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# Supplemental Data

# SSEA-1 Is an Enrichment Marker for Tumor-Initiating Cells in Human Glioblastoma

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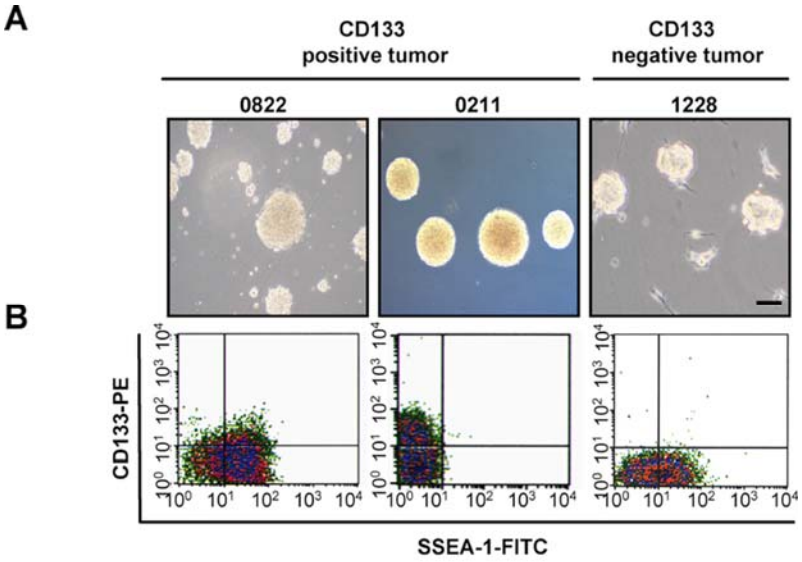


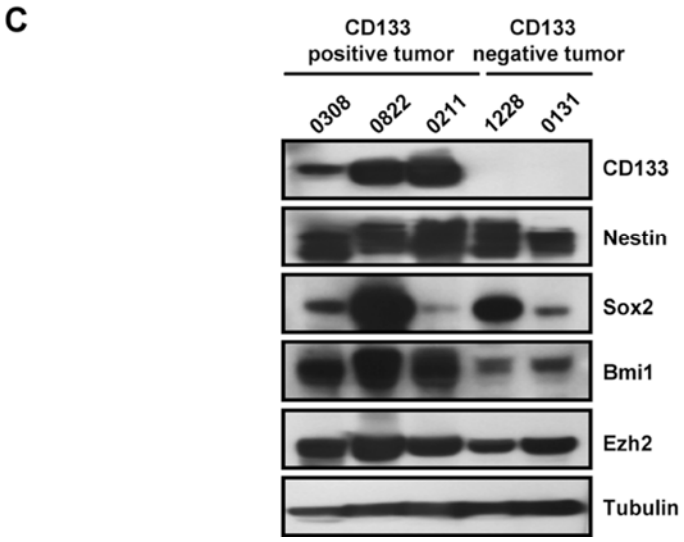

**Figure S1** **Expression of CD133 and SSEA-1 in TSC/TIC lines derived from various GBM tissues.** (A) Microphotographs showing typical morphology of neurosphere-like cells in TSC/TIC cultures. Scale bar represents 50 micron. (B) Flow cytometry analysis of CD133 (PE labeled, y-axis) and SSEA-1 (FITC labeled, x-axis) in various TSC/TICs. (C) Western blot analysis of several stem cell associated proteins in various TSC/TIC cell lines. CD133 proteins are detected in 0308, 0822, and 0211 lines, but not in 1228 and 0131. Alpha tubulin was used as a loading control.

