Exploratory analysis of single cell RNA sequencing experiments using the Loupe browser
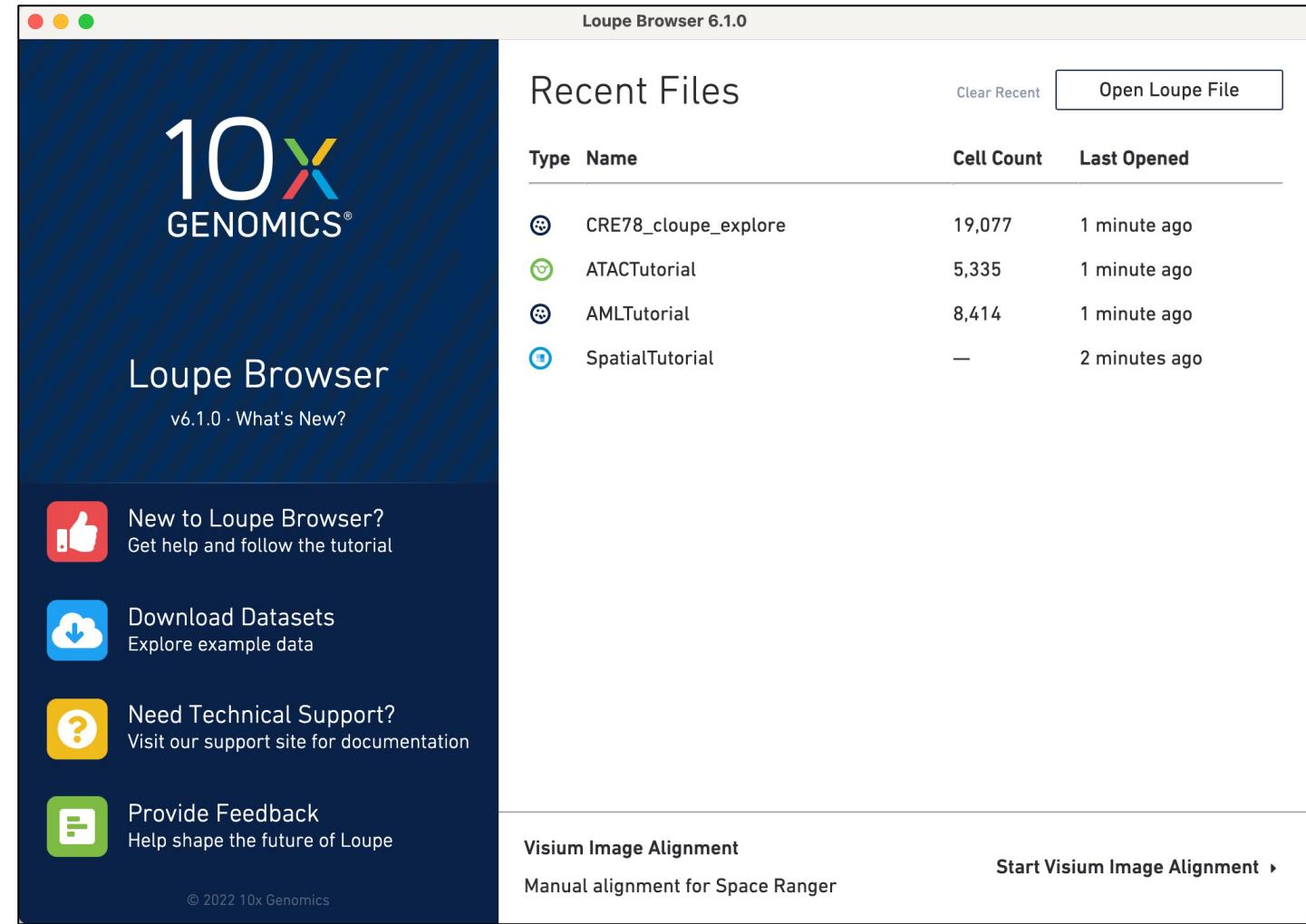
Roderik Kortlever

Abigail Edwards

Loupe Browser: a 10X Genomics tool for analysis of single cell data

Loupe Browser is a desktop application that provides interactive visualization functionality to analyse data.

It allows you to easily interrogate different views of your data to quickly gain insights into the underlying biology.

(source: https://support.10xgenomics.com)

# Loupe Browser: a 10X Genomics tool for analysis of single cell data
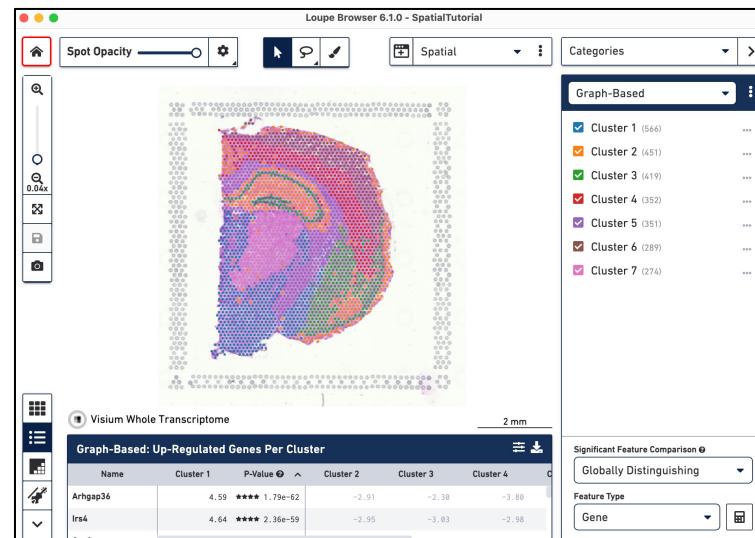
Loupe Browser Aims:

