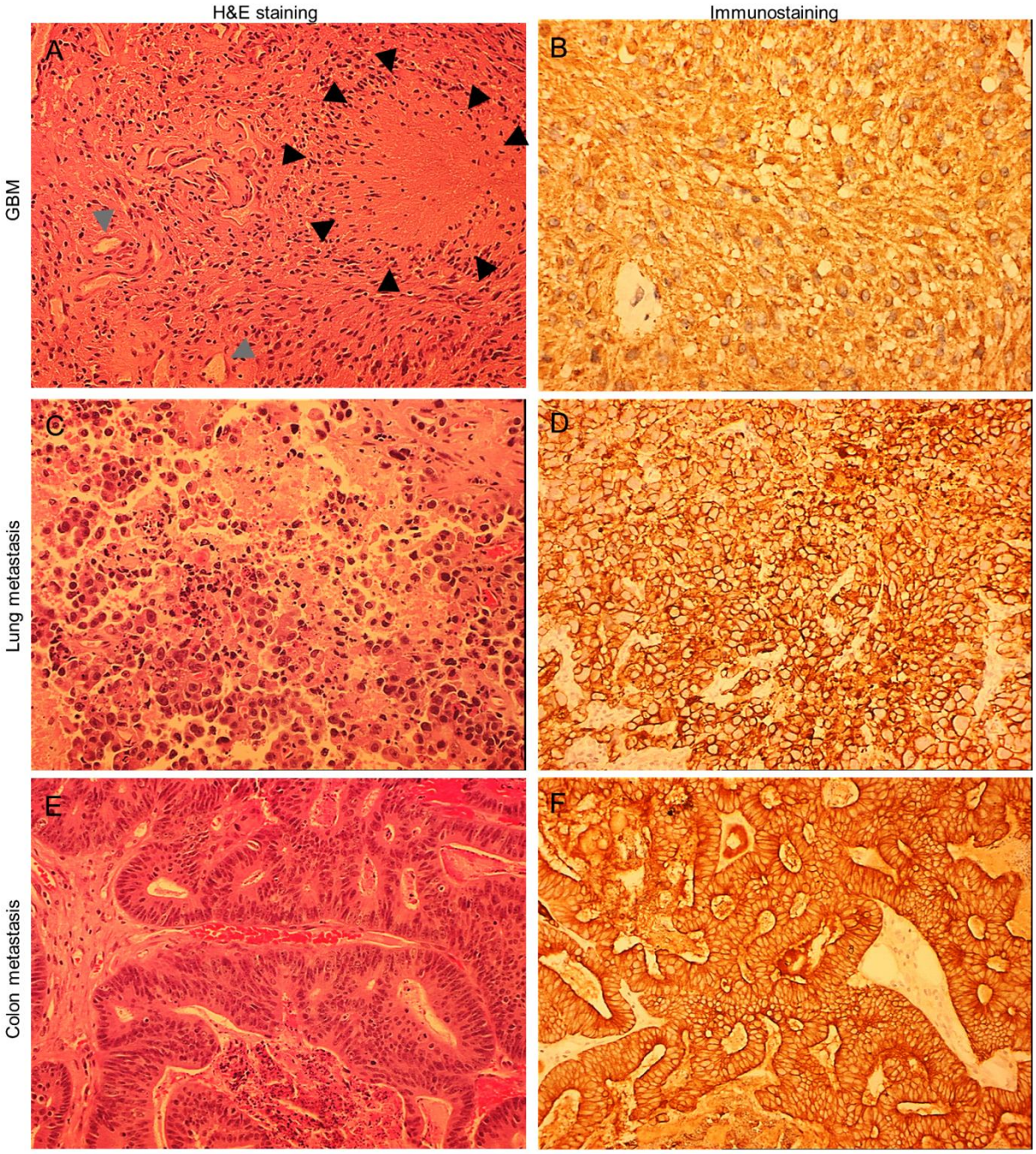
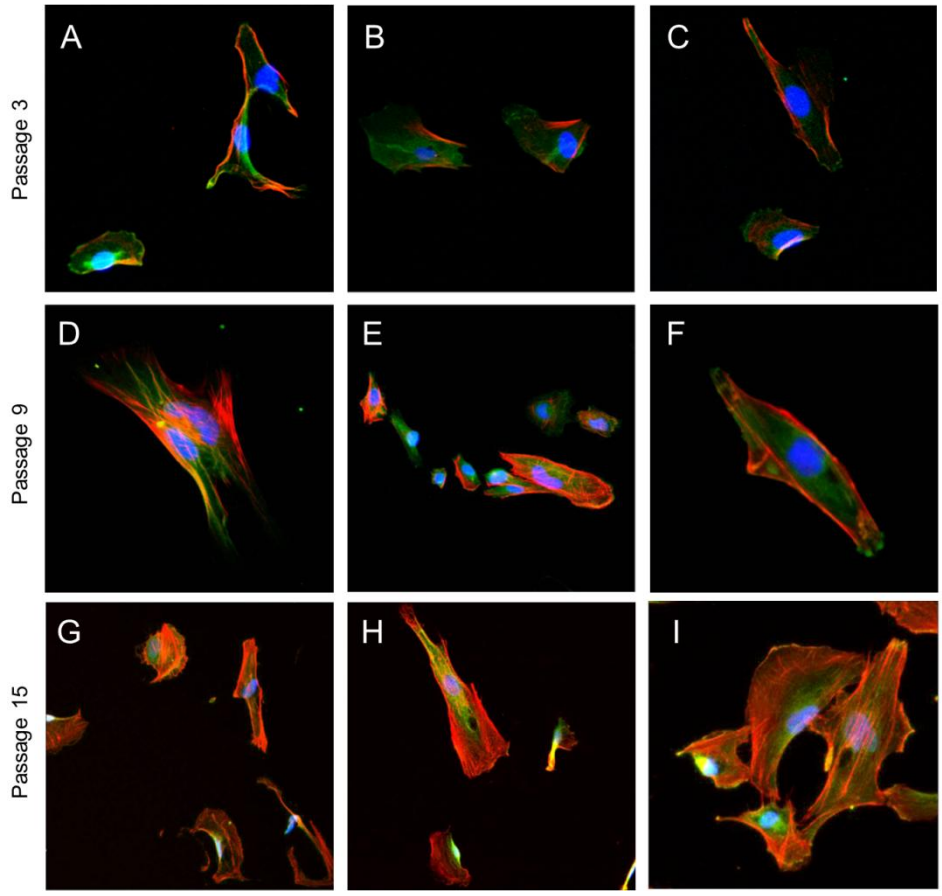
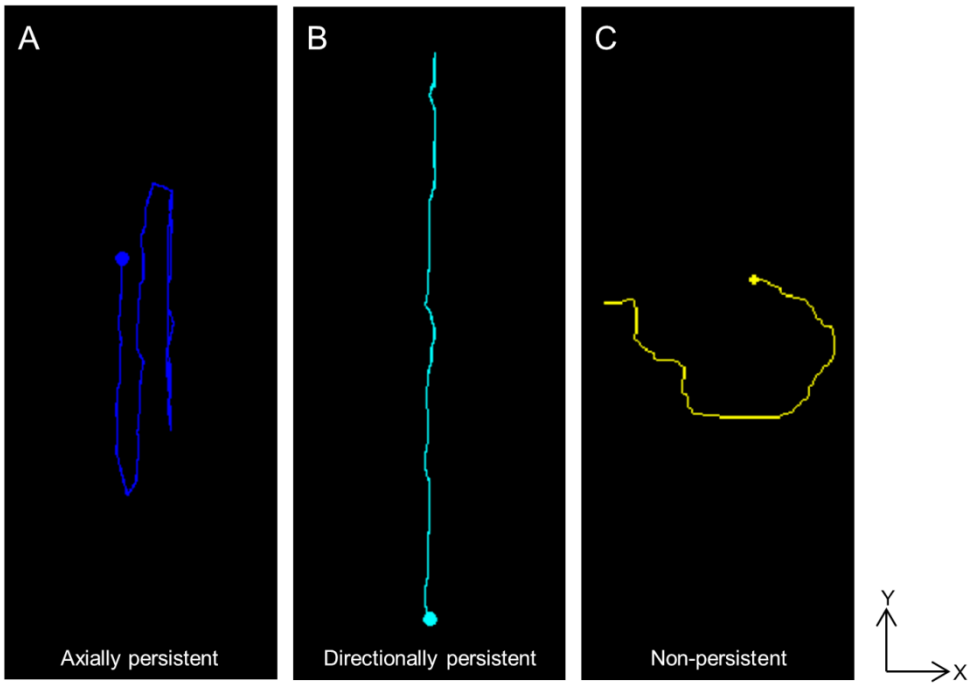


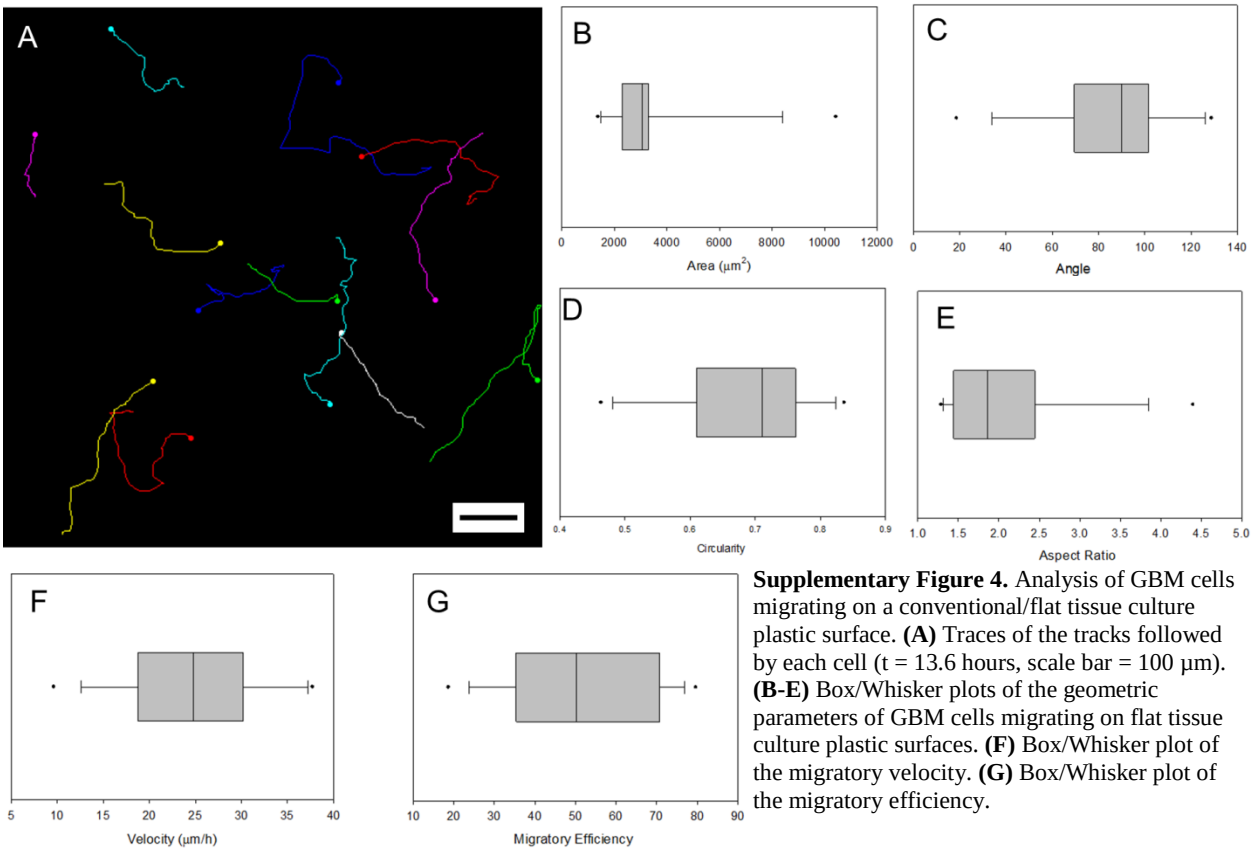
Supplementary Figure 1. H&E staining and immunostaining of the three different intracranial tumors analyzed in this study. **(Top)** Glioblastoma Multiforme: **(A)** Hypercellular field devoid of normal brain parenchyma; single grey arrow: endothelial proliferation; black arrows: necrosis; **(B)** Glial fibrillary acidic protein (GFAP) showing most of the cells staining positive (cytoplasmic, stained brown). **(Middle)** Lung metastatic tumor: **(C)** Lung metastatic tumor cells shown with a background of necrosis; **(D)** Diffuse positive staining for cytokeratin (carcinoma marker). **(Bottom)** Colon metastatic tumor: **(E)** Metastatic adenocarcinoma of colon showing complex glandular structures; **(F)** Diffuse positive staining for cytokeratin.



