Supplementary Figures

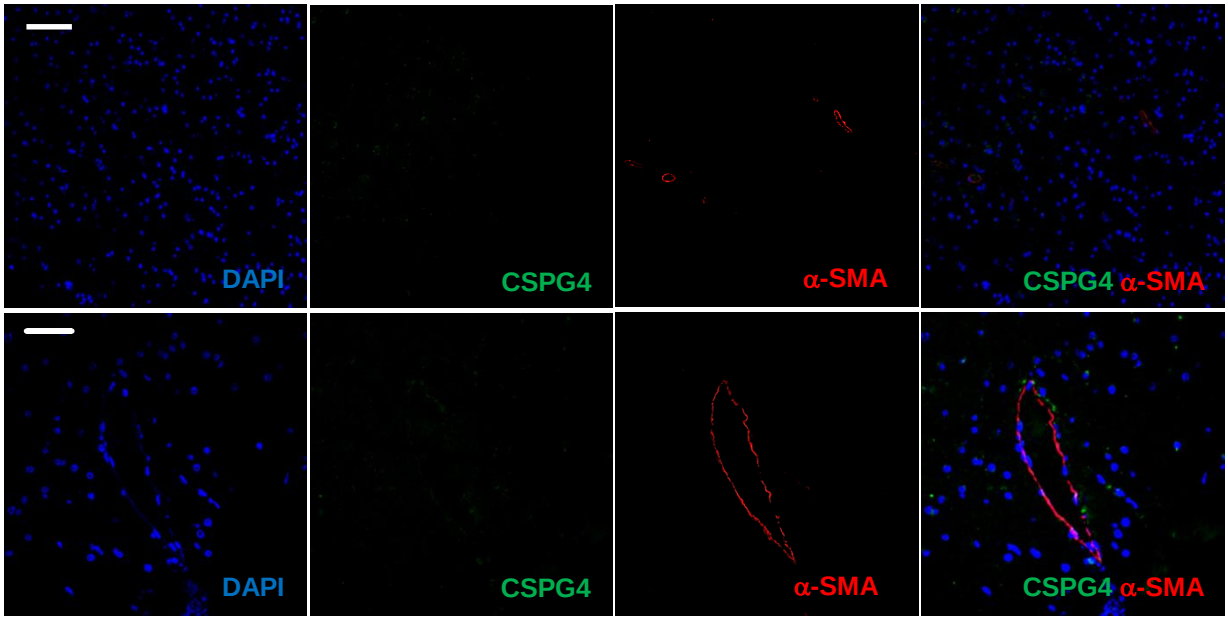

Fig. S1. Normal brain vessels do not express CSPG4.

Normal brain samples obtained from diagnostic biopsies of epileptic patients were stained with anti-α-SMA and anti-CSPG4 mAbs. Nuclei are visualized by DAPI staining. Two different areas from a representative normal brain sample show the presence of small and well-organized vessels expressing α-SMA, but not CSPG4 (scale bar 50 μm).