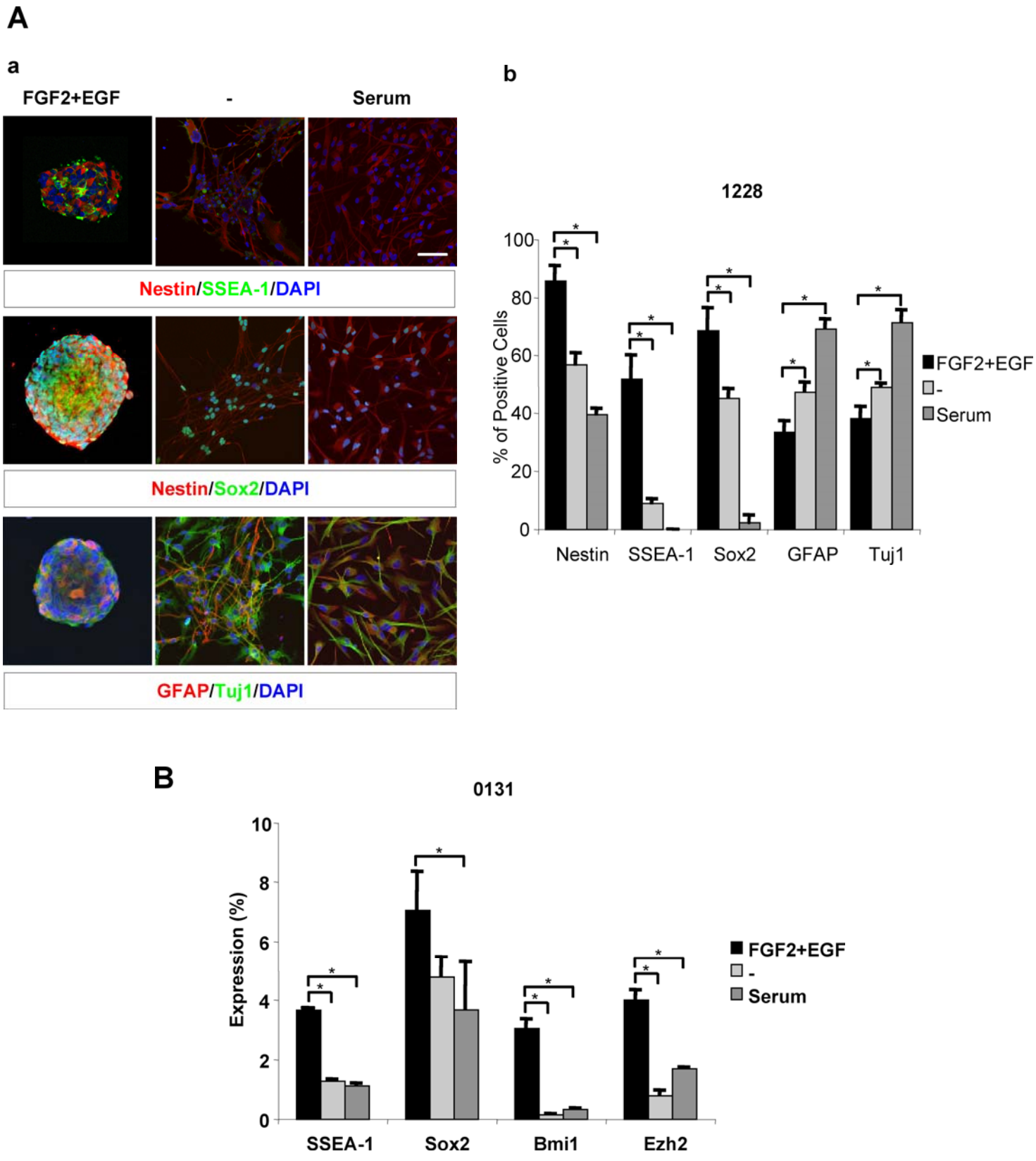
A
a
FGF2+EGF
-
Serum
Nestin/SSEA-1/DAPI
Nestin/Sox2/DAPI
GFAP/Tuj1/DAPI
b
1228
% of Positive Cells
100
80
60
40
20
0
Nestin
SSEA-1
Sox2
GFAP
Tuj1
FGF2+EGF
-
Serum
B
0131
Expression (%)
10
8
6
4
2
0
SSEA-1
Sox2
Bmi1
Ezh2
FGF2+EGF
-
Serum

**Figure S2** **Differentiation potentials of SSEA-1 positive TSC/TICs *in vitro*.** **(**A) Immunocytochemical analysis of 1228 GBM line cultured in differentiation-inducing condition (a). Each column represents the same culture condition. “FGF2+ EGF” indicates the NBE condition used in the proliferation of these cells. “–” and “serum” indicate the growth factor withdrawal and the addition of serum, respectively. Antibodies used in each condition are shown below. DAPI staining (blue) was used to identify nuclei. Quantitation of immunopositive cells in (b). Scale bar represents 50 micron. **(**B) FACS analysis of stem cell associated proteins in 0131 GBM lines during differentiation. Error bars represent SD. *$p < 0.05$.

