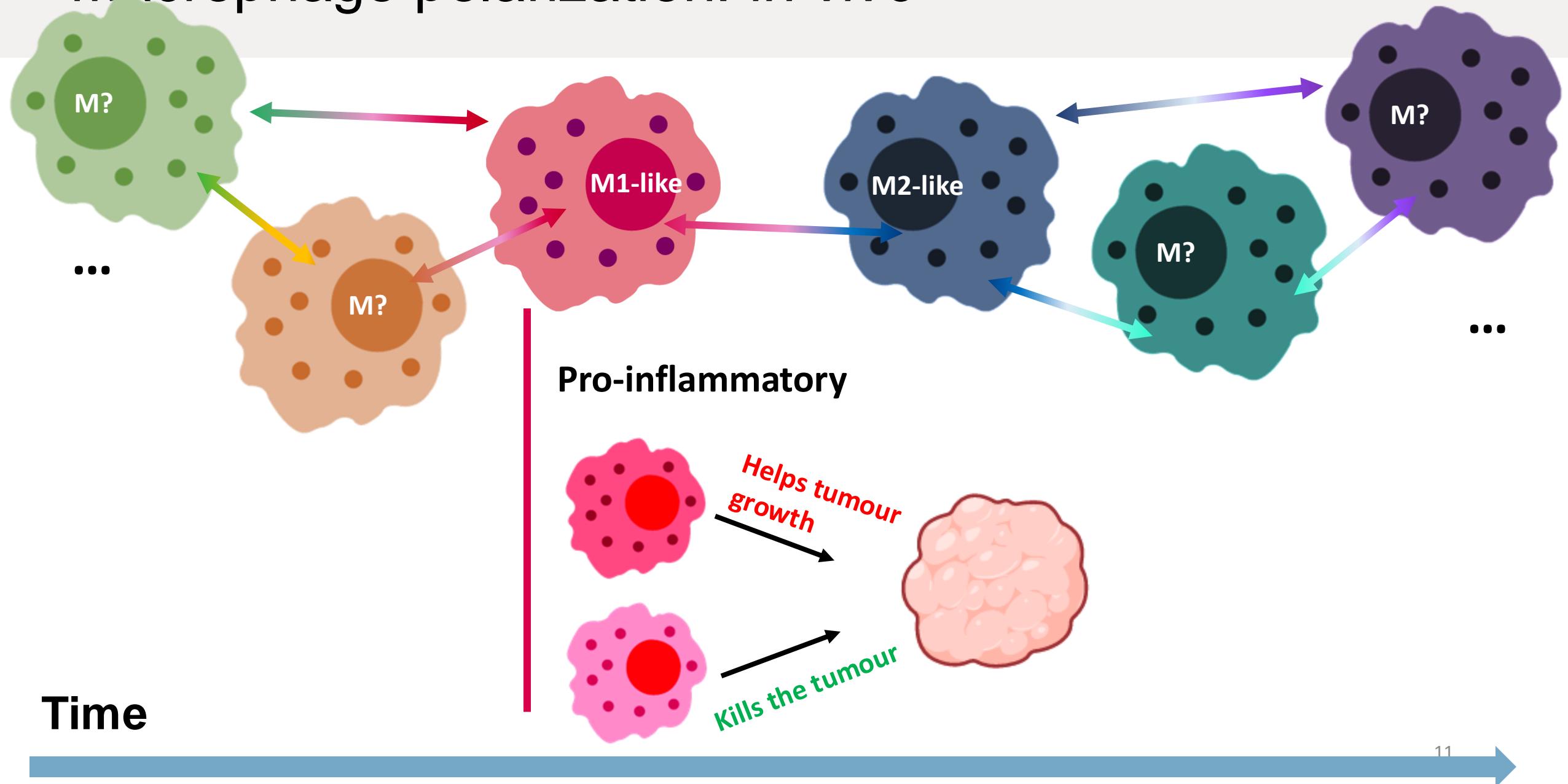
Macrophage polarization: *in vivo*



Macrophage polarization: *in vivo*



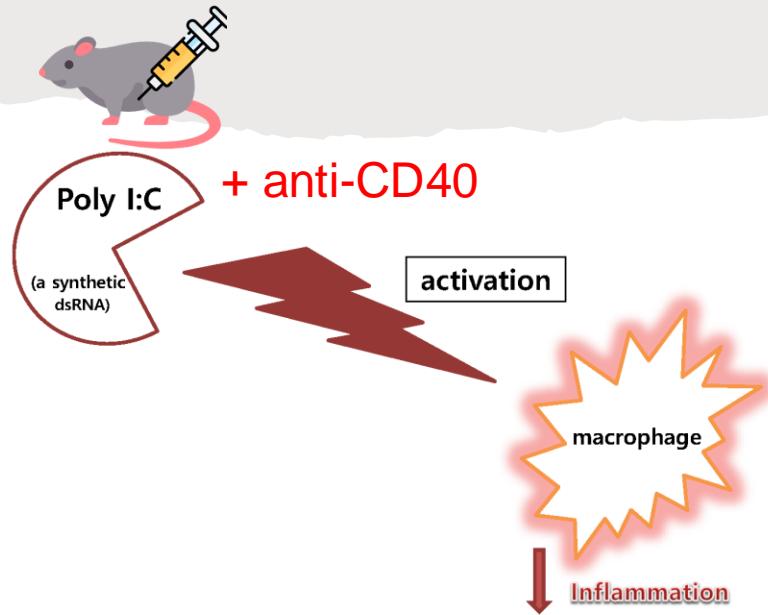
Macrophage polarization: *in vivo*



In vivo myeloid cell treatment



Karine Serre Carolina Jardim

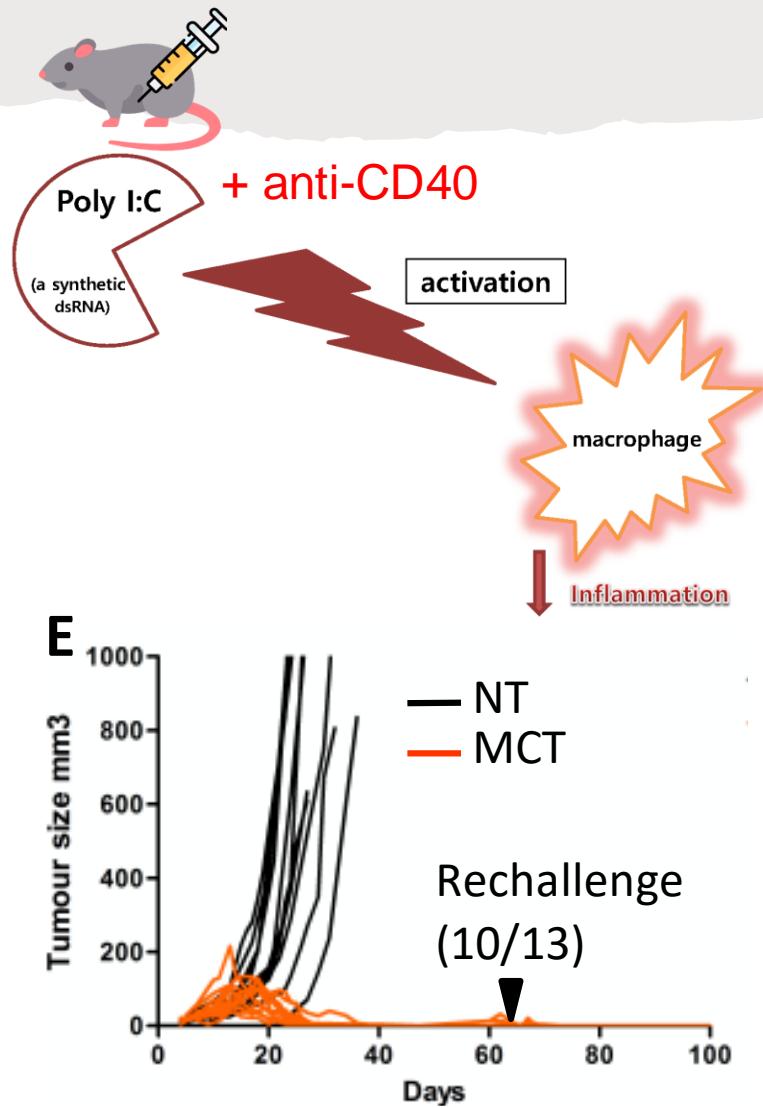


In vivo myeloid cell treatment

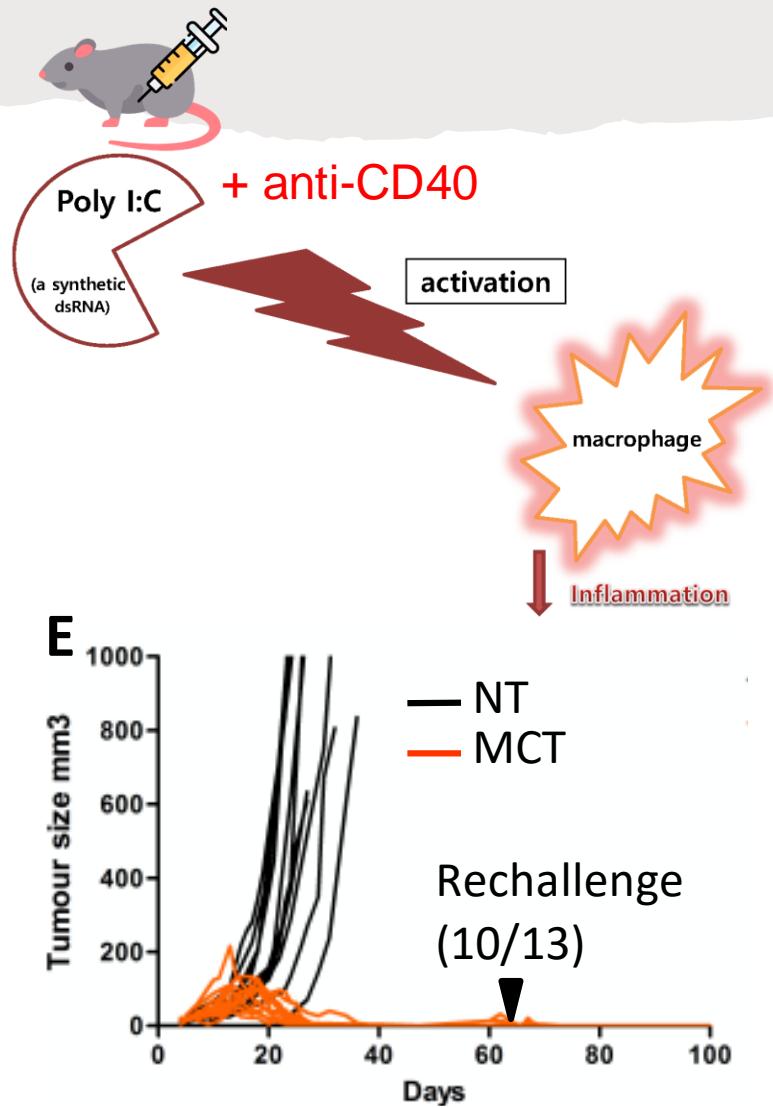


Karine Serre

Carolina Jardim

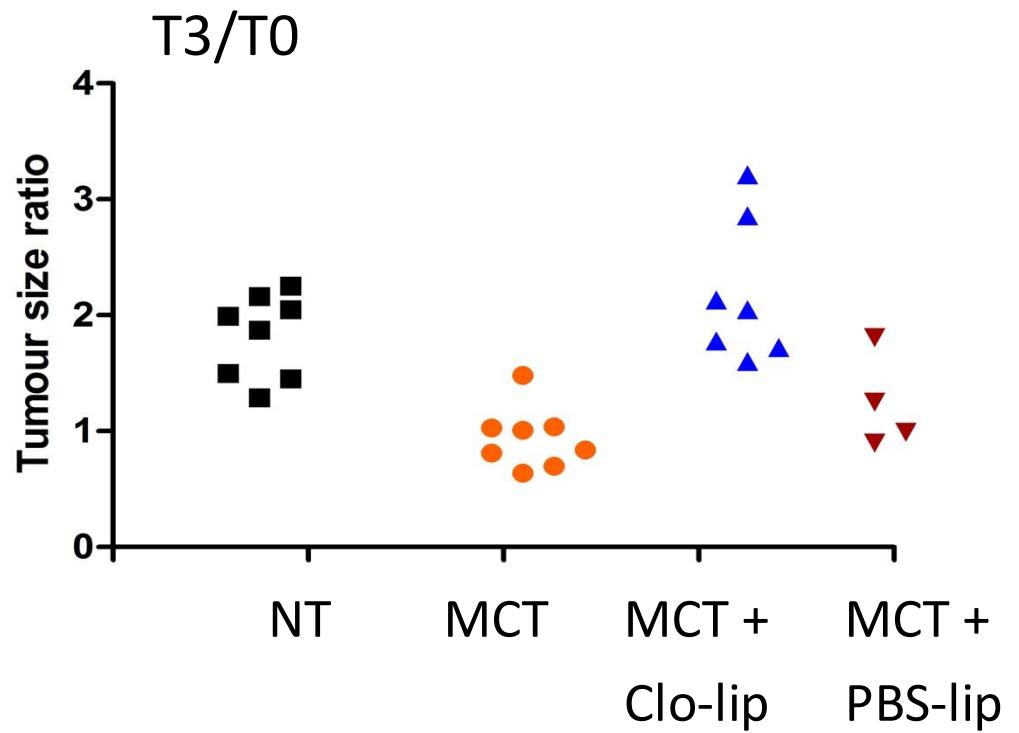


In vivo myeloid treatment



Karine Serre

Carolina Jardim



Our questions

How does the macrophage landscape evolve
during tumour progression?

Early vs
Late

What are the macrophage-mediated mechanisms
that control tumour growth post-treatment?

