

Settima edizione di



AIEOP..

...in Lab

**Single-nucleus RNA Sequencing
reveals chemotherapy-induced
remodeling of the tumor
microenvironment in Neuroblastoma**

Michele Minopoli

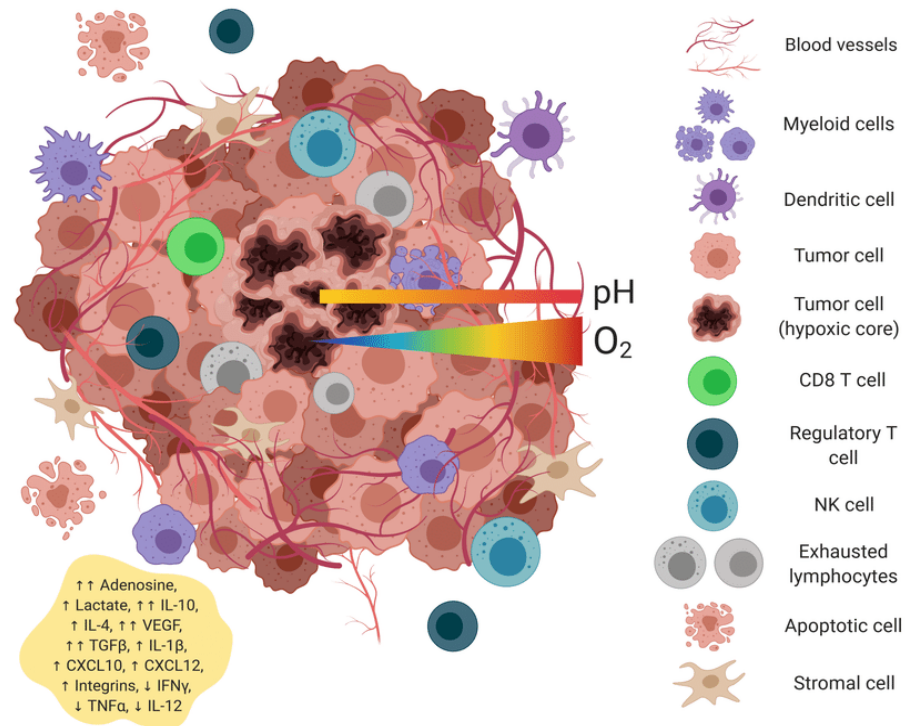
Milano, 22 e 23 maggio 2026

Disclosures of Michele Minopoli

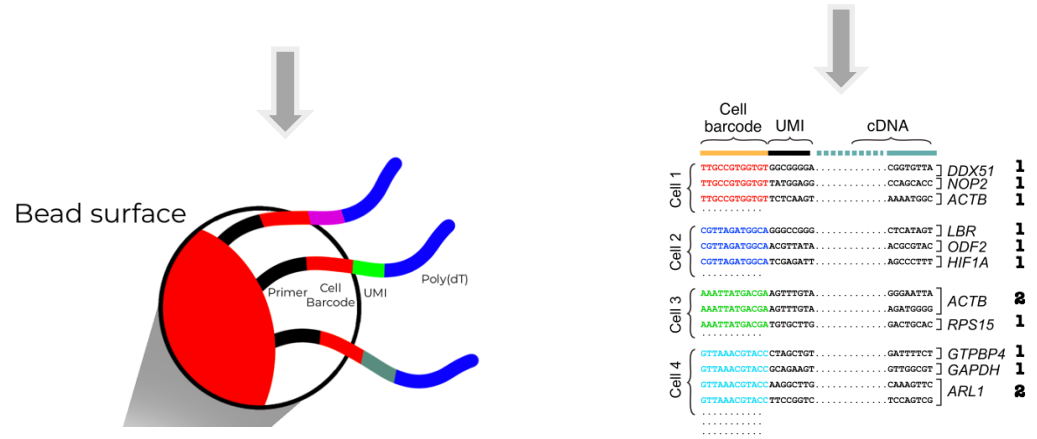
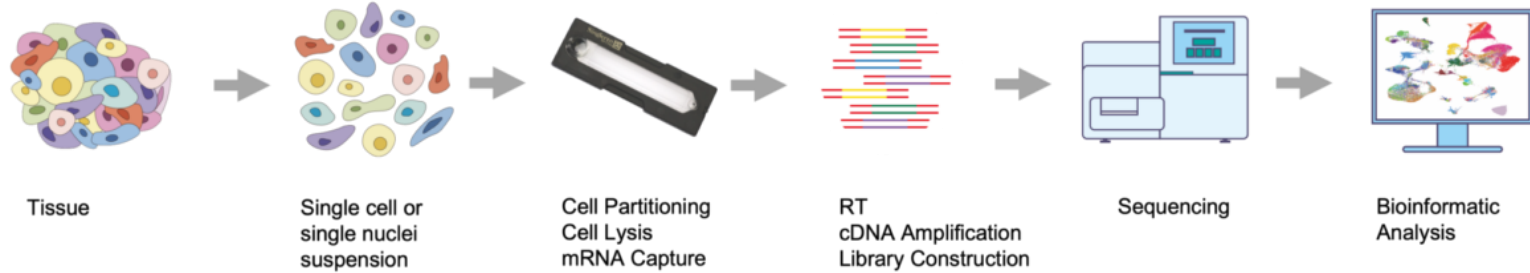
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

The tumor microenvironment (TME) is the silent architect of cancer progression and resistance

- Includes immune cells, cancer-associated fibroblasts (CAFs), endothelial cells, pericytes, and other resident cell types.
- Composition and function vary by tumor origin, stage, and patient characteristics.
- TME cells can have tumor-promoting or tumor-suppressive roles.
- Growing understanding of the TME reveals new therapeutic targets for overcoming tumor progression and therapy resistance.

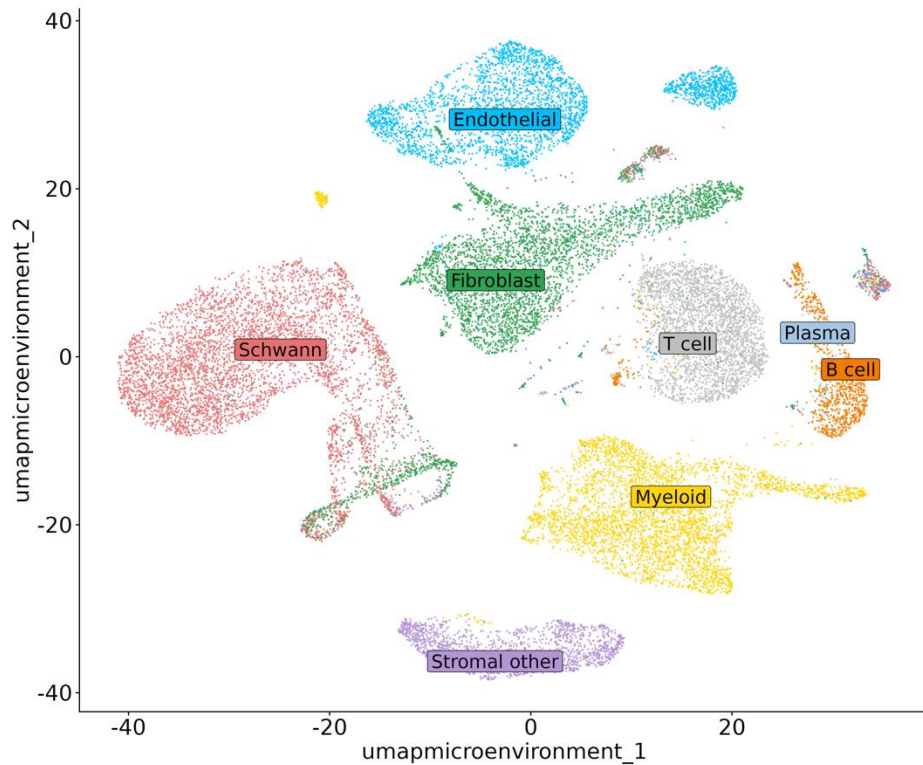


Single-cell RNA sequencing (scRNA-seq) enables the characterization of the cellular heterogeneity within the tumor microenvironment, revealing distinct populations and their transcriptional profiles



How did we select TME cells?

