# Probing Spatial Myeloid Heterogeneity in Glioblastoma


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## Introduction

- Glioblastoma, the most common type of malignant brain tumor, has evaded conventional adaptive immunotherapeutic efforts.[1]
- Little is understood about the myeloid composition in the glioma microenvironment. Modulating glioma-associated macrophage (GAM) activity presents an alternative immunotherapeutic strategy.
- $Qk^{L/L}$;$Pten^{L/L}$;$Trp53^{L/L}$ (*QPP*) mice develop glioma with immune environments resembling that of human glioma.[2-3] They are thus ideal in determining myeloid composition across a tumorigenic brain.
- We sought to probe the following:
    1. Myeloid cell morphology in non-tumor and tumor regions
    2. Distinction between resident microglia and circulation-derived macrophages (CDMs)
    3. Presence of pro- or anti-phagocytic markers

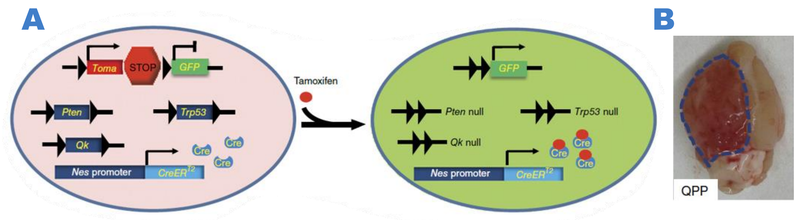


**Figure 1. A.** Design of *Nes-CreER$^{T2}$*;*Qk$^{L/L}$*;*Pten$^{L/L}$*;*Trp53$^{L/L}$* (*QPP*) mouse model. **B.** Representative image of QPP murine brain after harvesting.[2]

## Methods

**Mouse models:**

- *QPP7* (genetic tumors), injected with tamoxifen at P7, and harvested when moribund
- *Cx3cr1-CreER$^{T2}$* adult mice injected with *QPP7* tumor cells (implanted tumors), harvested when moribund

**Slide preparation:** Following euthanasia, brains were removed, fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 µm via microtome.

**Immunofluorescence (IF) staining or co-staining:** Sections were stained with 1:250 primary antibody dilution and 1:1000 secondary antibody dilution (488 nm or 594 nm). The following antibodies were used: Iba1, TMEM119, CD45, CD47, Arg1, and GFP. Images were taken via widefield microscopy.

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## References


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2. Shingu T. et. al. *Nat. Genet.* 2017; 49(1); 75-86.
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## Results

- **Microglia change morphology based on location. Morphotypes have obvious but unexplained functional differences.**

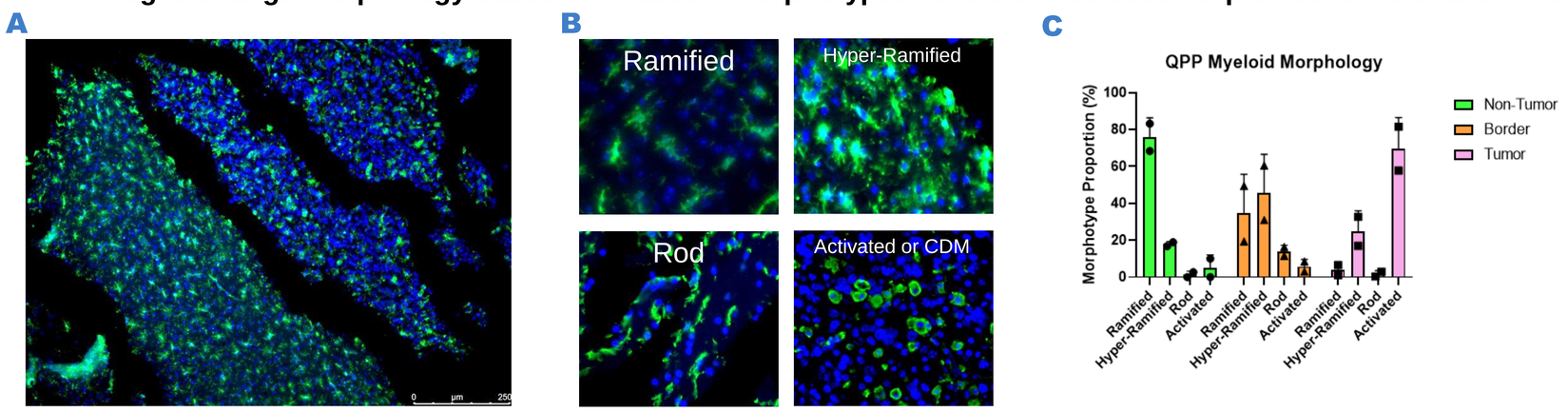


**Figure 2. A.** Representative 10x IF staining of implanted *QPP* with Iba1 (green). **B.** Classification of Iba1$^+$ myeloid cells based on morphology. **C.** Proportion of myeloid cell morphotypes by brain region.

