Table S1: Patient History for PBT lines.

Patient history for other PBT lines characterized in this manuscript is described in (Brown et al. 2009).

Tumor ID	Patient ID	Age	Gender	Diagnosis	Prior Teatment
PBT015	UPN033	57	M	Glioblastoma Multiforme grade IV	Radiotherapy, temozolomide
PBT030	De-Identified	59	M	Glioblastoma Multiforme grade IV; right temperal lobe	Newly diagnosed

Table S2: Primary Antibody Details

Antibody	Clone	Source: Catalogue Number	Usage
Goat polyclonal anti-IL13Rα2	N/A	R&D Systems: AF146	FC; Western: P-IHC
Mouse monoclonal anti-IL13Rα2	B-D13	Diaclone/Cell Sciences Inc: 852.120.00	FC
Chicken polyclonal anti-IL13Rα2	N/A	Sigma-Aldrich: GW22455A	Western
PE-conjugated mouse anti-CD133/1	AC133	Miltenyi Biotec Inc.: 130-080-801	FC
PE-conjugated mouse anti-CD133/2	293C3	Miltenyi Biotec Inc.: 130-090-853	FC
Mouse monoclonal anti-CD133	W6B3C1	Miltenyi Biotec Inc.: 130-092-395	Western
Goat polyclonal anti-Olig2	N/A	R&D Systems: AF2418	Western, P-IHC
Rabbit polyclonal anti-ß-Actin	N/A	Rockland Immunochemicals: 600-401-866	Western
Mouse monoclonal anti-ß-III Tubulin	TU-20	Millipore: CBL412	Western; IF
Rabbit polyclonal anti-GFAP	N/A	Sigma-Aldrich: G9269	Western; IF
Rabbit Polyclonal anti-GFAP	N/A	Dako Corp: Z0334	P-IHC
Rabbit polyclonal anti-SOX2	N/A	Abcam, Inc.: ab15830	IF
Mouse monoclonal anti-SOX2	245610	R&D Systems: MAB2018	P-IHC
Mouse monoclonal anti-Nestin	10C2	Millipore: MAB5326	IF; P-IHC
FITC-conjugated mouse monoclonal anti-TCR α/β	WT31	Becton Dickenson: 347773	FC
FITC-conjugated mouse monoclonal anti-CD3	SK7	Becton Dickenson: 349201	FC
PE-conjugated mouse monoclonal anti-CD8	SK1	Becton Dickenson: 340046	FC
Anti-CD3zeta	8D3	BD Biosciences: 51-6527GR	Western

N/A, Not applicable
FC, Flow cytometry
IF, Immunofluorescence
P-IHC, Immunohistochemistry of paraffin embedded tissue

Table S3: Real-time PCR Primer Details.

Primer target	Primer Sequences	Amplification Efficiency*
IL13Rα2 exons 1-2	5'TGAAGTCGCCATAACCTG 5'AAGCATCCGATAGCCAAG	85 ± 6.5%
IL13Rα2 exons 6-7	5'ATGCAGATTTCCCTATTTGGAGG 5'TGGCGGCAAAGGTTTAACTAT	80% ± 4.4%
β-actin	5'CCGCCGATCCACACGGAGTACTTG 5'CAGGATGCAGAAGGAGATCACTGCCCTG	84% ± 2.4%

*, PCR products were evaluated by melting curve analysis and gel electrophoresis to verify amplification of a single product of the correct size.

