

# Squamous cell carcinomas (SCCs) harboring both p53 deletion and PIK3CA hyperactivation sensitivity to Immune Checkpoint Inhibitor (ICI) Treatment is dictated by its Tumor Microenvironment (TME)



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## Abstract

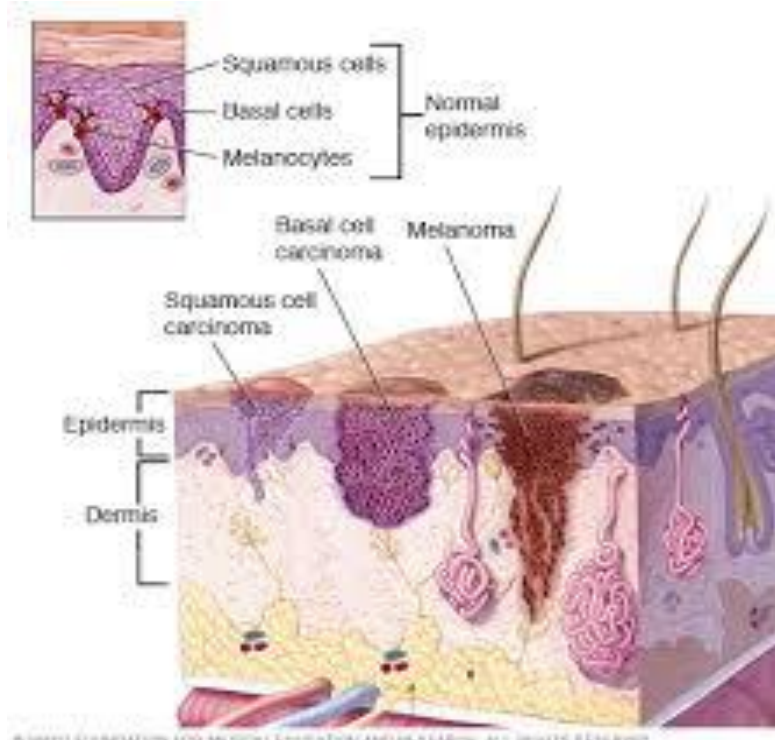
Head and neck squamous cell carcinomas (HNSCCs) make up 90% of all HNCs, and has been found to disproportionately affect Veterans than the civilian populations. In addition, HNSCC is a service-connected disease and the Veteran population has been found to have a worse prognosis compared to the civilian population. Due to genetic heterogeneity of HNSCC, the major concerns for treatment are the resistance and low response rate to immune checkpoint inhibitor (ICI) therapy. I recently created a murine model containing two most commonly mutated genes in HNSCC, PIK3CA and p53, termed keratin-15-p53-PIK3CA (KPPA). This SCC model may contribute to our understanding of HNSCC's heterogeneous nature in tumor progression and evasion. In addition, using our KPPA model, we have a method to potentially identify HNSCC patients who may not respond to ICI therapy by determining tumor-intrinsic cues and profiling the tumor microenvironment (TME) for therapeutic development to enhance responsiveness and individualize treatment for Veterans with HNSCC.

## Introduction

Head and Neck Cancer (HNC) is the sixth most common cancer, with 90% of those being squamous cell carcinoma (SCC). Common cancer-driving mutations of HNC are TP53 inactivation and PIK3CA over-expression.

Different patients' tumors vary widely in immune infiltration and response to immunotherapy

