Suppl. Figure 1. GIC cultures used for this work fulfill the criteria of tumor-initiating cells.

Stem cell characteristics of the used GIC cultures are shown exemplary for T269. **(A)** T269 forms spheres when cultured in serum free medium supplemented with the growth factors EGF and bFGF. **(B and C)** T269 is positive for the progenitor marker nestin and can be differentiated by growth factor removal and serum supplementation. In response to differentiation, Tuj1 and GFAP are upregulated as demonstrated with immune fluorescence microscopy and flow cytometry. **(D)** T269 is highly tumorigenic after orthotopic implantation of as few as 50 cells into CD1 *nu/nu* mouse brains and forms a highly invasive tumor as demonstrated by HE staining. **(E)** Shows tumor initiation capacity of GIC cultures T269, T325, T323 and T1 after orthotopic implantation of 10^3 and 5 x 10^4 cells in CD1 nu/nu mice.

Suppl. Figure 2. Regulation of 4IgB7H3-expression and cleavage of soluble 4IgB7H3.

(A) Relative 4IgB7H3 mRNA or protein levels in lysates and supernatant as well as protein surface levels were evaluated in LN-229 after treating the cells with hypoxia, ilomastat, a furin-inhibitor and PMA (grey bars). Control treatments (white bars) show no significant differences.

(B) Ilomastat does not alter the content of B7H3 on the surface of GIC culture T325 in flow cytometry.

(C) Irradiation with 8 Gy, treatment with H_2O_2, dexamethasone and IFN-γ do neither increase 4IgB7H3 expression on mRNA level nor in the supernatant.

Suppl. Figure 3.

Anti-human B7-Homolog3 antibody from R&D is directed against Leu29-Pro245 (red color) of 2IgB7H3. The illustration demonstrates sequence homology (red) of this section in 2Ig and 4IgB7H3 explaining detection of both isoforms by the polyclonal antibody ...

Suppl. Figure 4.

In order to prove the specificity of the 4IgB7H3 stains for flow cytometry, 4IgB7H3-containing supernatant of LN-229 wild type cells was added to the cells during the staining procedure. This measurement led to a reduction of the fluorescence intensity (grey curve) compared with the untreated probes (black curve) while the isotype control remained unchanged.