- **TMEM119 is downregulated in glioma conditions, supporting its identity as a marker of homeostatic conditions.[4]**
- **Iba1$^+$ CD45$^{high}$ cells are increasingly found towards tumor core, suggesting higher likely CDM infiltration.**

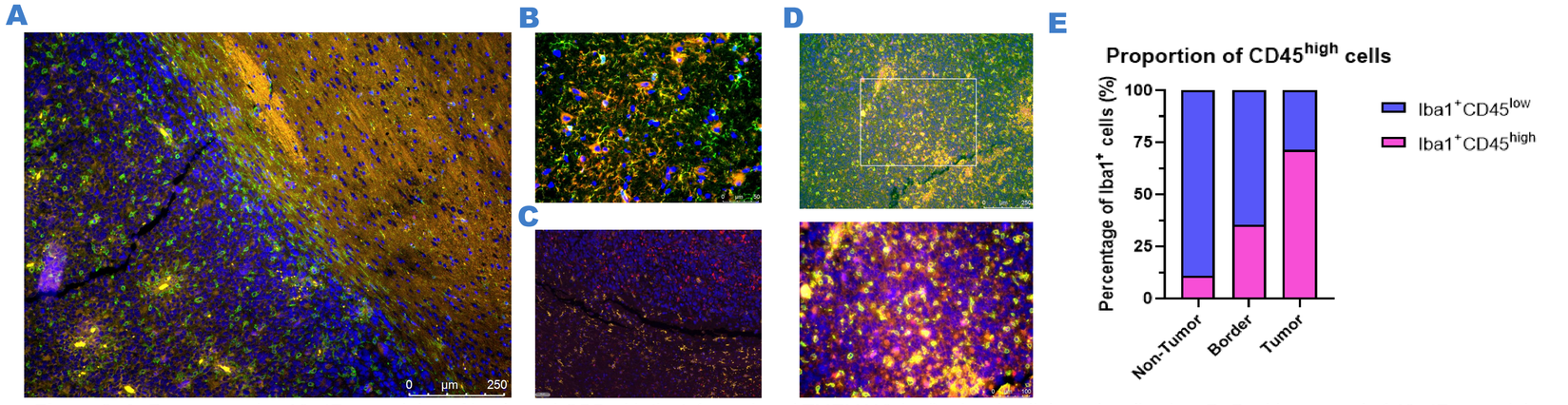


**Figure 3. A.** Representative 10x IF co-staining of implanted *QPP* with Iba1 (green) and TMEM119 (red), showing little co-localization. **B.** Positive control of 40x IF co-staining using genetic *QPP* brain harvested at 7 weeks with Iba1 (green) and TMEM119 (red). **C.** Representative 20x multiplex IF staining using Vectra Polaris slide scanner (more powerful imaging) of implanted *QPP* with 1:2000 CD11b (red) + TMEM119 solution (yellow), showing co-localization. **D.** Representative 10x (top) and 20x (bottom) IF co-staining of implanted *QPP* with Iba1 (green) and CD45 (red). **E.** Proportion of CD45$^{high}$ cells by brain region.

- **Tumor cells are largely CD47$^+$, indicating phagocytic suppression.**
- **Arg1$^+$ expression increases towards tumor core, suggesting increasing myeloid polarization to "M2" subtype.**
- **GFP signal was found only in some Iba1$^+$ cells, suggesting high phagocytic heterogeneity. It remains unclear whether there is downregulation of uptake and/or endolysosomal activity, and at what point of development.**