Checkpoint blockade targeting PD1 and PDL1 have been used as immunotherapy



## Questions

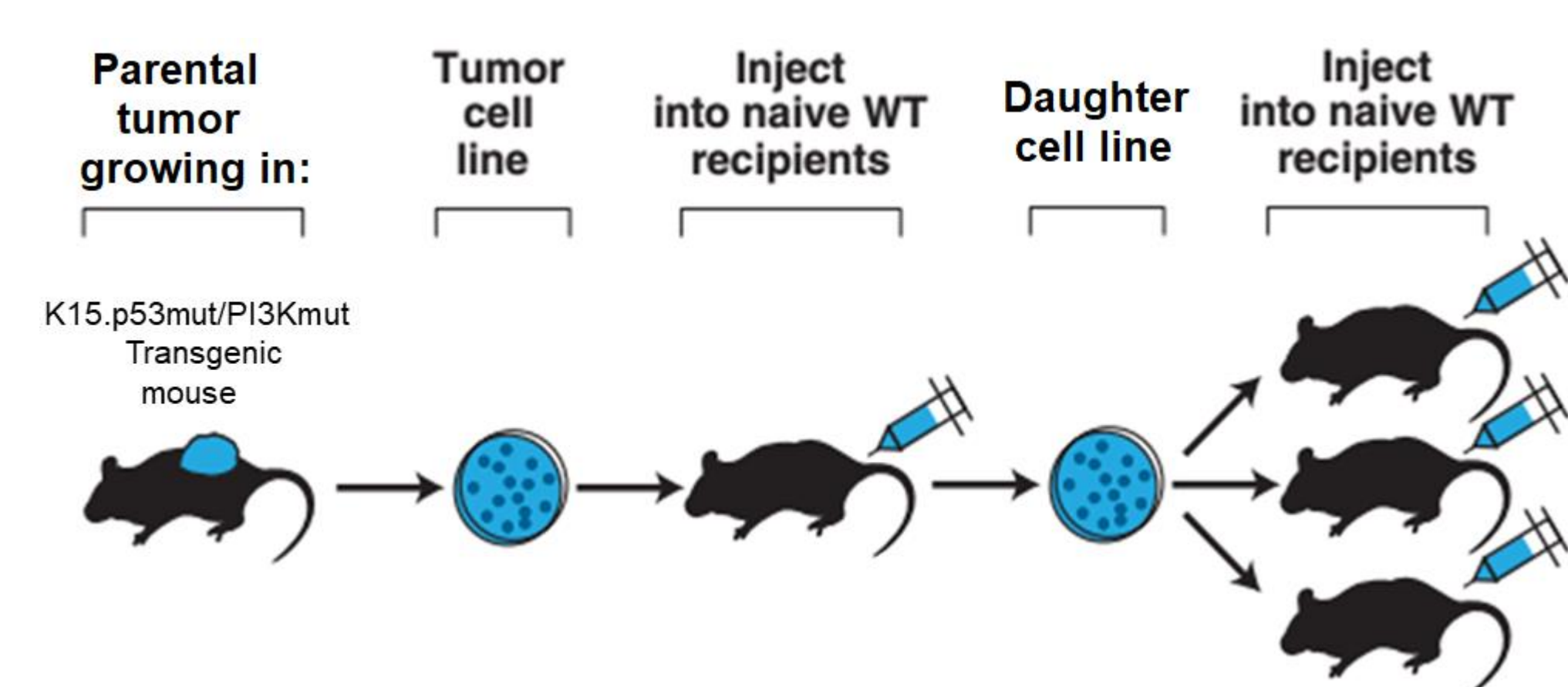
Can cancers be stratified according to their genetic alterations?

What are the different infiltrating immune cell subsets present in the tumor microenvironment as a result of the host's immune response to transplanted KPPA tumors?