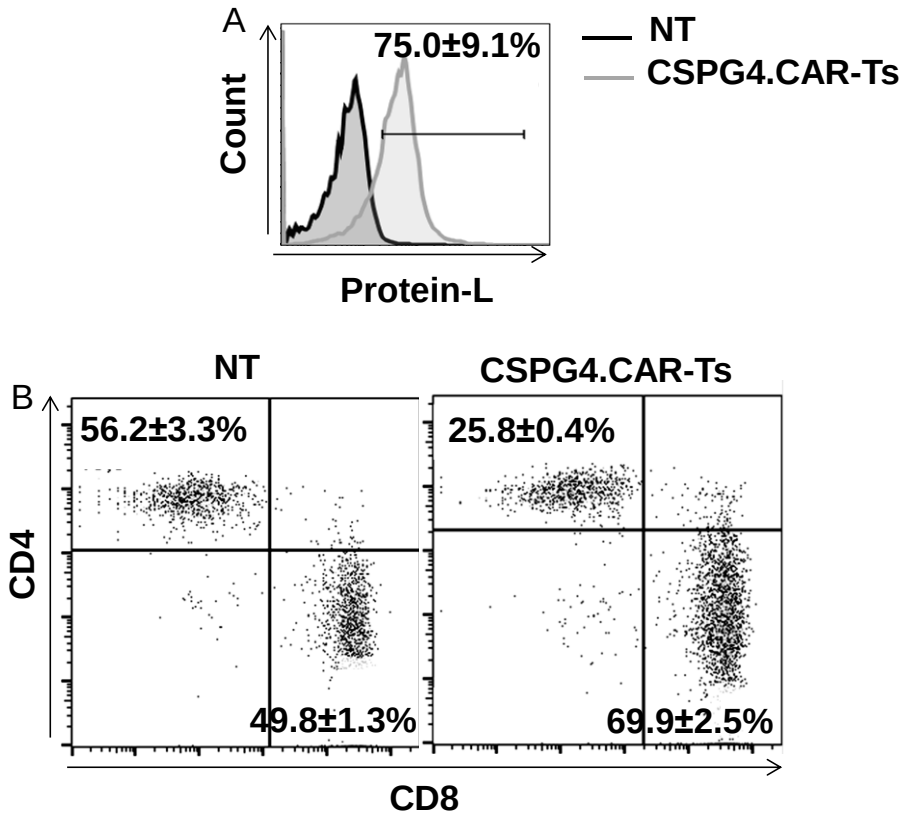

Fig. S2. CSPG4.CAR is expressed in T cells.

(**A**) CAR expression in T cells transduced with the vector encoding the CSPG4.CAR containing the 4-1BB co-stimulatory endodomain (CSPG4.CAR-Ts) was assessed by flow cytometry using protein L staining. (**B**) Frequency of transduced $CD8^+$ and $CD4^+$ T cells was measured on gated $CD3^+$ T cells (CSPG4.CAR-Ts, left panel), and compared to the frequency of $CD8^+$ and $CD4^+$ T cells in non-transduced T cells (NT, right panel). The percentages reported as mean ± SD in the flow cytometry dot plots are representative of three different donors.

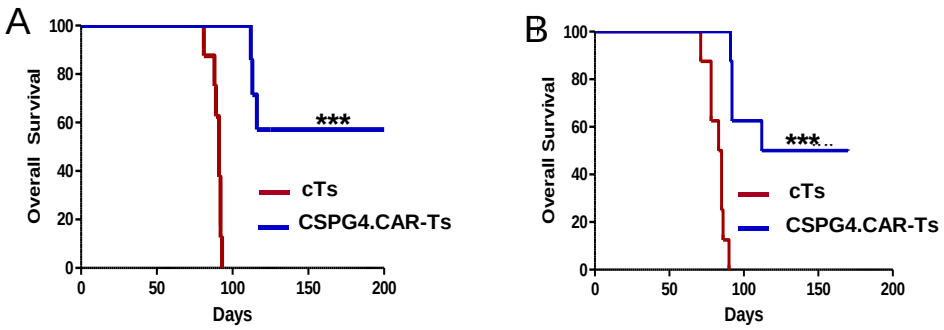

Fig. S3. CSPG4.CAR-Ts prolonged the survival of mice injected with BT275-NS and BT462-NS.

Kaplan-Meier survival curves of nude mice implanted intracranially with the GBM-NS CSPG4^{H} (97%, BT275-NS) (**A**) and GBM-NS CSPG4^{ML} (42.5%, BT462-NS) (**B**) and injected intratumorally with either CSPG4.CAR-Ts or cTs (*** $P < 0.0001$).