Treated vs
Untreated

Experimental design



Karine Serre

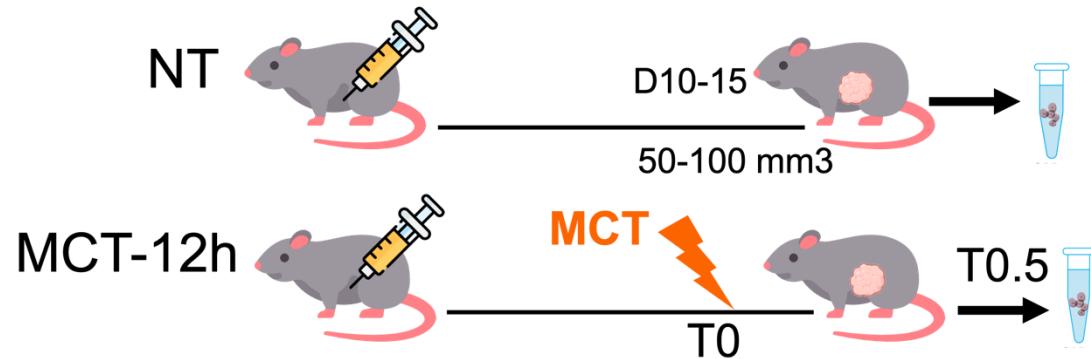


Carolina Jardim



Neuza Sousa

Experimental design



Karine Serre



Carolina Jardim



Neuza Sousa

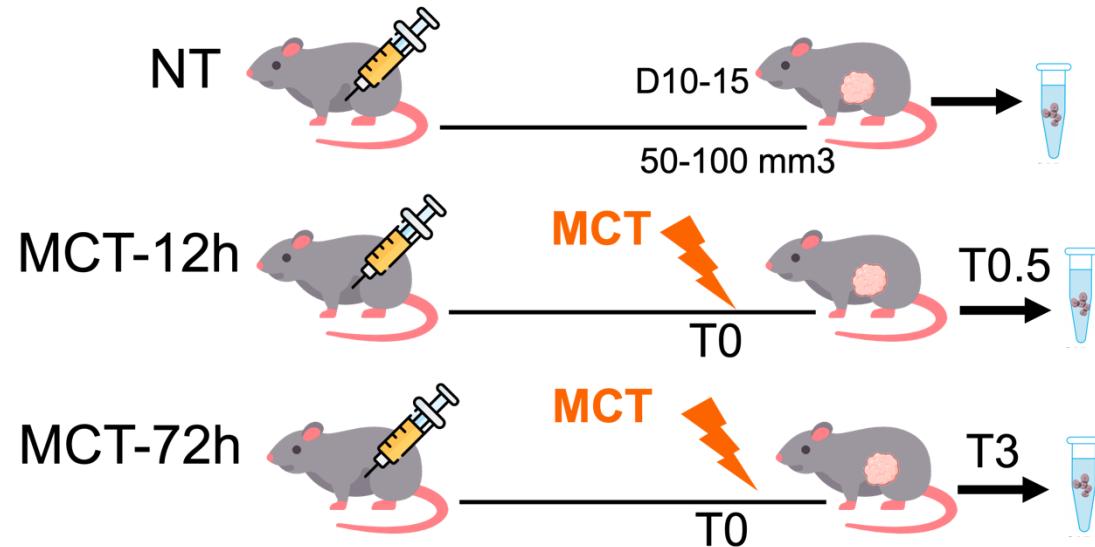
Experimental design



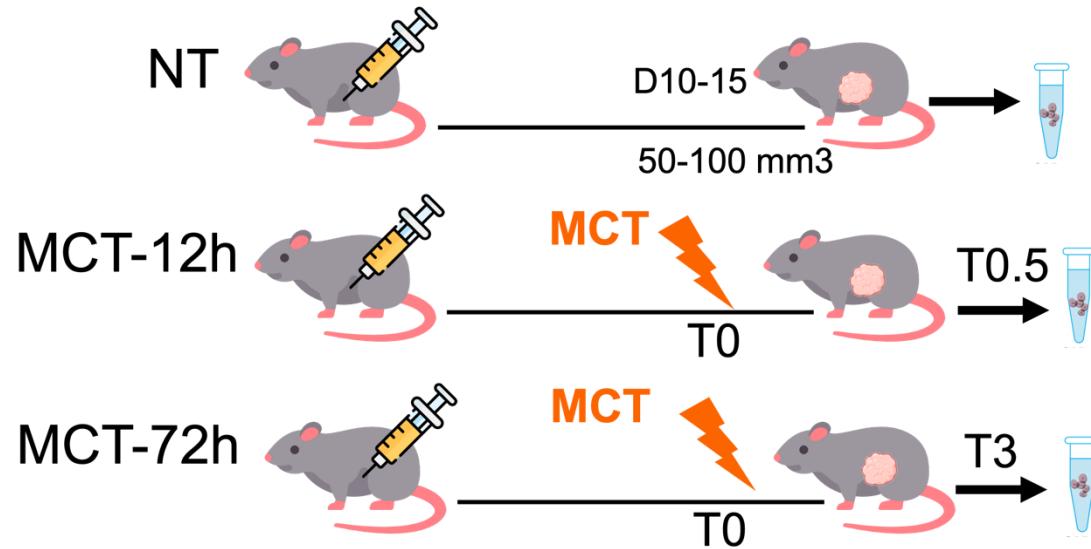
Karine Serre

Carolina Jardim

Neuza Sousa



Experimental design



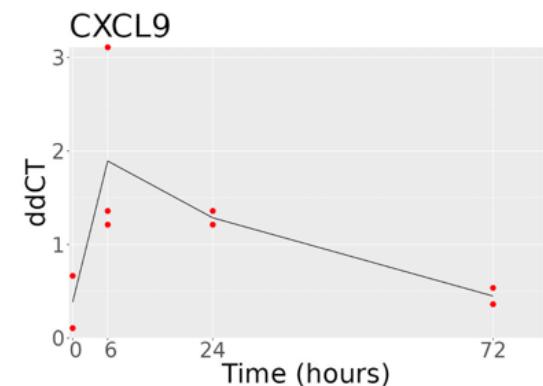
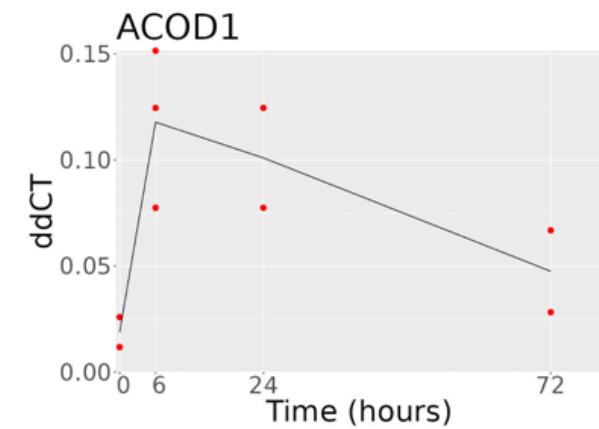
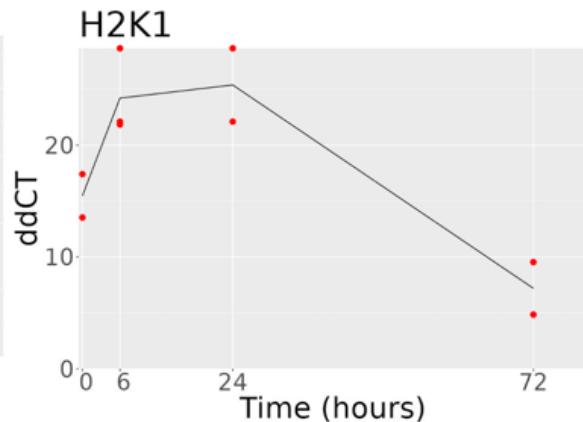
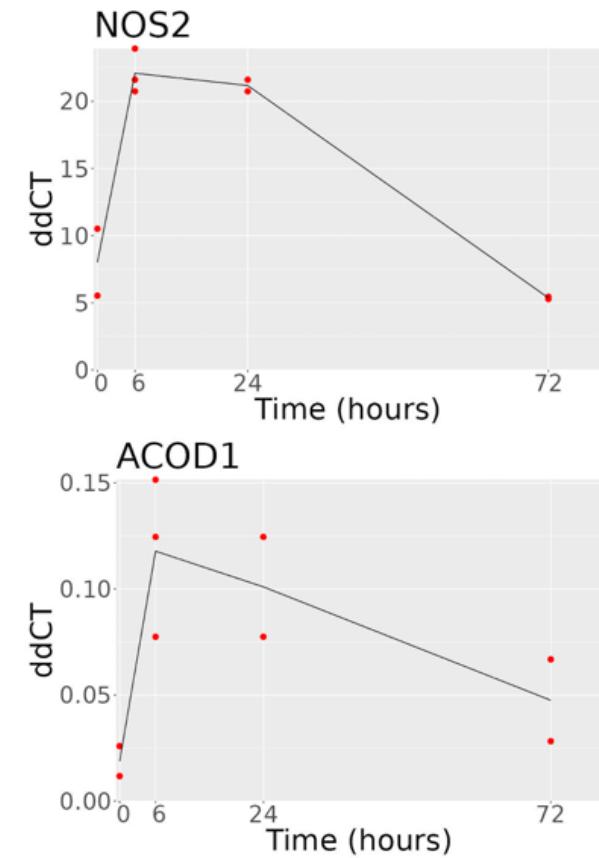
Karine Serre



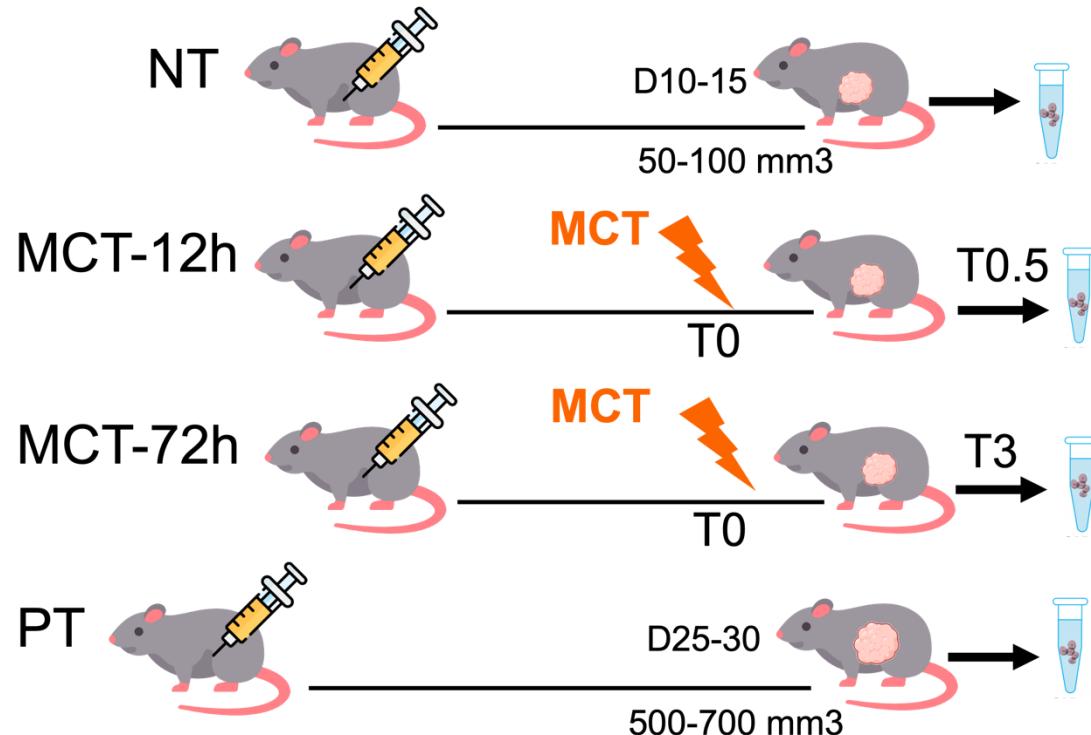
Carolina Jardim



Neuza Sousa



Experimental design



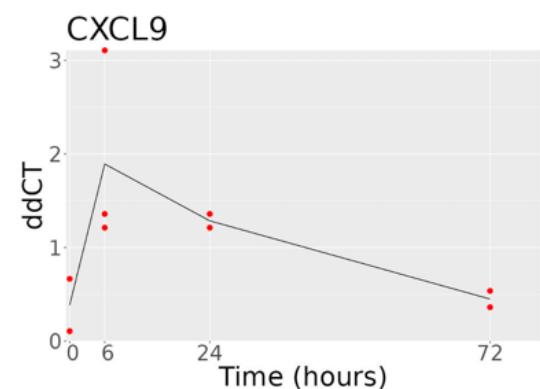
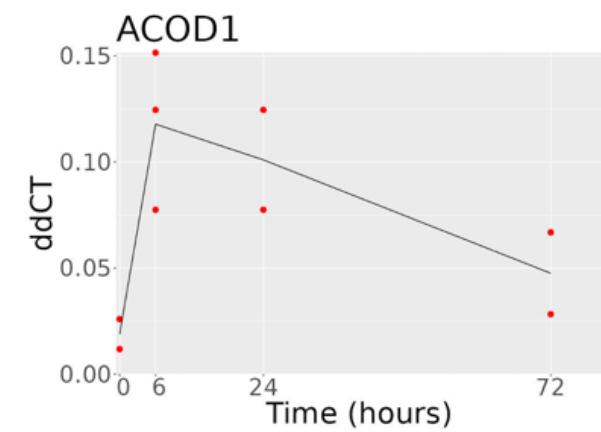
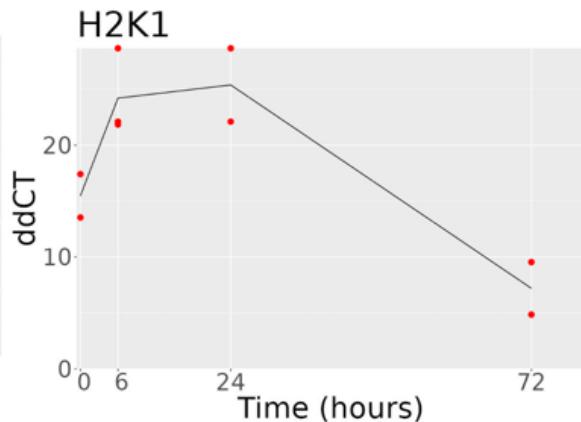
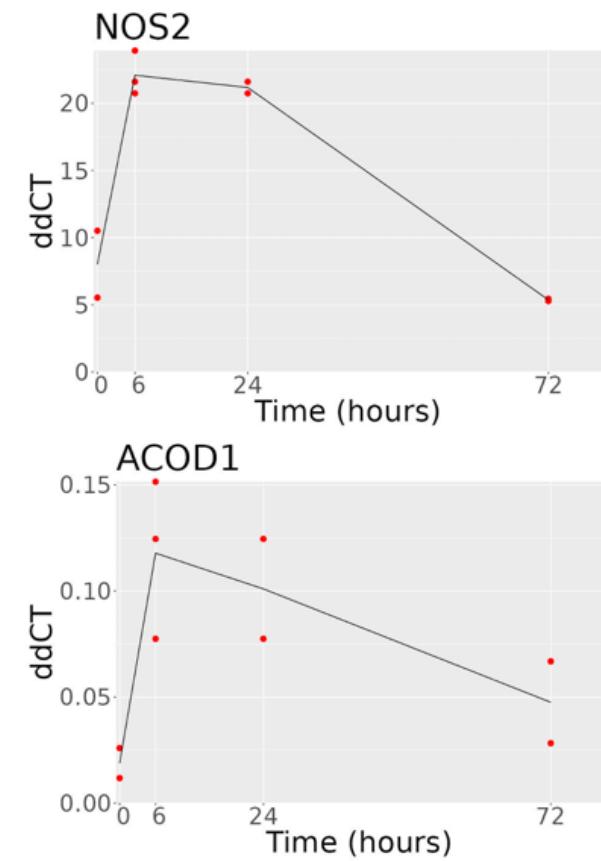
Karine Serre