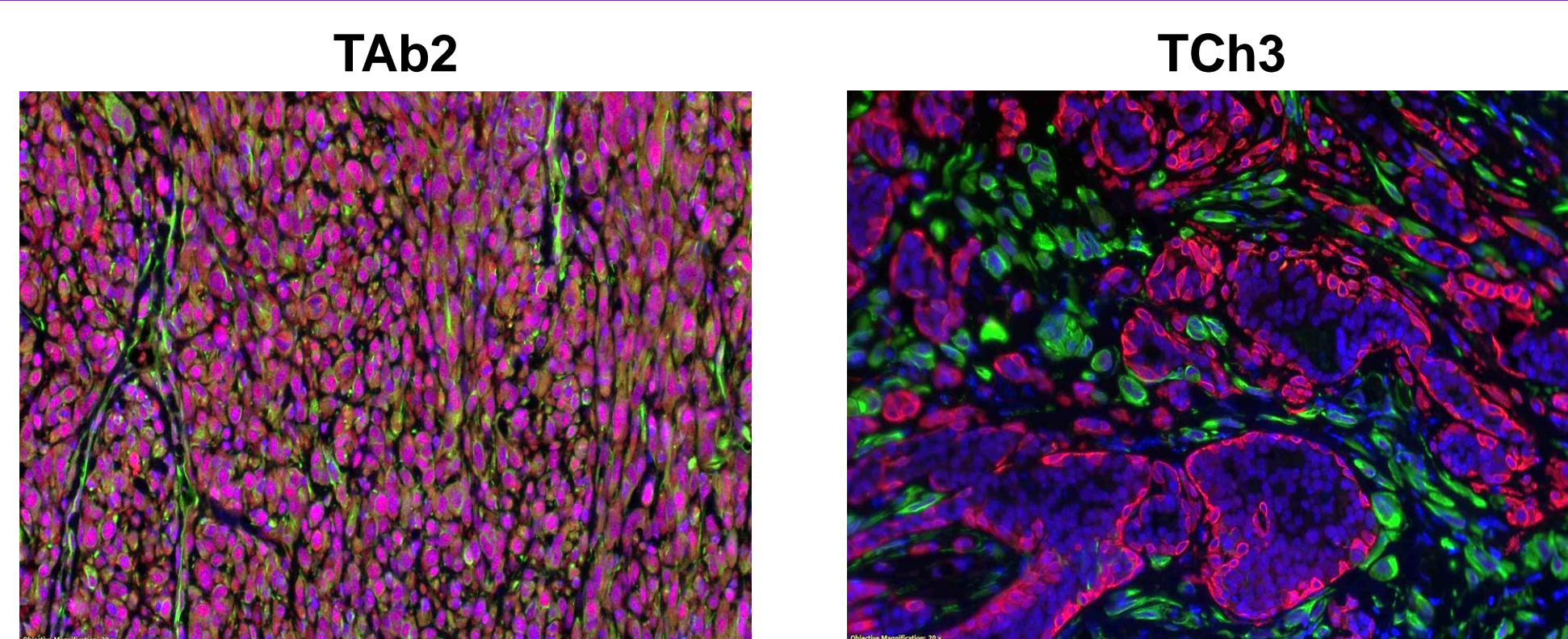
What are the potential markers that can be identified to determine ICI therapy response?

Does treatment with anti-PD-L1 antibodies inhibit transplanted KPPA tumor growth and prolong survival?

## Results



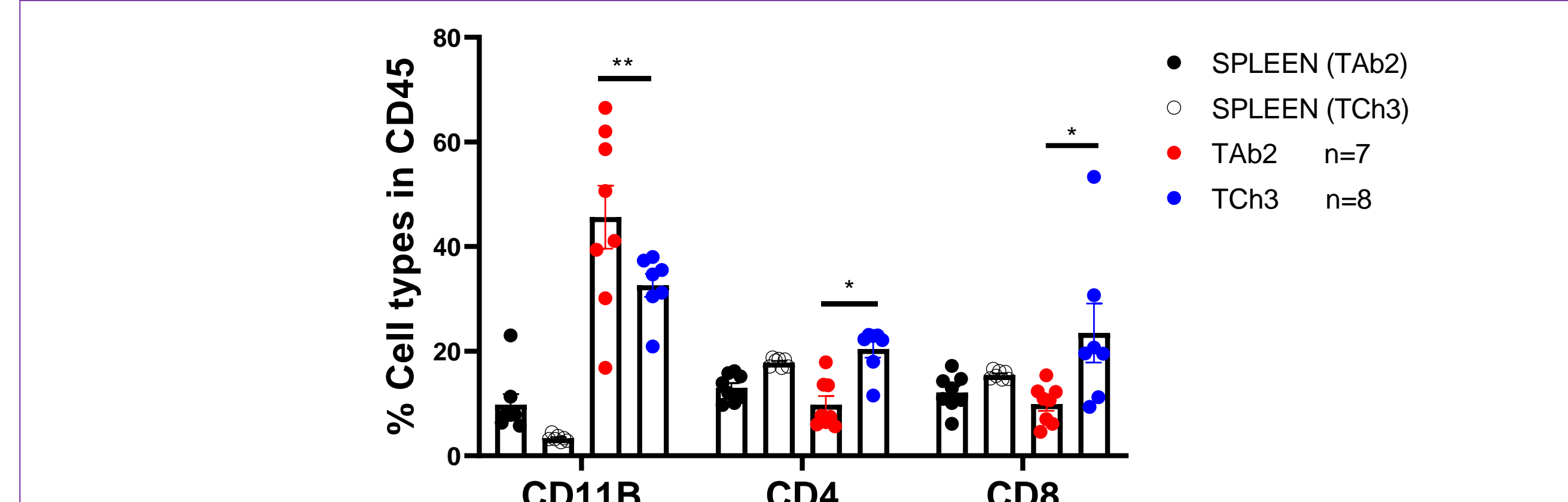
**Figure 1. Schematic representation of KPPA tumor cell lines.** Spontaneous Keratin-p53-PIK3CA (KPPA) tumors were harvested from transgenic mice and multiple cell lines were created and transplanted into wildtype (WT) C57BL/6 (B6) recipients. Tumors were again harvested to develop daughter cell lines and transplanted into WT mice. Tumors and infiltrating immune cells were stained for immune profiling.