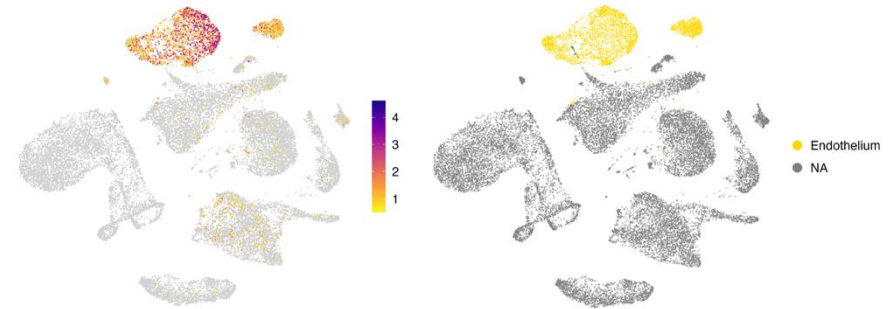
- Absence of neuroblastoma-associated CNAs (1p, 11q, 17q) based on CNV score
- Annotation as non-malignant cells using projection onto reference neuroblastoma datasets (NBAtlas and Patel et al.)



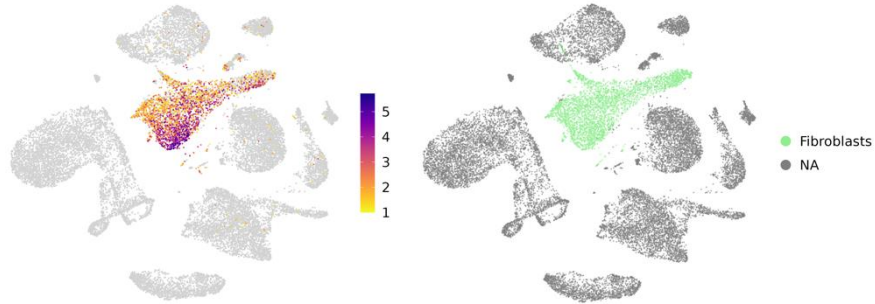
UMAP of 22k TME cells from 33 samples with cell annotation

Focusing on 3 TME macro-compartments

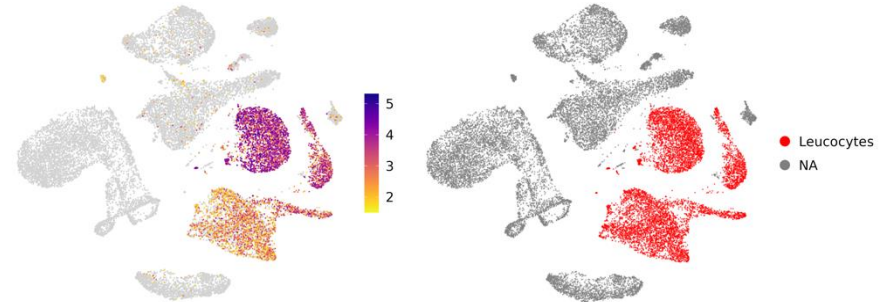
ENDOTHELIUM
EGFL, PLVAP EXPRESSION



FIBROBLASTS
COL1A1, COL1A2, COL3A1 EXPRESSION



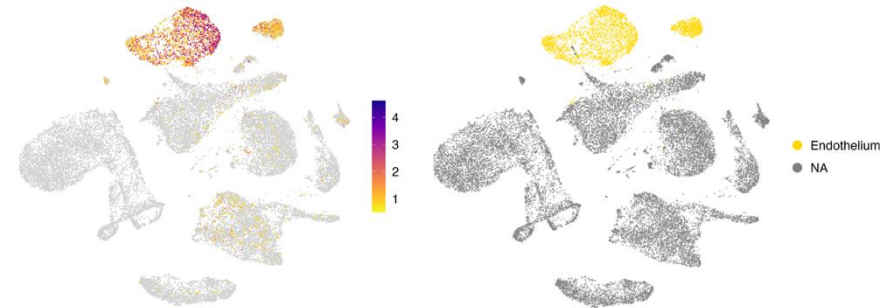
LEUCOCYTES
CD45 EXPRESSION



Focusing on 3 TME macro-compartments

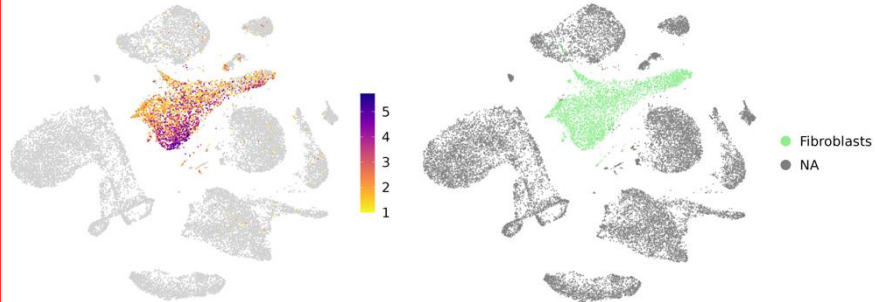
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EGFL, PLVAP EXPRESSION