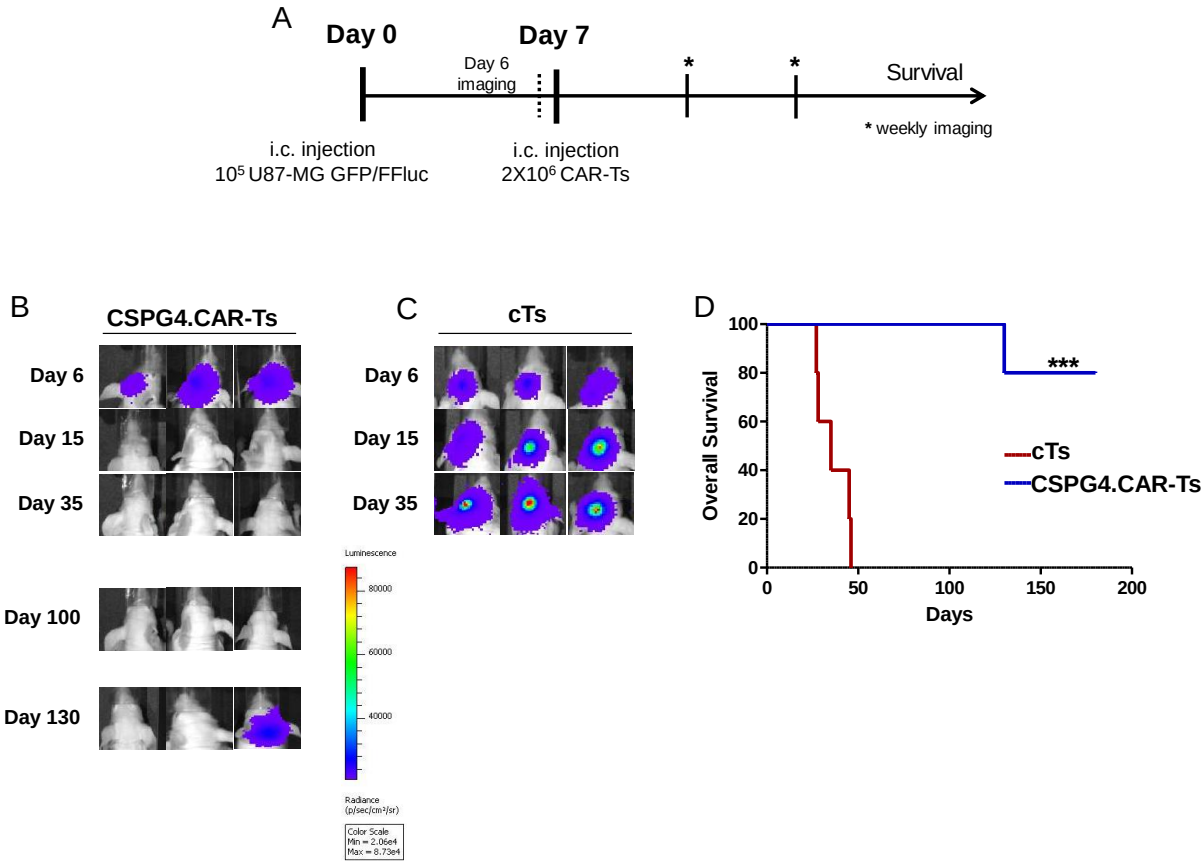

Fig. S4. CSPG4.CAR-Ts controlled tumor growth in the U87-MG xenograft model.

(**A**) Experimental schema of nude mice bearing the human GBM tumor cell line U87-MG labeled with GFP/FFluc and injected intracranially, then infused intratumorally with either CSPG4.CAR-Ts or cTs. (**B, C**) Representative in vivo imaging of mice treated with either CSPG4.CAR-Ts (**B**) or cTs (**C**). (**D**) Kaplan-Meier survival curves of treated mice (*** P = 0.002).