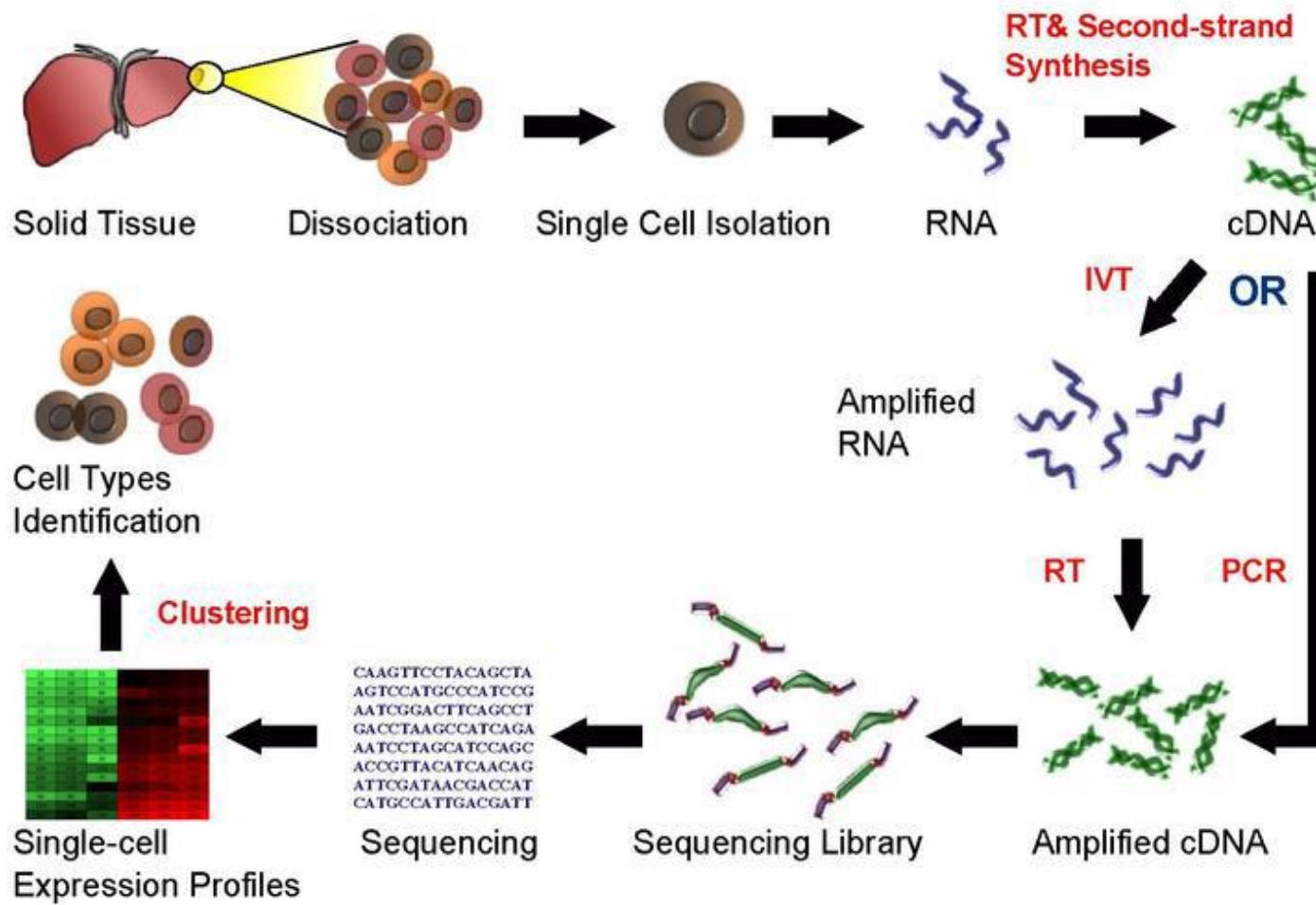
Carolina Jardim

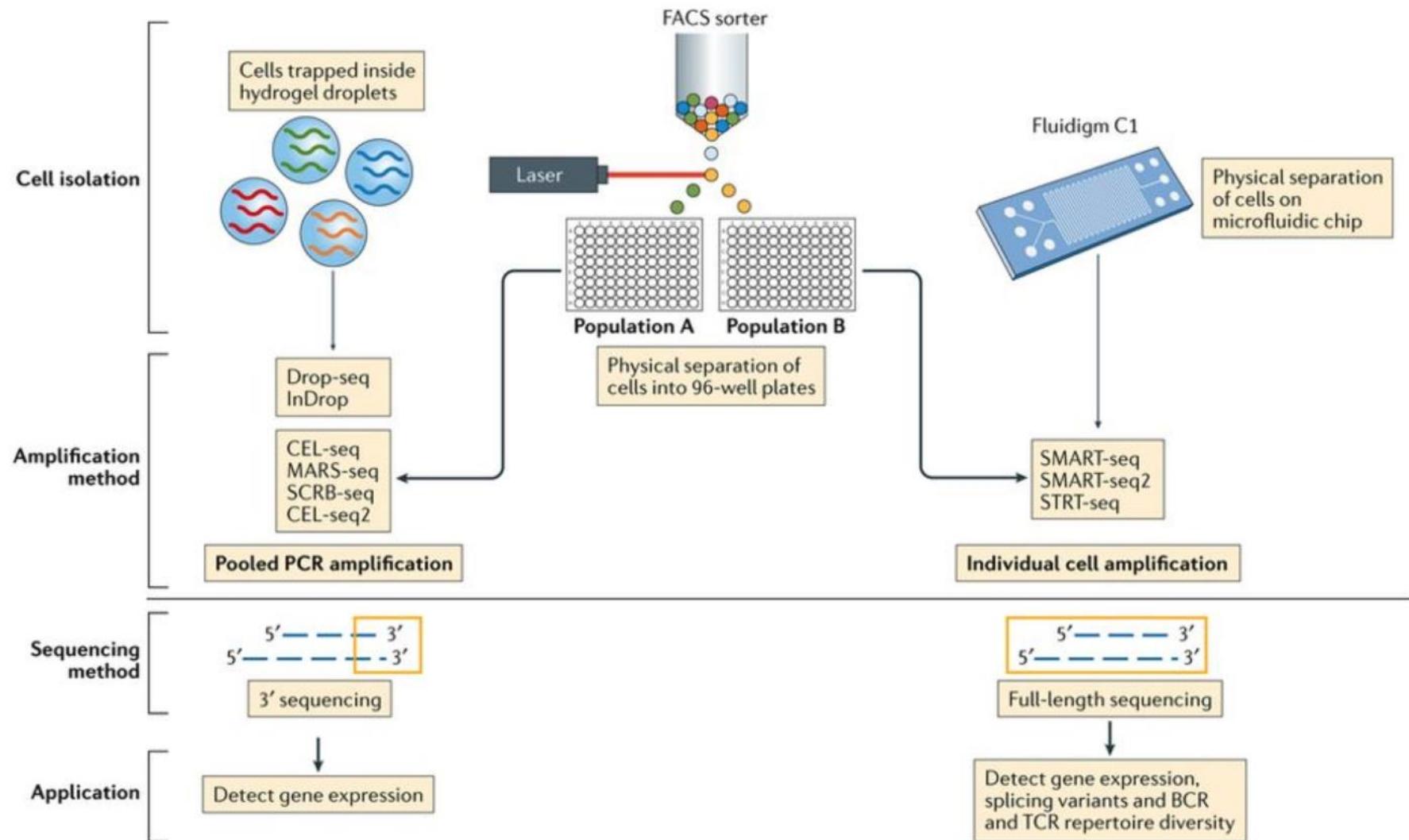


Neuza Sousa

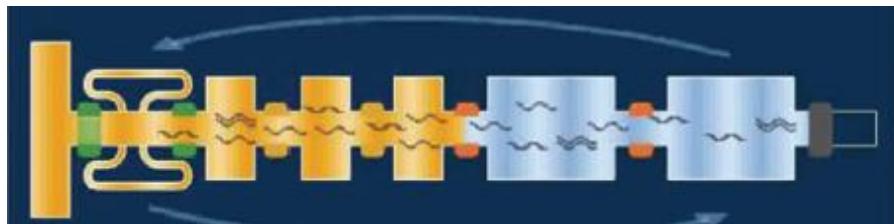
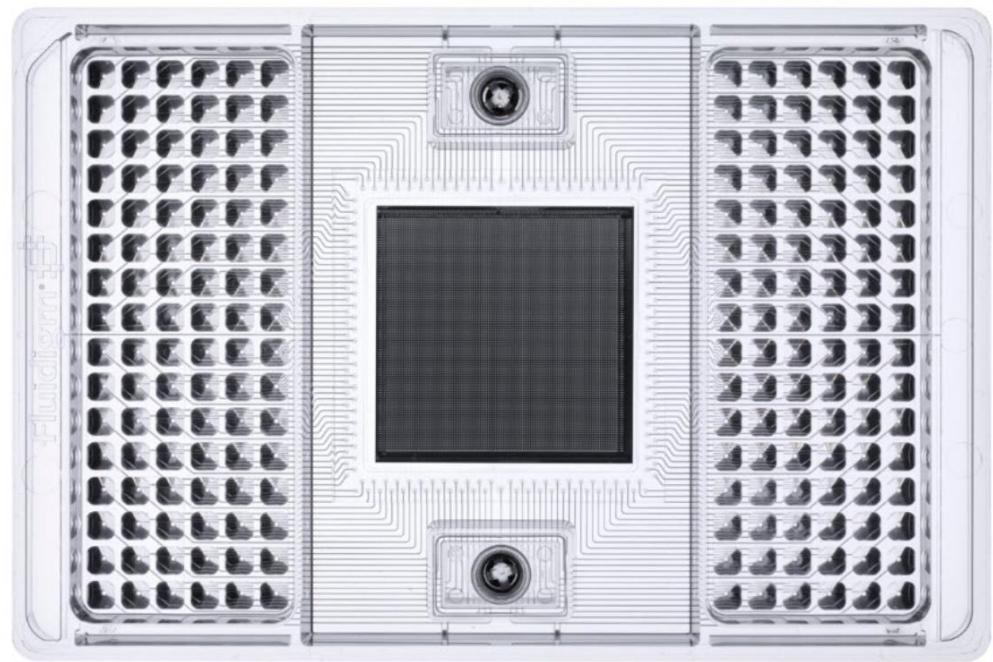
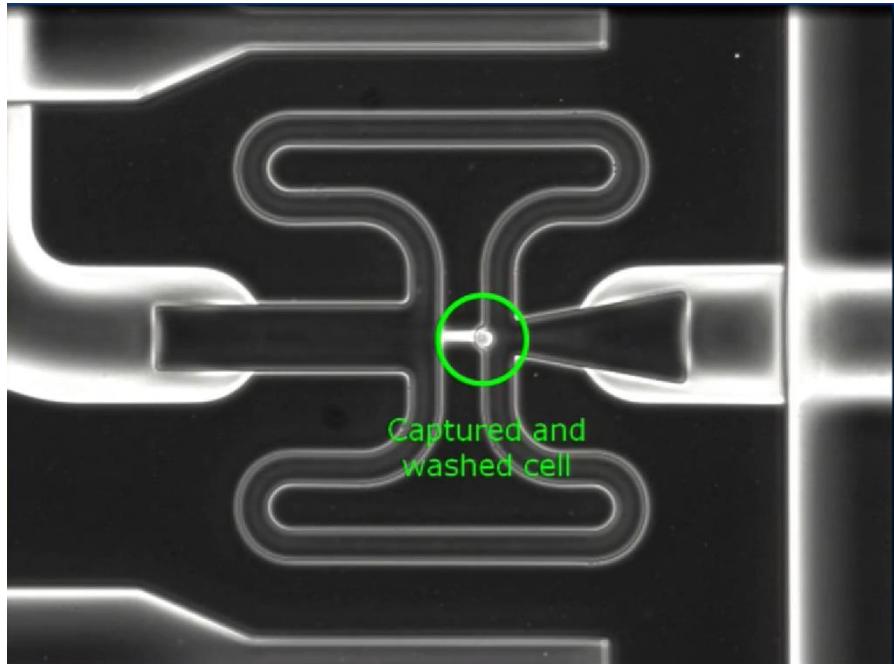


Typical workflow



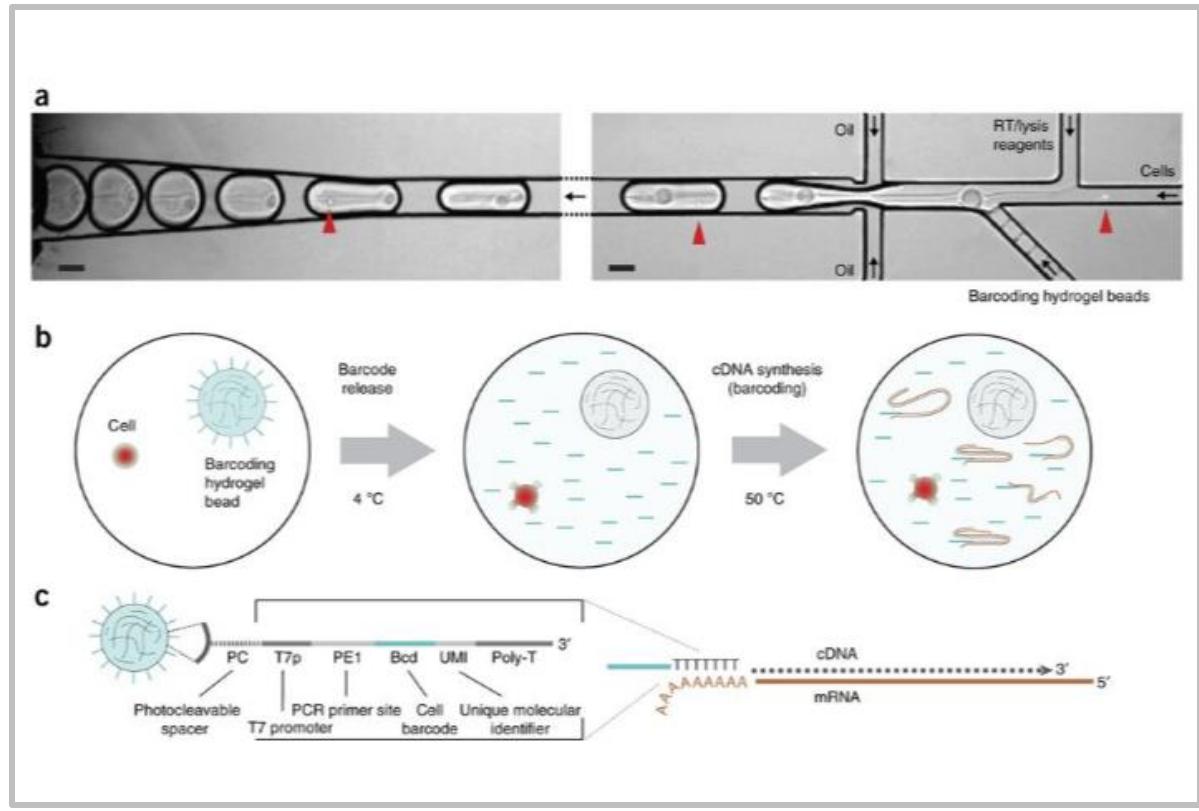


Microfluidics



Single-Cell Gene Expression Profiling using TaqMan® Gene Expression Assays with the C1™ Single-Cell Auto Prep System Technical Note

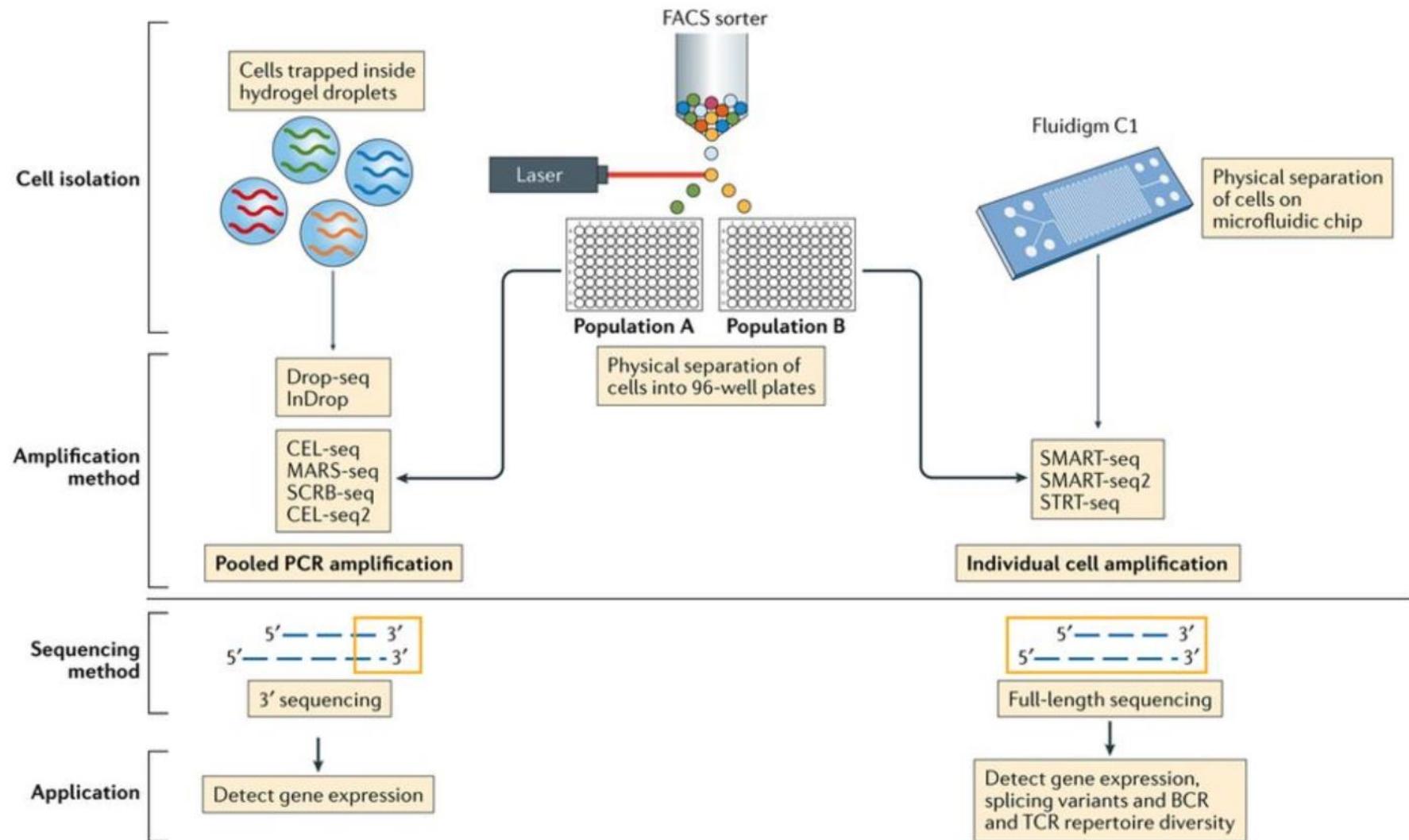
Microdroplet

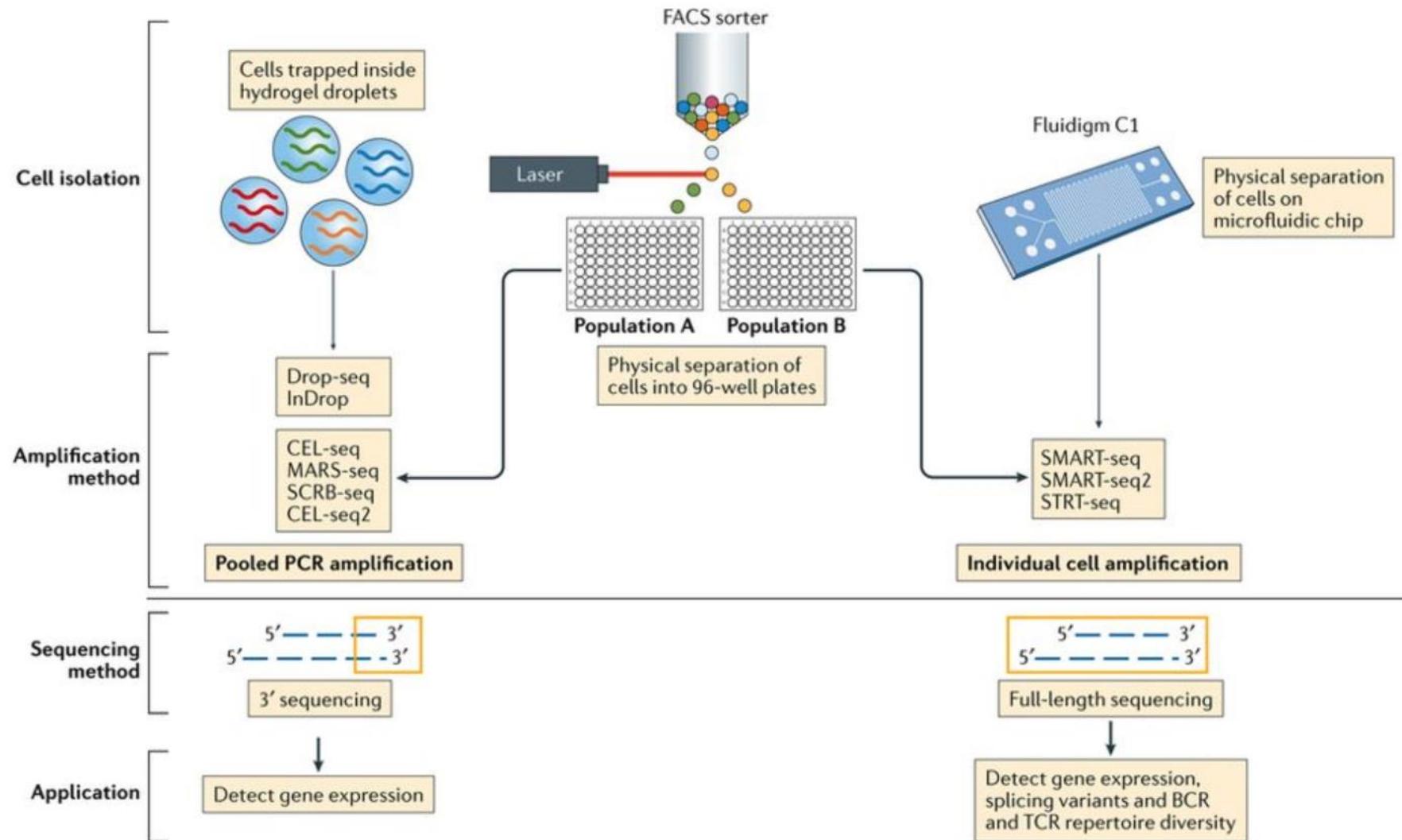


Zilionis, R., Nainys, J., Veres, A., Savova, V., Zemmour, D., Klein, A. and Mazutis, L. (2016). Single-cell barcoding and sequencing using droplet microfluidics. *Nature Protocols*, 12(1), pp.44-73.

Single-use microfluidics chip

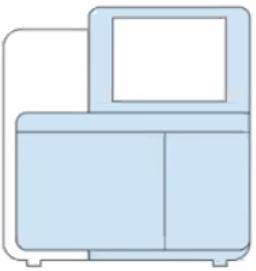
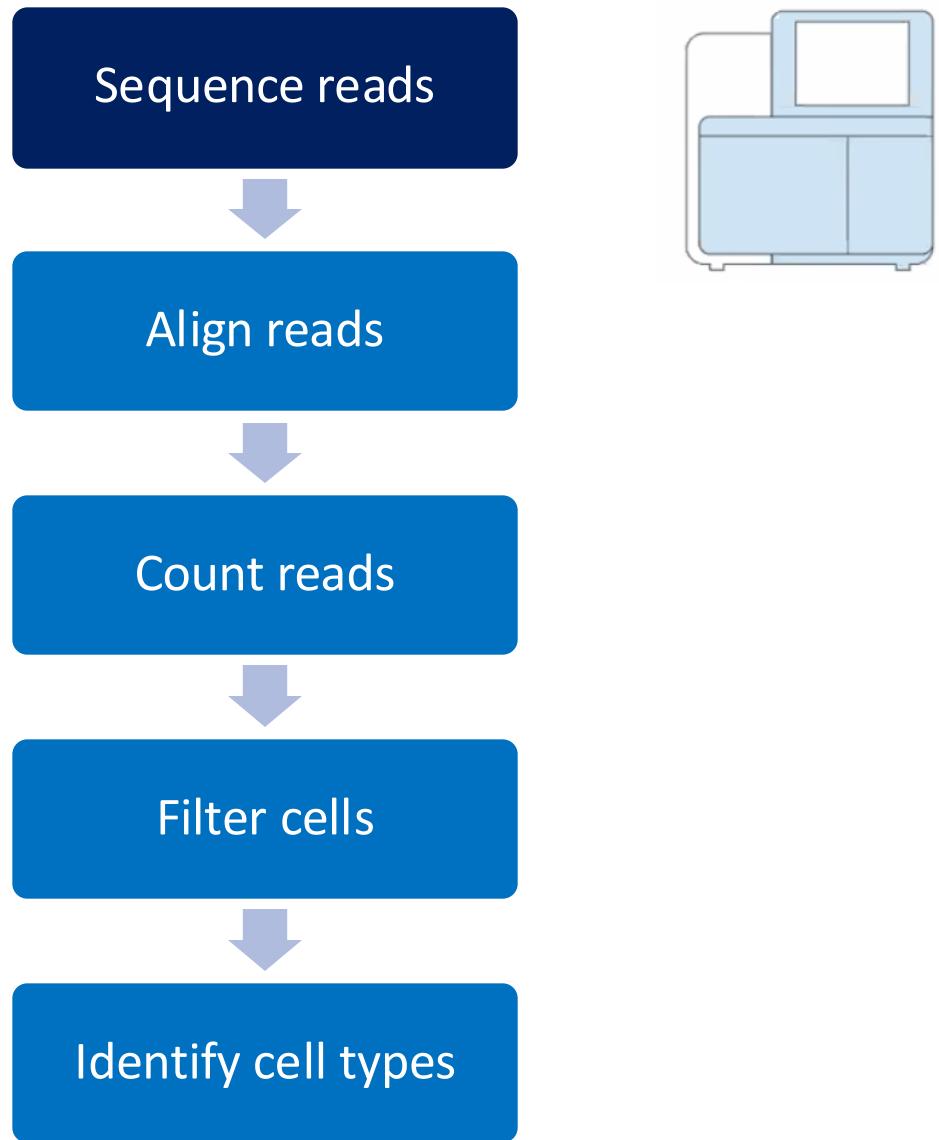




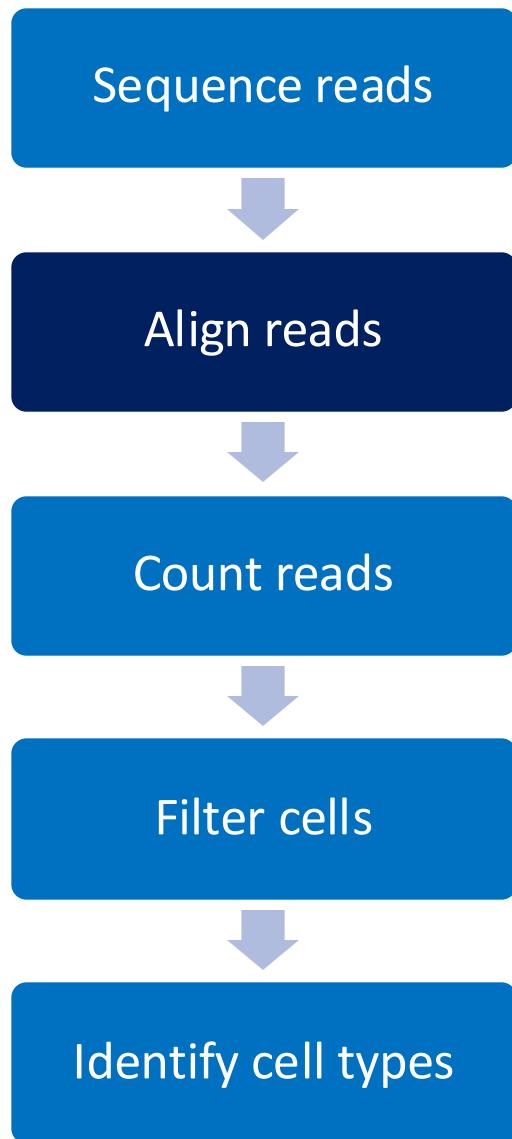


Different scRNA-seq protocols with different cell number and sequencing depth capacities can be used in an integrated approach

