

**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 $\gamma$ R higher on HSPCs from glioma-bearing mice than non-tumor-bearing controls.** A) IFN $\gamma$ R1 and IFN $\gamma$ R2 expression in scRNAseq of HSPCs from non-tumor-bearing and glioma-bearing mice. B) Representative flow cytometry plots of IFN $\gamma$ R1 and IFN $\gamma$ R2 expression. gMFI of C) IFN $\gamma$ R1 and D) IFN $\gamma$ R2 on lineage- cells from non-tumor-bearing and glioma-bearing mice. Percentage of E) IFN $\gamma$ R1+ and F) IFN $\gamma$ 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 $\gamma$ R Expression.** A) gMFI of IFN $\gamma$ R1 and IFN $\gamma$ R2 on lineage- cells. B) Frequency of IFN $\gamma$ R1 and

IFN $\gamma$ 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.