# Single-cell analysis of the stromal cells of neuroblastoma

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## Introduction

### Background

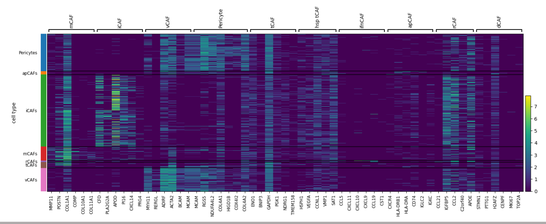
- Neuroblastoma, the most common extracranial solid tumour in children worldwide[1], has a 50% 5-year survival rate for high-risk cases[2].
- Relapses due to residual tumour cells post-treatment necessitates the need to understand the role of the tumour microenvironment (TME) in treatment resistance.
- The TME is enriched with stromal cells such as fibroblasts and endothelial cells, which have polarizing effects as either pro- or anti-tumorigenic[3,4].

### Aims

- Leveraging recent advances in single-cell analyses, this study seeks to elucidate these heterogeneous cell types from neuroblastoma patient samples' transcriptomes, particularly fibroblast and endothelial cells.
- To describe the stromal cells' involvement in the neuroblastoma microenvironment across various treatment stages from their gene expression profiles

## Methods

1. Biopsies were taken from five different anatomical sites (abdomen, bone marrow, lymph node, bone) from 5 neuroblastoma patients at **diagnosis (Dx)**, **post-3-month chemotherapy (Rx)** and at **first (Rel)** or **second (Rel2) timepoint** of **relapses** if applicable (total n = 21).
2. The samples were dissociated into single cells for **10x Genomics 3' single-cell sequencing**.
3. Data was then processed using 10x Genomics CellRanger software, and further analysed using the singe-cell analysis framework **Scanpy** (v1.9.3)[5].
4. Established marker genes from public databases (CELLxGENE) and literature (Cords et al.[6]; fibroblast gene list shown in heatmap below) were used to annotate cell types.



## Results and Discussion

### 1. Initial clustering reveal immune, neural, adrenal and stromal cell types present across the neuroblastoma samples

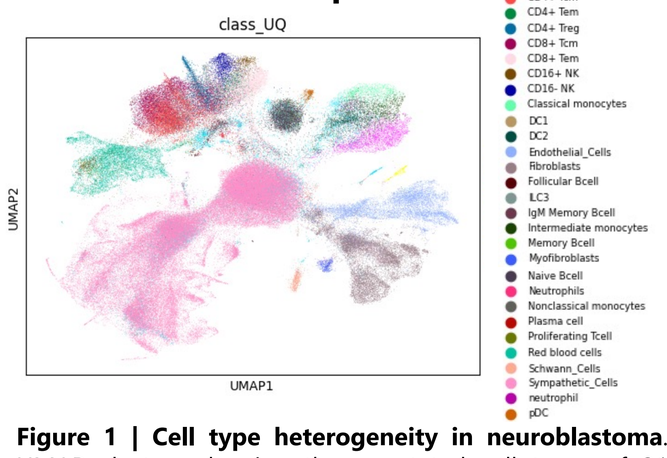



**Figure 1 | Cell type heterogeneity in neuroblastoma**. UMAP clusters showing the annotated cell types of 21 patient samples identified based on gene markers. This is prior to subclustering the fibroblast and endothelial cell subtypes further.