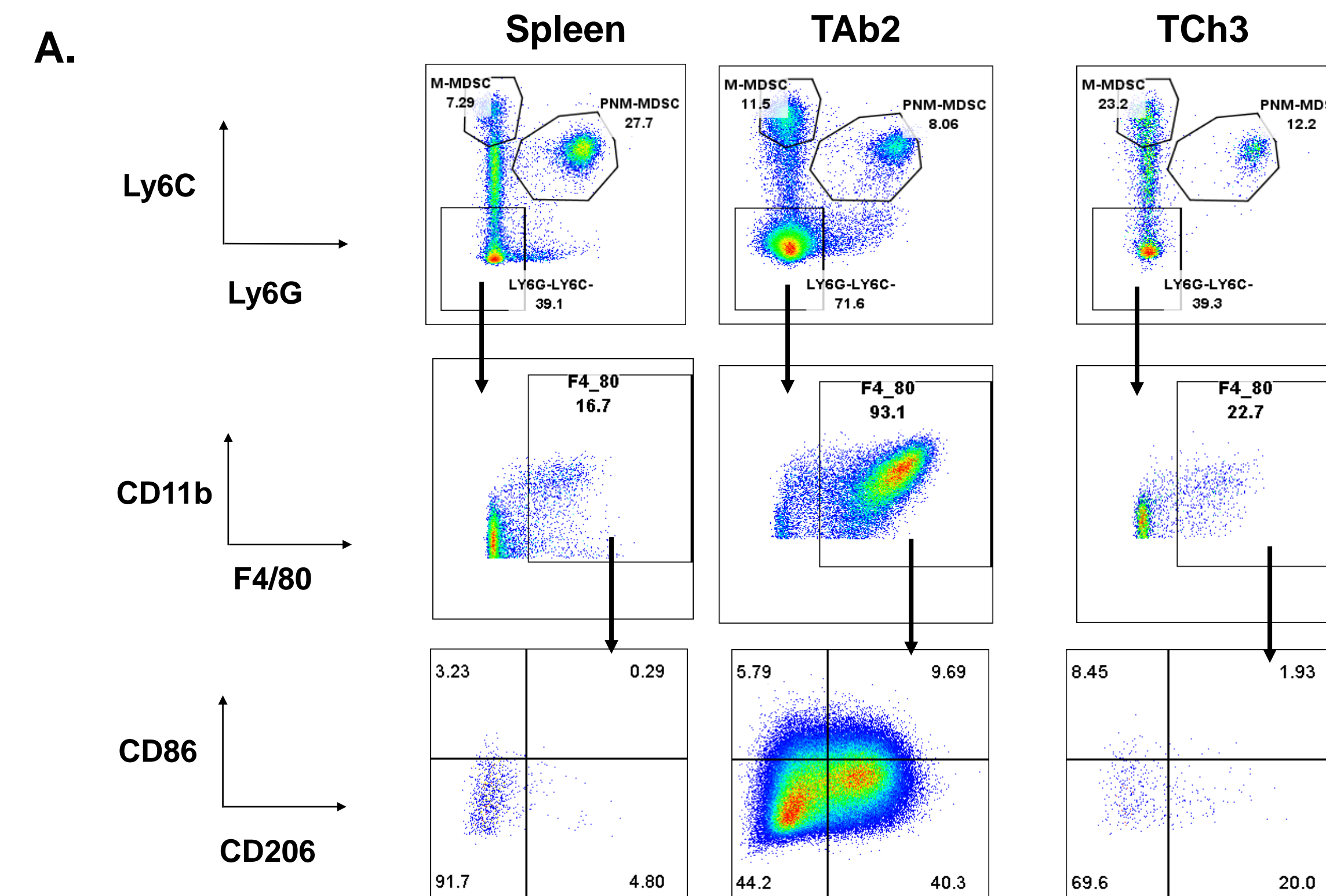
**Figure 2. Morphological changes of transplanted KPPA tumors.** Both parental KPPA tumors were SCCs, and after transplantation, Tab2 underwent epithelial-mesenchymal transition (EMT) and was poorly differentiated, while TCh3 maintained its SCC morphology.

