

Supplementary Table 1 Gene markers excluded in scRNAseq analysis.

Supplemental Figure 1. scRNAseq data quality assurance and cluster marker gene expression. A)

Representative visualization of nFeature RNA, nCount RNA, and percent.mt filtering was performed as described in methods. B) Heatmap showing scaled, log-normalized expression of top 5 marker genes for each cluster. (n=4 biological replicates per group).

Supplemental Figure 2. Visualization of pseudotime quantification separated by cluster.

Supplemental Figure 3. HSPC differentiation and gating strategy. A) A brief overview of HSPC differentiation. Image created on biorender.com. B) Gating strategy used to determine frequency of progenitor populations.

Supplemental Figure 4. HSPC phenotype in glioma-bearing mice. A) Glioma-bearing mice possess a slight but significant reduction in lineage- cells relative to non-tumor-bearing control mice. Glioma-bearing mice possess similar frequencies of B) $\text{lin}^{-}\text{cKit}^{+}\text{Sca-1}^{-}\text{CD16/32}^{\text{lo}}$ CMPs, C) $\text{lin}^{-}\text{cKit}^{+}\text{Sca-1}^{+}\text{M-CSFR}^{+}\text{Flt3}^{+}$ MDPs D) $\text{lin}^{-}\text{cKit}^{+}\text{M-CSFR}^{+}\text{Flt3}^{+}$ CDPs. E) Glioma-bearing mice have higher frequencies of $\text{lin}^{-}\text{cKit}^{+}\text{Sca-1}^{-}\text{CD16/32}^{\text{hi}}$ GMPs relative to non-tumor-bearing, age-matched mice. Data represents mean +/- SD. * $P < .05$ by Mann-Whitney t test (n \geq 3 biological replicates).

Supplemental Figure 5. No myeloid cell expansion is found in the splenic compartment of glioma-bearing mice. A) Representative flow cytometry plots of MDSC phenotyping. B – G) Splensens were collected from non-tumor-bearing, age-matched mice and glioma-bearing mice 28 days after implantation. No difference in CD11b+F4/80+ macrophages, CD11c+MHC II+DCs, or CD11b+Gr-1+MDSCs is seen in glioma-bearing mice relative to non-tumor-bearing control mice. Data represents mean +/- SD. *P<.05, **P<.01, ***P<.001, ****P<.0001, by Mann-Whitney test (n=5 biological replicates).

Supplemental Figure 6. MDSC purity and experimental layout for MDSC functional assays. A) Representative flow plots of purity of CD11b+ Gr-1+ MDSCs after isolation. B) Experimental layout for MDSC suppression assay using MDSCs from healthy and glioma-bearing mice and CTV-labeled T cells. C) Experimental layout for MDSC killing assay using MDSCs from healthy and glioma-bearing mice, tumor-specific T cells, and target KR158Bluc glioma cells.

Supplemental Figure 7. Higher IFN γ R higher on HSPCs from glioma-bearing mice than non-tumor-bearing controls. A) IFN γ R1 and IFN γ R2 expression in scRNAseq of HSPCs from non-tumor-bearing and glioma-bearing mice. B) Representative flow cytometry plots of IFN γ R1 and IFN γ R2 expression. gMFI of C) IFN γ R1 and D) IFN γ R2 on lineage- cells from non-tumor-bearing and glioma-bearing mice. Percentage of E) IFN γ R1+ and F) IFN γ R2+ cells on cKit+ Sca-1- cells from non-tumor-bearing and glioma-bearing mice. *P<.05, **P<.01, ***P<.001, ****P<.0001, by Mann-Whitney test (n=10 biological replicates).

Supplemental Figure 8. Irradiation abrogates myeloid cell expansion in glioma-bearing hosts and drives IFN γ R Expression. A) gMFI of IFN γ R1 and IFN γ R2 on lineage- cells. B) Frequency of IFN γ R1 and

IFN γ R2 on cKit+Sca-1- myeloid precursors. Frequency of HSPC-derived A) CMPs, B) macrophages, and C) DCs in mice that received either non-tumor-bearing or glioma HSPCs. *P<.05, **P<.01, ***P<.001, ****P<.0001, by Mann-Whitney test (n=7 biological replicates).

Supplemental Figure 9. Reduction in GMPs with ACT. A) Representative flow cytometry plots of CMP and GMP frequencies in mice that received adoptive cellular therapy.