### 2. Four endothelial cell subtypes were identified based on their respective cluster's highly ranked genes expression

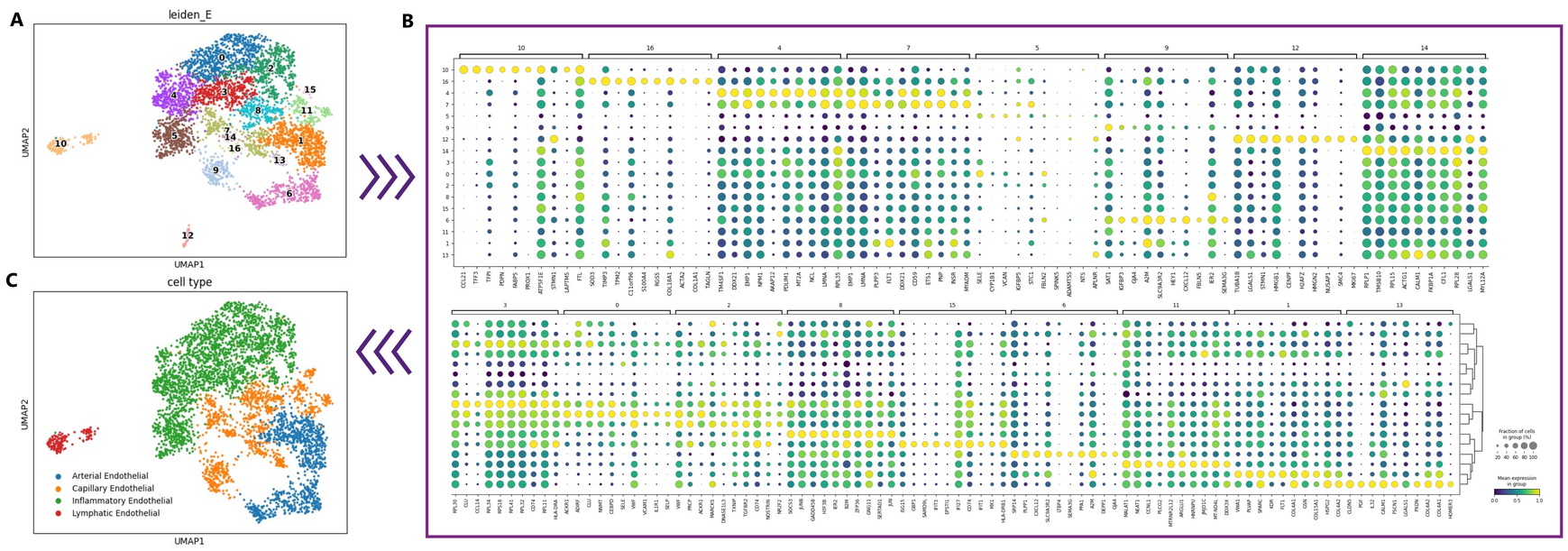



**Figure 2 | Endothelial cell heterogeneity in neuroblastoma**. **A.** UMAP of all endothelial cells coloured by their clusters with the **B**. dotplot showing the expression level of the highly ranked genes of the respective clusters. **C.** The final annotated clusters of endothelial subtypes identified.

### 3. Six fibroblast subtypes and pericytes were identified based on their respective clusters' highly ranked gene expressions

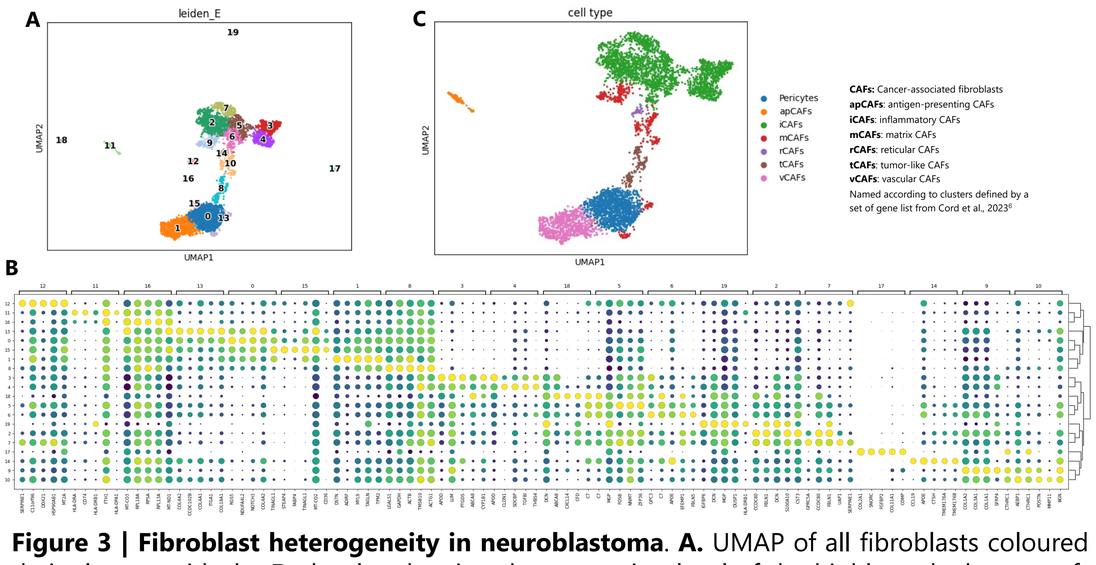



**Figure 3 | Fibroblast heterogeneity in neuroblastoma**. **A.** UMAP of all fibroblasts coloured by their clusters with the **B**. dotplot showing the expression level of the highly ranked genes of the respective clusters. **C.** The final annotated clusters of fibroblast subtypes identified.