## Funding:

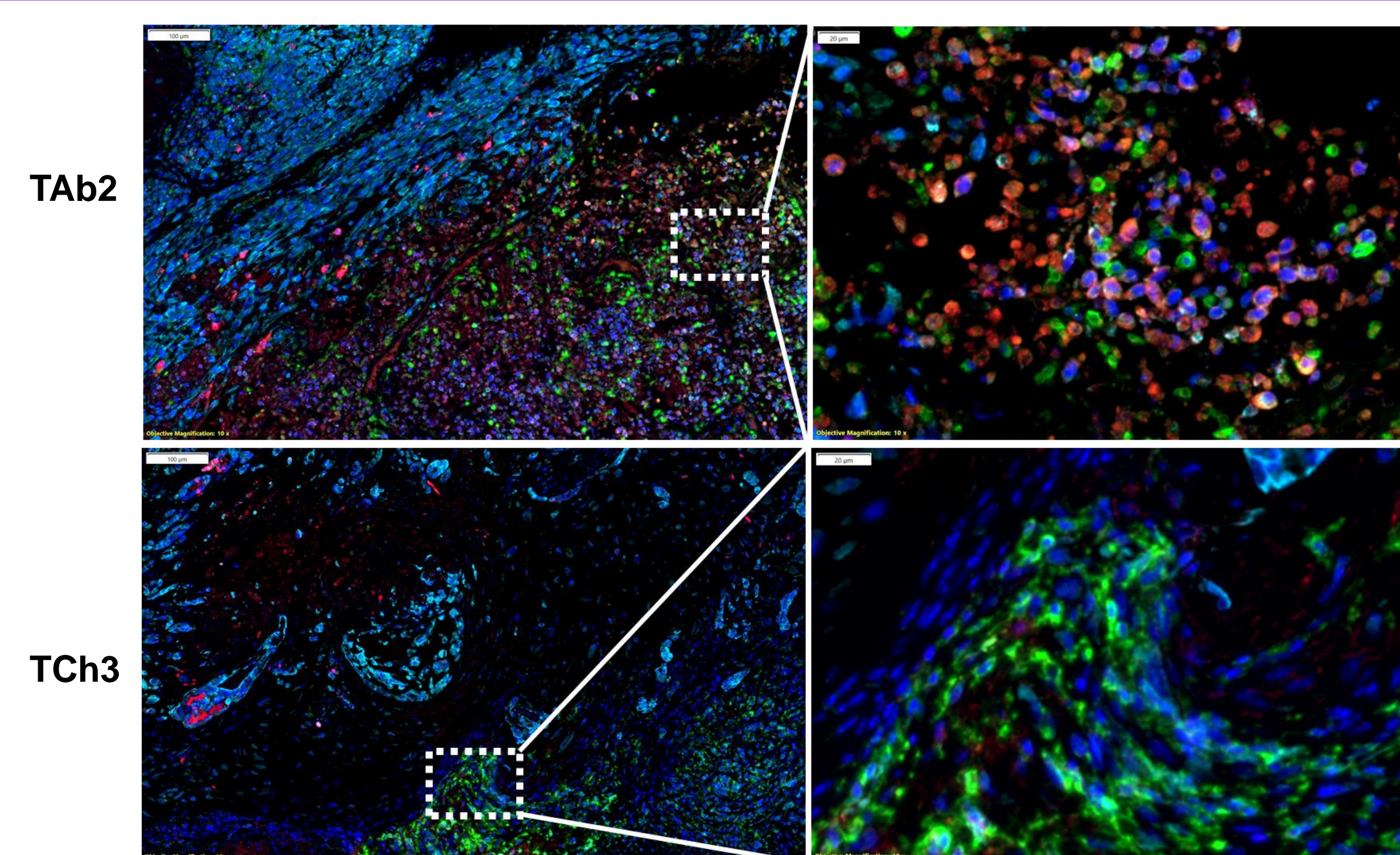
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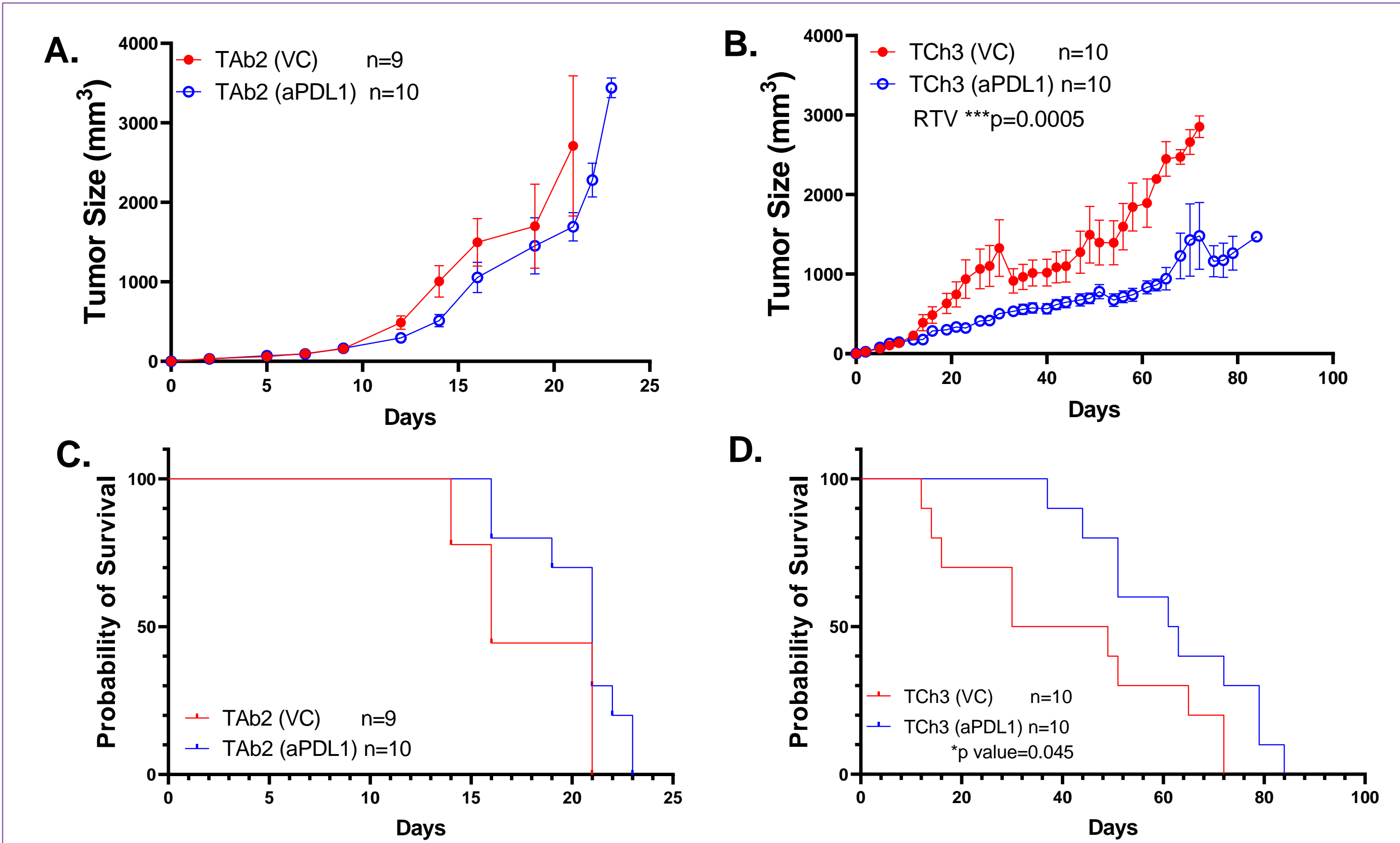
**Figure 3. Profiling the Tumor Microenvironment (TME).** Quantification of the percentage of CD11b<sup>+</sup>, CD4<sup>+</sup>, or CD8<sup>+</sup> cells in CD45<sup>+</sup> population of spleen controls, Tab2 and TCh3 tumors. Significance was calculated using two-way ANOVA with Tukey's multiple comparison test.