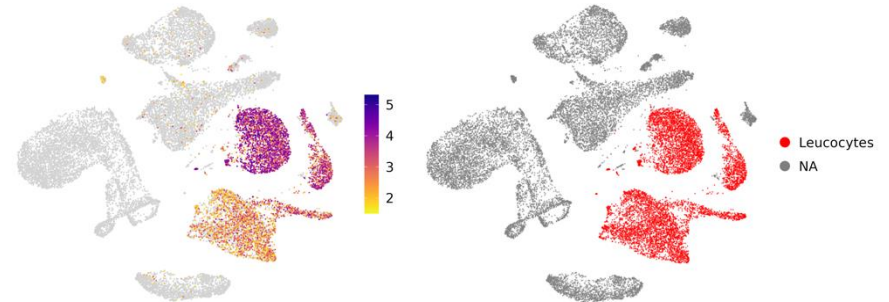
FIBROBLASTS

COL1A1, COL1A2, COL3A1 EXPRESSION



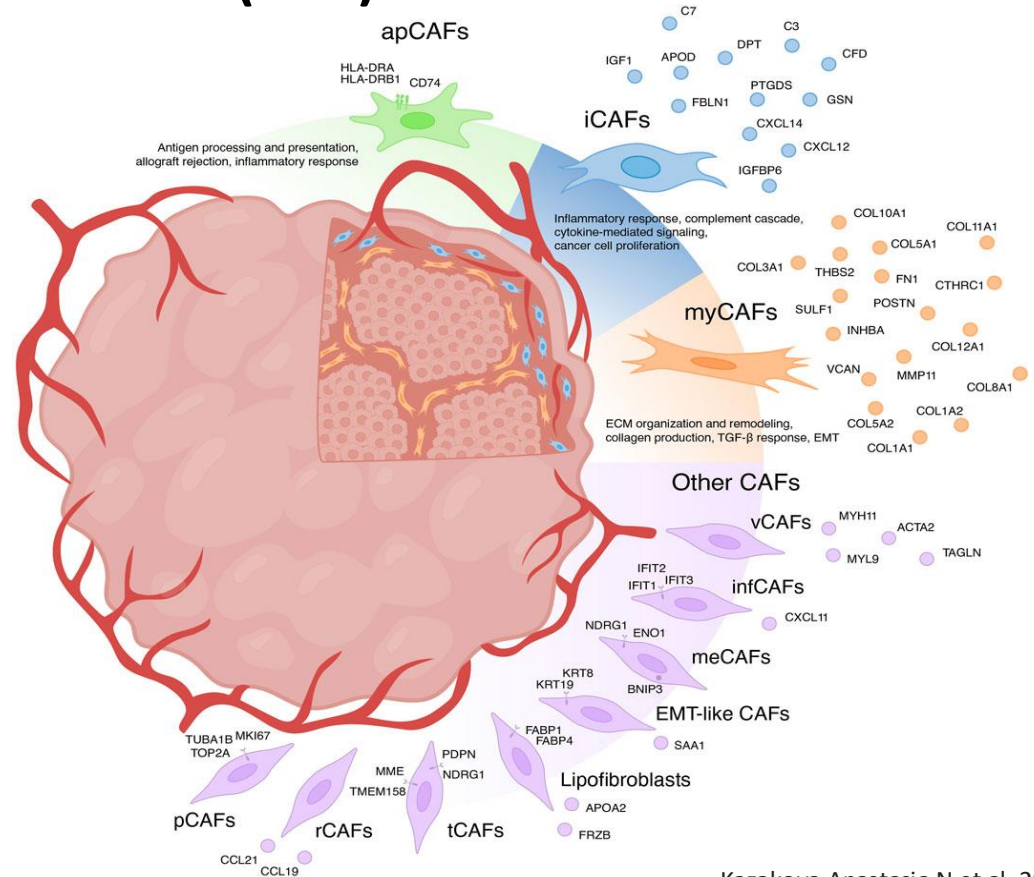
LEUCOCYTES

CD45 EXPRESSION

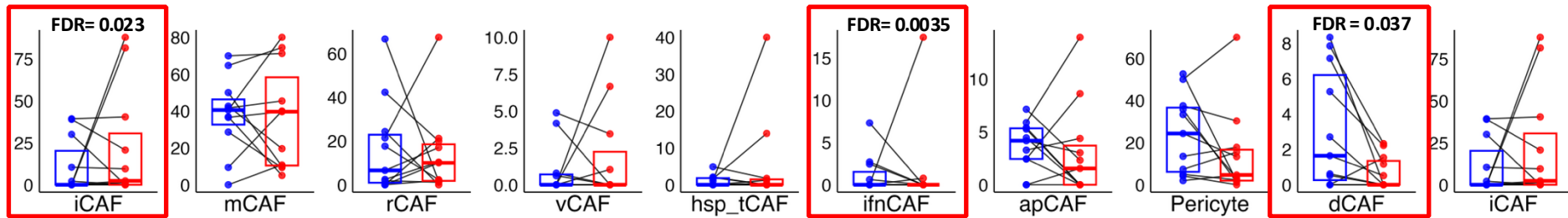
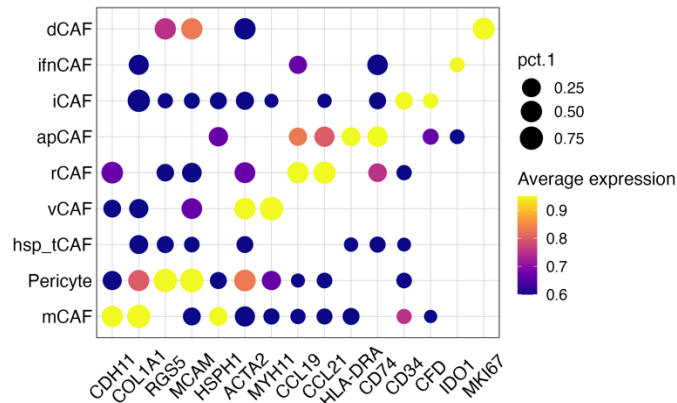
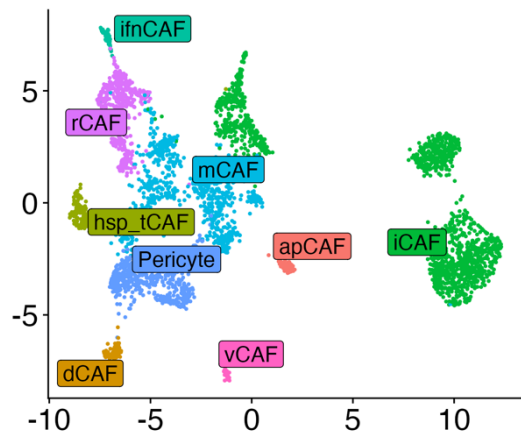


CANCER ASSOCIATED FIBROBLASTS (CAF)

- Heterogeneous population of stromal cells within the TME.
- Support of **tumor growth** and invasion, modulation of immune responses and **inflammation**, remodeling of the extracellular matrix (ECM).
- Subtypes include: matrix (mCAFs), inflammatory (iCAFs), and antigen-presenting (apCAFs), reticular (rCAFs), vascular (vCAF).
- Promising CAF targets have demonstrated **potent effects in preclinical models**



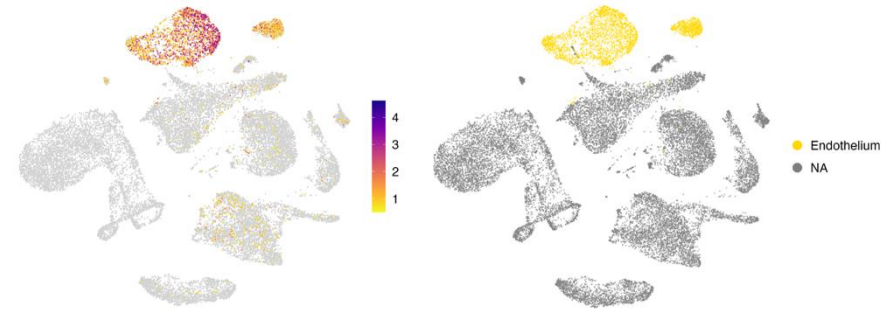
Chemotherapy reshapes CAF state composition



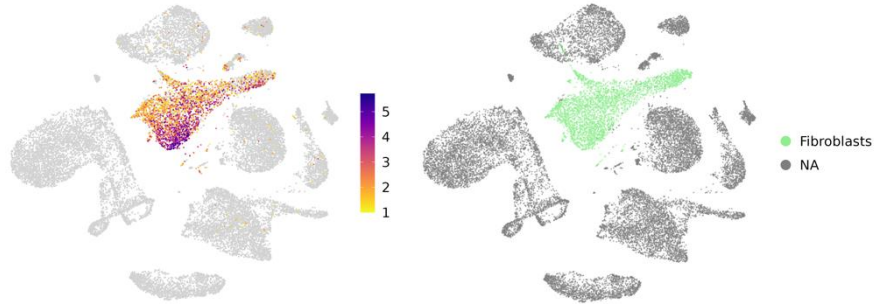
Post-treatment samples showed significant enrichment of iCAF and reduction of dCAF and ifnCAF

Focusing on 3 TME macro-compartments

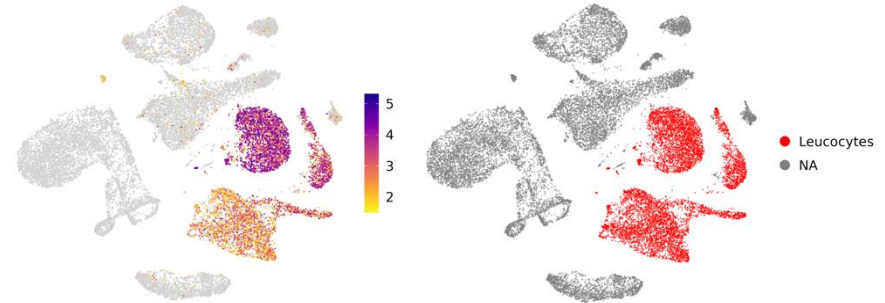
ENDOTHELIUM
EGFL, PLVAP EXPRESSION



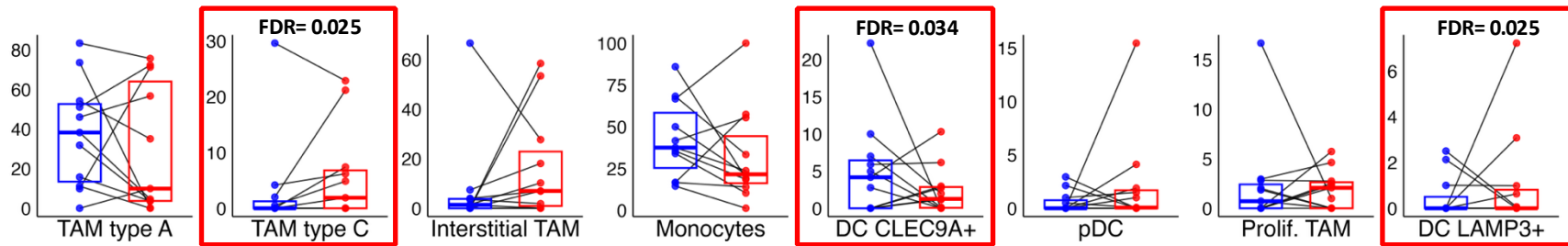
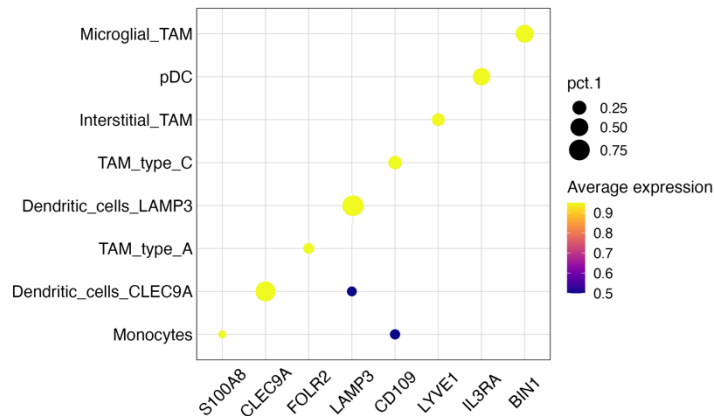
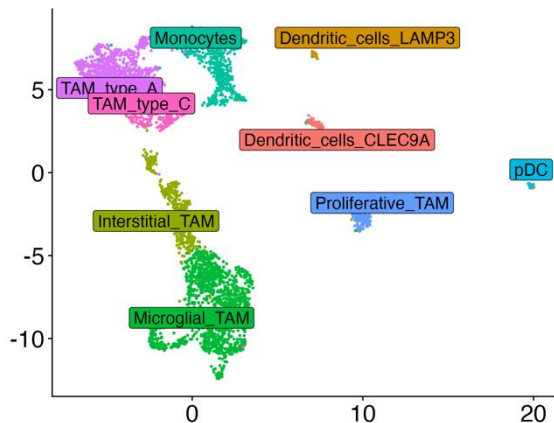
FIBROBLASTS
COL1A1, COL1A2, COL3A1 EXPRESSION