1. Finding cells of interest

2. Finding significant genes

3. Identifying cell types

4. Exploring substructure

5. Exploring cell subtypes

6. Sharing results

(source: https://support.10xgenomics.com)

Chromium Platform

Visium Platform

- Supports quick insight in the quality of the samples used

- Provides a detailed view of the array of cell types from a particular tumour or tissue

- Allows for easy identifiable cell-specific transcription

- Differential gene expression gives insight into changes as a consequence of experimental conditions

- The dynamics of a biological system are uniquely exposed
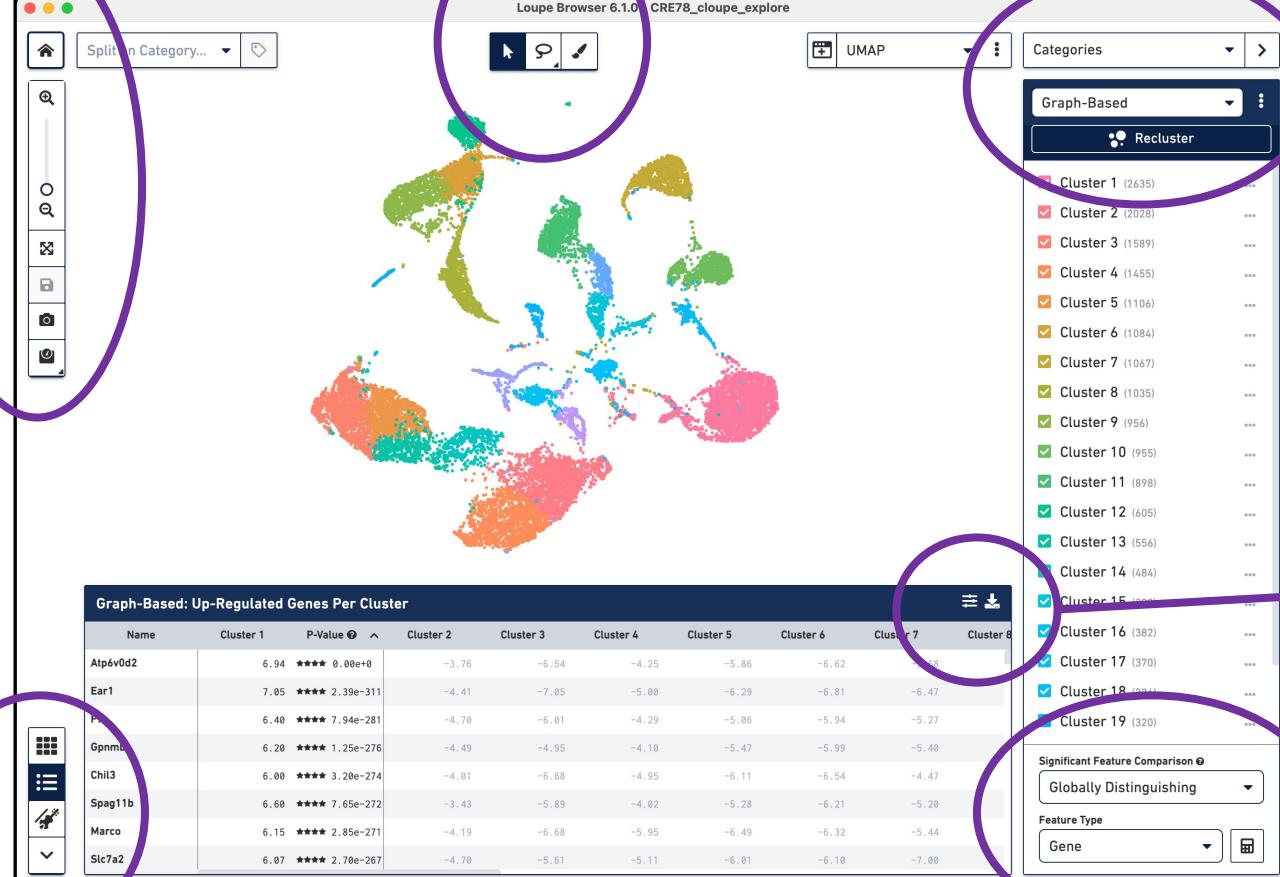
Remember: cloupe browser is an exploratory tool!

- From unprocessed data at the beginning of the quality check pipeline

- No formal data quality checks yet

- No data normalisation yet

- No batch effect and integration correction yet

- Etc...

How to exploit the cloupe functions?