### 4. Inflammatory endothelial subtype is the dominant subtype at Dx, Rx and at first relapse timepoint (Rel)

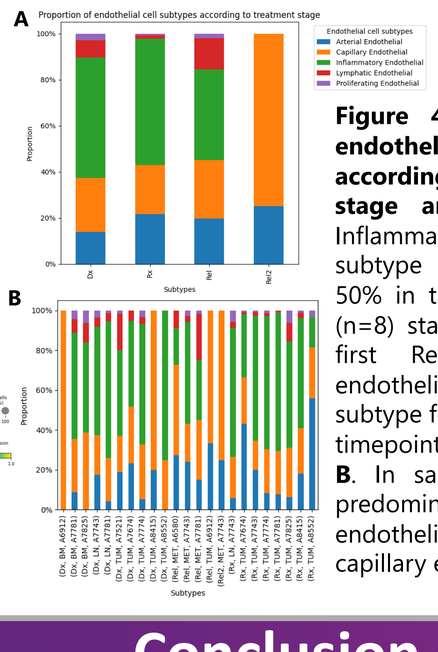



**Figure 4 | Proportion of endothelial subtypes according to A. treatment stage and B. patient**. **A.** Inflammatory endothelial subtype makes up around 50% in the Dx (n=9) and Rx (n=8) stages and 40% at the first Rel (n=4); Capillary endothelial is the predominant subtype for the second relapse timepoint (Rel2) which is n=1. **B**. In samples that are not predominantly inflammatory endothelial, they are majority capillary endothelial.

### 5. iCAFs are the predominant subtype in the Dx, Rx and Rel stages while it is predominantly pericytes at Rel2

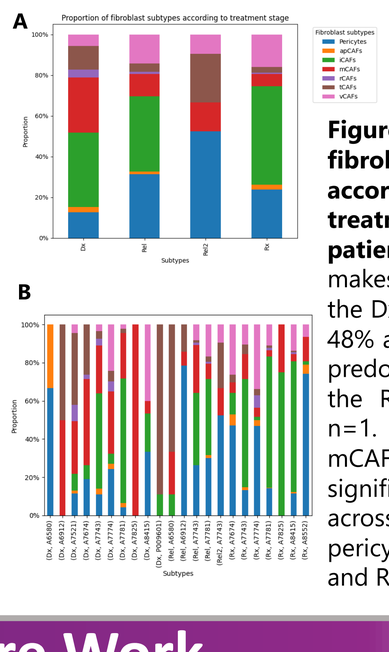



**Figure 5 | Proportion of fibroblast subtypes according to A. treatment stage and B. patient**. **A.** iCAFs subtype makes up around 35% in the Dx and Rel stages and 48% at Rx; Pericytes is the predominant subtype for the Rel2 stage which is n=1. **B**. Across patients, mCAFs also form a significant proportion across the Dx stages and pericytes across Rel, Rel2 and Rx stages.

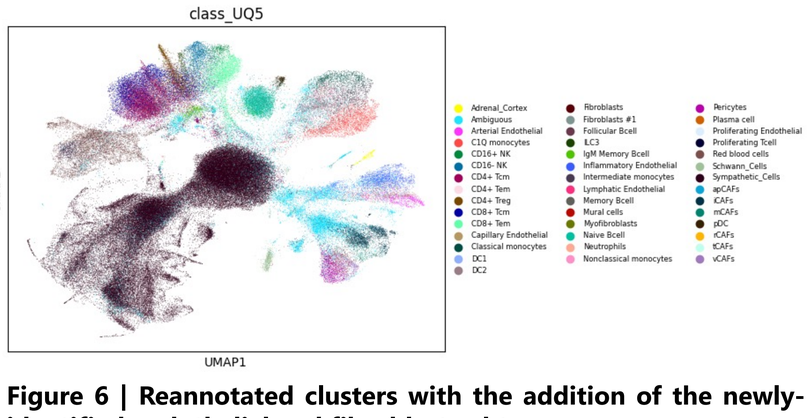



**Figure 6 | Reannotated clusters with the addition of the newly-identified endothelial and fibroblast subtypes.**

## Conclusion and Future Work

- Preliminary results from single-cell analyses highlight the heterogeneity of endothelial and fibroblast populations, comprising five and six subtypes respectively – mapped back onto the original UMAP cluster with all cell types present in neuroblastoma samples (**Figure 6**)
- Each subtype has their distinct gene expression programs.
- Notably, six fibroblast clusters displayed inflammatory gene expression, whereas other subpopulations exhibited vascular and antigen-presentation genes.
- Future work include will explore signalling pathway analyses and cell-cell interactions.
- These findings hold potential for tailoring personalised treatments such as immunotherapy for future neuroblastoma patients.

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