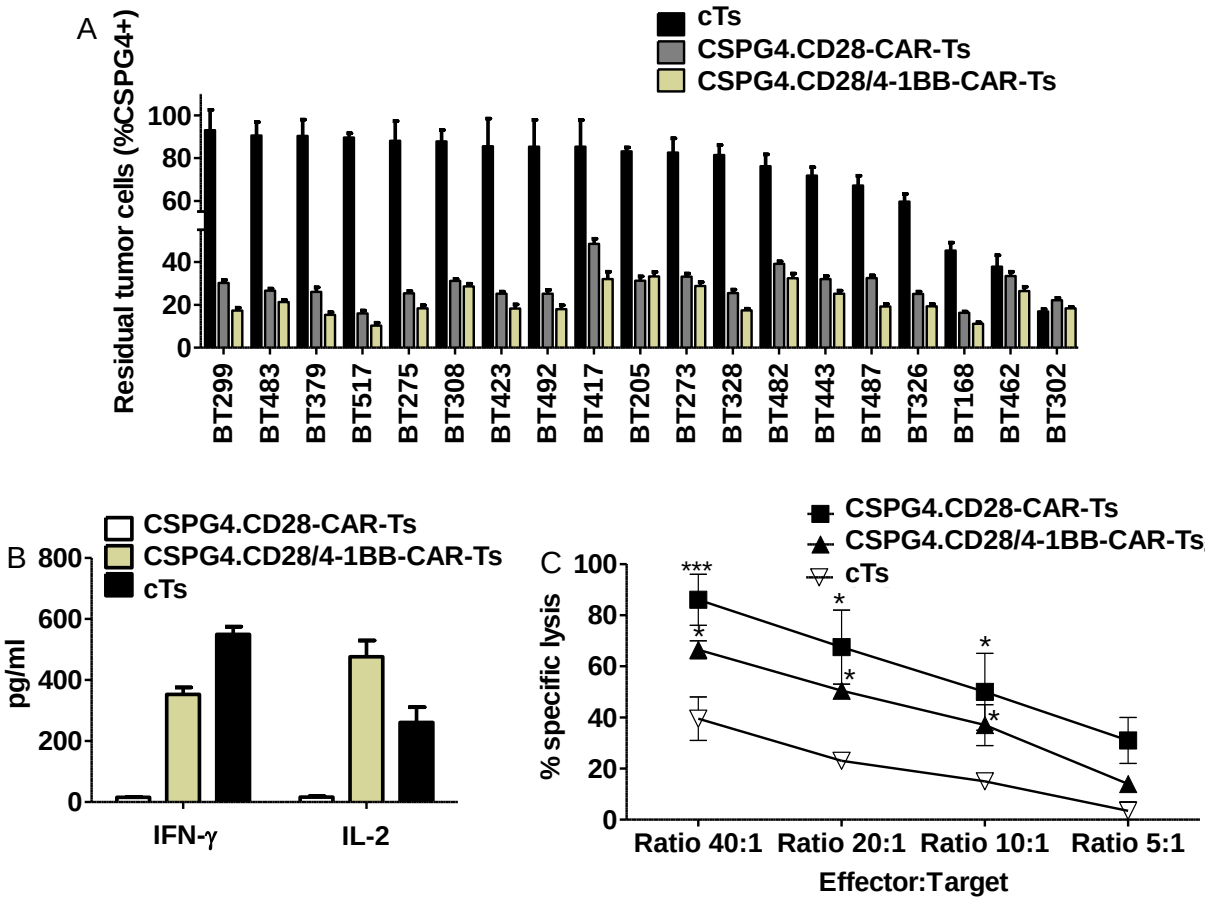

Fig. S5. CSPG4.CAR encoding either CD28 or CD28/4-1BB showed lower antitumor activity as compared to CSPG4.CAR encoding 4-1BB.

(**A**) GBM-NS were co-cultured in vitro with either cTs or T cells expressing the CSPG4.CAR containing either CD28 or CD28/4-1BB co-stimulatory endodomains in GBM-NS serum-free medium (DMEM/F12 and B27 supplement). The E:T ratio used was 1:5. GBM-NS and T cells were assessed by flow cytometry on day 3 of co-culture, using CSPG4 and CD3 as markers for GBM-NS and T cells, respectively. Bar graph shows the residual GBM-NS. Data represent mean ± SD of six T cell products. (**B)** IFN-γ and IL-2 released by T cells in the supernatants of co-cultures with GBM-NS collected after 24 hours. Cytokines were measured by specific ELISA. (**C**) Cytotoxic activity of cTs and T cells expressing the CSPG4.CAR containing either CD28 or CD28/4-1BB co-stimulatory endodomains and evaluated in a 6-hour ^{51}Cr release assay. Data show the mean ± SD of four GBM-NS and T cells generated from two donors (* $P = 0.01$, *** $P < 0.005$).