Selecting cells / groups of cells of interest

tSNE / UMAP presentation

Identifying cell types
Exploring cell subtypes

Data export

Differential gene expression presentation
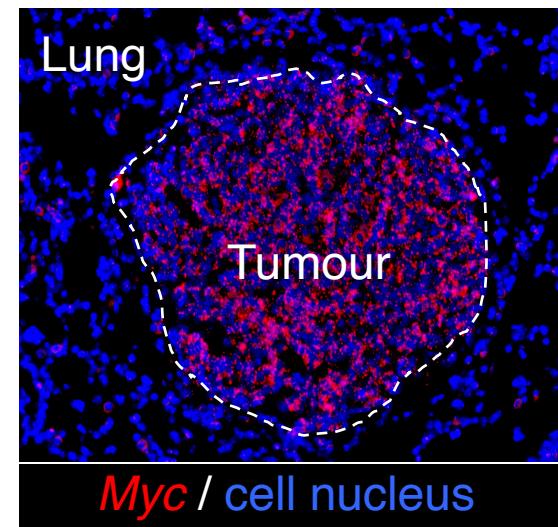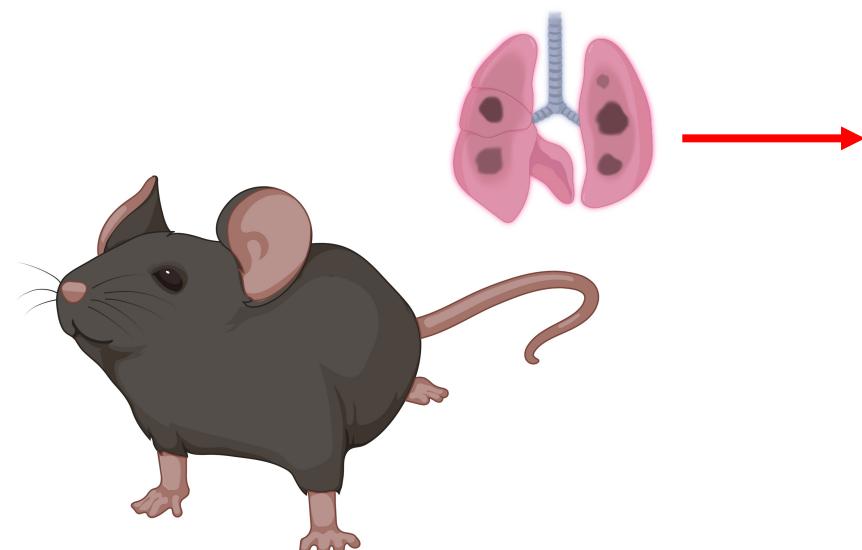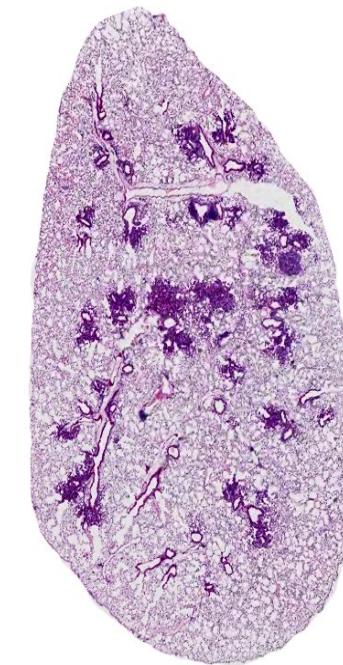
Finding significant and differentially expressed genes

Our model to study cancer growth

Genetically engineered mouse in which we can - at will - activate oncogene Myc in lung tumours

Myc OFF

Myc ON

Lung

Tumour

Myc / cell nucleus
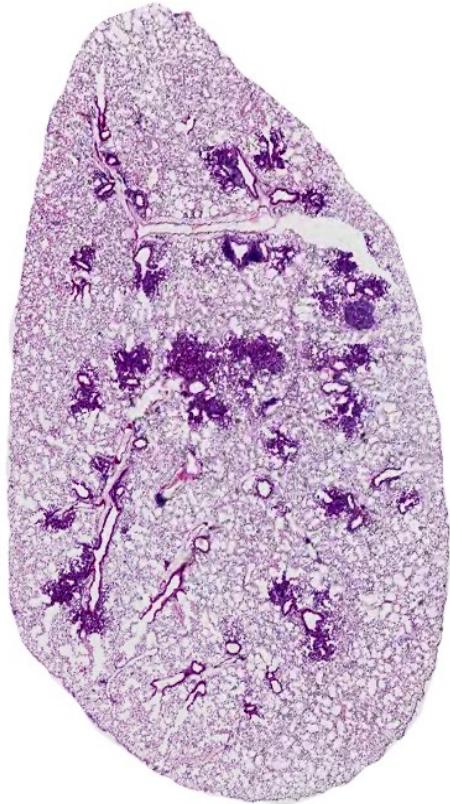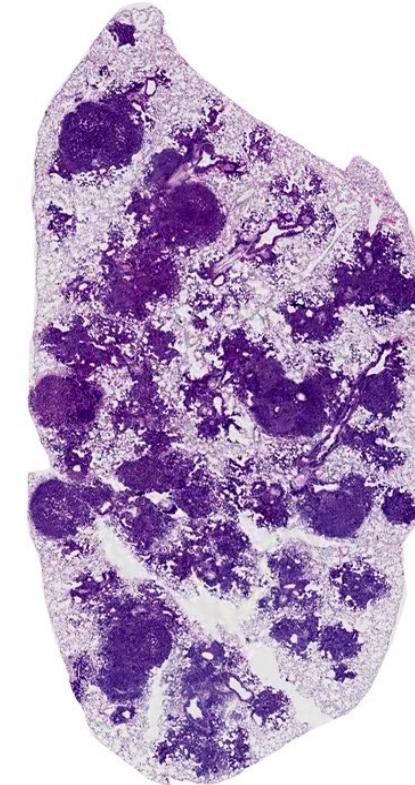
Myc is a transcription factor that regulates genes involved in cellular growth and tissue expansion

How does Myc do it: what are the molecules involved that make the tumours grow?