LEUCOCYTES
CD45 EXPRESSION

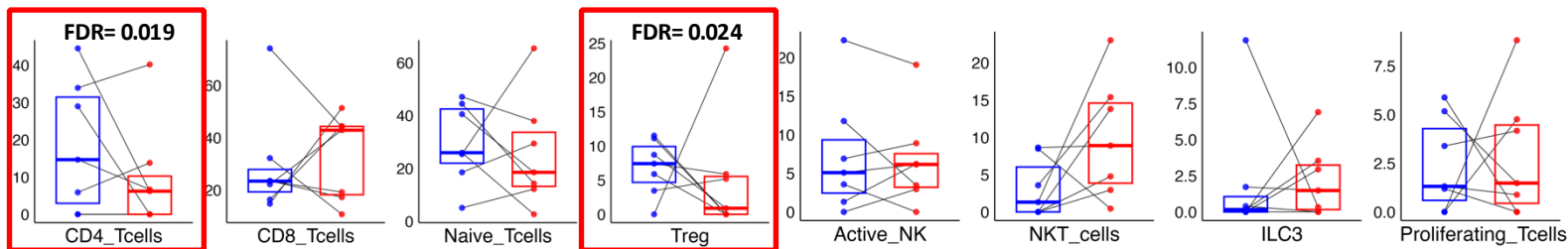
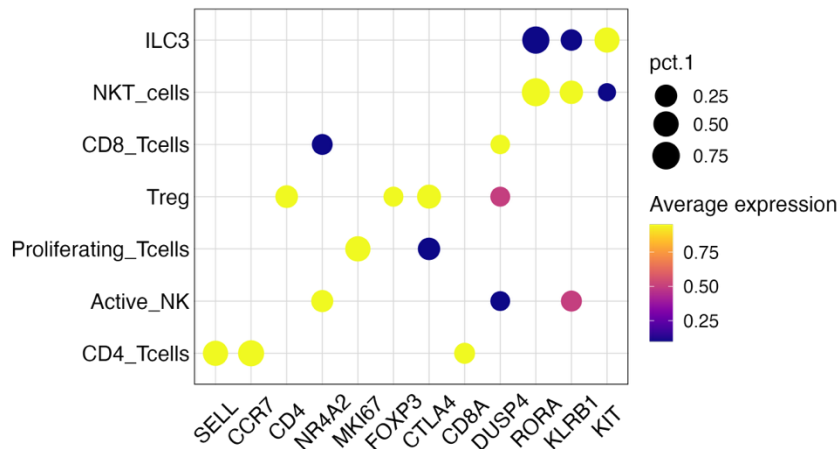
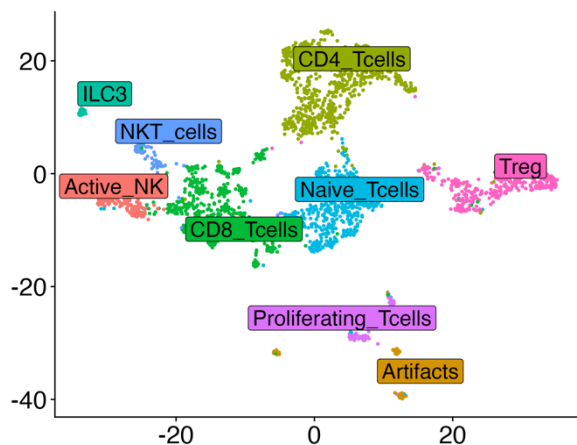


Myeloid remodeling identifies TAM type C enrichment after chemotherapy



Among myeloid populations, TAM type C showed marked enrichment in post-treatment samples, whereas DC CLEC9A and DC LAMP3 decreased

Post-treatment T cells display features of functional remodeling

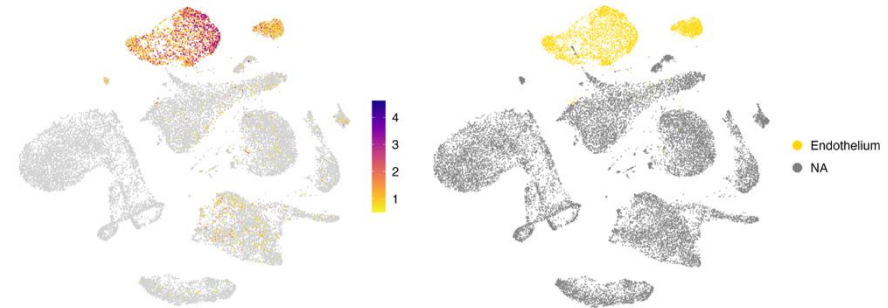


Chemotherapy is associated with shifts in T cell composition, suggesting altered immune activation and dysfunction states