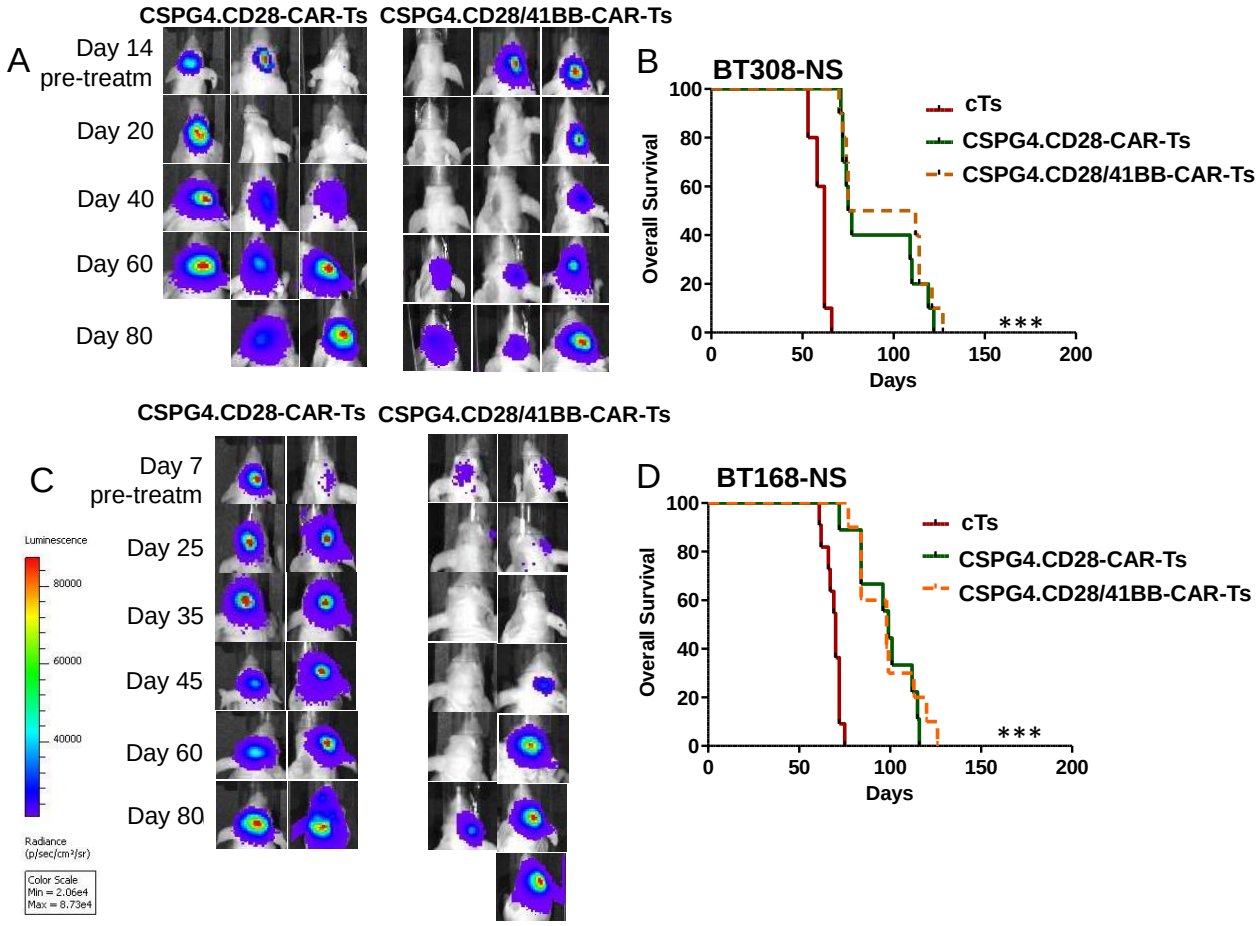

Fig. S6. CSPG4.CAR encoding either CD28 or CD28/4-1BB co-stimulatory endodomains showed limited antitumor activity in vivo.

(**A-D**) Nude mice bearing intracranial GBM-NS CSPG4^{H} (BT308-NS) (**A**, **B**) or GBM-NS CSPG4^{ML} (BT168-NS) (**C**, **D**) were injected intratumorally with either cTs or T cells expressing the CSPG4.CAR encoding either CD28 or CD28/4-1BB co-stimulatory endodomains. (**A**, **C**) Representative in vivo imaging of treated mice. (**B**, **D**) Kaplan-Meier survival curves of treated mice (** $P < 0.001$).