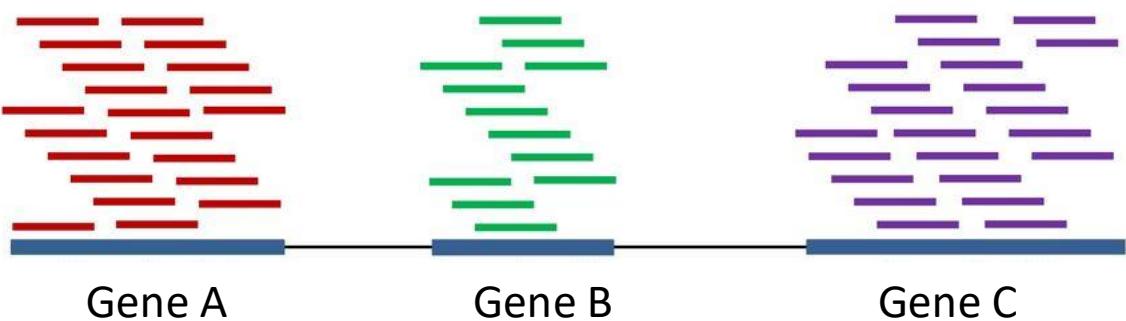
Computational analysis



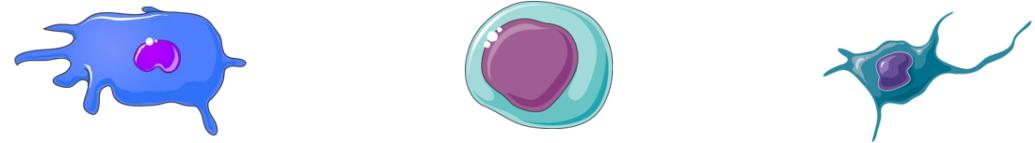
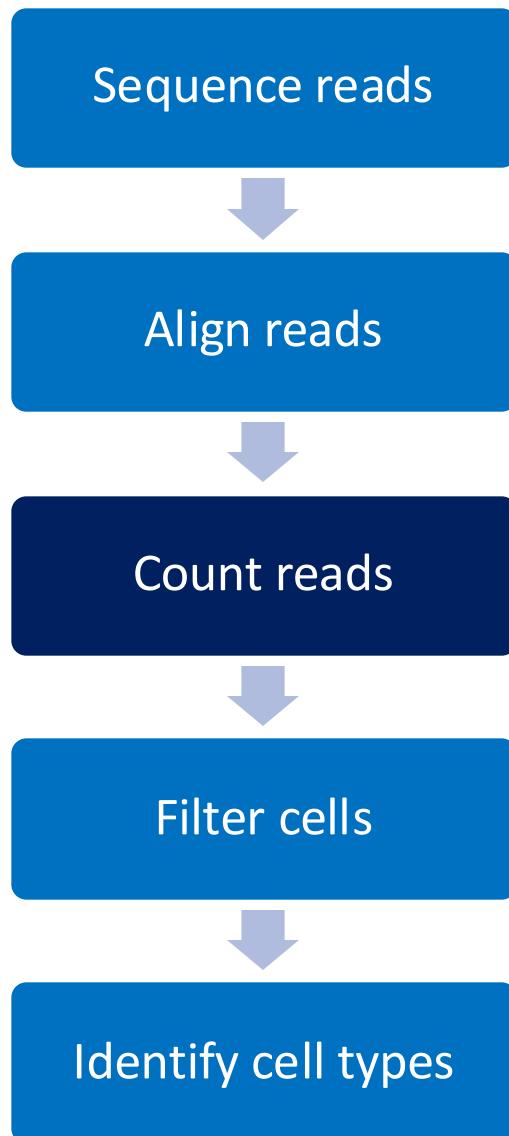
Computational analysis



Read Alignment and Counting

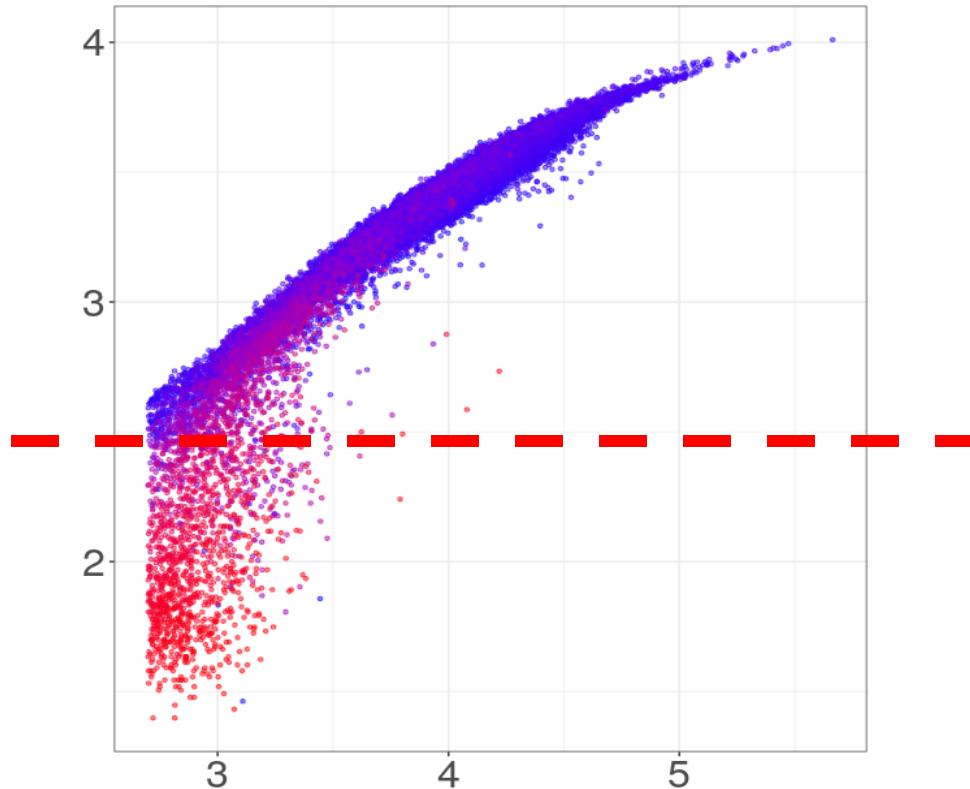
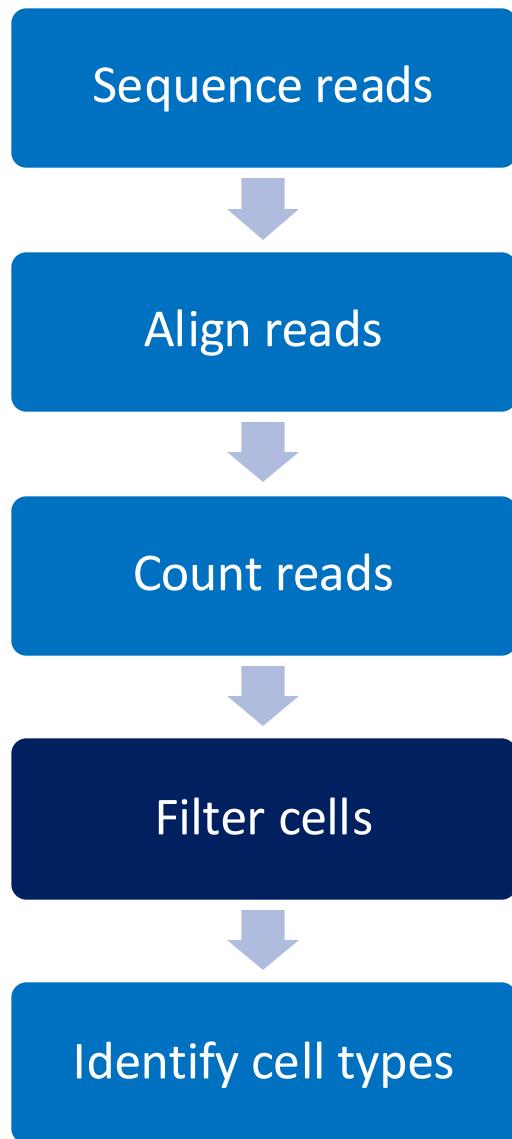


Computational analysis

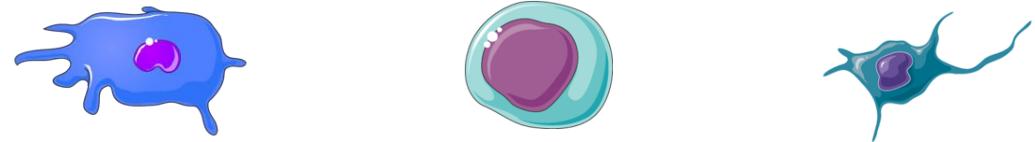
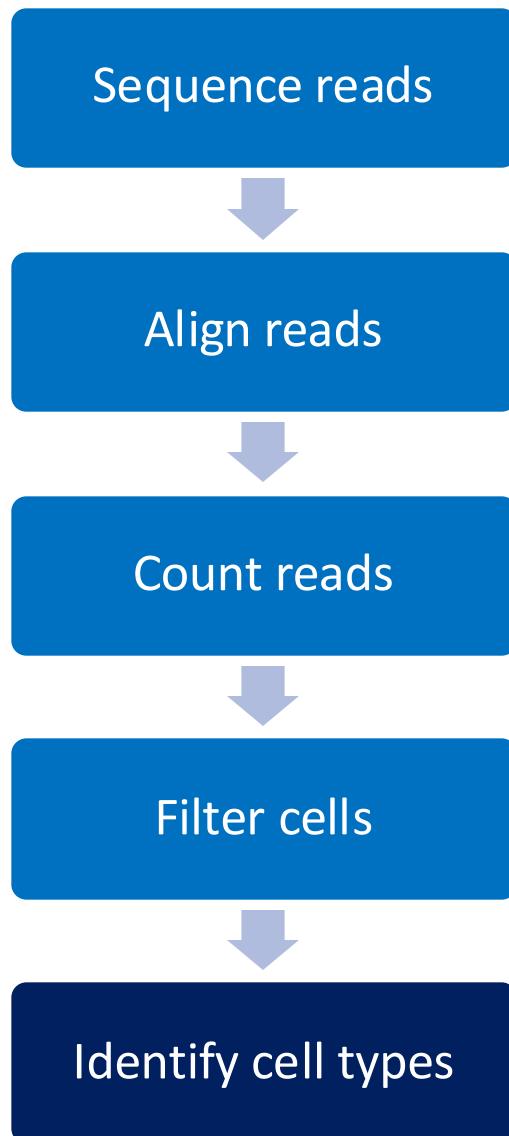


| Genes | Cell 1 | Cell 2 | Cell 3 | ... |
|-------|--------|--------|--------|-----|
| A | 0 | 0 | 0 | ... |
| B | 0 | 0 | 1 | ... |
| C | 0 | 10 | 2 | ... |
| D | 5 | 0 | 0 | ... |
| E | 1 | 3 | 0 | ... |
| F | 0 | 0 | 0 | ... |
| G | 2 | 1 | 1 | ... |
| H | 0 | 0 | 0 | ... |
| I | 0 | 22 | 5 | ... |
| ... | ... | ... | ... | ... |

Computational analysis

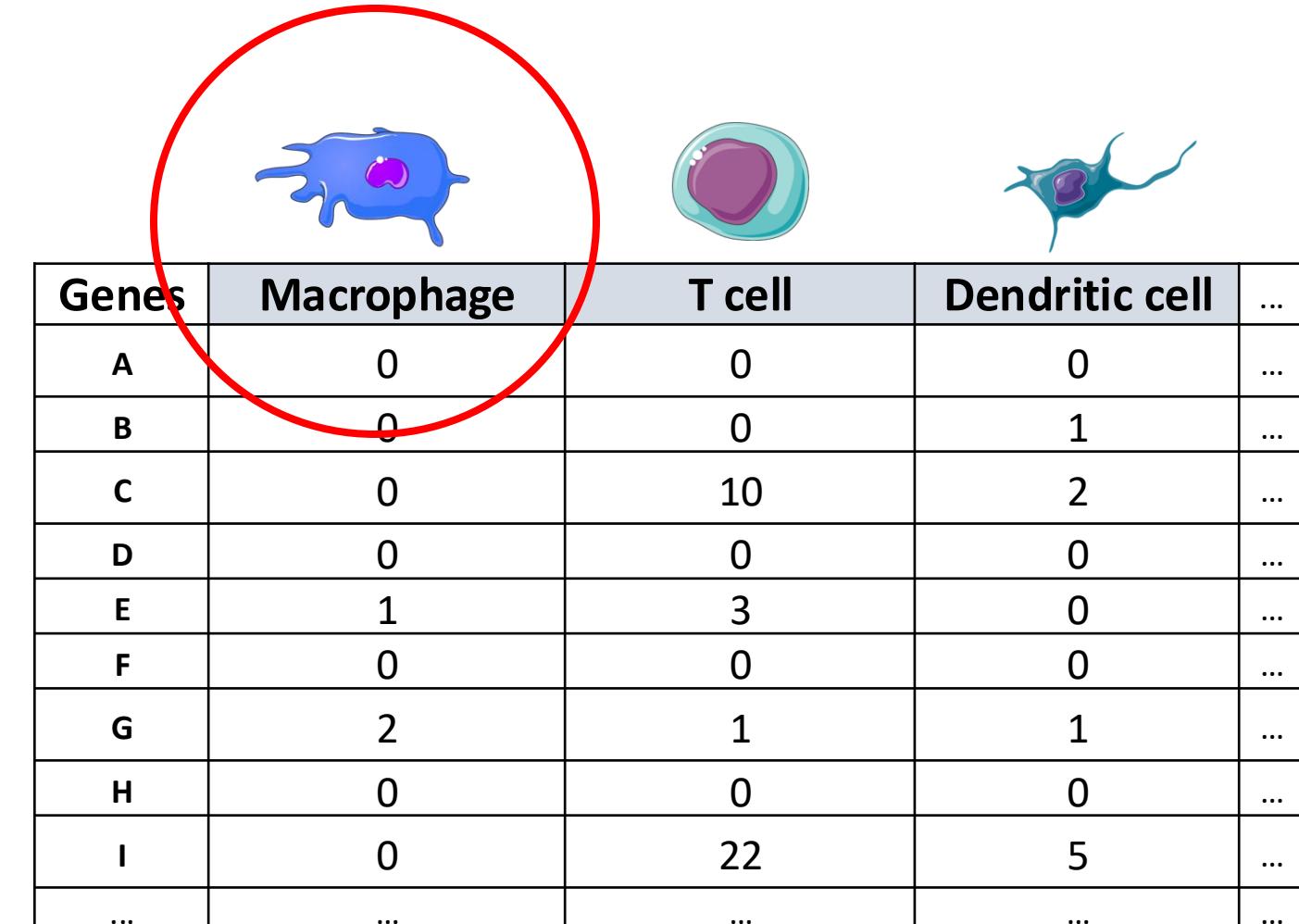
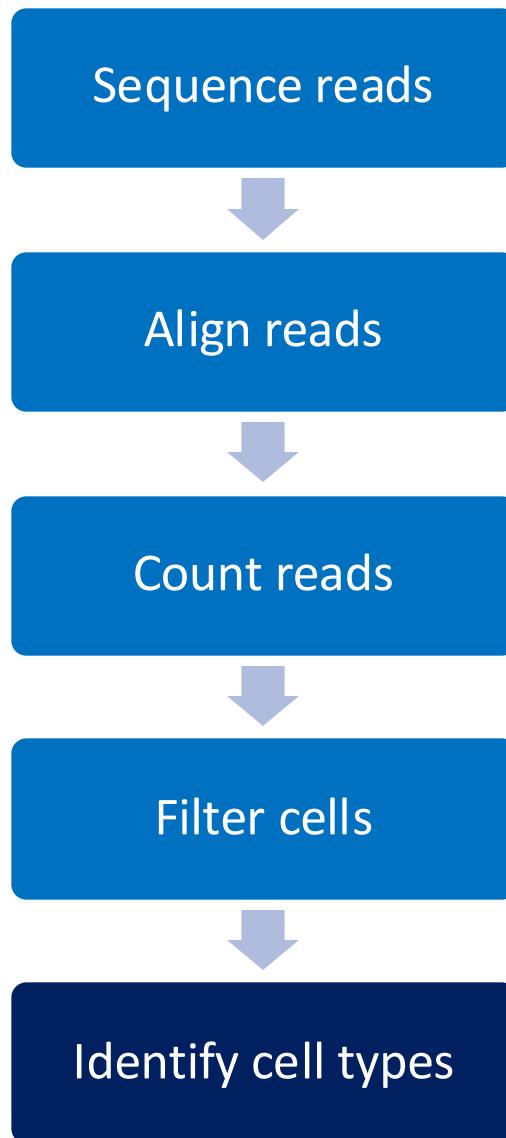


Computational analysis



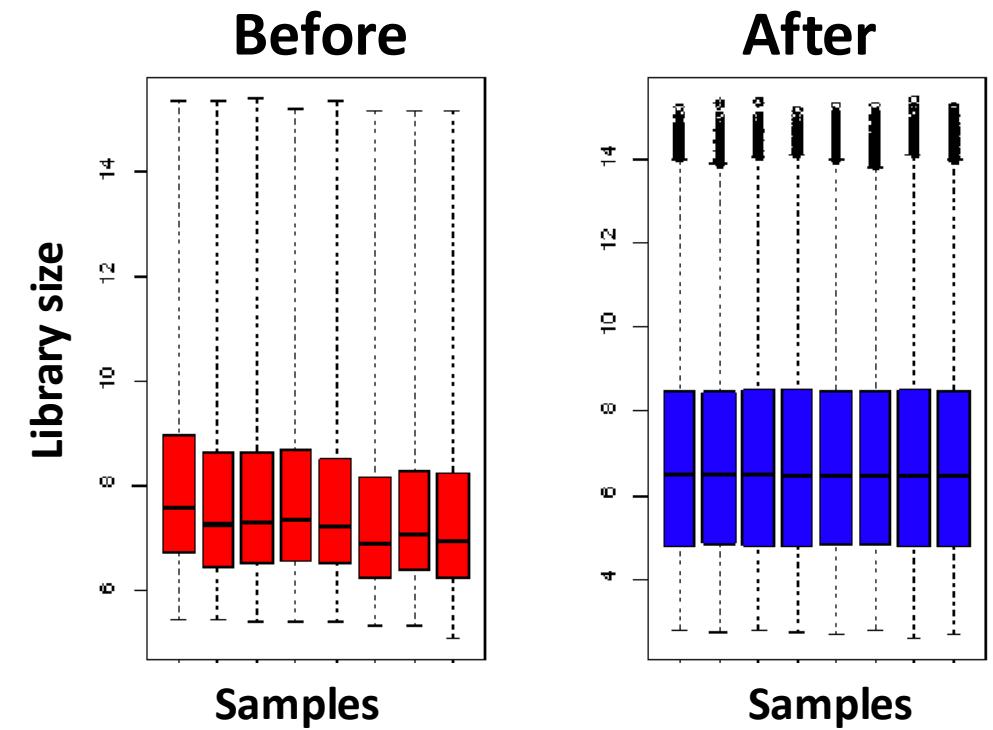
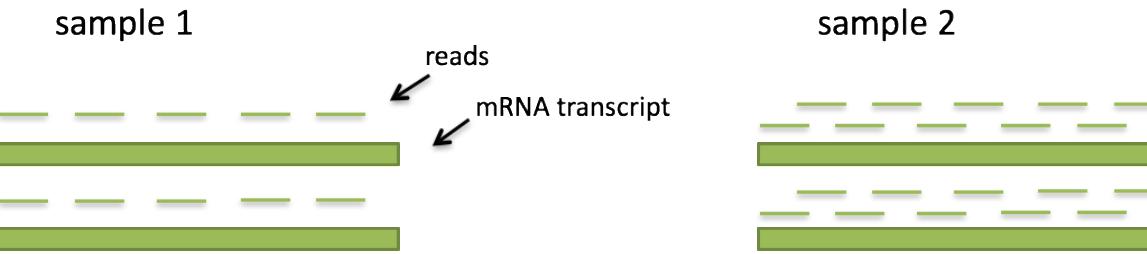
| Genes | Macrophage | T cell | Dendritic cell | ... |
|-------|------------|--------|----------------|-----|
| A | 0 | 0 | 0 | ... |
| B | 0 | 0 | 1 | ... |
| C | 0 | 10 | 2 | ... |
| D | 0 | 0 | 0 | ... |
| E | 1 | 3 | 0 | ... |
| F | 0 | 0 | 0 | ... |
| G | 2 | 1 | 1 | ... |
| H | 0 | 0 | 0 | ... |
| I | 0 | 22 | 5 | ... |
| ... | ... | ... | ... | ... |

