**Supplemental Figure 1: Representative whole tumor image.** Regions of high (Evans blue, red) and low flow around the tumor (human nuclear antigen, green) in an implanted G34 glioma.
Supplemental Figure 2: Fold change in flow-stimulated invasion changes with blocking in GSCs. (A) Invasion of G34 in IFF with blocking by anti-CXCR4 (5 µg/mL), anti-CXCL12 (50 µg/mL) and anti-CD44 (10 µg/mL) inhibits glioma stem cell invasion response to interstitial flow in a 3D cell culture model. Same for (B) G2, (C) G62, and (D) G528. Data shown are mean ± SEM, with n=6 independent experiments. *p<0.05 paired t-tests.
**Supplemental Figure 3:** Subpopulations of glioblastoma stem cells expressing CD44, CXCR4, or CXCL12 partially explains blocking responses. (A) Representative gating of CD44 positive cells (gated on live cells). (B) Percentage of live cells expressing CD44. (C) Mean fluorescence intensity of CD44 positive cells. (D) Representative gating of CXCR4 positive cells (gated on live cells) (E) Percentage of live cells expressing CXCR4. (F) Mean fluorescence intensity of CXCR4 positive cells. (G) Representative gating of CXCL12. (H) Percentage of live cells expressing CXCL12. (C) Mean fluorescence intensity of CXCL12 positive cells. Data represented as mean +/- SEM of six biological replicates. *p<0.05 by paired t-tests.
Supplemental Figure 4: ELISA for CXCL12 from cells in suspension culture. Data shown are mean ± SEM.*p<0.05; **p<0.01 compared to 528 by unpaired t-test.
Supplemental Figure 5: Flowchart detailing agent-based model decisions and process.
**Supplemental Figure 6: Agent-based model predicts experimental results for G2, G62, and G528.**

(A) Table of ABM input ranges for each protein positive subpopulations for each cell type. (B) G2 agent based model and experimental invasion data. (C) G62 agent based model and experimental invasion data. (D) G528 agent based model and experimental invasion data. Statistical significance represented for the agent-based model data (n=6 independent simulation runs). (E) Characterizing ABM directionally migrating cell type behavior by varying the number of random movements relative to directional movements in the flow direction for CXCR4+/CXCL12+ cell types. *p<0.05, **p<0.01, ***p<0.001 (B-D) by unpaired t-tests. *p<0.05, **p<0.01, ****<0.0001 by unpaired t-test to static at each time point. #p<0.01 by unpaired t-test compared to all flow conditions.
Supplemental Figure 7: Ionizing radiation effects on subpopulations of CXCR4+/CD44+/CXCL12+ cells. (A) Percentage of live cells expressing CD44. (B) Mean fluorescence intensity of CD44 positive cells. (C) Percentage of live cells expressing CXCR4. (D) Mean fluorescence intensity of CXCR4 positive cells. (E) Percentage of live cells expressing CXCL12. (F) Mean fluorescence intensity of CXCL12 positive cells. (G) Co-expression of CXCR4+CD44+ cells. Data represented as mean +/- SEM of 6 independent experiments and compared by paired t-tests.