Focusing on 3 TME macro-compartments

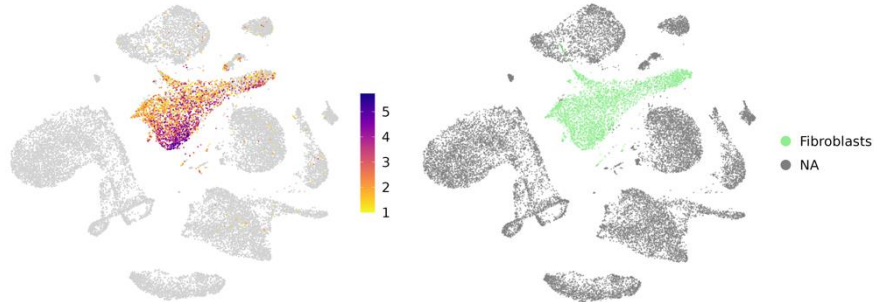
ENDOTHELIUM

EGFL, PLVAP EXPRESSION



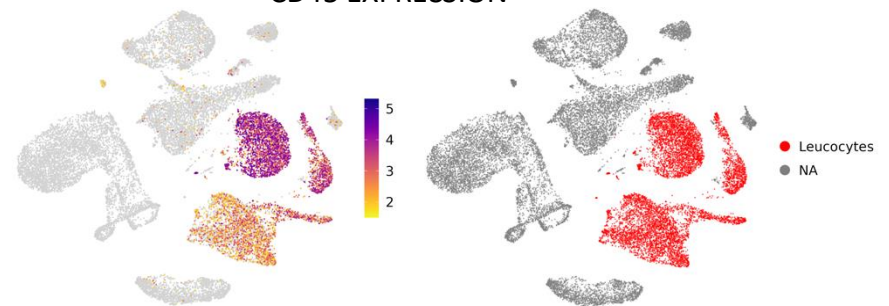
FIBROBLASTS

COL1A1, COL1A2, COL3A1 EXPRESSION

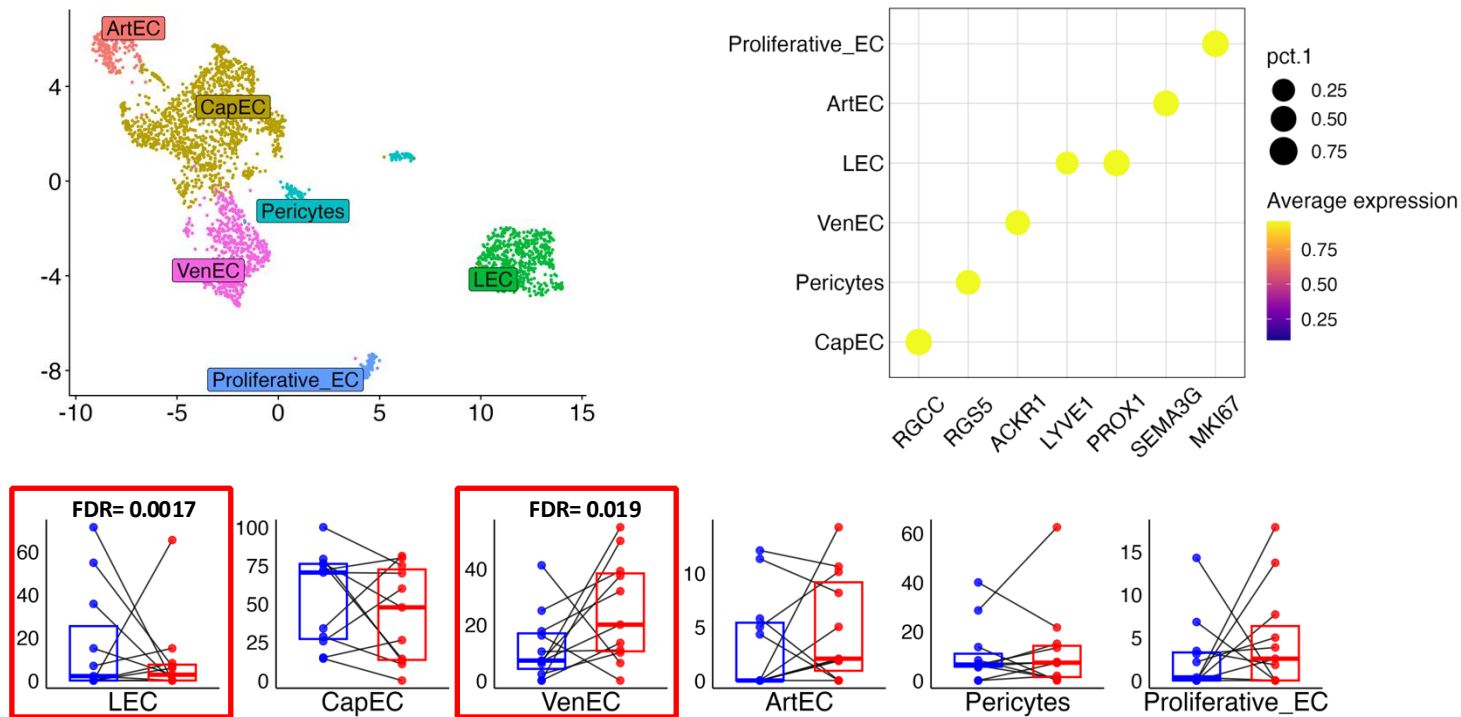


LEUCOCYTES

CD45 EXPRESSION

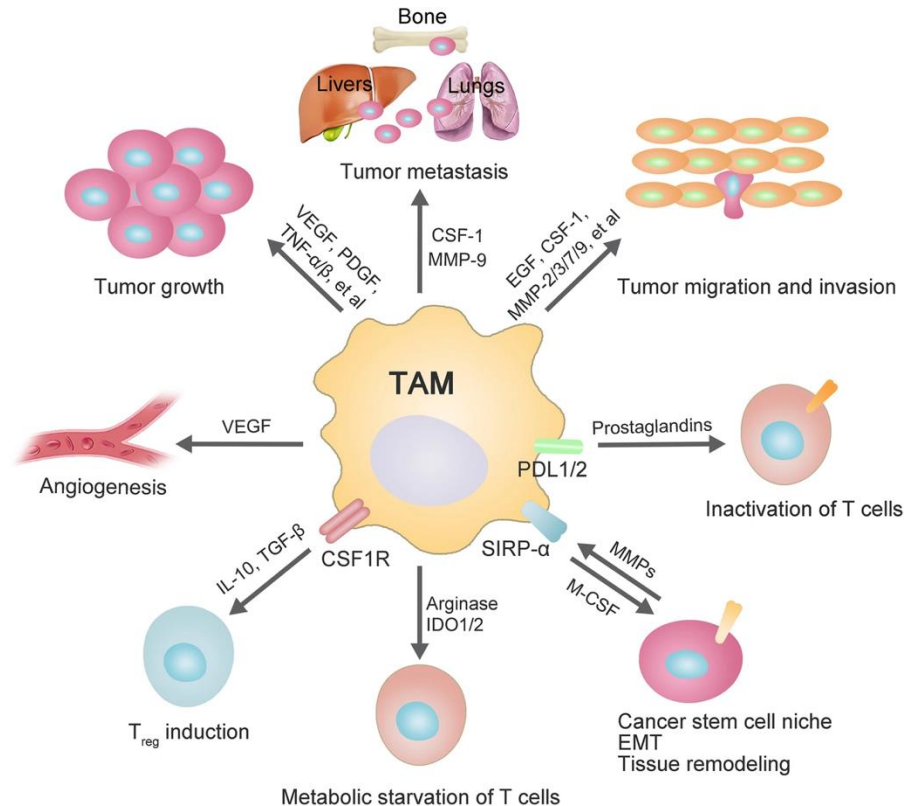


Chemotherapy differentially remodels endothelial compartments

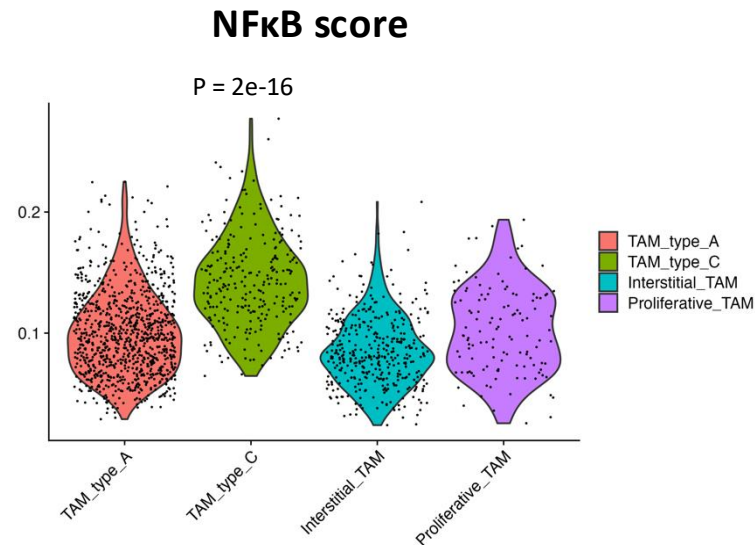
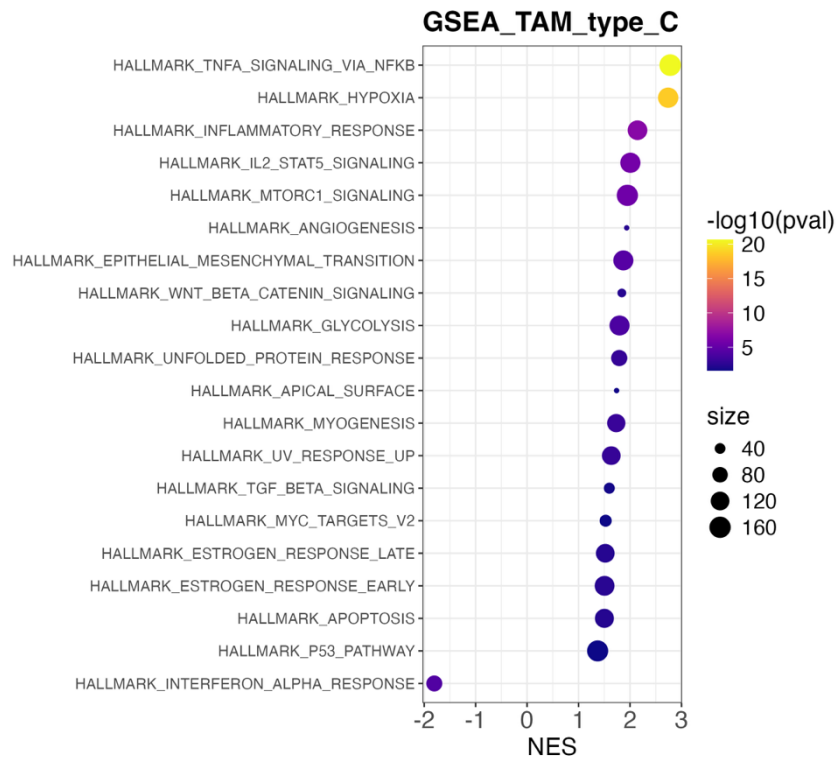