Computational analysis

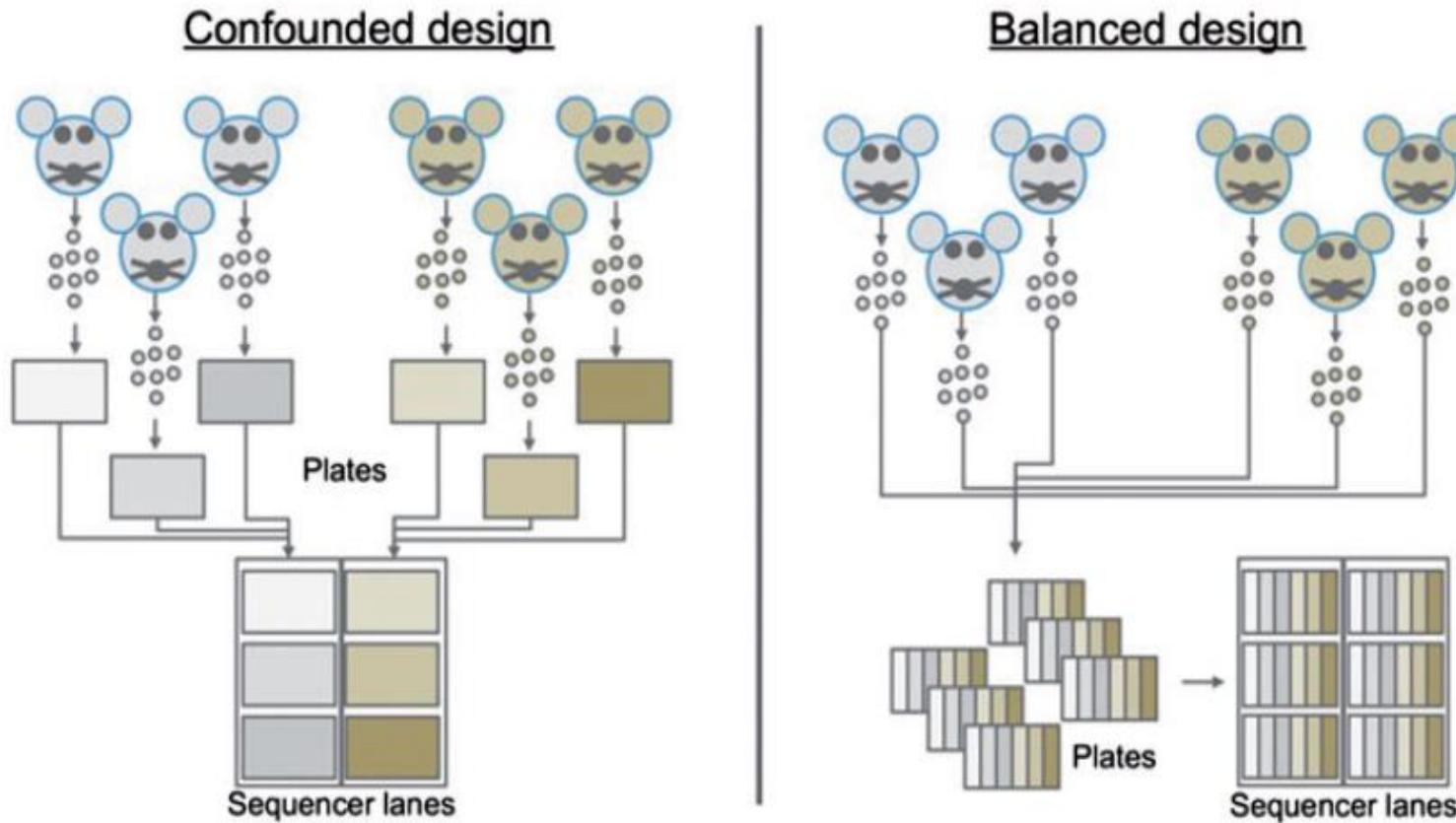


Library size normalization

Different sequencing depth

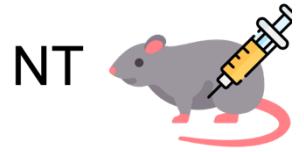


Batch effect

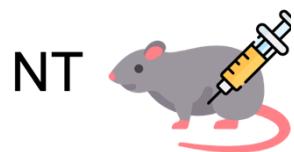


Sort cells from different biological conditions into different wells of the same plate.

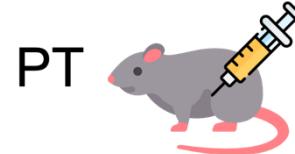
12/04/2019



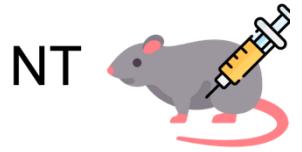
15/07/2019



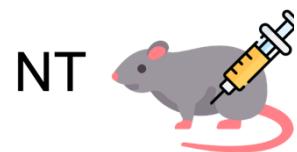
25/09/2019



12/04/2019



15/07/2019



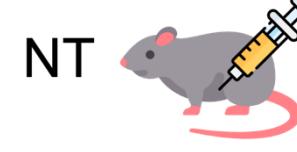
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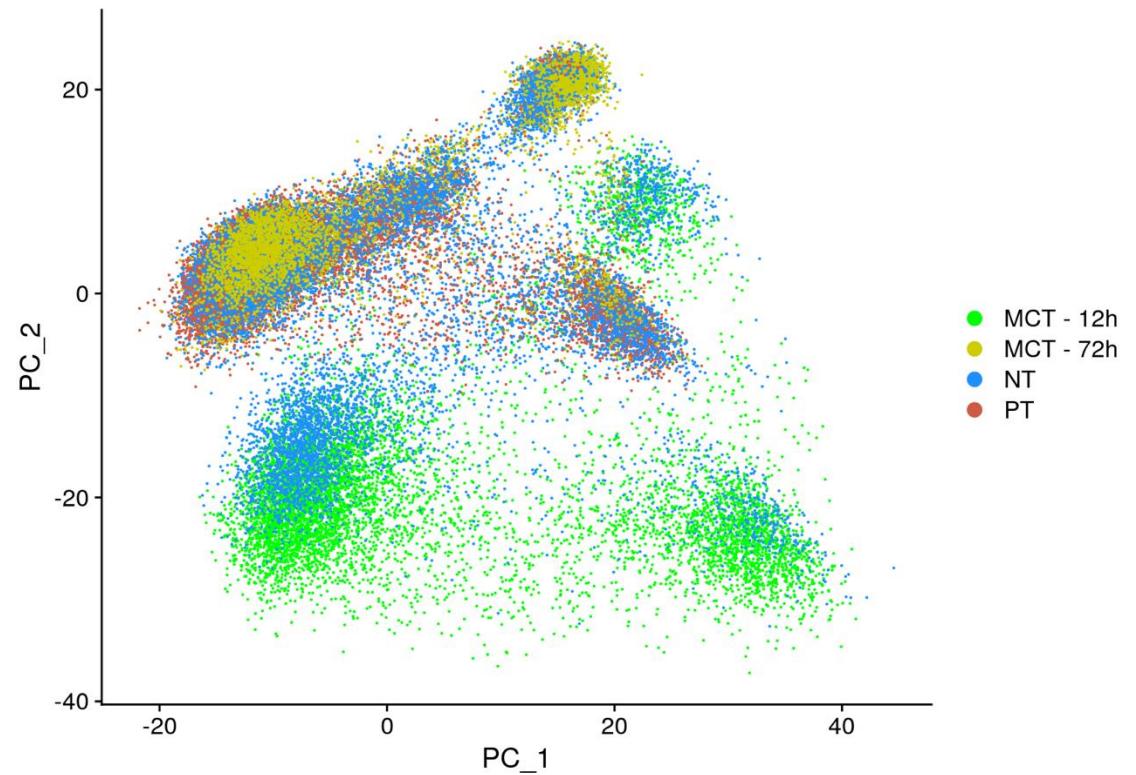
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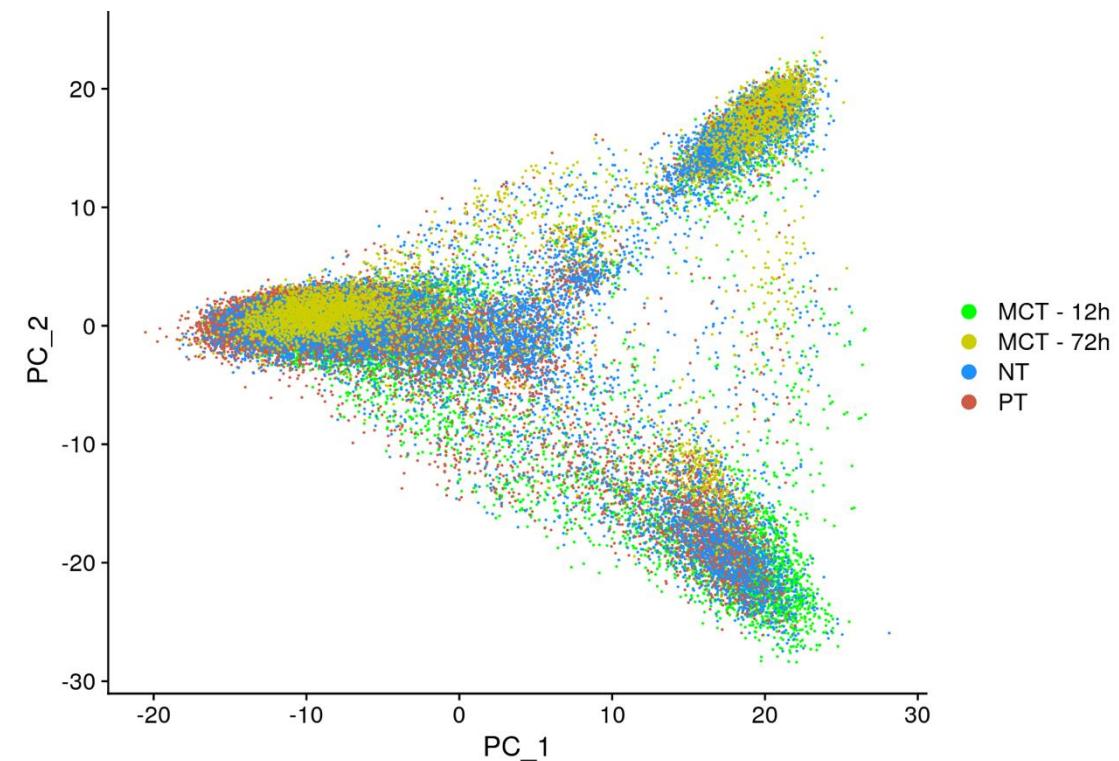
15/05/2023



