

**Figure S2 (related to Figure 1): Characterization of A2B5<sup>+</sup> cells in GBM-derived TIPC lines**

(A-D) A2B5<sup>+</sup> and A2B5<sup>-</sup> cells isolated from 2 different GBM-derived cell lines (GBM-268 and GBM-233) were plated into 96-well plates with a FACS-automated cell deposition unit (ACDU), at seeding densities of either 5, 10 or 100 cells per well (n=6-18 wells/density level per line). The cells were cultured in serum-free media for 14 days, and resultant glial spheres were individually counted using an inverted microscope. Results are expressed following a non-linear regression analysis. Graphed as mean  $\pm$  SEM. N = Number of replicate wells. (E) Flow cytometry analysis of A2B5 expression in 4 representative primary gliomas and their derived cell lines. (F) Flow cytometry (\*) and immunocytochemical analysis of GBM-derived cell line (n=3) for A2B5, CD133, Sox2, Survivin, Olig2 and Ki67 expression. (G) Flow cytometry analysis of CD133 expression cells in a total of 21 primary gliomas and 5 GBM cell lines. Horizontal lines indicate mean percentages. (H) Representative scatter plot of a GBM-derived cell line, stained with A2B5 (bottom right), CD133 (upper left), A2B5 and CD133 together (upper right), or their isotype-matched controls (bottom left). *AST: Astrocytoma; OLG: Oligodendroglioma; OLG-AST: Oligo-astrocytoma; GBM: Glioblastoma; GSC: Gliosarcoma; p: passage; TIPC: tumor initiating precursor cell.*