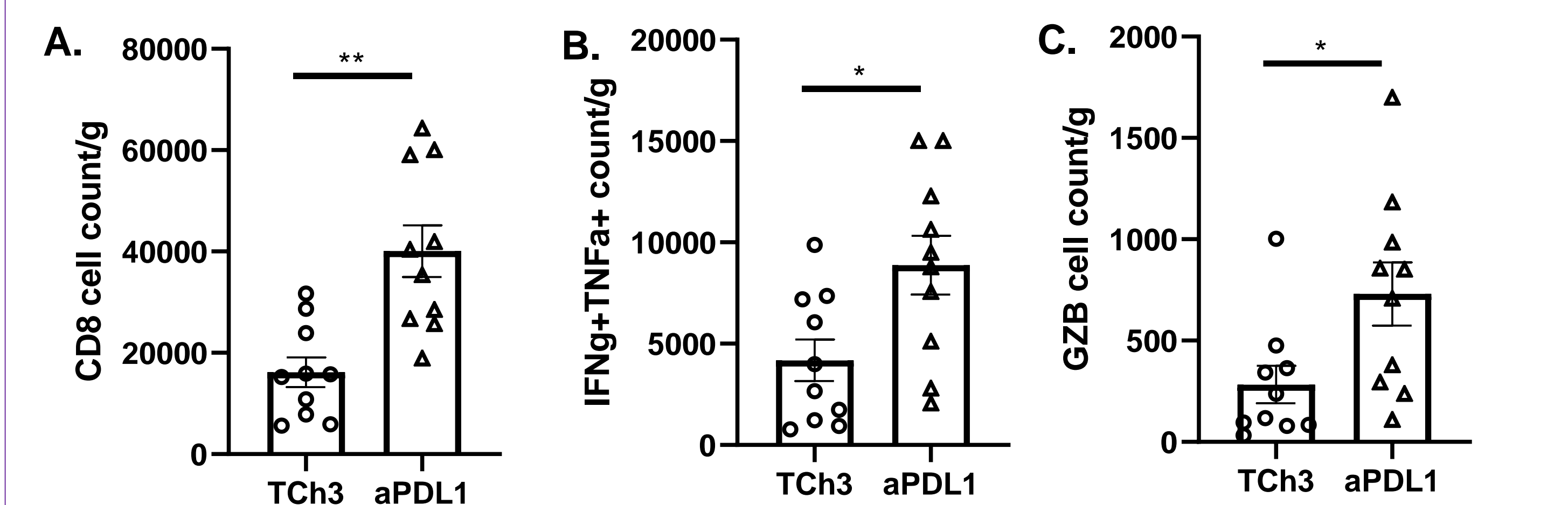
**Figure 4. Characterization of Tumor Associated Macrophages (TAMs) population.** (A) Representative flow plots of different MDSC populations (top panel), TAMs population defined by CD11b<sup>+</sup>Ly6C<sup>+</sup>Ly6G<sup>-</sup>F4/80<sup>+</sup> expression (middle panel), and TAMs expressing CD86 and/or CD206 (bottom panel). (B) Quantification of the percentages of TAMs in CD45<sup>+</sup> population of spleen, Tab2 and TCh3 tumors. (C) Quantification of the percentages of M1 (CD86<sup>+</sup>CD206<sup>-</sup>) and M2 (CD206<sup>+</sup>CD86<sup>+</sup>) TAMs in F4/80<sup>+</sup> population. P values are shown for one-way ANOVA and Tukey's multiple comparisons by two-way ANOVA.