Myc OFF
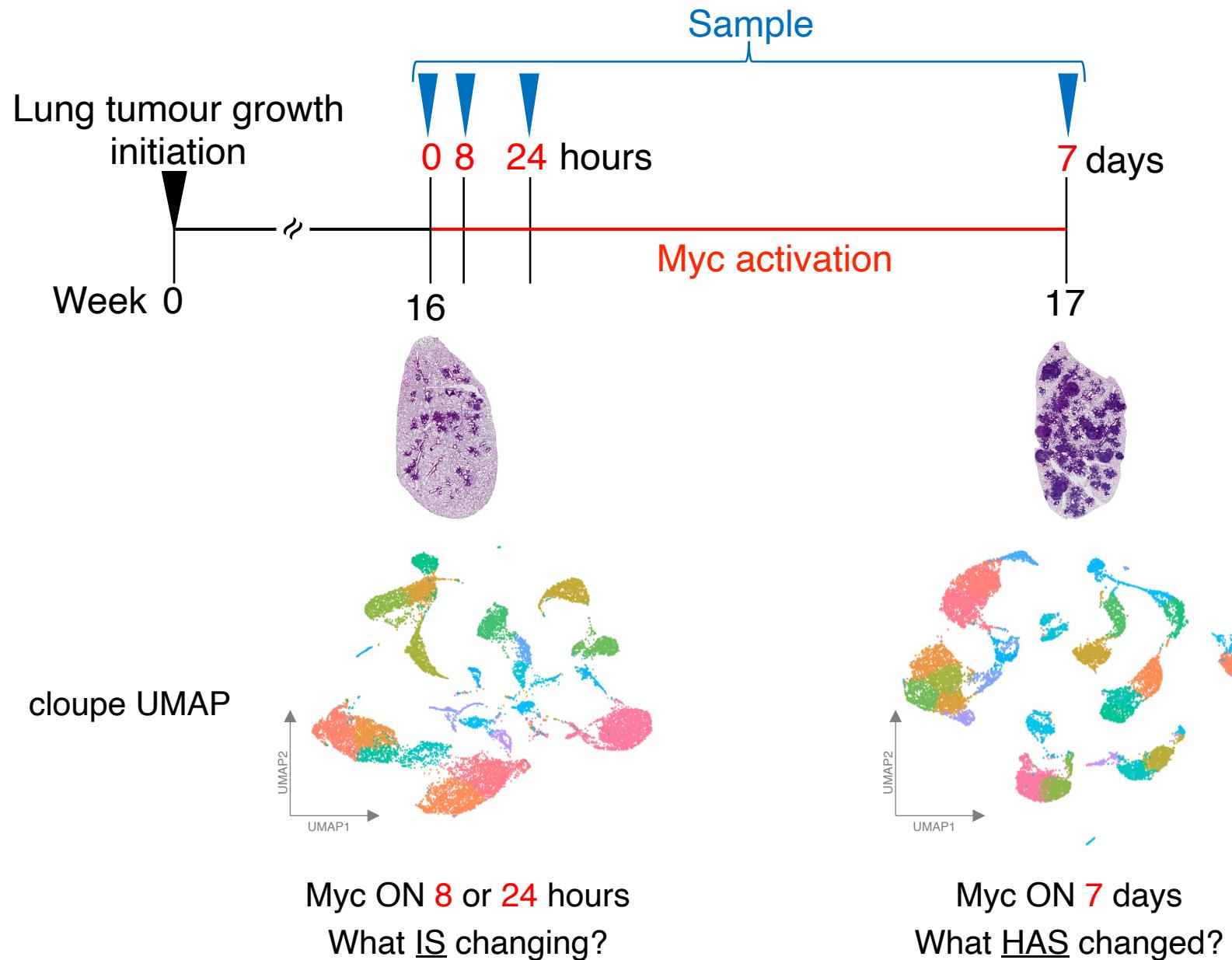
Myc ON

?

Experimental analysis: study the consequences of Myc activation

7 days after Myc ON there are many more larger tumours, but how does that come about?
What molecules are changing, and what could that mean?

What significant changes in gene expression and, consequently, secreted signaling molecules are coming from the tumour cells? (with a focus on immune signaling)