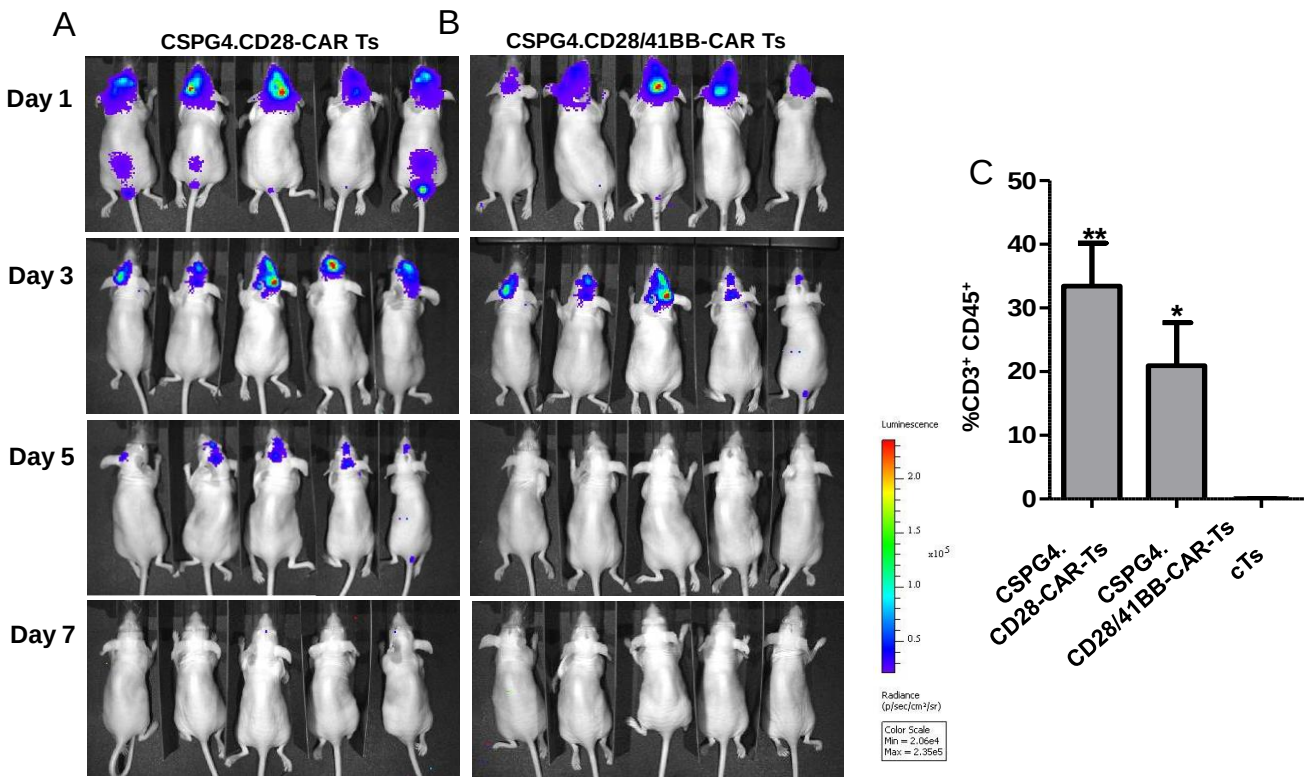

Fig. S7. CSPG4.CAR-Ts encoding either CD28 or CD28 and 4-1BB persisted for less than five days.

(**A**, **B**) CSPG4.CAR-Ts encoding either CD28 (**A**) or CD28/4-1BB (**B**) persisted for 5 and 3 days, respectively, upon intratumoral inoculation in tumor-bearing mice. (**C**) CSPG4.CAR-Ts were detected within the tumor 1 week after infusion, as measured by flow cytometry, whereas T cells were undetectable in mice infused with cTs (* $P < 0.01$, ** $P = 0.005$).