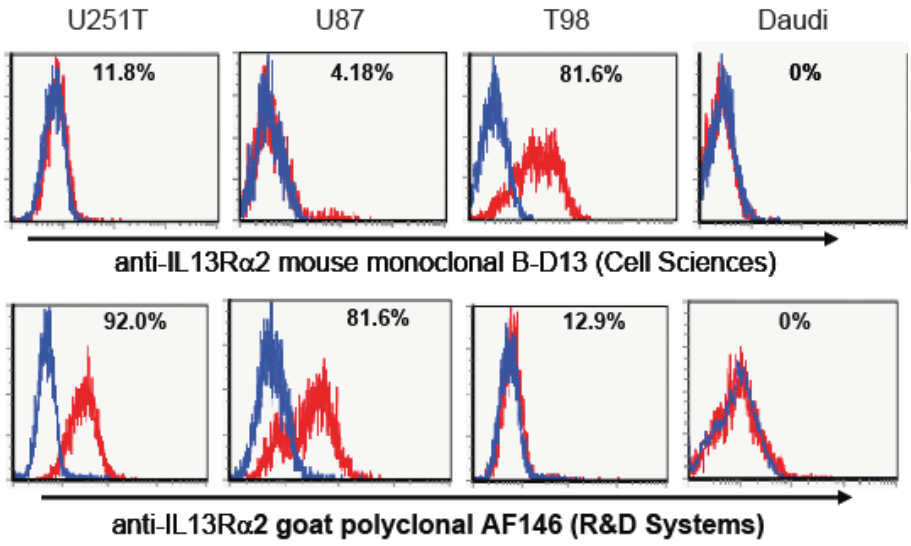
Table S4: *In Vitro* Self-Renewal and *in Vivo* Tumorigenicity.

TS Line	Number of heterotopic passages	Secondary TS Formation	Min. # cells for tumor initiation (2-4 mo)	Time to Morbidity (10^5 cells)	Histological Features
UPN033-PBT015	0	4.9 ± 0.6%	10^5§	ND	Diffuse cells, no tumor mass
PBT030	0	11.8 ± 0.4%	10^5§	80 ± 9 (n = 12)	Tumor mass with highly infiltrative edges
PBT030-2	2	5.2 ± 1.4%	10^5§	76 ± 2 (n = 4)	Tumor mass with highly infiltrative edges

§, Lower numbers of cells were not tested.
ND, not done.
Characterization of other PBT lines is reported in (Brown et al. 2009).

Figure S1: Differential recognition of IL13Rα2 by commercial antibodies. (A) Flow cytometry analysis of IL13Rα2^{pos} U251T and U87, and IL13Rα2^{neg} T98 glioma cell lines using the mouse monocloncal (Cell Sciences, B-D13) and goat polyclonal (R&D Systems, AF146) antibodies. Daudi lymphoma serves as an IL13Rα2^{neg} control cell line. Percent positive cells are indicated in each histogram. Note that monoclonal anti-IL13Rα2 B-D13 antibody (Cell Sciences) did not recognize IL13Rα2 protein by flow cytometry on the well documented IL13Rα2^{pos} U251T or U87 cell lines, which express high levels of IL13Rα2 mRNA (**Fig. 2**), and are potently killed by the IL13Rα2-specific IL13-zetakine-engineered CTL (**Fig. 4**). Moreover, this antibody detects an antigen expressed by the IL13Rα2^{neg} T98 cell line, which express very low levels of IL13Rα2 mRNA (**Fig. 2**), and are not recognized and killed by the IL13Rα2-specific IL13-zetakine-engineered CTL (**Fig. 4**). (B) Western analysis of established cell lines U251T, U87, T98 and Daudi using chicken polyclonal anti-IL13Rα2 antibody (Sigma, GW22455A). Note that IL13Rα2 expression is detected for IL13Rα2^{neg} T98 glioma cell line (**Fig. 2**). Detection of a similar Western reactive protein was detected for the IL13Rα2^{neg} primary glioma lines PBT003, PBT008 and PBT009 (data not shown).

A



B

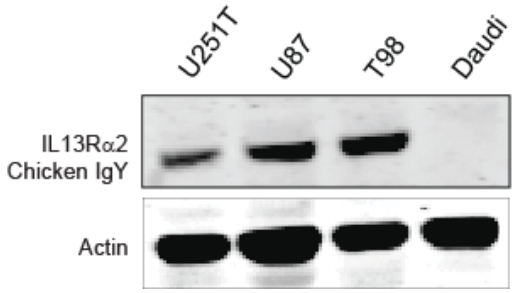


Figure S2: Cell surface phenotype and chimeric antigen receptor (CAR) expression of ex vivo engineered and expanded effector cytolytic T lymphocytes (CTLs). (A) CTLs were analyzed by flow cytometry using FITC or PE-conjugated antibody against α/ß T cell receptor (TCR), CD3, and CD8 (grey histograms; BD Biosciences) or isotype control antibody (solid line). Percent positive cells are indicated in each histogram. (B) Western analysis using anti-human CD3-zeta mAb detects both endogenous CD3-zeta (16 kDa) and IL13-zetakine (52 kDa) or CD19R-zeta (67 kDa) CAR expression for engineered CTL lines. IL13-zeta CAR migrates as diffuse band consistent with glycosylation of human IL-13 (KS Kahlon et al, *Cancer Res* (2004) 64:9160-9166).