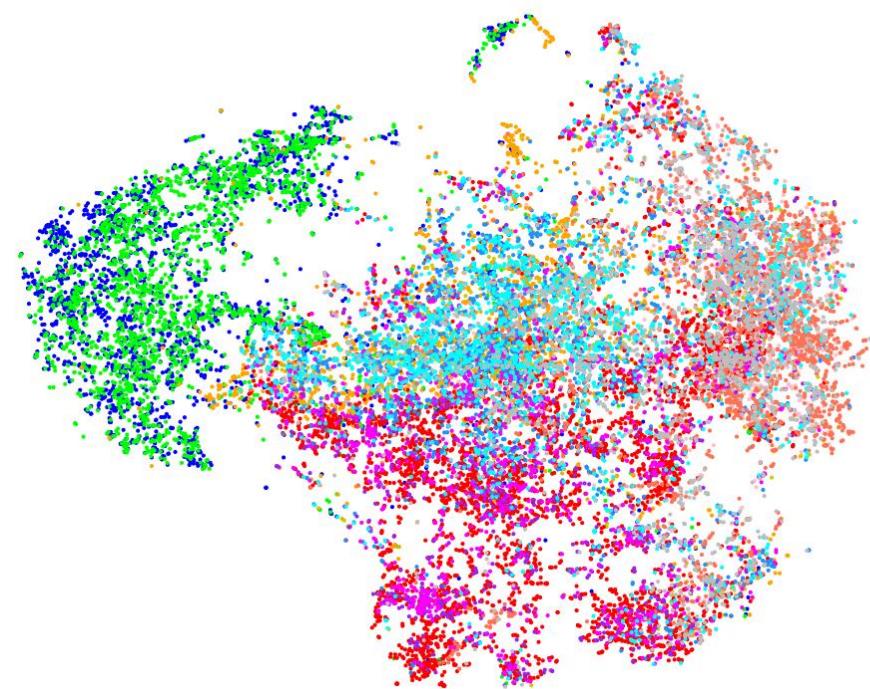
Before correction



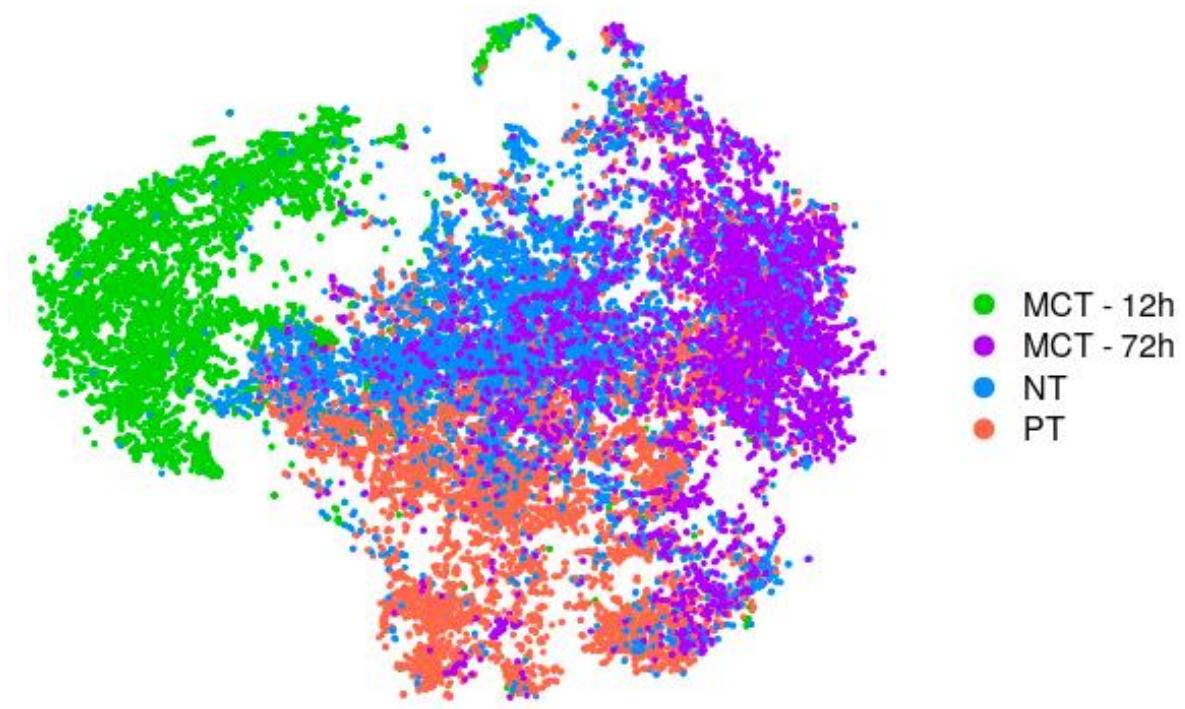
After correction



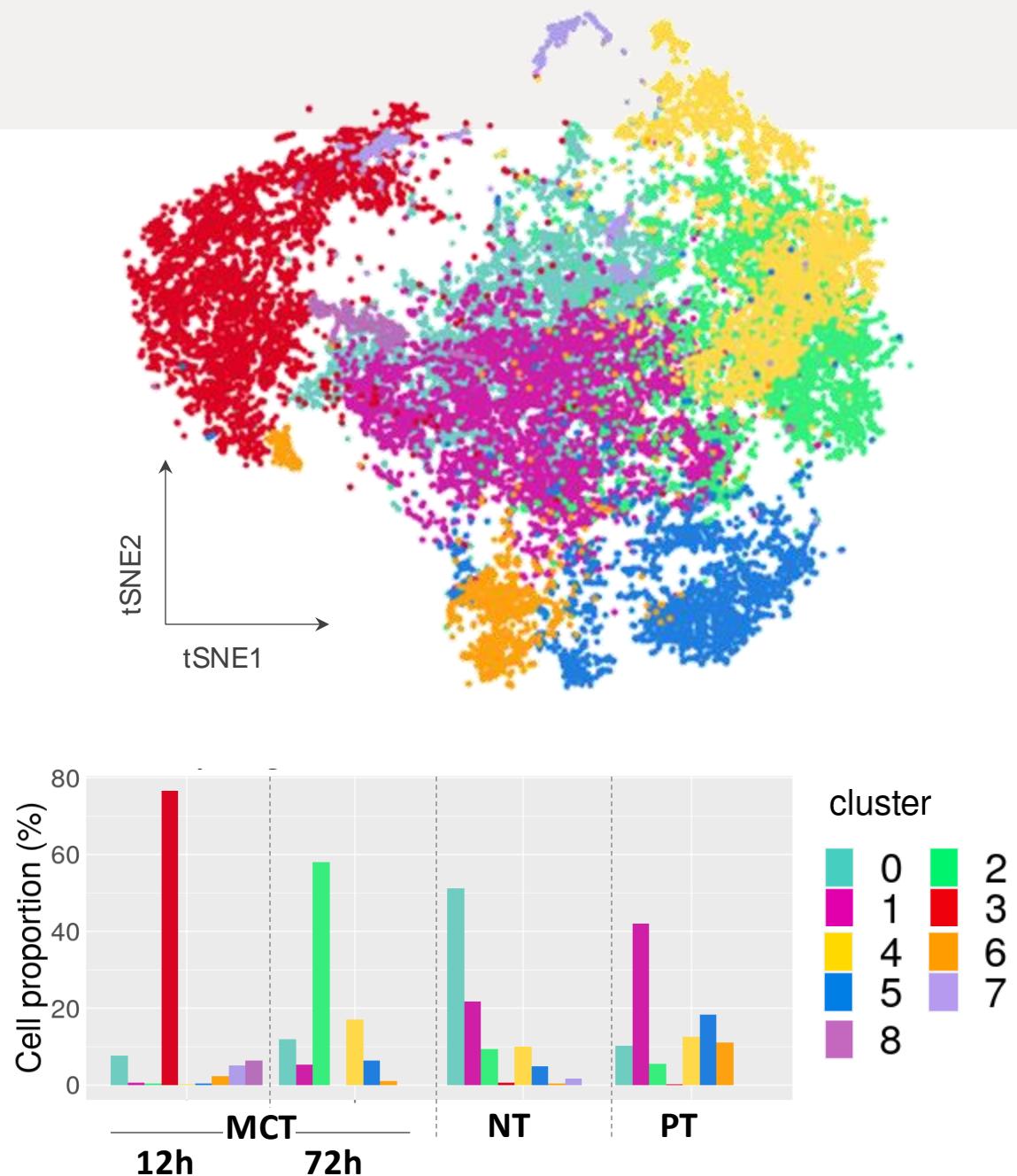
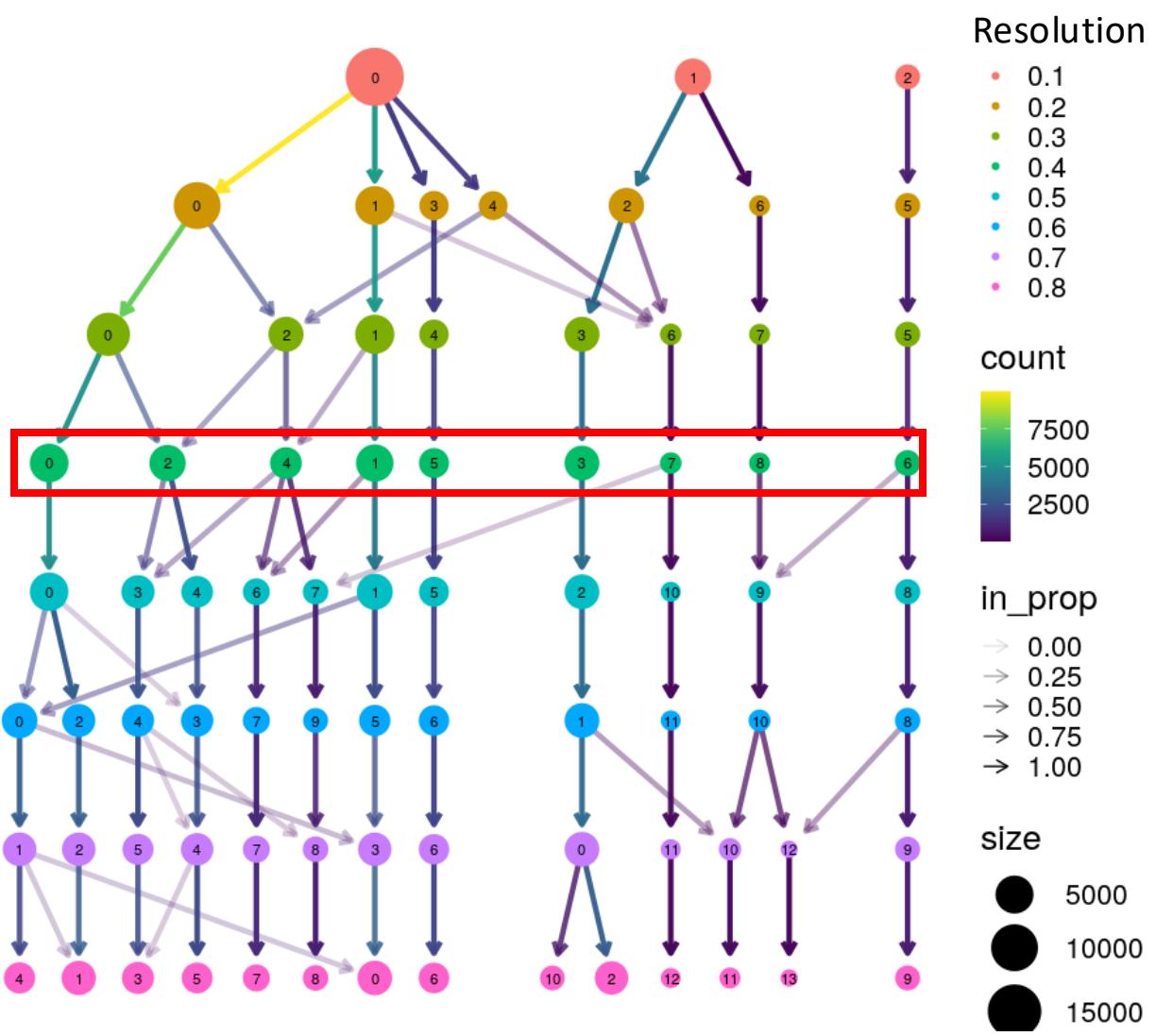
scRNA-seq data analysis of tumour-infiltrating macrophages



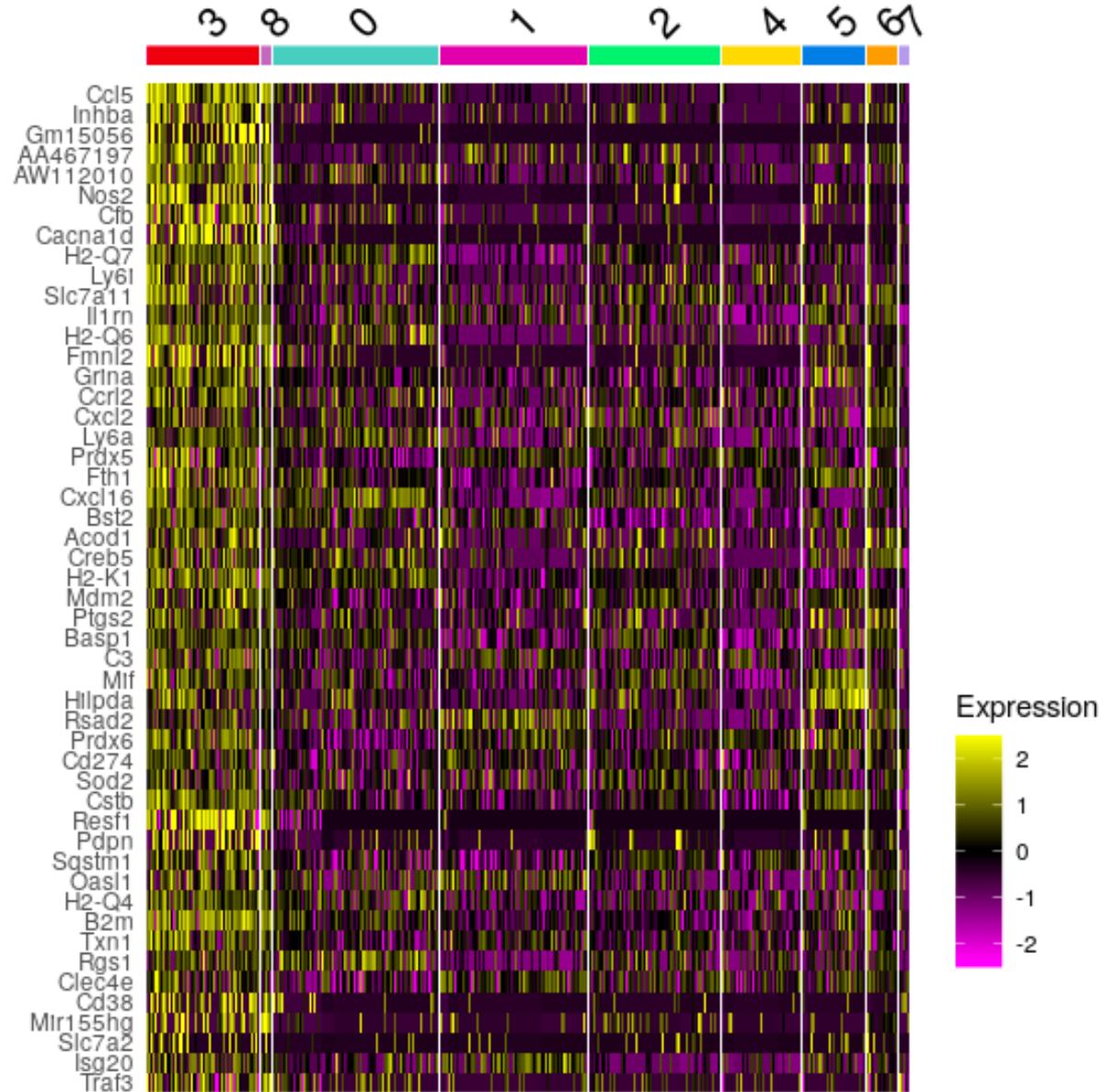
- MCT1-12h
- MCT3-12h
- NT1
- S1-NT
- S2-MCT-72h
- S3-PT
- S4-NT
- S5-MCT-72h
- S6-PT
- S7-PT
- S8-NT
- S9-MCT-72h



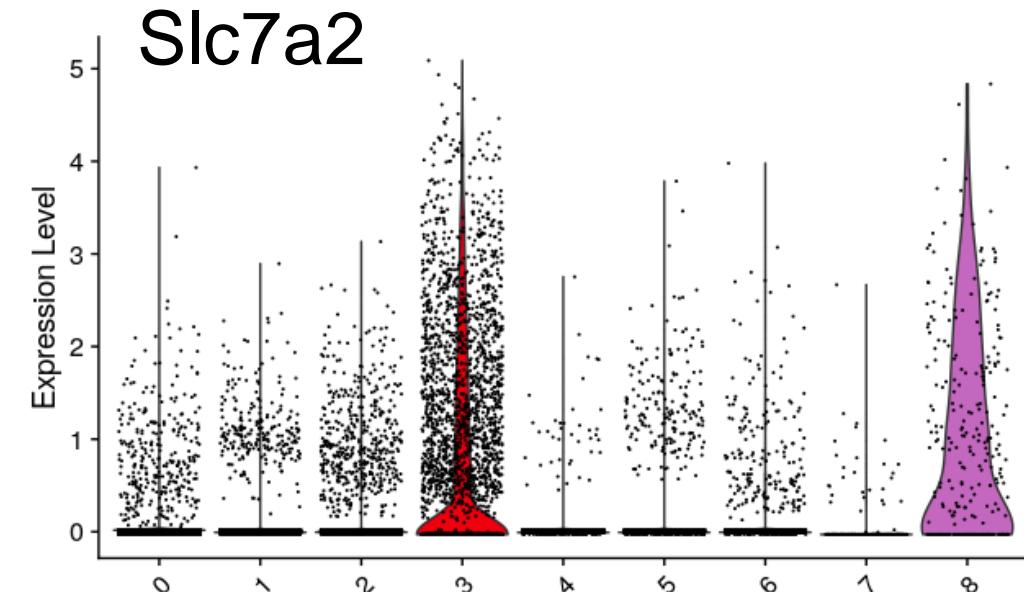
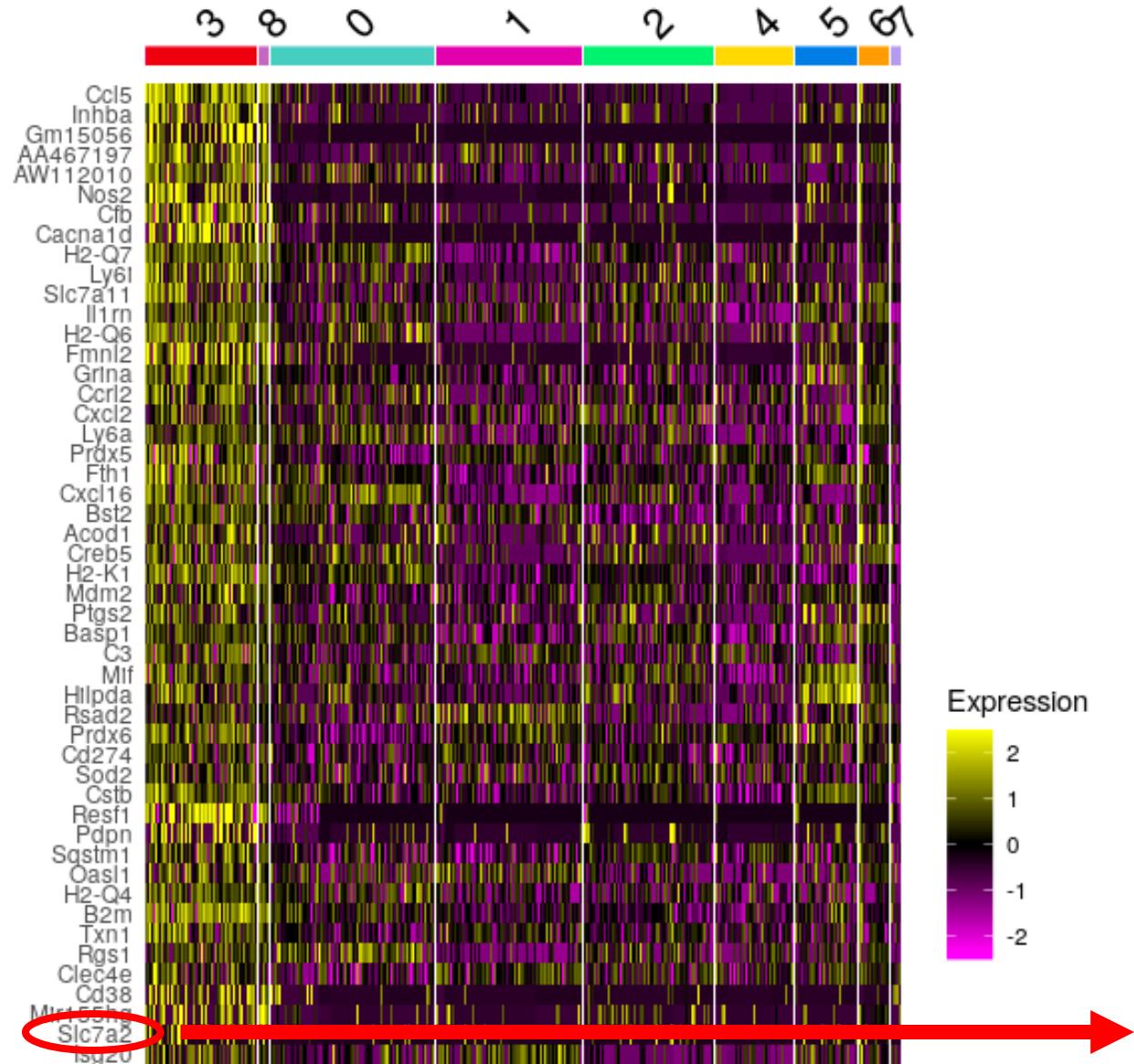
Clustering analysis