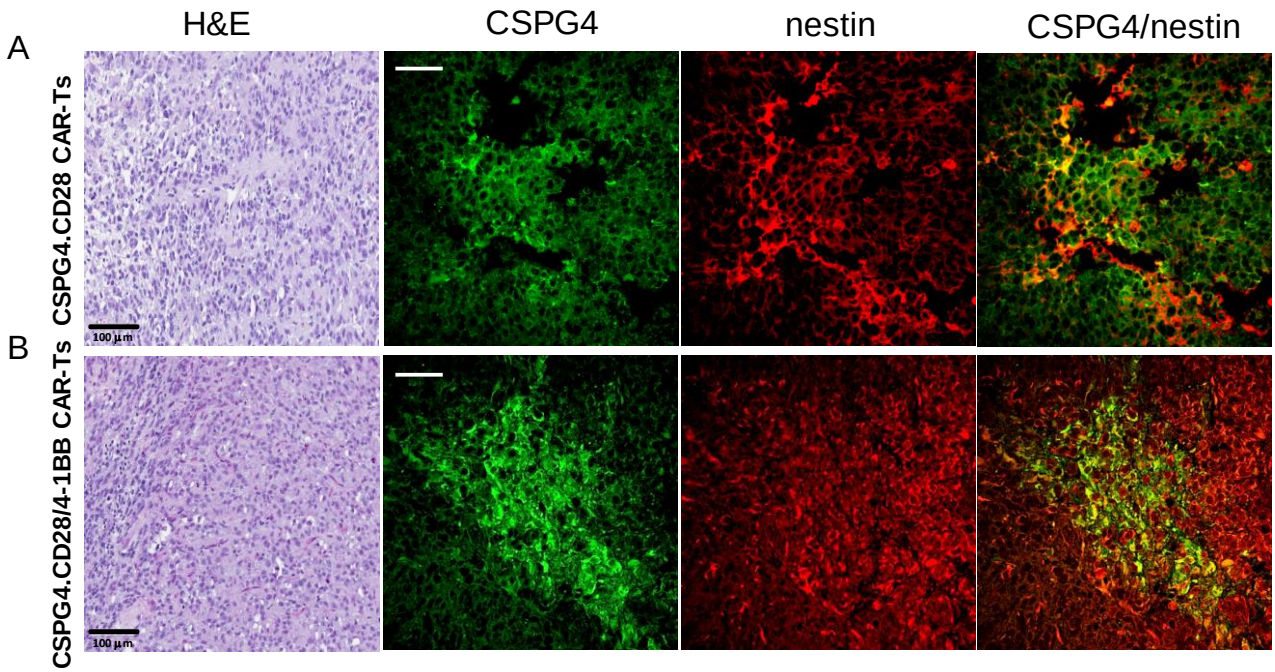

Fig. S8. GBM-NS-derived gliomas recurring in vivo retained CSPG4 expression.

GBM-NS-derived gliomas were removed from mice previously treated with T cells expressing the CSPG4.CAR containing CD28 (**A**) or CD28/4-1BB (**B**) co-stimulatory endodomains. Hematoxylin and eosin (H&E) showed that gliomas are partially proliferating based on the low density of tumor cells (scale bar 100 μm). Tumor masses co-expressed high amounts of CSPG4 and nestin, recapitulating the phenotype of the originating implanted GBM-NS (scale bar 50 μm).

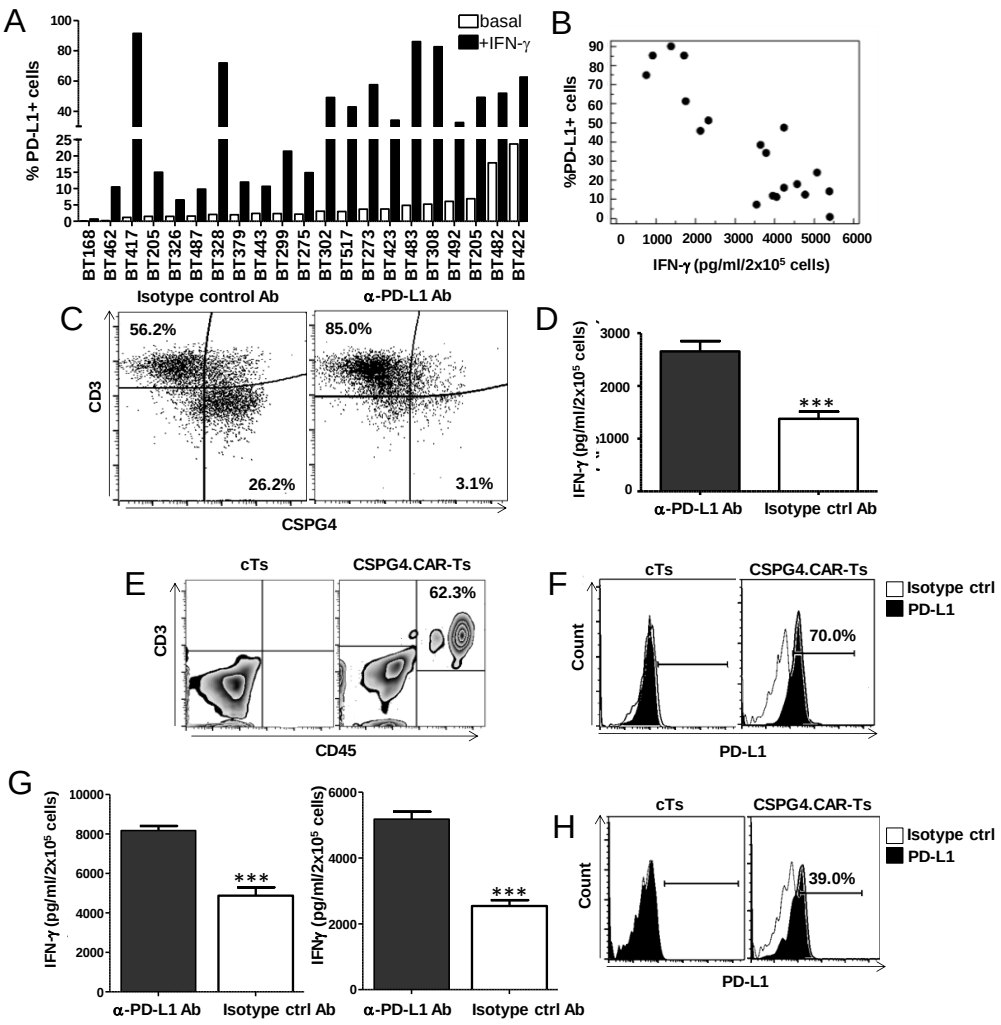

Fig. S9. GBM-NS upregulated PD-L1 in response to IFN-γ.