Supplementary Figure 2. Dual immunofluorescence staining of cell cultures derived from the excised GBM tumor (actin –red-, glial fibrillary acidic protein –green-, and cell nuclei –blue-) after 3 (A-C), 9 (D-F), and 15 (G-I) passages. The micrographs show representative cells from different locations of the culture surface for each passage number. As can be seen in the micrographs, all of the cells had a tendency to stain positive for GFAP, which is an astrocytic marker commonly used in the pathological diagnosis of glioblastoma multiforme. The absence of any actin(+)/GFAP(-) cells in the cultures suggests that the cells derived from the brain stroma and parenchyma (e.g., neurons, other glial cells) succumbed to the isolation/culture conditions at very early stages (passage $\leq$3), thus resulting in the establishment of short-term and/or permanent lines exclusively derived from the tumorous tissue.



Supplementary Figure 3. Tracks followed by patient-derived tumor cells on the microfabricated platform **(A,B)** and flat TCPS **(C)**. The cell in panel **(A)** maintained a motility pattern along the same axis throughout the entire experiment (axially persistent migration), but reversed direction twice (on separate tracks). The cell in panel **(B)** not only moved along the same axis, but also maintained the same direction during the full experiment (directionally persistent migration). The cell in panel **(C)** on the other hand had a tendency to move randomly over the flat surface (non-persistent migration). Both axial and directional persistence migrations were greatly influenced by the aligned microfeatures on the surface of the platform.



Supplementary Figure 4. Analysis of GBM cells migrating on a conventional/flat tissue culture plastic surface. **(A)** Traces of the tracks followed by each cell (t = 13.6 hours, scale bar = 100 μm). **(B-E)** Box/Whisker plots of the geometric parameters of GBM cells migrating on flat tissue culture plastic surfaces. **(F)** Box/Whisker plot of the migratory velocity. **(G)** Box/Whisker plot of the migratory efficiency.