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.²
- Qk−/−,Pten−/−,Trp53−/− (QPP) mice develop glioma with immune environments resembling that of human glioma.³ They are thus ideal in determining myeloid composition across a tumorigenic brain.¹

- 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

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

![Image 1](https://via.placeholder.com/150)
Figure 1. A. Design of Nes-CreERT²;Qk−/−,Pten−/−,Trp53−/− (QPP) mouse model. B. Representative image of QPP murine brain after harvesting.

![Image 2](https://via.placeholder.com/150)
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.

![Image 3](https://via.placeholder.com/150)
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 CD45high cells by brain region.

- TMEM119 is downregulated in glioma conditions, supporting its identity as a marker of homeostatic conditions.⁴
- Iba1+ CD45high cells are increasingly found towards tumor core, suggesting higher likely CDM infiltration.

![Image 4](https://via.placeholder.com/150)
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.

Methods
Mouse models:
- QPP7 (genetic tumors), injected with tamoxifen at P7, and harvested when moribund
- Cx3cr1-CreERT² 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