What cells are they talking to?
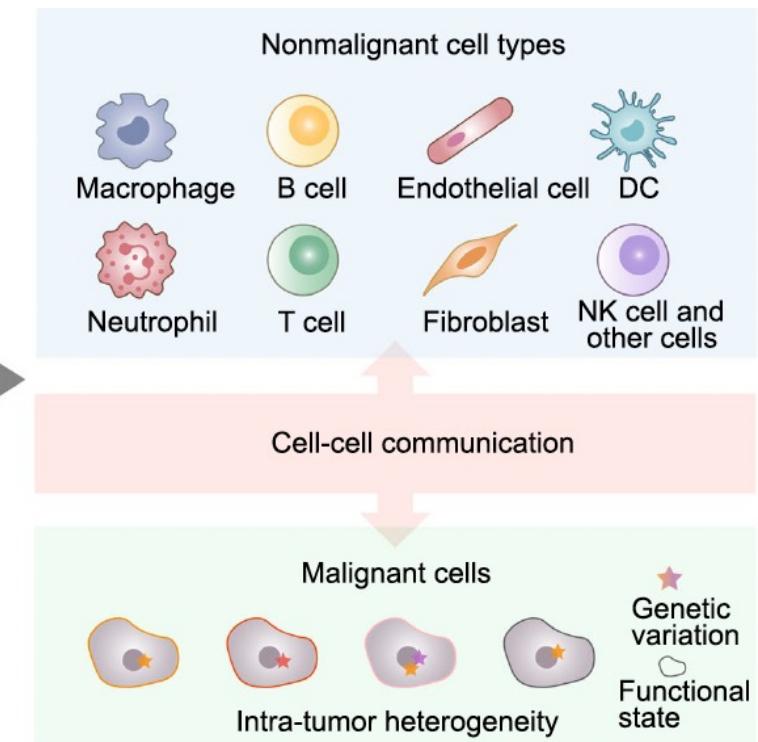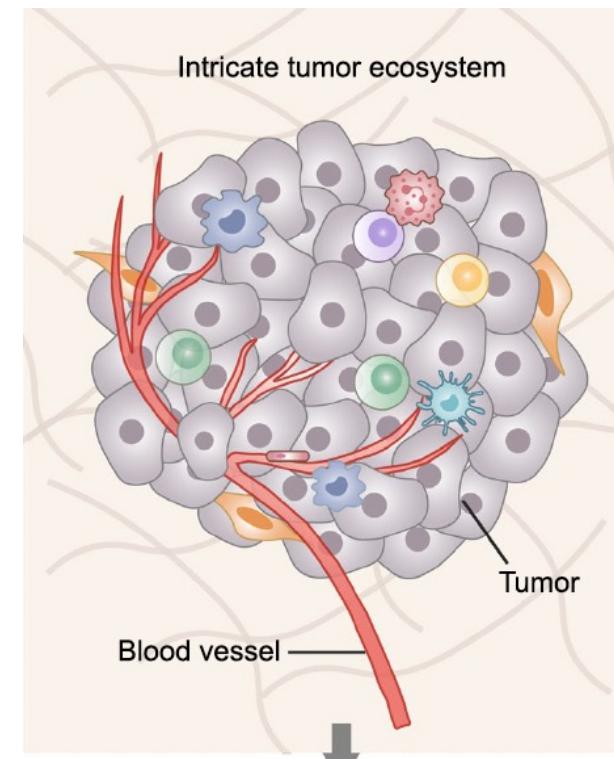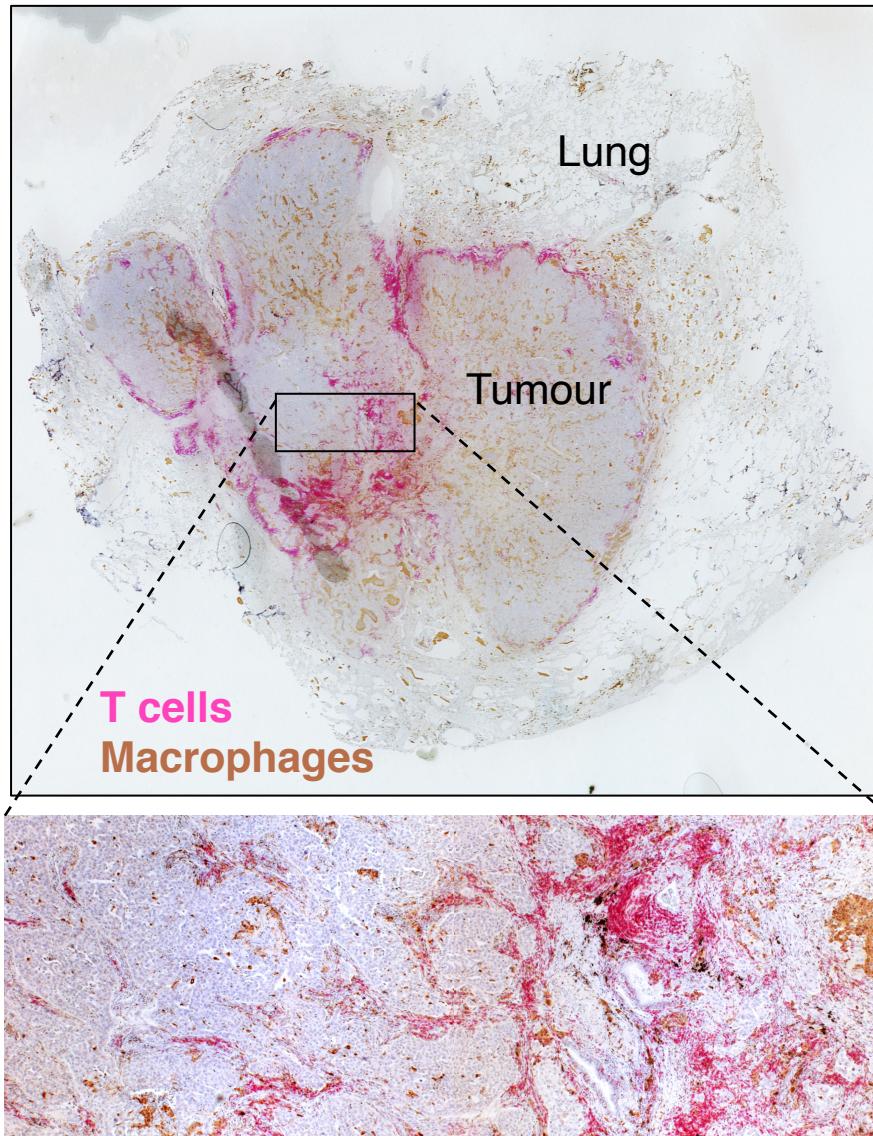
How are these cells responding?