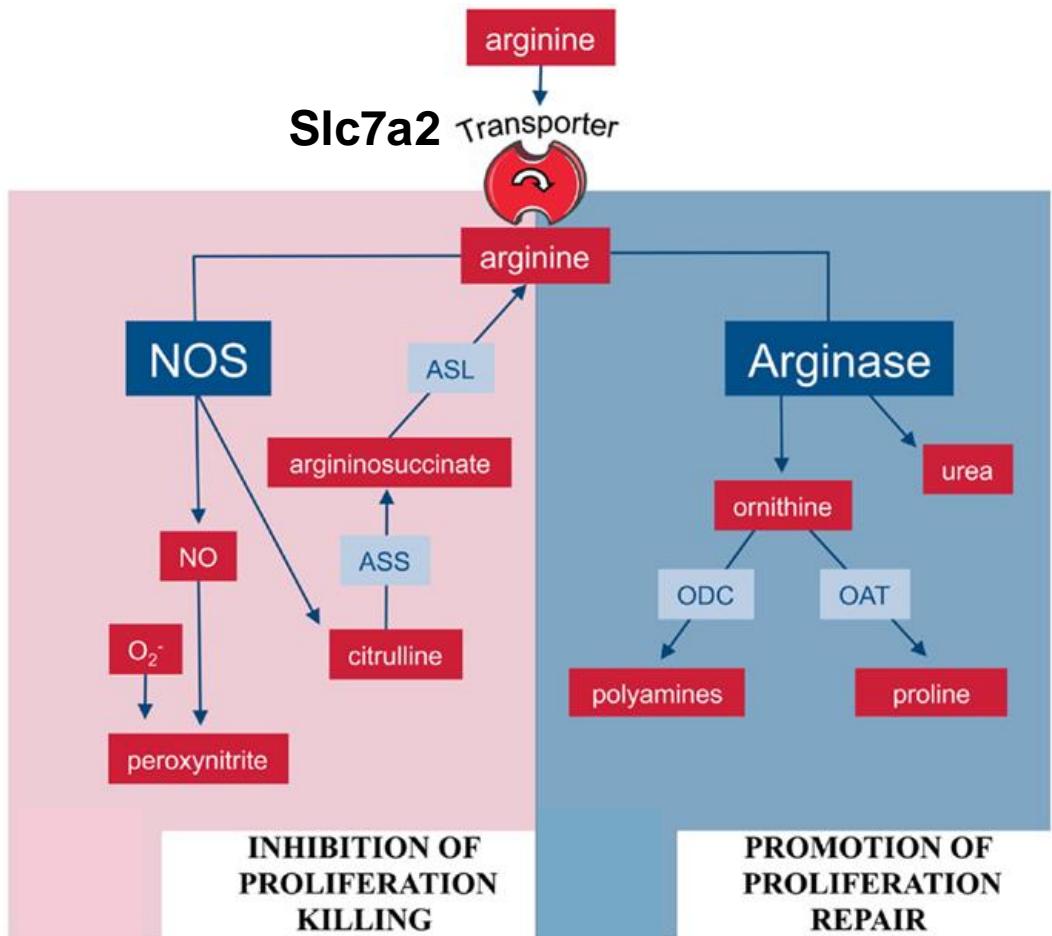
Upregulated genes in MCT-12h vs others



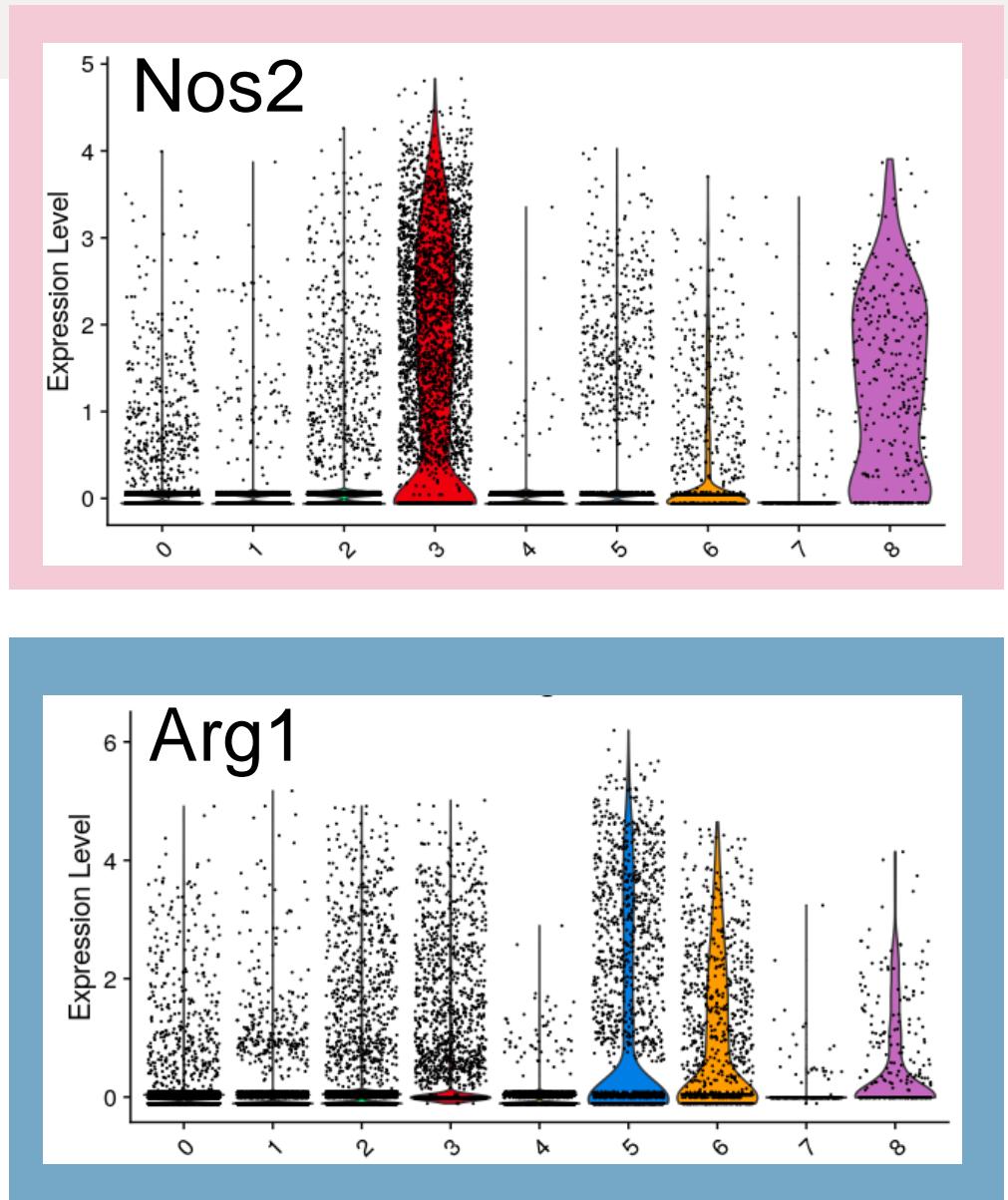
Upregulated genes in MCT-12h vs others



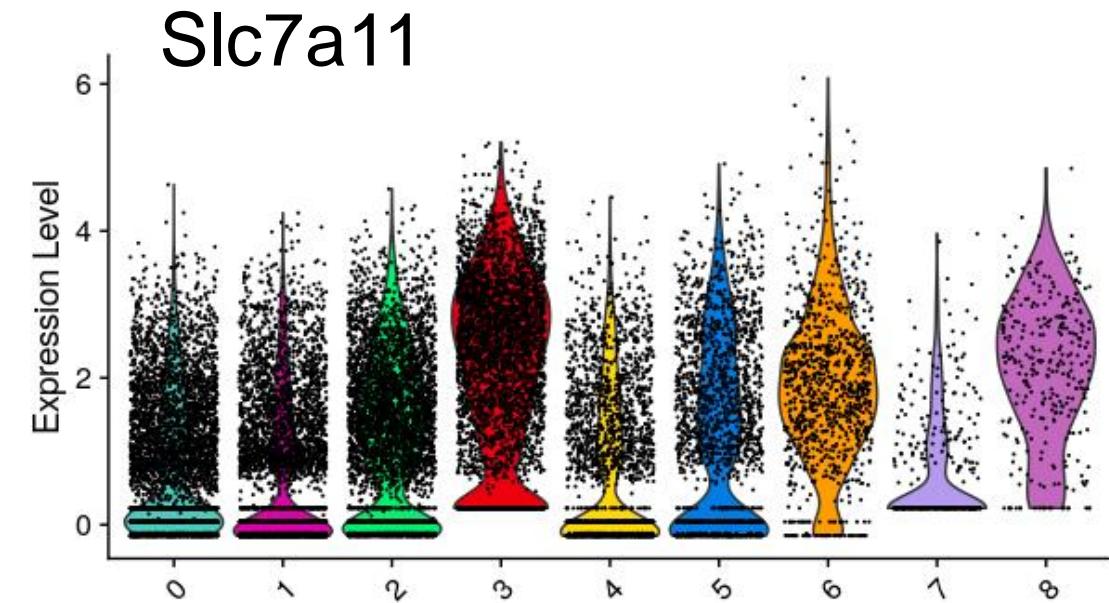
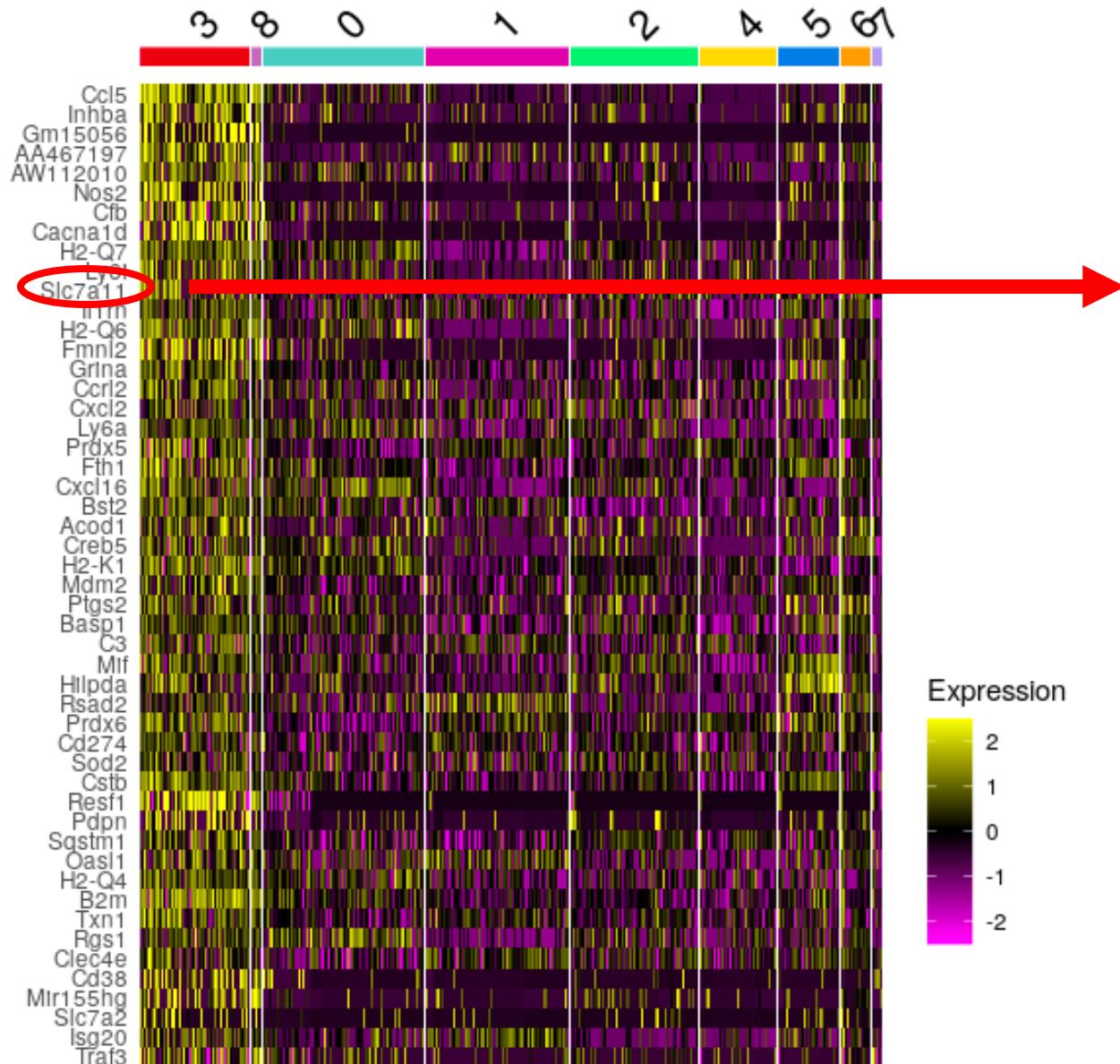
Arginine intake



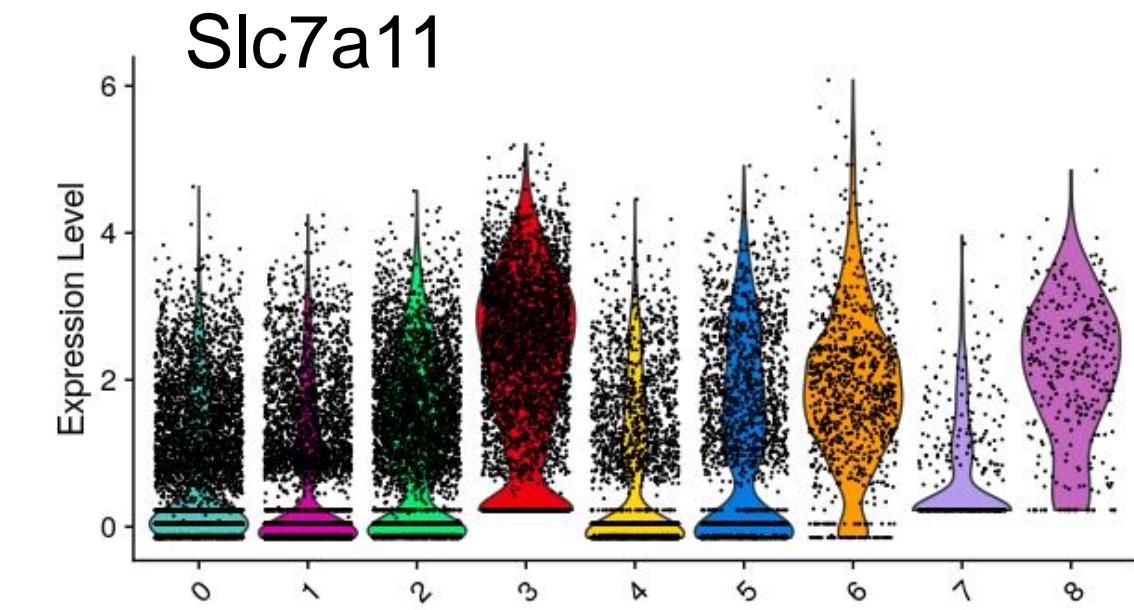
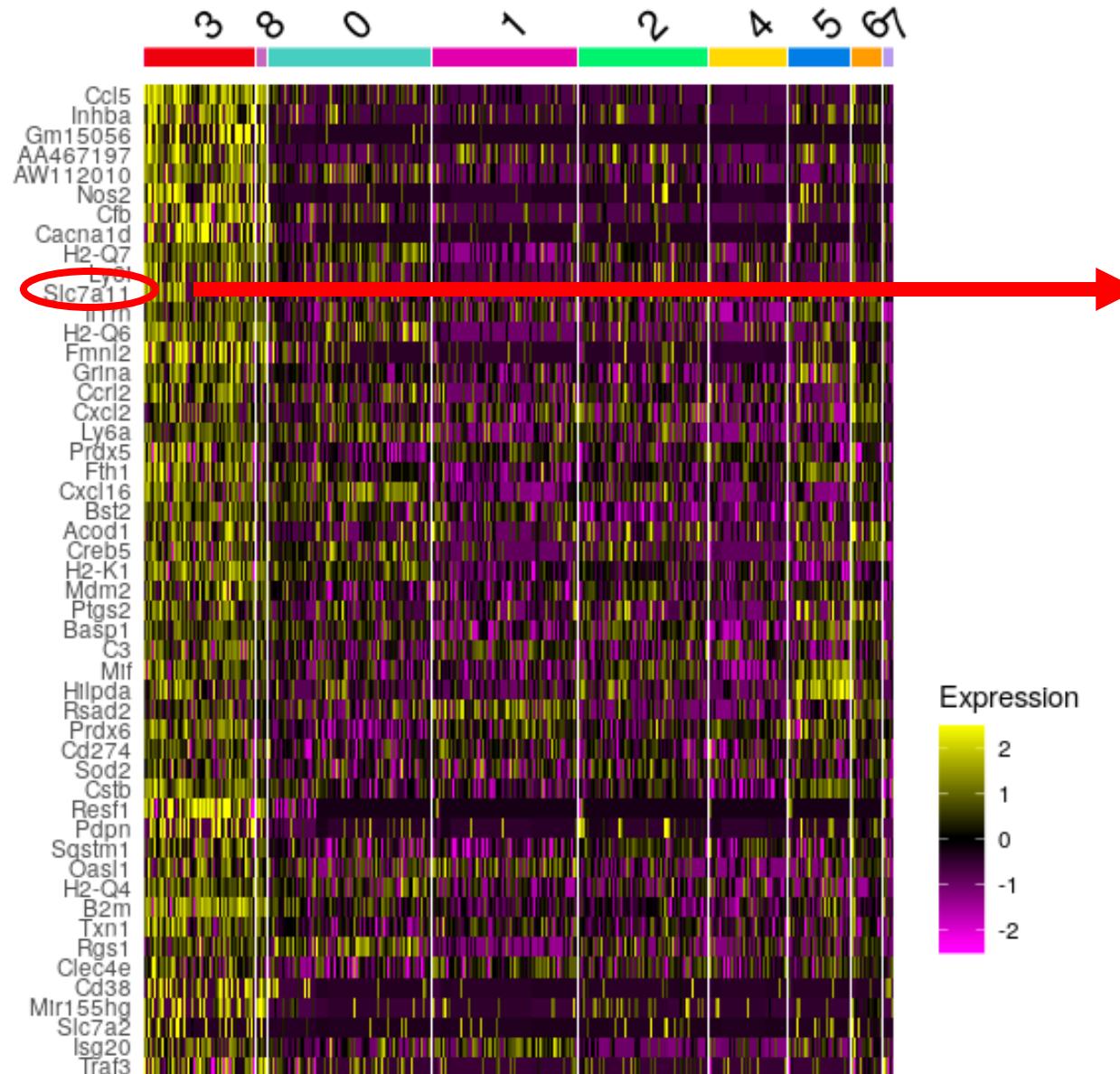
Rath et al., 2014



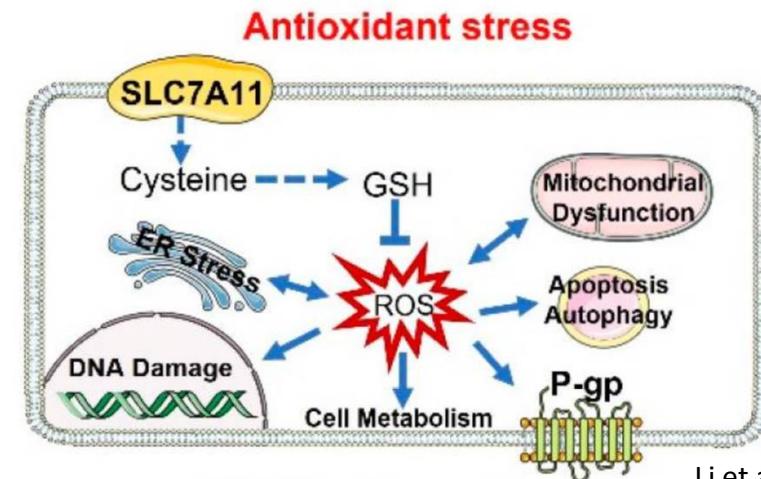
Upregulated genes in MCT-12h vs others



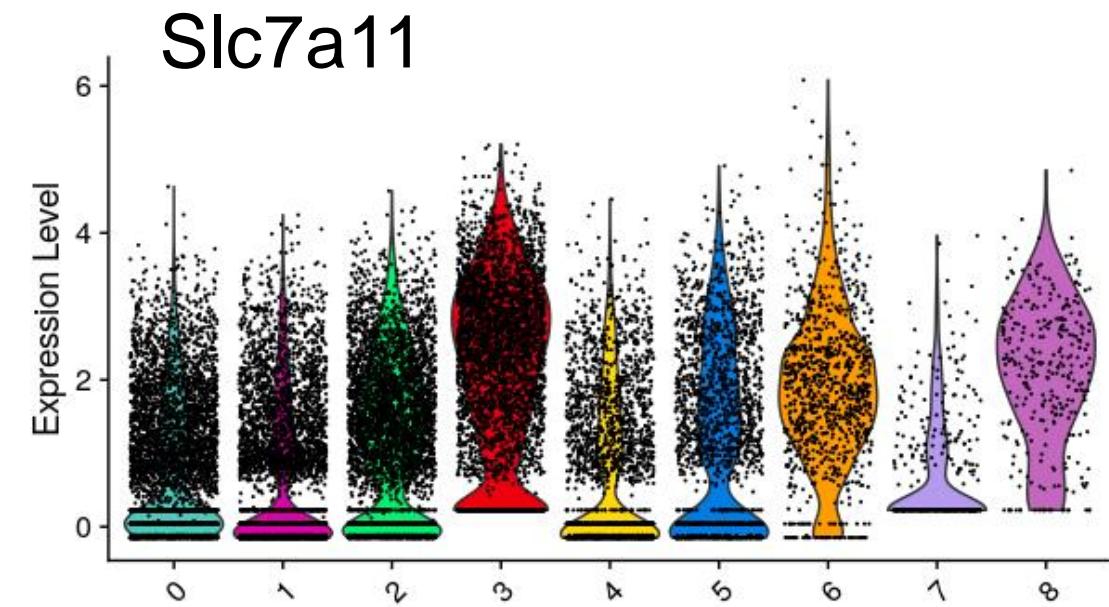
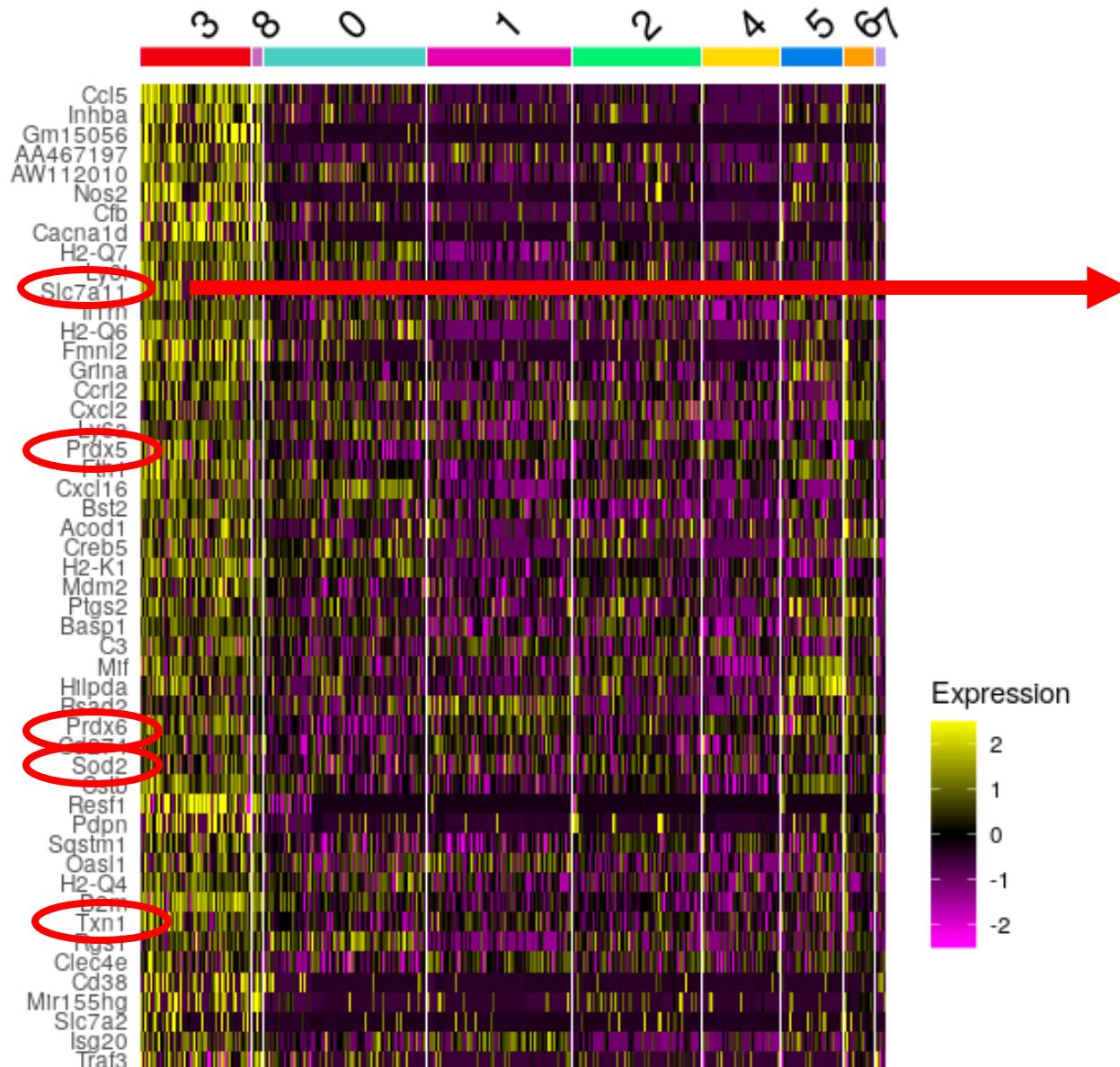
Upregulated genes in MCT-12h vs others