Lymphatic endothelial cells decreased after treatment, whereas vascular endothelial populations were enriched, suggesting angiogenic reprogramming of the tumor microenvironment,

Macrophages as key players of the neuroblastoma TME

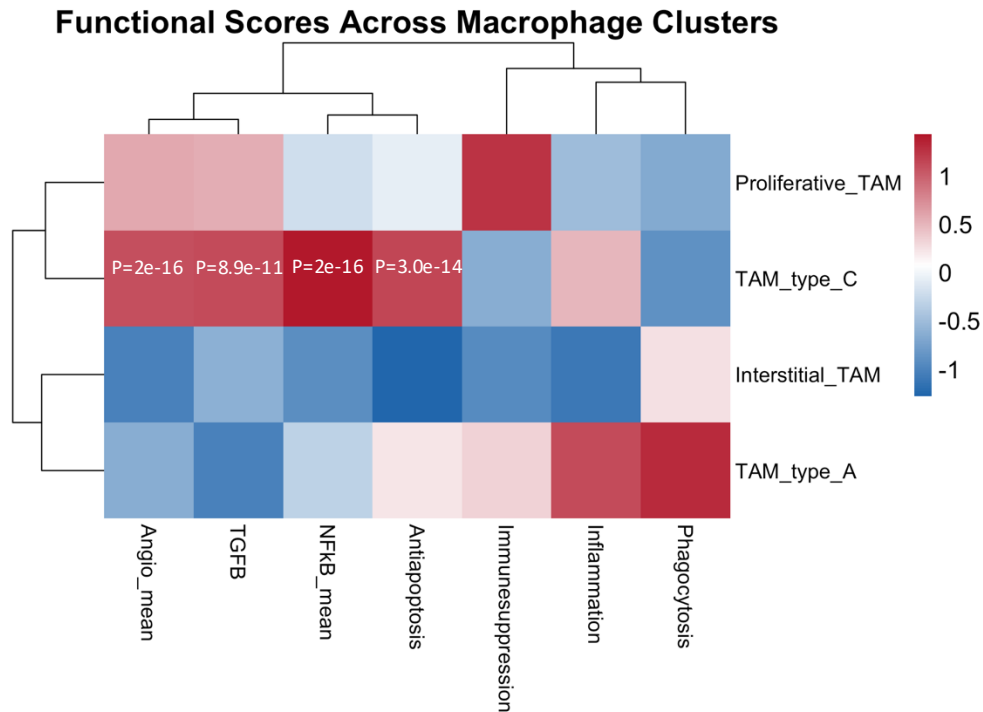


NFkB signaling defines the TAM type C transcriptional state



TAM type C shows the highest NFkB activity

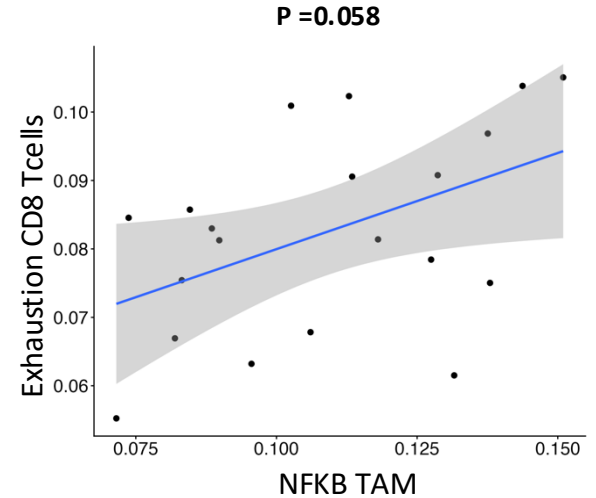
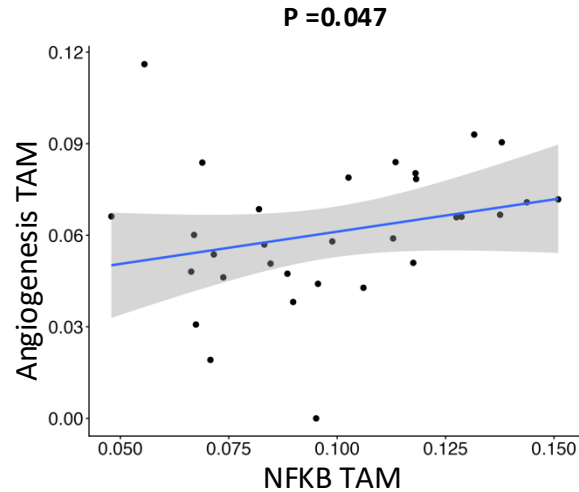
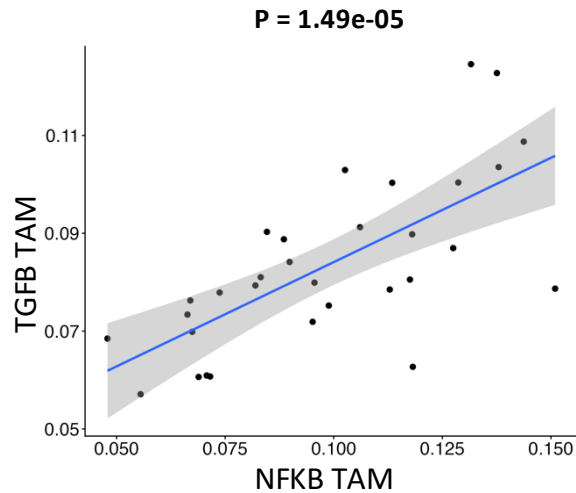
Functional programs distinguish macrophage states



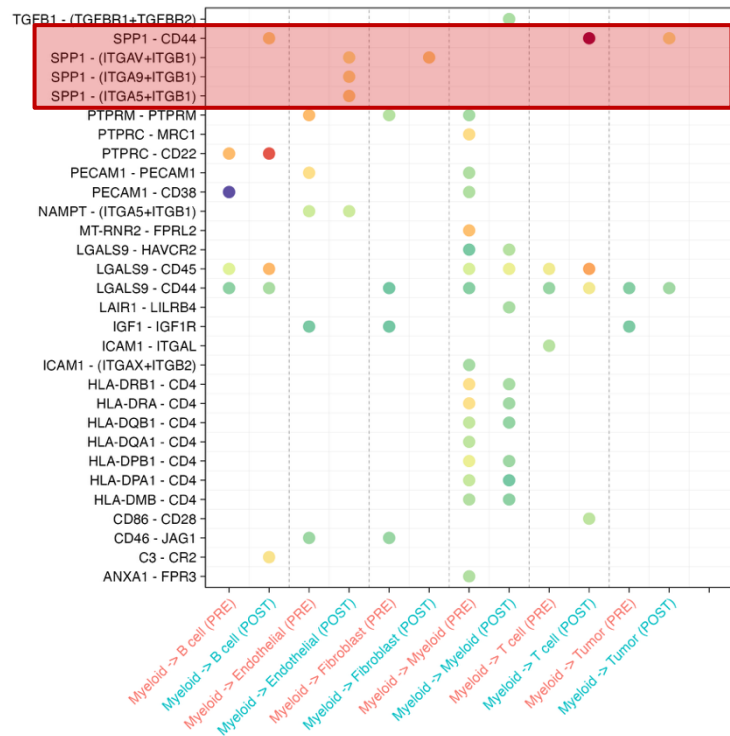
TAM C is characterized by:

- High NF κ B signaling
- Enhanced anti-apoptotic programs
- TGF β -associated activity
- Angiogenesis