The overarching aim here:
From our single cell RNA data, can we extract insights into the molecules, cells and molecular pathway(s) that support tumour growth?
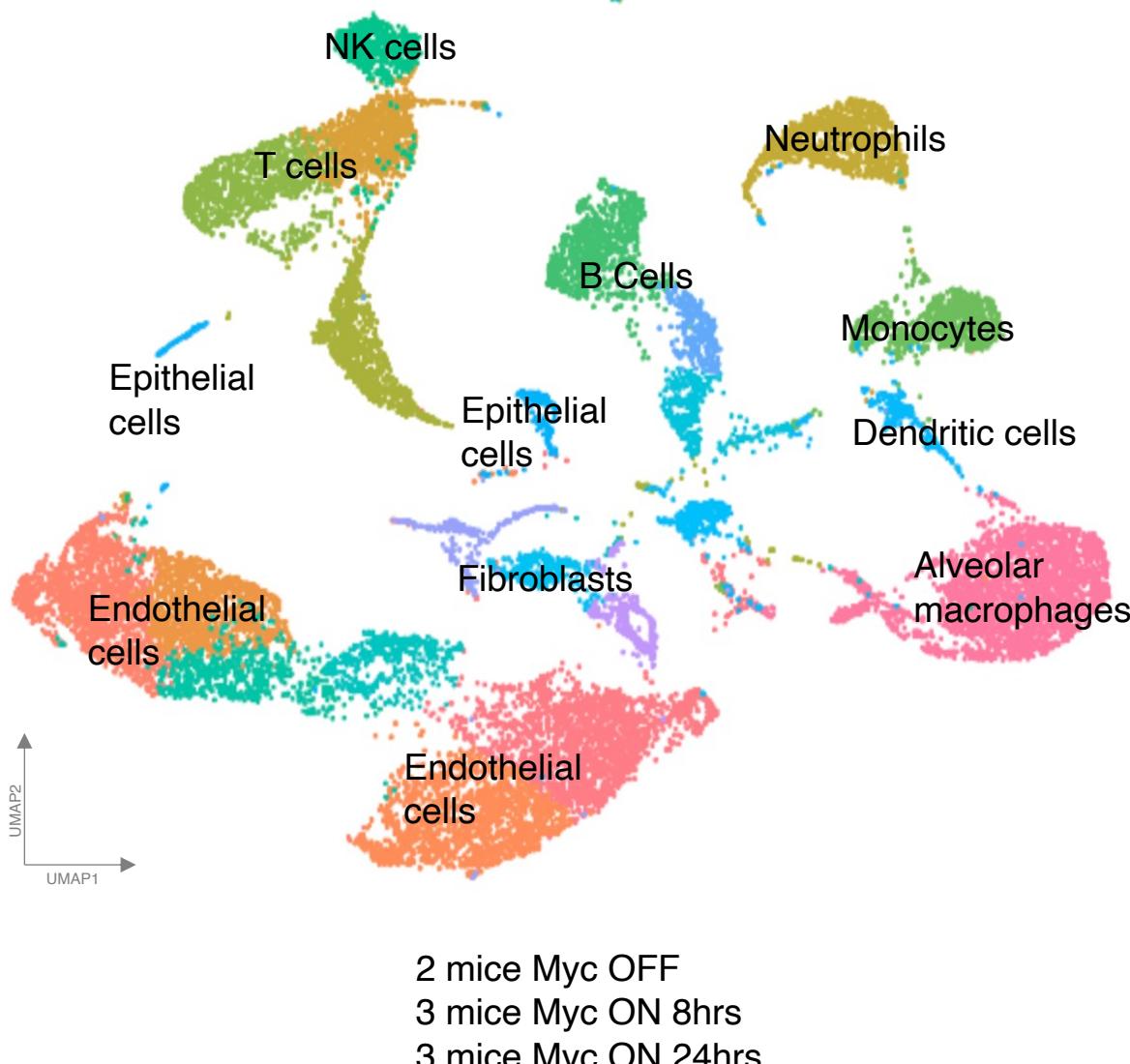
Human lung tumour cross section

Lung

Tumour

T cells
Macrophages

Intricate tumor ecosystem

Blood vessel

Tumor

Nonmalignant cell types

Macrophage   B cell   Endothelial cell   DC

Neutrophil   T cell   Fibroblast   NK cell and other cells

Cell-cell communication

Malignant cells

Genetic variation

Functional state

Intra-tumor heterogeneity

Cancer biology deciphered by single-cell transcriptomic sequencing,
Li *et al*, 2022, Protein Cell 13

What cells do we capture in our cloupe analysis?