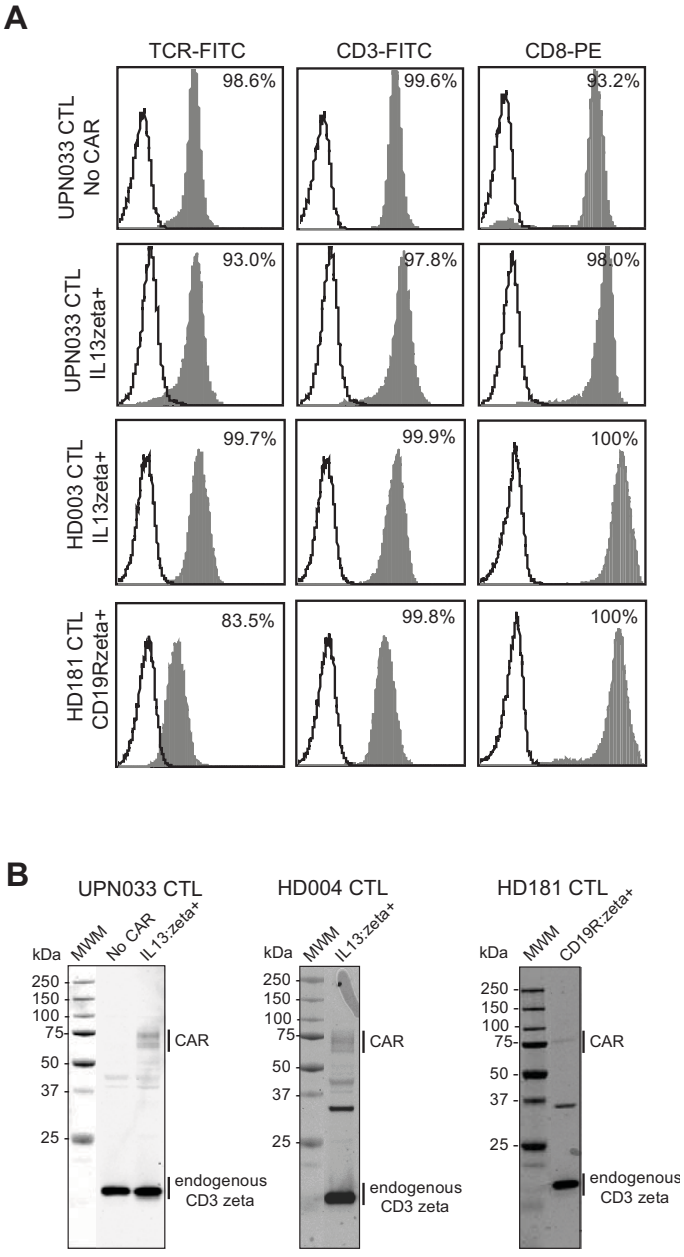


Figure S3: IL13Rα2-specific CTLs kill IL13Rα2-expressing PBT017 brain tumor stem and differentiated cell populations. (A) CRA measuring the lysis of IL13Rα2^{pos} PBT017-4 TS and 7-day serum-differentiated (DIF), or serum-expanded (p7; ADH) cells at increasing effector:target ratios (x-axes). The IL13Rα2^{pos} U251T established glioma line served as a positive control. The IL13Rα2^{neg} CD19^{pos} Daudi lymphoma served as a control target. Effector lines tested include the allogeneic CD8^{+} IL13-zetakine^{+} CTL clone 2D7 (HD003 IL13zeta^{+} CTL) and the CD19-specific CD19R^{+} CTL clone E8 (JD10 CD19R^{+} CTL). (B) CRA measuring lysis of IL13Rα2^{pos} PBT030 TS and 7-day serum-differentiated (DIF). The IL13Rα2^{pos} U87 and LCL-OKT3 lines served as a positive controls. Effector lines tested are as described in (A). Mean ± S.D. values of 6 wells are depicted.