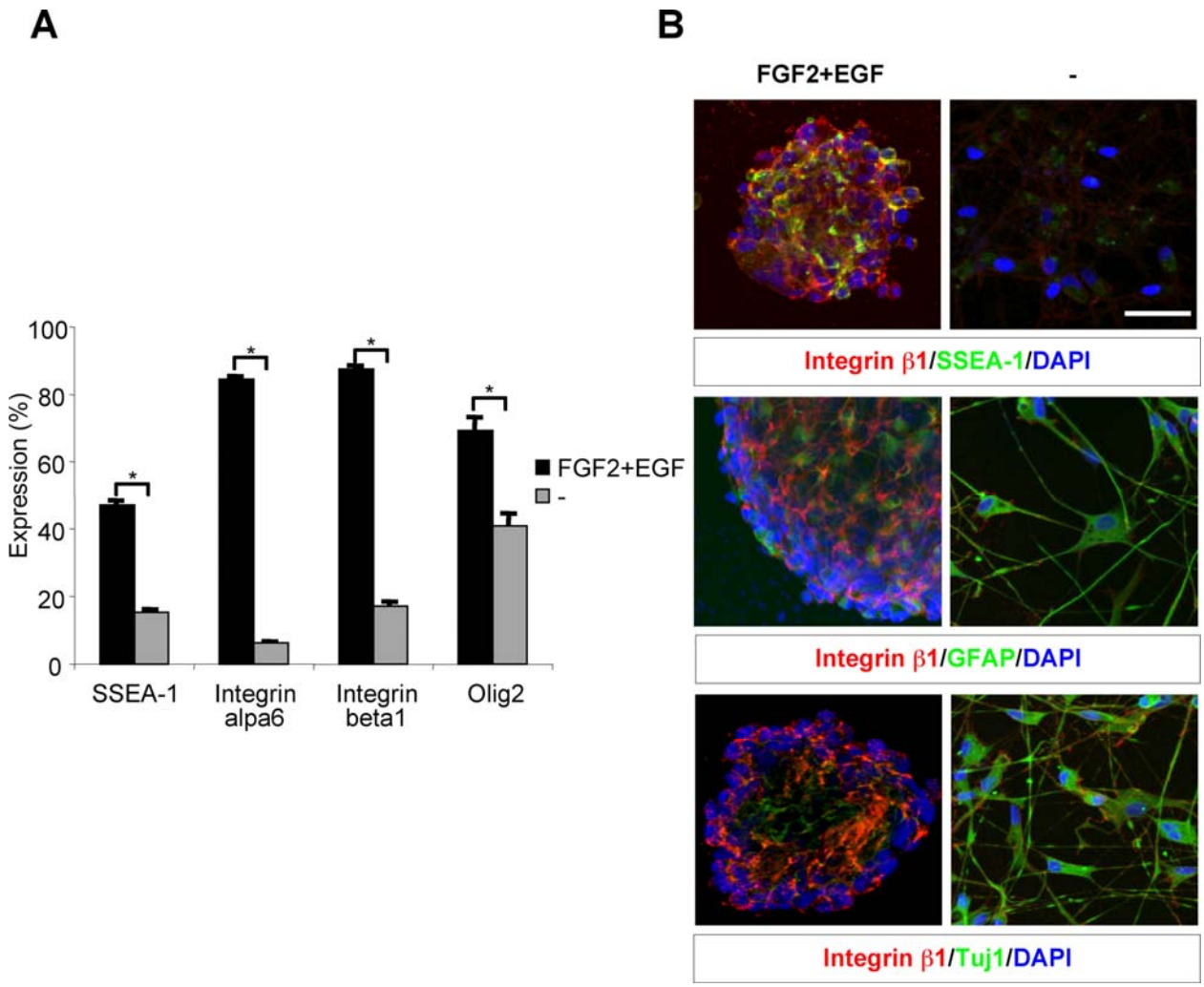


**Figure S3** **Expression of normal neural stem/progenitor associated proteins in SSEA-1+ cells.** (A) Expression of SSEA-1, Integrin alpha6, Integrin beta1 and Olig2 following differentiation. 1228 cells were cultured either in the presence of FGF2/EGF or in the absence of FGF2/EGF (-) and then processed for flow cytometry analysis. Error bars represent SD. *p < 0.05. (B) Representative microphotographs of immunocytochemical analysis of 1228 GBM line cultured in differentiation-inducing condition. Cells were cultured with or without FGF2 and EGF for 7 days and processed for immunocytochemical analysis using the indicated antibodies. DAPI staining (blue) was used to identify nuclei. White bar represents 20 micron.