(**A**) GBM-NS express low amounts of PD-L1, but they differentially upregulate PD-L1 when stimulated in vitro with recombinant IFN-γ (500 pg/mL) for 24 hours. GBM-NS are ordered from the lowest to the highest basal PD-L1 expression. BT417-NS, showed the highest expression of PD-L1 upon IFN-γ stimulation. (**B**) We correlated the expression of PD-L1 by GBM-NS after IFN-γ stimulation with the amount of IFN-γ secreted by CSPG4.CAR-Ts co-cultured with GBM-NS,and found a significant inverse correlation (n=19, $P < 0.0001$). (**C, D**) Co-culture of CSPG4.CAR-Ts with BT417-NS in the presence of α-PD-L1 Ab improved both the killing of BT417-NS (**C**) and IFN-γ production (**D**) compared to the isotype control at an E:T ratio 1:5. (**E, F**) BT417-NS were implanted intracranially in nude mice. The tumor mass removed one week after the infusion of either cTs or CSPG4.CAR-Ts showed the presence of infiltrating T cells (**E**) and upregulation of PD-L1 (from 0% to 70%) (**F**) only in mice inoculated with CSPG4.CAR-Ts. Dot plots showing the presence of CSPG4.CAR-Ts within the tumor one week after T cell inoculation are in (**E**). Flow cytometry histograms showing the expression of PD-L1 in tumors from mice treated with CSPG4.CAR-Ts compared to mice treated with cTs are shown in (**F**). (**G**) Blocking PD-L1 during the co-culture of CSPG4.CAR-Ts with two other GBM-NS (BT275-NS and BT308-NS) in the

presence of α-PD-L1 Ab improved IFN-γ production compared to the isotype control at an E:T ratio 1:5. (**H**) Tumor mass derived from BT308-NS implantation and explanted one week after CSPG4.CAR-T infusion upregulated PD-L1 compared to cTs (from 0% to 39%).

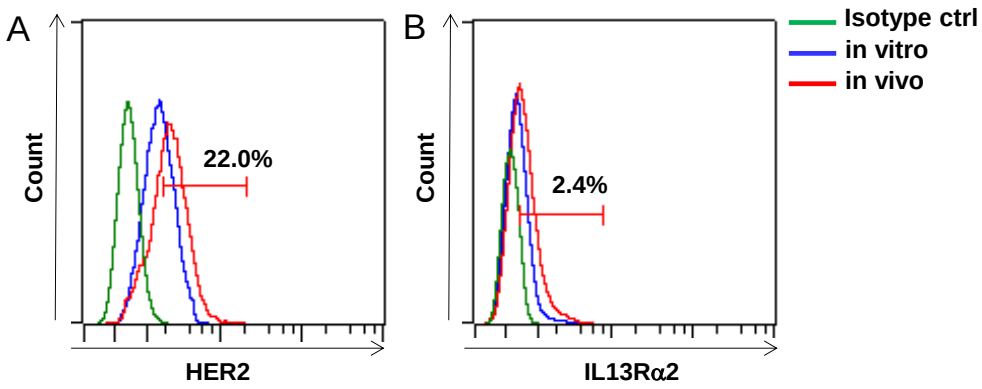

Fig. S10. HER-2 and IL13Rα2 expression is not upregulated in GBM-NS in xenograft models. Tumors from BT168-NS xenografts were explanted 1 week after intracranial inoculation in mice. Engrafted GBM-NS showed similar expression of HER2 (**A**) and IL13Rα2 (**B**) as compared to cells growing in vitro (HER2: 23.4 vs 22.0%; IL13Rα2: 2.7 vs 2.4%). Blue and red lines indicate antigen expression in GBM-NS growing in vitro and in vivo, respectively. Green lines indicate the isotype controls.

Table S1. GBM characterization based on CSPG4 expression and molecular subtypes

	High expression of CSPG4		Moderate/low expression of CSPG4	
Subtype	N. of GBM (tot. 28)	% subtype	N. of GBM (tot. 14)	% subtype
MES	10	35.7	3	21.4
CLAS	11	39.3	6	42.9
PN	7	25.0	5	35.7

Abbreviations. MES: mesenchymal; CLAS: classical; PN: proneural