Lungs of 8 mice combined

2 mice Myc OFF
3 mice Myc ON 8hrs
3 mice Myc ON 24hrs

## List of mouse lung cell type marker genes
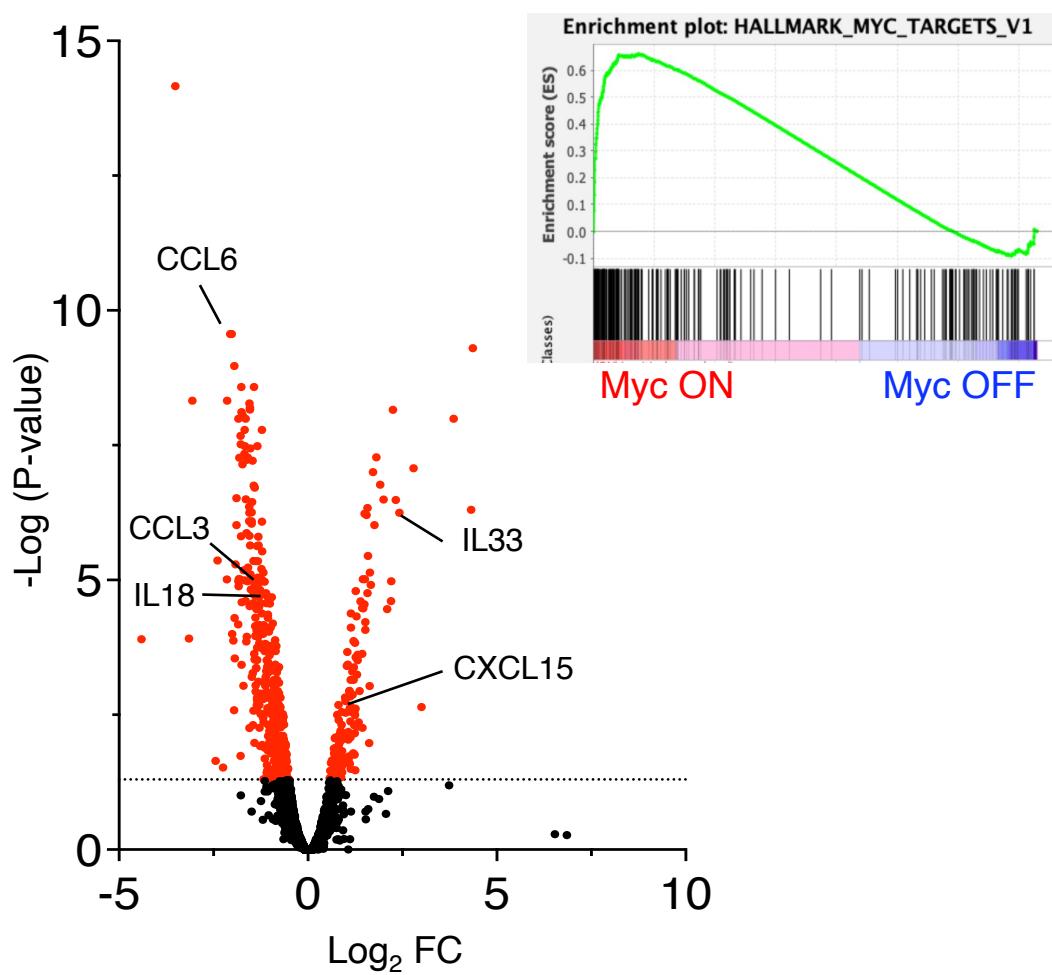
Cell type	Single marker gene	Combination of marker genes (more stringent unle
Cycling cells	Top2a or Cenpf	Stmn1 and Top2a/Cenpf
All 'non-blood' cells	Malat1	
Myc targets	Cad or Ncl	Ncl and Rasd2
Epithelial club (secretory) cells	Scgb1a1 or Scgb3a2	Cyp2f2 and Scgb3a2
Epithelial AT1	Rtkn2	
Epithelial AT2	Lamp3 or Sftpc	Lamp3 and Sftpc/Sftpd
Goblet cells	Tulp3	
(Mucous) ciliated epithelial cells	Lrriq1 or Dnah12	
Submucosal (gland) epithelial cells	Azgp1	S100b
Lymphatic cells	Mmrn1 or Ccl21a	
Endothelial	Tmem100 or Cdh5	Tmem100 and Cdh5
Lymphatic (capillary) endothelium	Edn1 or Sema3g	Ptgs2 and Lyve1/Sox17
Activated (vein) endothelium	Sele	Vwf and Bst1 (less stringent)
Alveolar type 1 (a capillary) endothelium	Ednrb	Car4 and Ednrb
Fibroblasts	Col1a1 or Col1a2	
Smooth muscle fibroblast	Lgr5 or Lgr6	Acta2 and Lgr5/Lgr6
Pericyte/vascular smooth muscle	Gucy1a1 or Cspg4	Gucy1a1 and Pdgfrb
Interstitial Fibroblasts	Dcn	Dcn and Pdgfra
All Immune cells (CD45+ cells)	Ptprc	
Myeloid	Csf2ra	
Monocytes	Ly6i	Ly6i and Plac8
Alveolar Macrophages	Marco or Ear1	
Interstitial Macrophages (FN1+)	Fcgr1	Fcgr1 and Fn1/Ccr2
Granulocytes/neutrophils	S100a8 or S100a9 or Retnlg	S100a8/S100a9 and Retnlg
cDC1 Dendritic cells	Xcr1 or Itgae or Clec9a	Xcr1 and Itgae/Clec9a
cDC2 Dendritic cells	C1qa or C1qb	C1qa and C1qb (both are already quite stringent)
cDC3 Activated Dendritic cell (mreg)	Ccr7	Fscn1
pDC Dendritic cells	Ccr9 or Siglech	Ccr9 and Siglech
MoDC Monocytic Dendritic cells	Cd14	Cd14 & Itgam
B cells	Cd79a or Cd19	Cd79a and Cd19
T cells	Cd3e	
CD4+ T cell	Cd4	
CD8+ T cell	Cd8a	Cd8a and Dusp2
gammadelta T cell	Tcrg-C1	
Treg	Foxp3	
ILC1	-	Ncr1 and Tbx21 together
ILC2	-	Gata3 and Rora together
ILC3	Rorc?	Rorc and IL2ra together
NK cell	Ncr1	Ncr1 (and Klrk1) and Eomes together
NKT cell	-	Cd3e and Tbx21 together OR Cd8a and Fcer1g together
Basophils	Mcpt8	
Eosinophils	Prg2 or Rnase3 or Ccr3	
Mast cells	Ms4a2	Gata2 and Ms4a2
Megakaryocytes	Ppbp	Ppbp and Gata1
Erythrocytes	Hba-a2 or Alas2	Snca and Hbb-bt

Lavin, 2017, Cell
Ordovas-Montanes, 2018, Nature
Travaglini, 2020, BioRXiV
Goveia, 2020, Cancer Cell
Montoro, 2018, Nature
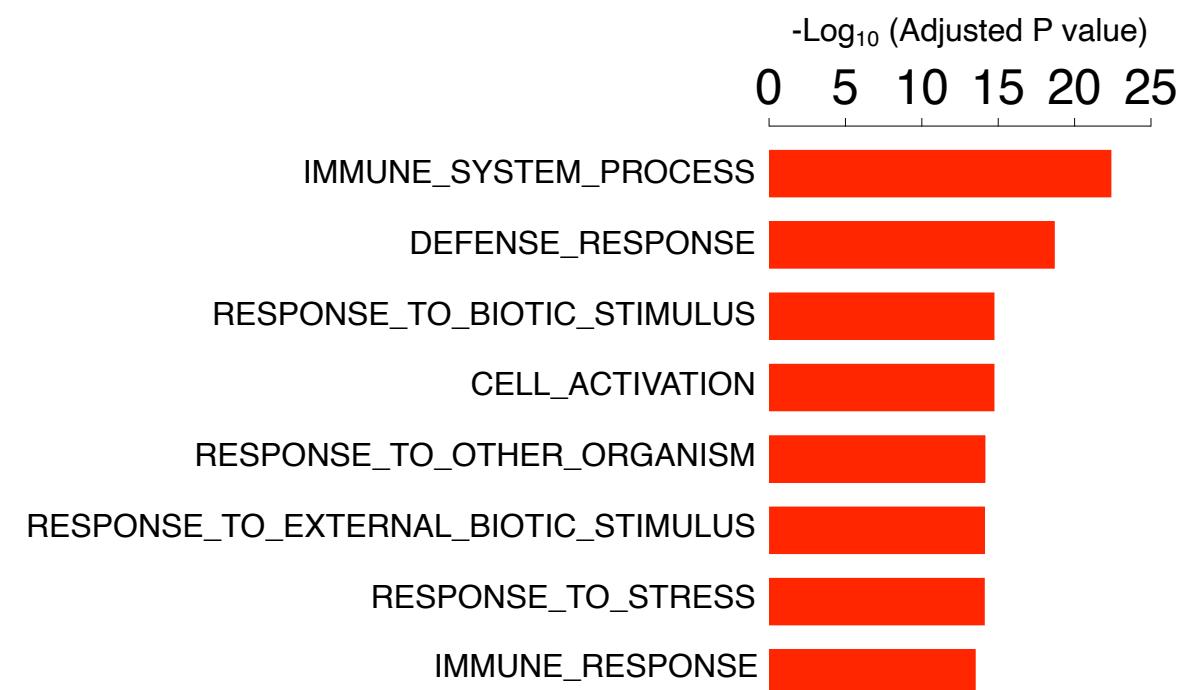
Plasschaert, 2018, Nature
VieiraBraga, 2019, Nature Medicine
Leylek, 2019, Cell Reports
Maier, 2020, Nature