**Figure 5. Representative images of multispectral imaging (MSI) analysis of Tab2 (top) or TCh3 (bottom) tumors stained for Arginase-1 (red), F4/80 (green), Keratin 5 (cyan), and DAPI (blue).** The white bars on the top left corners of the image indicates scale, 100µm (left panel) and 20µm (right panel).



**Figure 6. Treatment of KPPA tumors.** Mice were injected with KPPA Tab2 or TCh3. Once tumor size reached ~150mm<sup>3</sup>, mice were grouped into vehicle control (VC) or treatment groups (either treated with PBS or 200µg anti-PD-L1 (i.p.) every other day for two weeks). Tumor growth curves for (A) Tab2 and (B) TCh3 compared with VC (relative tumor volume: RTV). Survival curves of WT B6 mice bearing (C) Tab2 or (D) TCh3 tumors that were treated with VC or anti-PD-L1 were compared between the corresponding control and treated group using Gehan-Breslow-Wilcoxon test.



**Figure 7. Anti-PD-L1 treatment enhanced CD8 T cell number and effector functions in TCh3 tumors.** Flow cytometry analysis was performed for tumor-infiltrating immune cells from TCh3 VC (n=10) and TCh3 anti-PD-L1 (n=10) groups. Cell number count of CD8 T cells in TCh3 vs. TCh3 anti-PD-L1 group. Cell count of different CD8 populations present per gram of tumor tissue. (A) Total CD8, (B) double IFNγ<sup>+</sup>/TNFα<sup>+</sup> in CD8 and (C) Granzyme B<sup>+</sup> (GZB) in CD8. Statistical significance is calculated with unpaired two-tailed t test.

## Conclusions

- While both transplanted KPPA tumors (Tab2 vs. TCh3) harbor TP53 deletion and PIK3CA hyperactivation, they respond to ICI treatment differently.
- Our study indicates the limitations of stratifying cancers according to their genetic alterations alone.
- ICI non-responding Tab2 tumors are highly infiltrated with CD11b<sup>+</sup> myeloid cells.
  - These myeloid population are M2-TAMs, and express a higher level of CD206 and display immunosuppressive properties by expressing arginase-1 (Arg-1).
- Blockade using anti-PD-L1 antibodies significantly decreased TCh3 tumor growth and prolong survival.
  - TCh3 tumors have higher levels of pre-existing infiltrating CD8<sup>+</sup> T cells
  - After ICI therapy, CD8<sup>+</sup> T cells population further expanded with significantly higher effector function

## Future Directions

- Elucidate the mechanisms of PD-L1 blockade in KPPA tumors. What are the markers required for the anti-tumor effects of PDL1 in KPPA tumors?
- Define the tumor-intrinsic cues and immune profiles of ICI responders.
- Can other inhibitors along with anti-PD-L1 therapy improve treatment for tumors with highly infiltrating myeloid cells?