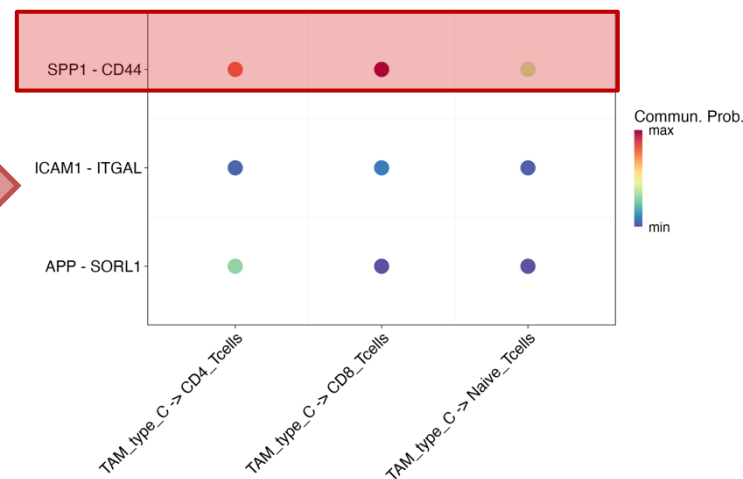
NFkB activity was associated with tumor-promoting and immunomodulatory programs, including TGFb signaling and a trend toward CD8 T cell exhaustion



SPP1-CD44 signaling guides T cell exhaustion

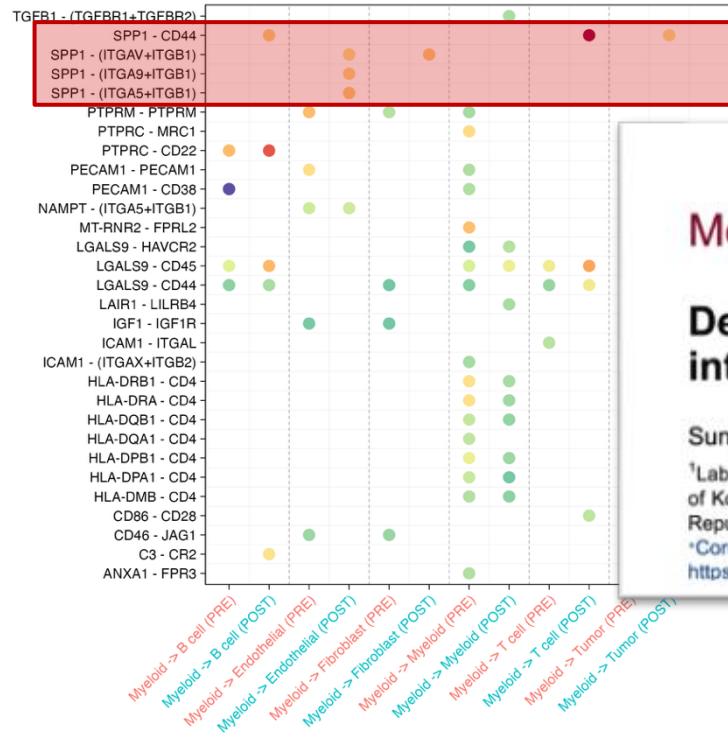


Zoom on TAM-C

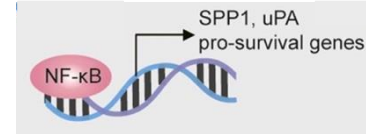


This suggests that TAM type C may act as a central hub of pro-tumoral communication in the post-chemotherapy microenvironment

SPP1-CD44 signaling guides T cell exhaustion



Molecules and Cells



Minireview

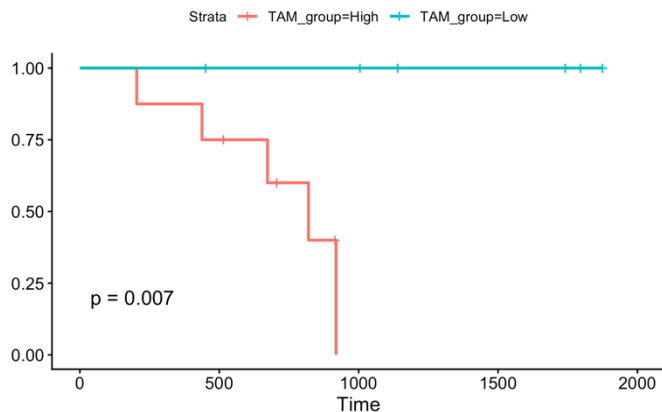
Decoding SPP1 regulation: Genetic and nongenetic insights into its role in disease progression

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*Correspondence: parkjh@sookmyung.ac.kr (J. H. Park), khryu@sookmyung.ac.kr (K.H. Yoo).
<https://doi.org/10.1016/j.mocell.2025.100215>

This suggests that TAM type C may act as a central hub of pro-tumoral communication in the post-chemotherapy microenvironment

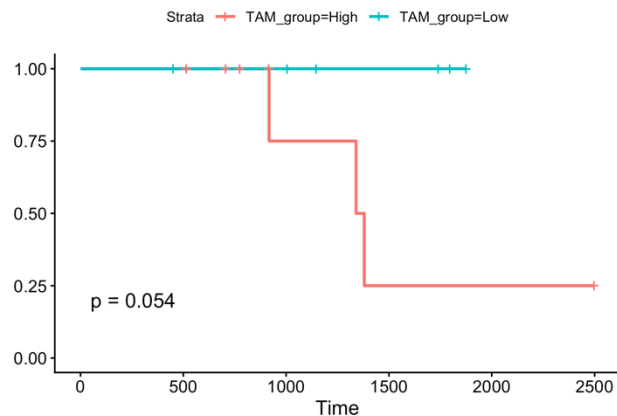
Patients with high TAM_C proportion show reduced progression-free survival



Number at risk

TAM_group=High	8	6	0	0	0
TAM_group=Low	6	5	5	3	0
	0	500	1000	1500	2000

PROGRESSION FREE SURVIVAL



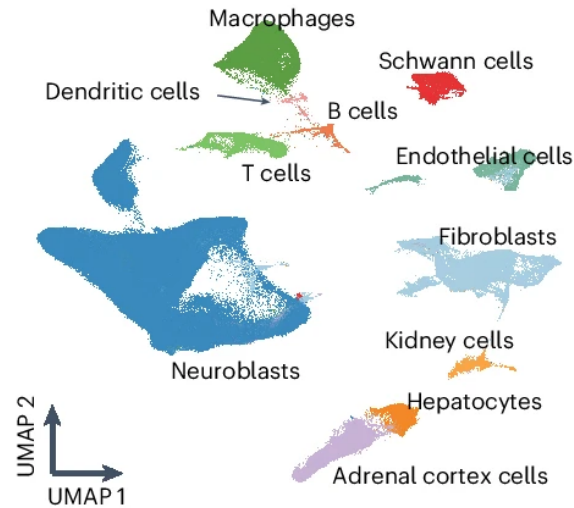
Number at risk

TAM_group=High	8	3	1	1	0
TAM_group=Low	5	5	3	0	0
	500	1000	1500	2000	2500

OVERALL SURVIVAL

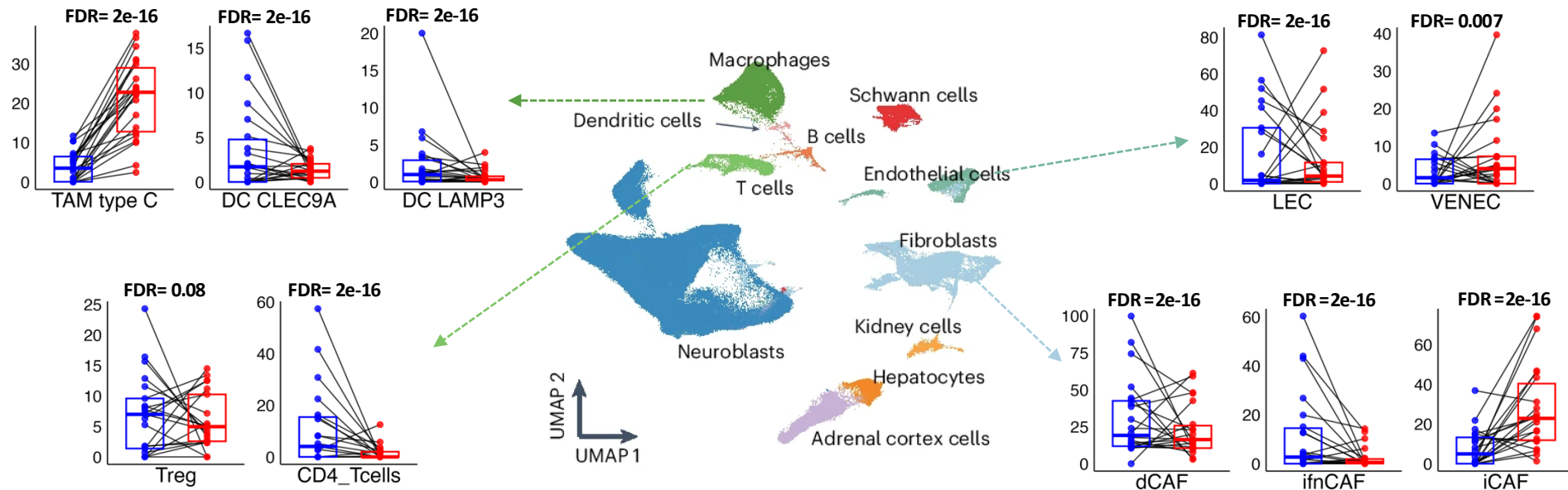
Results validated in an independent US cohort