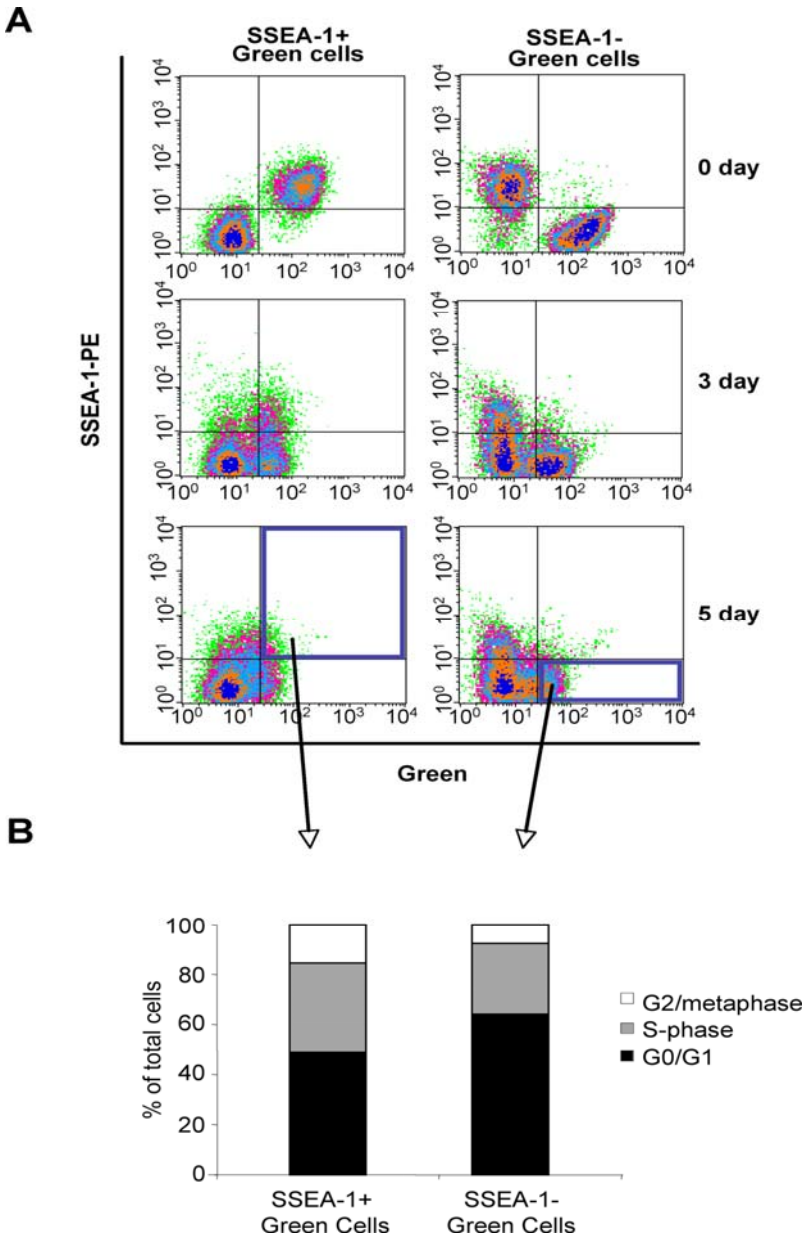


**Figure S4** **Lineage tracking of SSEA-1+ vs. SSEA-1- GBM cells by non-genetic labeling.** SSEA-1+ or SSEA-1- GBM cells were labeled with green color dye and then labeled SSEA-1+ (left panel) or SSEA-1- (right panel) cells were reconstituted with unlabeled SSEA-1- or SSEA-1+ cells, respectively. At each time point, cells were stained with SSEA-1-PE, and green-labeled cells were traced (A) and checked cell cycle (B) using flow cytometry.