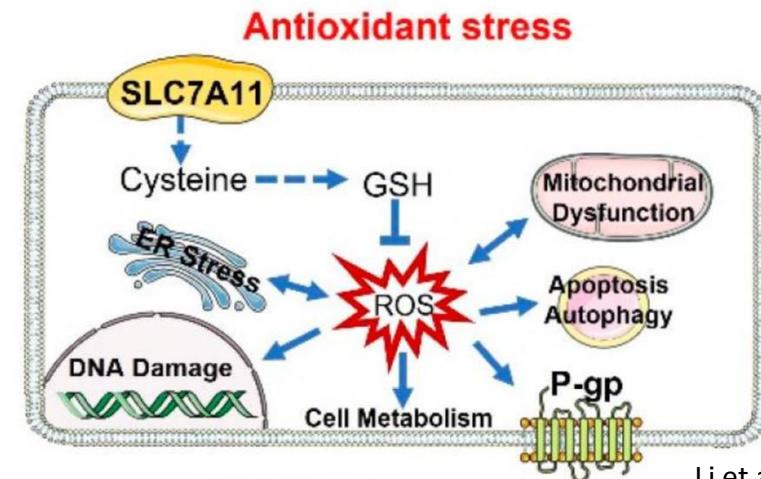
Expression
2
1
0
-1
-2



Upregulated genes in MCT-12h vs others

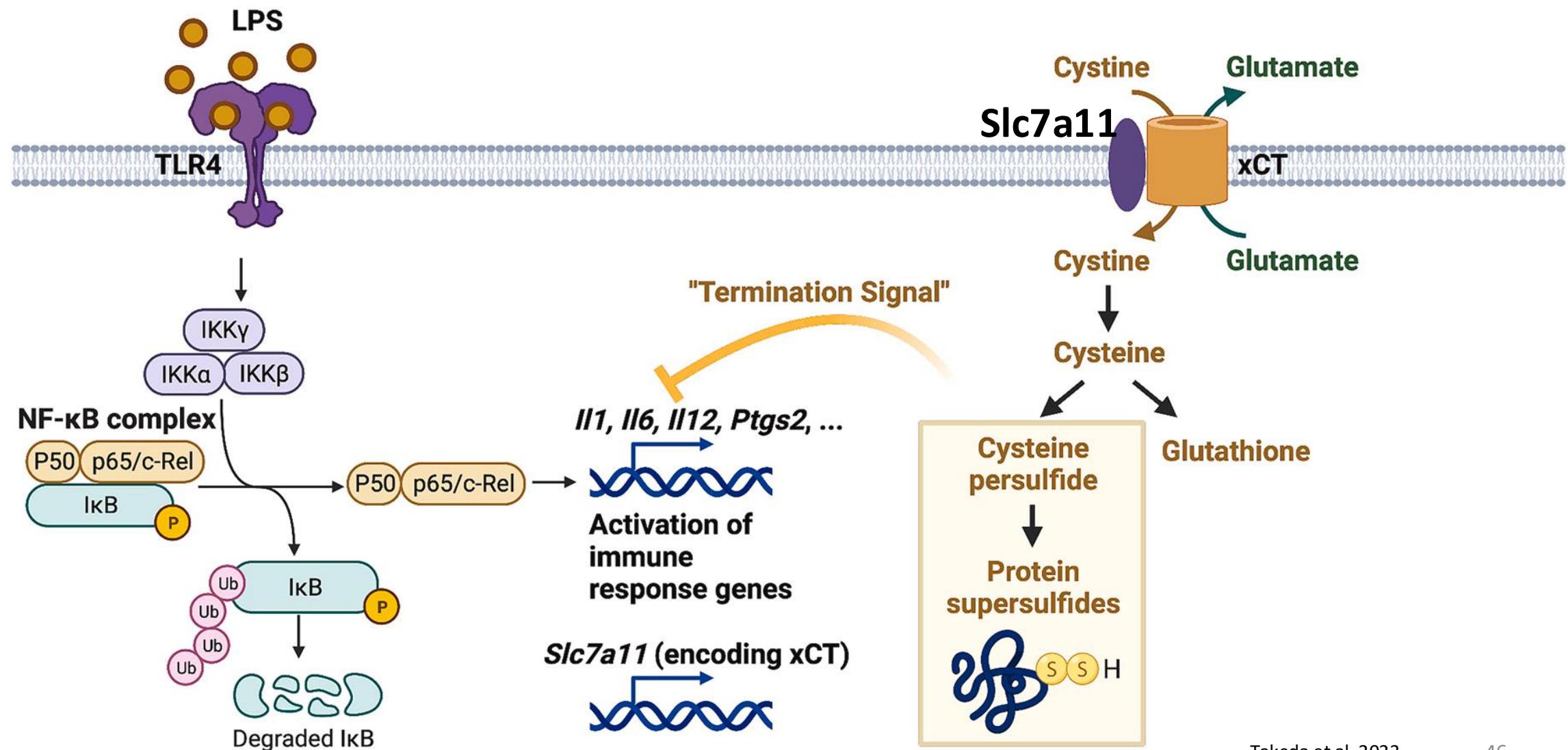


Expression
2
1
0
-1
-2

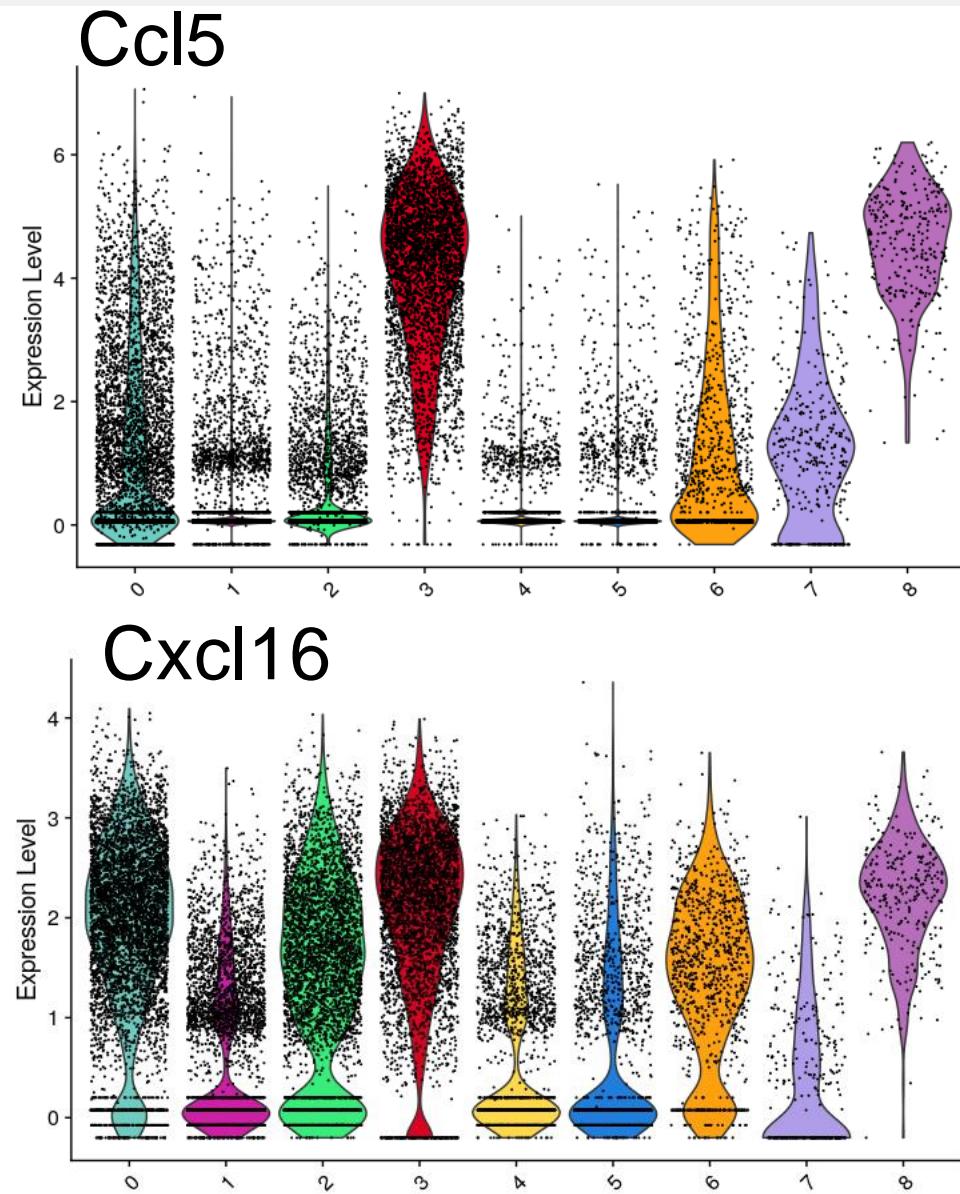
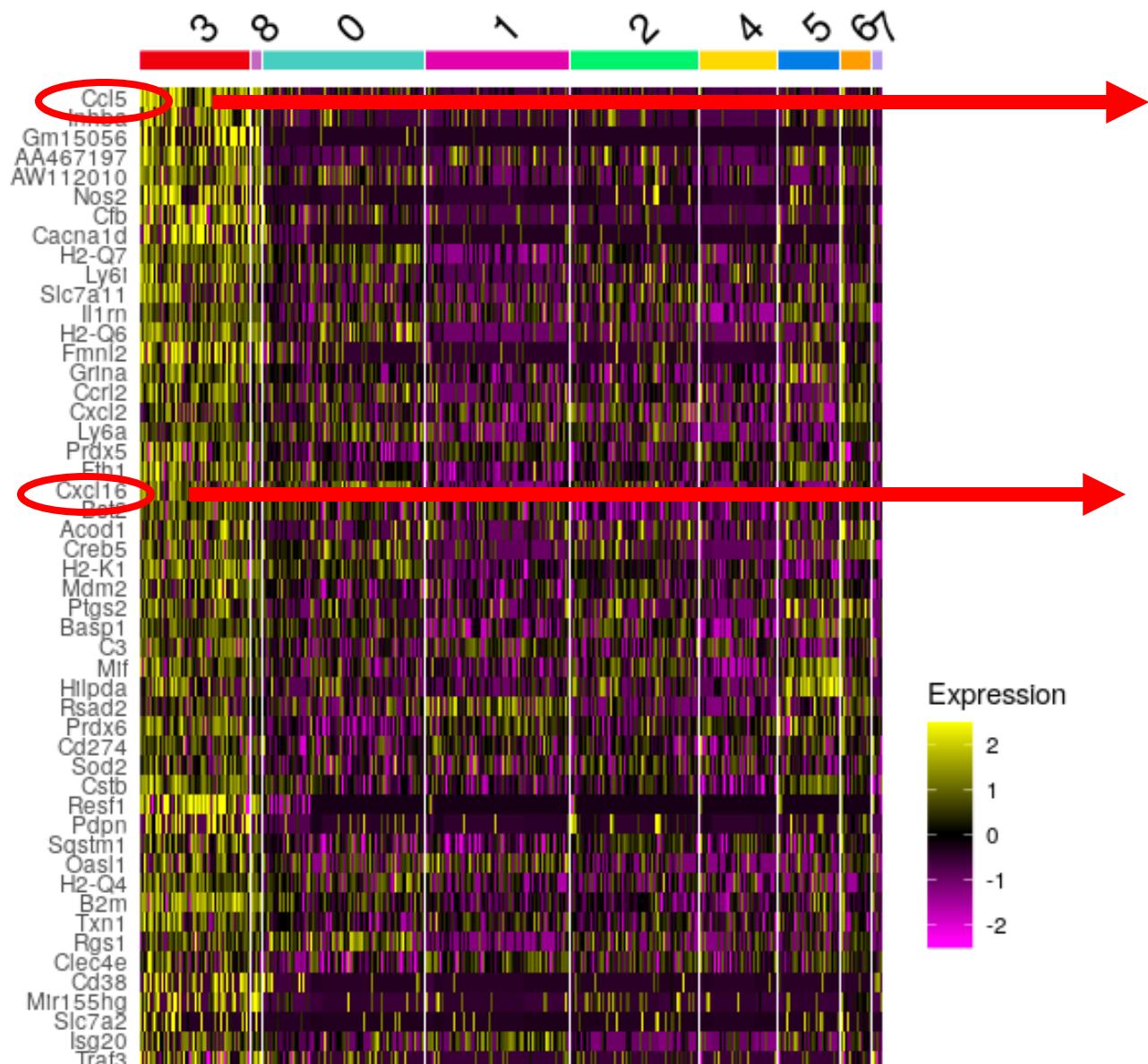


Li et al. 2022

Sulfur metabolic response in macrophages limits excessive inflammatory response by creating a negative feedback loop

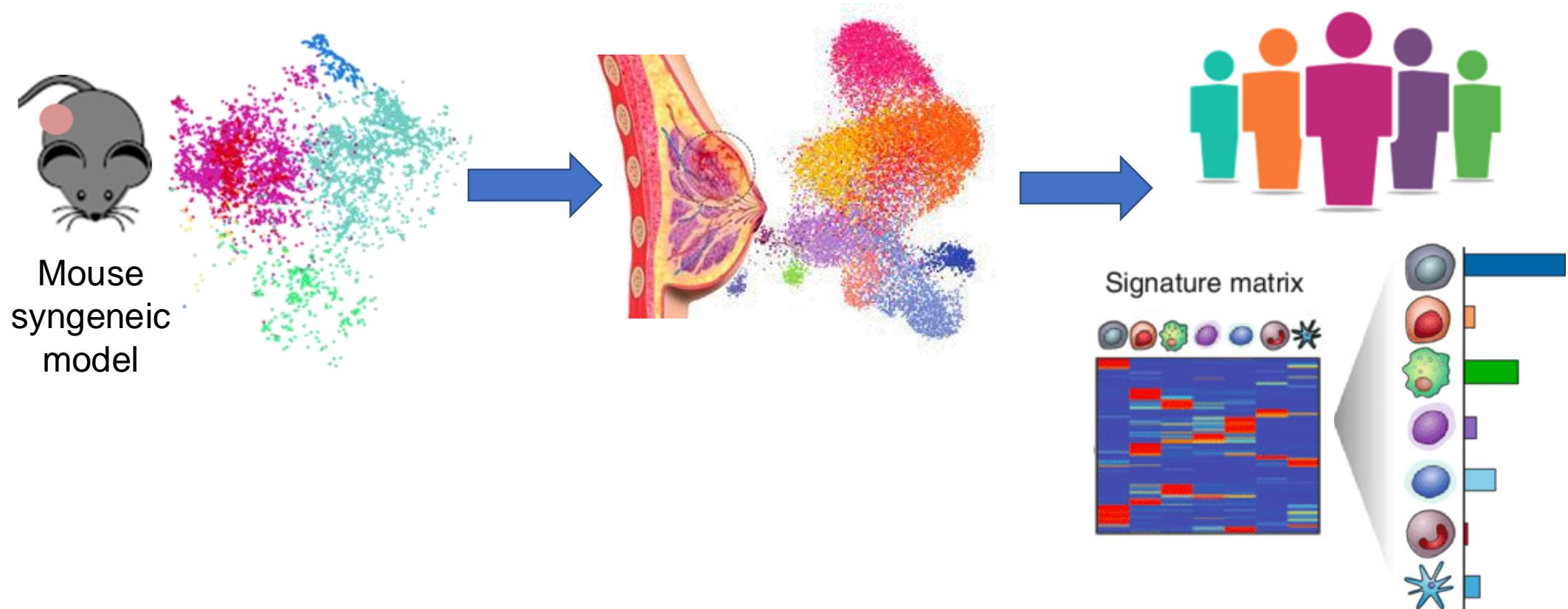


Upregulated genes in MCT-12h vs others: T cell recruitment



Ongoing

Evaluate the conservation of myeloid populations across individuals and species.



scStudio: flexible web-based scRNA-seq data analysis

Welcome to scStudio

by the Disease Transcriptomics lab

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|--|--|--|--|---|
|  <h3>Data</h3> <p>Access single-cell data and create a working session.</p> <p>></p> |  <h3>Quality Control</h3> <p>Discard poor quality cells using a set of indicators.</p> <p>></p> |  <h3>Normalization</h3> <p>Correct sequencing depth and batch effect.</p> <p>></p> |  <h3>Dimensionality Reduction</h3> <p>Choose highly variable genes and create low-dimensional visual representations.</p> <p>></p> |  <h3>Clustering</h3> <p>Perform clustering analysis to group similar cells.</p> <p>></p> |
|  <h3>Differential Expression Analysis</h3> <p>Compare and rank genes based on the expression levels between different conditions.</p> <p>></p> |  <h3>Functional Enrichment Analysis</h3> <p>Calculate gene set scores and identify up- or -down regulated pathways.</p> <p>></p> |  <h3>Feature Plots</h3> <p>Obtain visual representations of gene expression levels.</p> <p>></p> |  <h3>Settings</h3> <p>Optimize selection of colors and data annotation.</p> <p>></p> |  <h3>Report</h3> <p>Access methods and parameter annotation from all analysis performed in your session.</p> <p>></p> |



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