372,619 cells from 44 samples

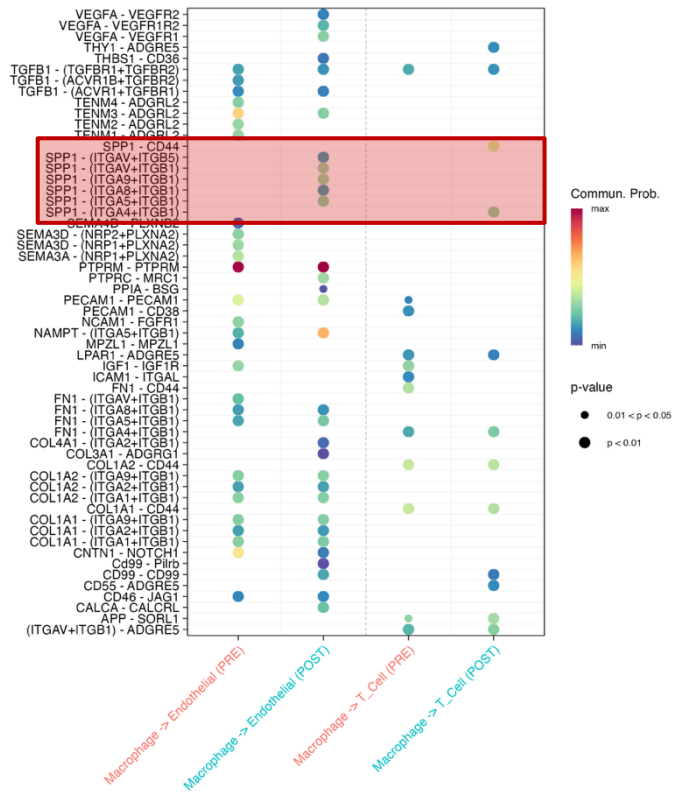
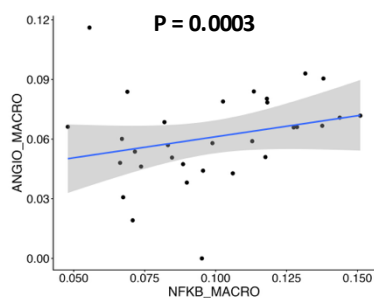
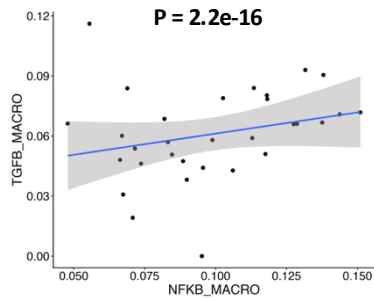
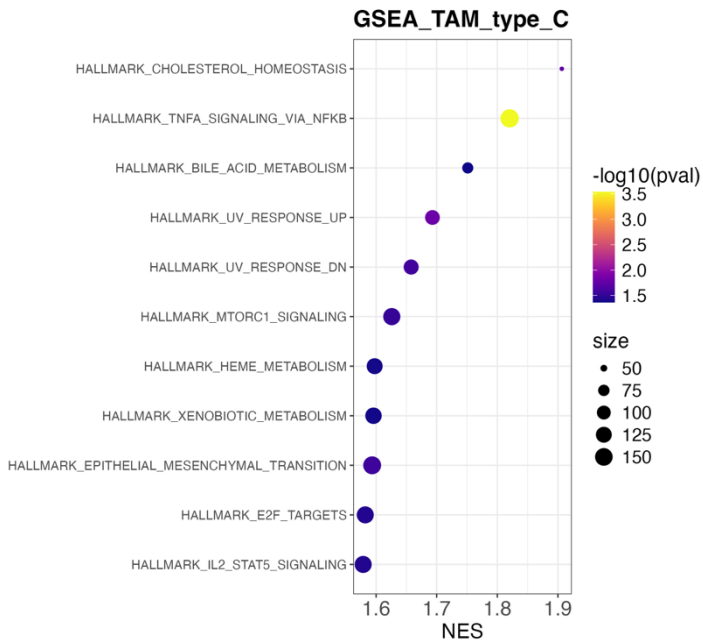


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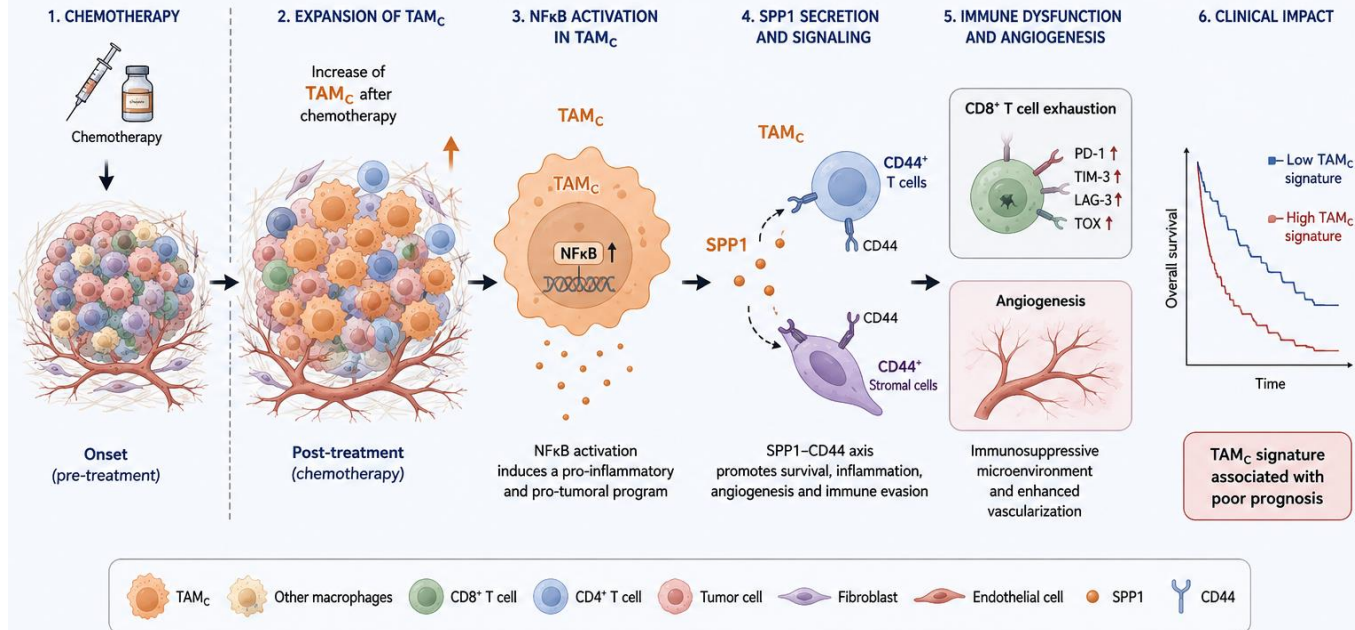


The NFkB-associated TAM program is reproduced in the US dataset



CONCLUSIONS

Chemotherapy reshapes the microenvironment by inducing $\text{NF}\kappa\text{B}^+$ TAM_C , driving SPP1-CD44 signaling, immune dysfunction and poor prognosis



TAKE-HOME MESSAGE Chemotherapy induces an $\text{NF}\kappa\text{B}$ -driven TAM_C program that rewires intercellular communication via SPP1-CD44 signaling, fostering immune suppression and contributing to treatment resistance and poor outcome.

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ITALIANA PER LA LOTTA
AL NEUROBLASTOMA
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