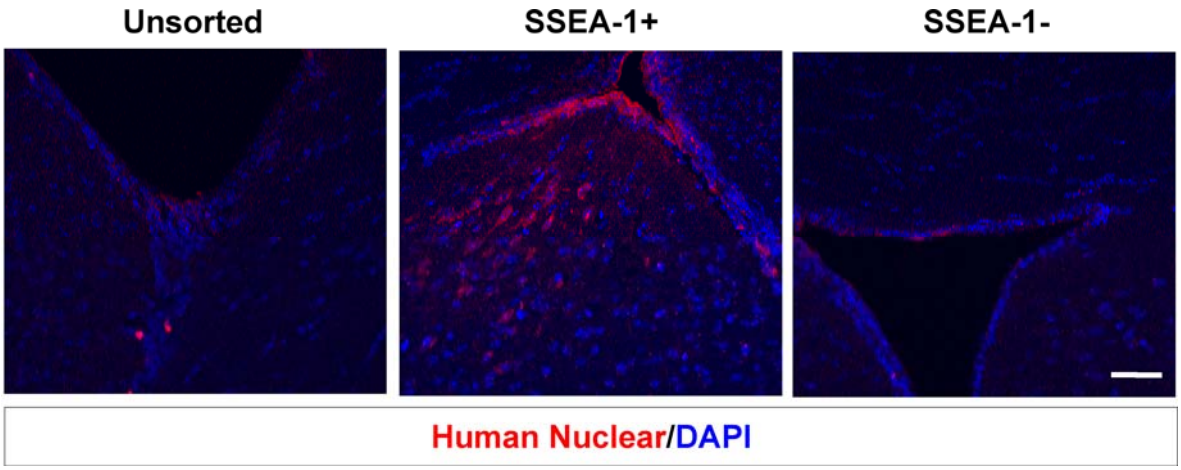


**Figure S5** **Representative immunohistochemical photographs of the SCID mice brains injected with 1228 total (unsorted), SSEA-1+ or SSEA-1- cells.** Human nucleus was stained with human specific antibody (red) at two months after injection. DAPI staining (blue) was used to identify nuclei. White bar represents 20 micron.

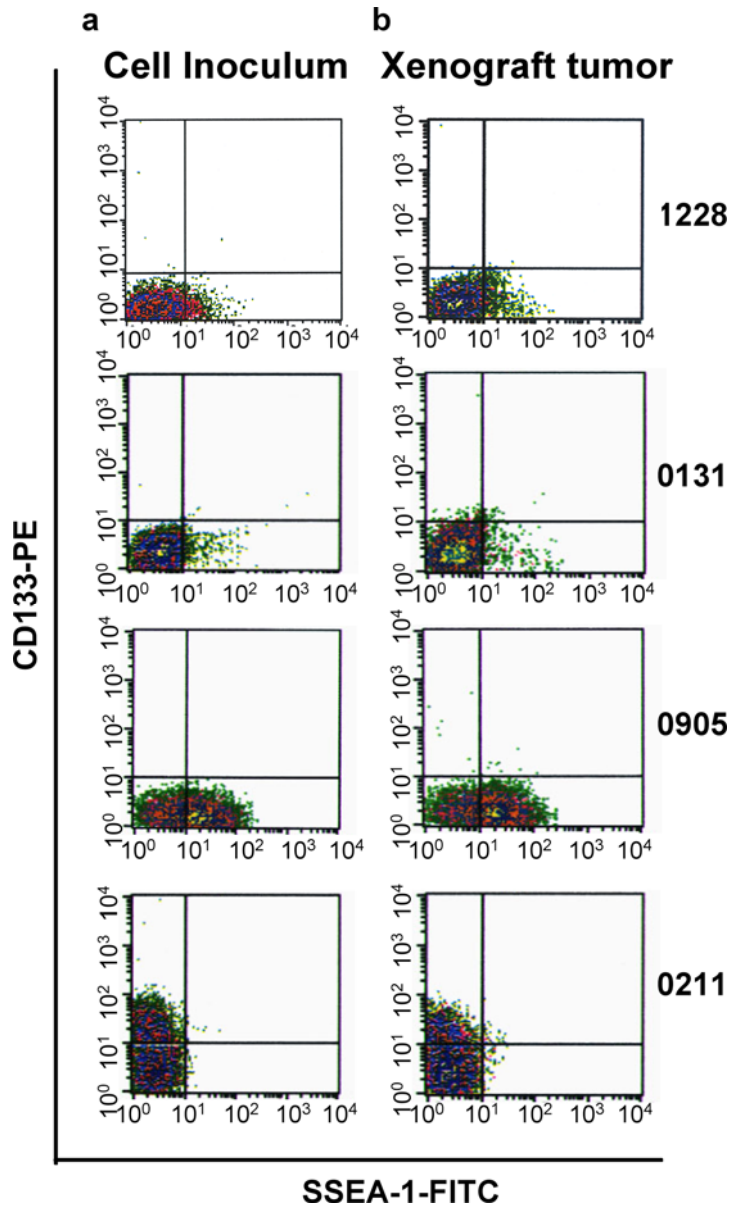


**Figure S6** **Expression of CD133 and/or SSEA-1 in various GBM-derived xenograft tumors.** FACS analysis to determine the percentage of cells expressing CD133 and/or SSEA-1. Cells prepared for the injection into mice (a) were compared to the tumor cells isolated from the resultant xenograft tumors (b). As a representative example, data from 1228, 0131, 0905, and 0211 line are shown.