Analysis of changes in expression of immune signaling molecules and biological pathways after Myc ON
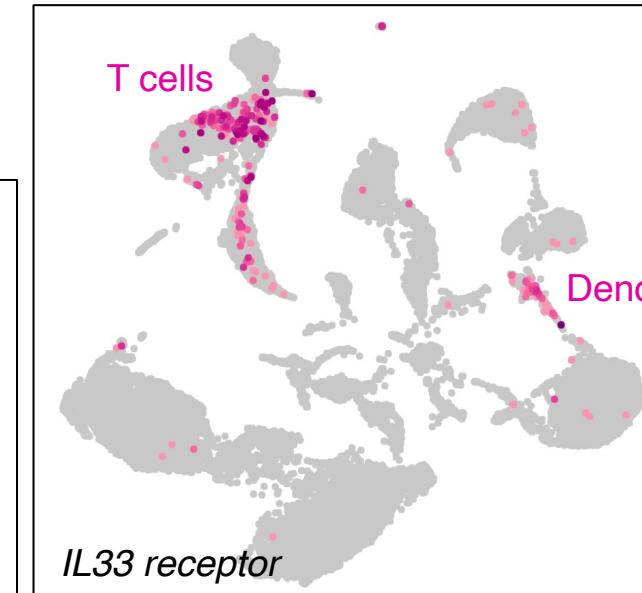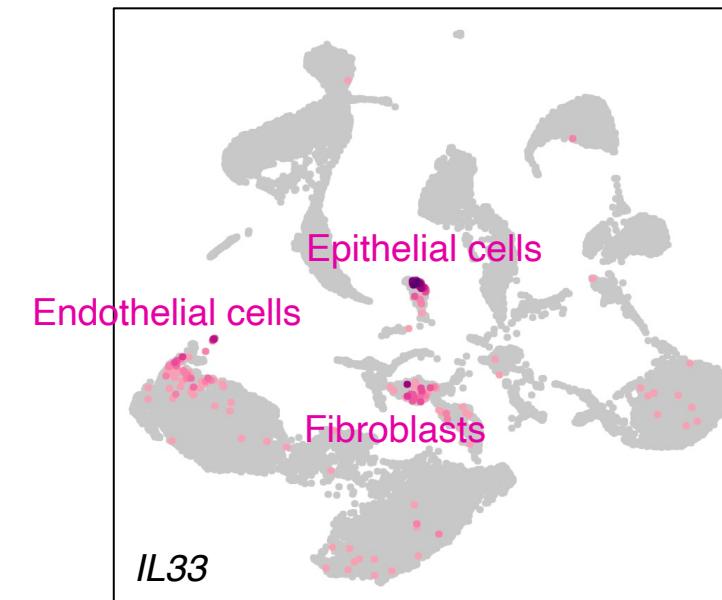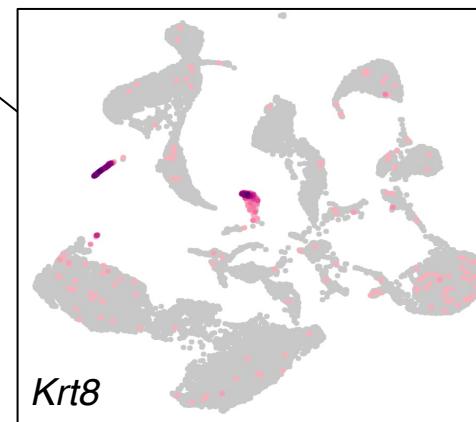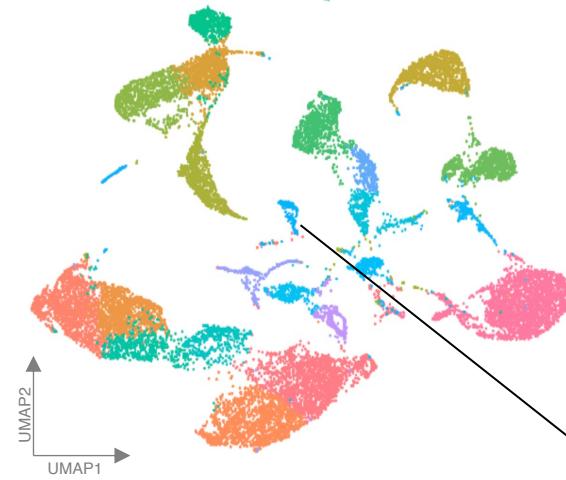
Kortlever *et al*, unpublished

# Analysis of changes in expression of immune signaling molecules and their targets after Myc ON

Myc is artificially expressed **only** in
Keratin 8 positive (Krt8+) epithelial tumour cells

On how to use cloupe to forward our experiments…

Selecting cells / groups of cells of interest

tSNE / UMAP presentation

Identifying cell types
Exploring cell subtypes

Data export

Differential gene expression presentation

Finding significant and differentially expressed genes