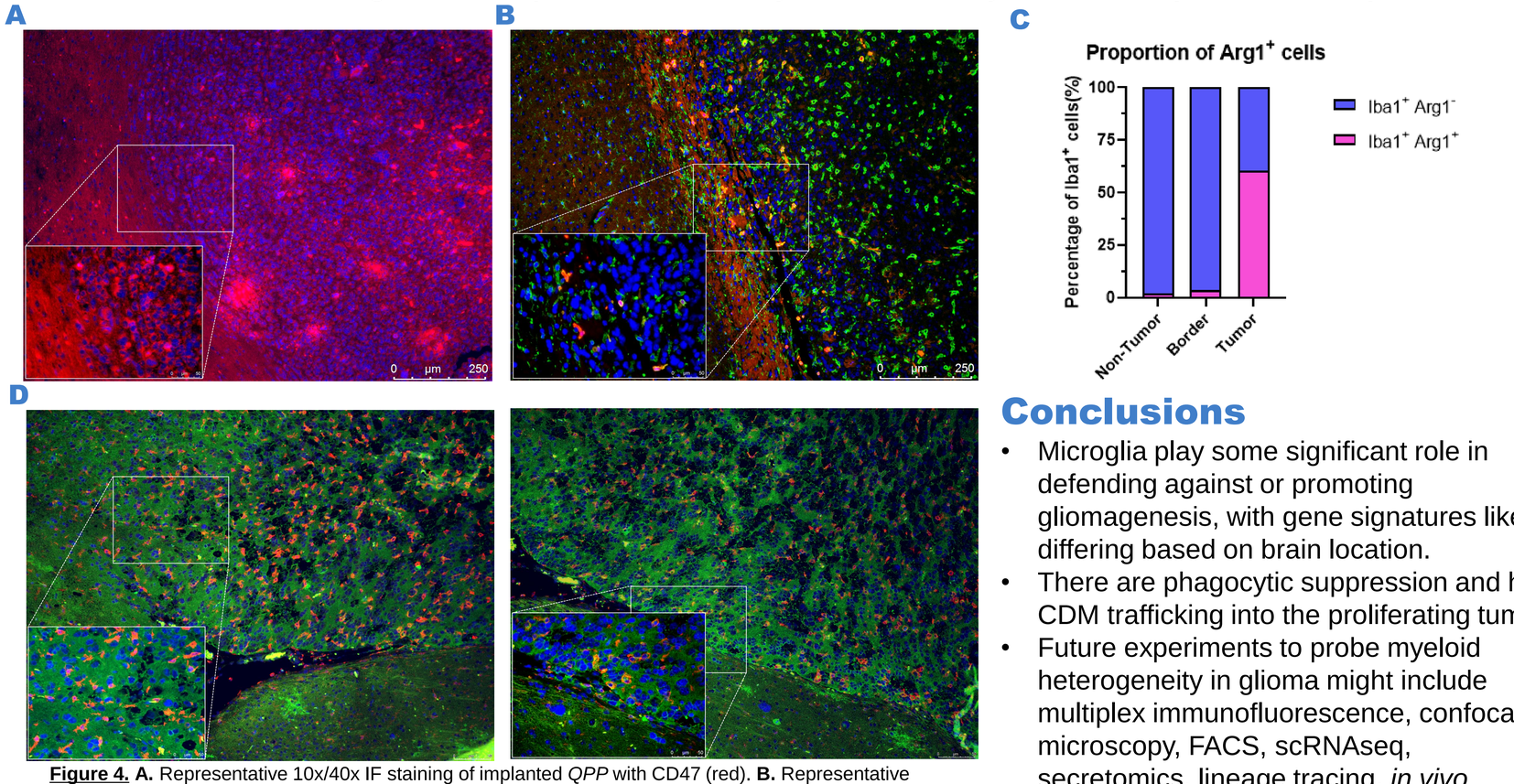


**Figure 4. A.** Representative 10x/40x IF staining of implanted *QPP* with CD47 (red). **B.** Representative 10x/40x IF co-staining of implanted *QPP* with Iba1 (green) and Arg1 (red). **C.** Proportion of Arg1$^+$ cells by brain region. **D.** Representative 10x and 40x IF co-stainings of genetic QPP with Iba1 (red) and GFP (green).

## Conclusions

- Microglia play some significant role in defending against or promoting gliomagenesis, with gene signatures likely differing based on brain location.
- There are phagocytic suppression and high CDM trafficking into the proliferating tumor.
- Future experiments to probe myeloid heterogeneity in glioma might include multiplex immunofluorescence, confocal microscopy, FACS, scRNAseq, secretomics, lineage tracing, *in vivo* tracking, and time course studies.