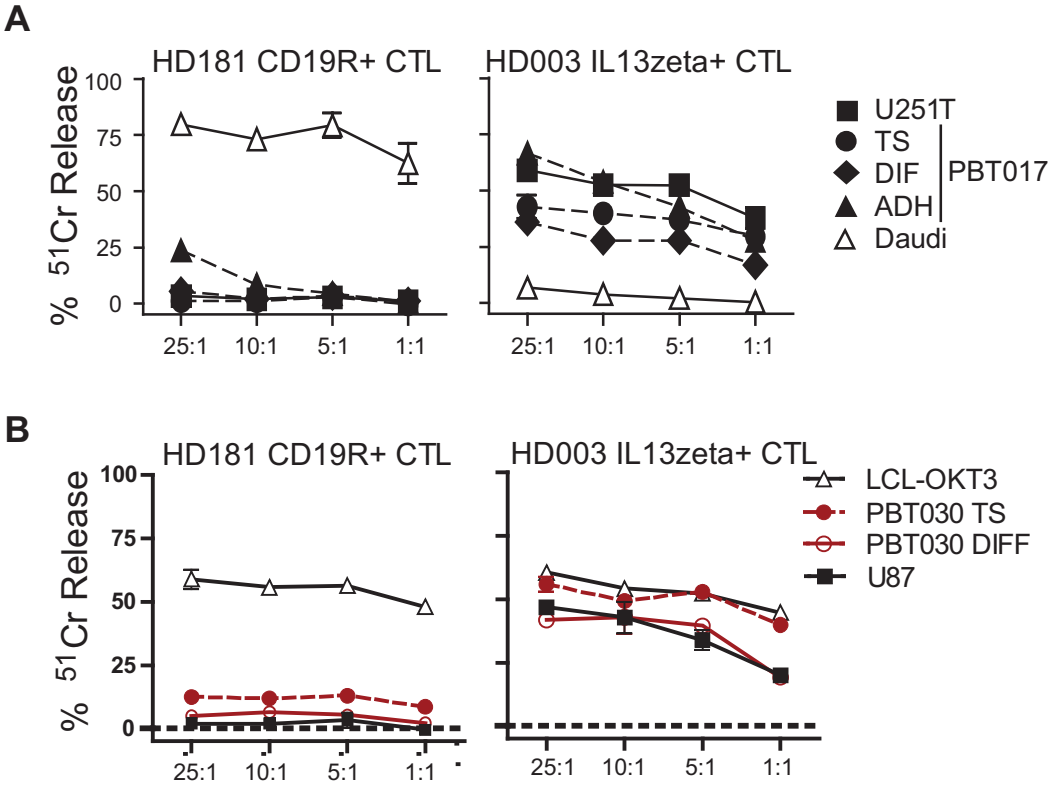


Figure S4: INF-γ and INF-α cytokine levels produced by IL13-zetakine^{+} CTL following co-culture with IL13Rα2^{pos} TS and DIF cells. (A) Representative experiment showing IFN-γ and TNF-α cytokine production by UPN033 IL13-zetakine+ CD8^{+} CTL clone 3C12 after overnight co-culture with IL13Rα2^{pos} GSC lines (TS) and matched serum-differentiated lines (DIF; 7 days serum-differentiation). Mean ± S.D. values of 3 replicate measurements from a single sample are depicted. Note that IL13-zetakine CTLs produced greater levels of cytokine when challenged with IL13Rα2^{pos} serum differentiated glioma lines (DIF) as compared to matched GSC TS lines. (B) Fold difference (DIF/TS) in cytokine release by two independent IL13-zetakine+ CTL clonal lines following co-culture with DIF and TS glioma lines. Each point represents an independent experiment. Line represents average fold increase in T cell cytokine production following engagement of DIF versus TS primary glioblastoma lines for all points (grand mean). Note that for the majority of experiments there was a 2-fold or greater increase in cytokine produced when targeting DIF versus matched TS targets (10 of 15 for INF-γ, and 11 of 14 for TNF-α). Upon comparison of all data sets, assuming all data points are independent, the difference between TS and DIF of PBT015 INF-γ and TNF-α (pg/mL) levels was determined to be statistically significant ($p < 0.05$, Wilcoxon matched-pairs signed rank test); differences between that of TS and DIF of the other PBT lines approached but did not achieve significance.

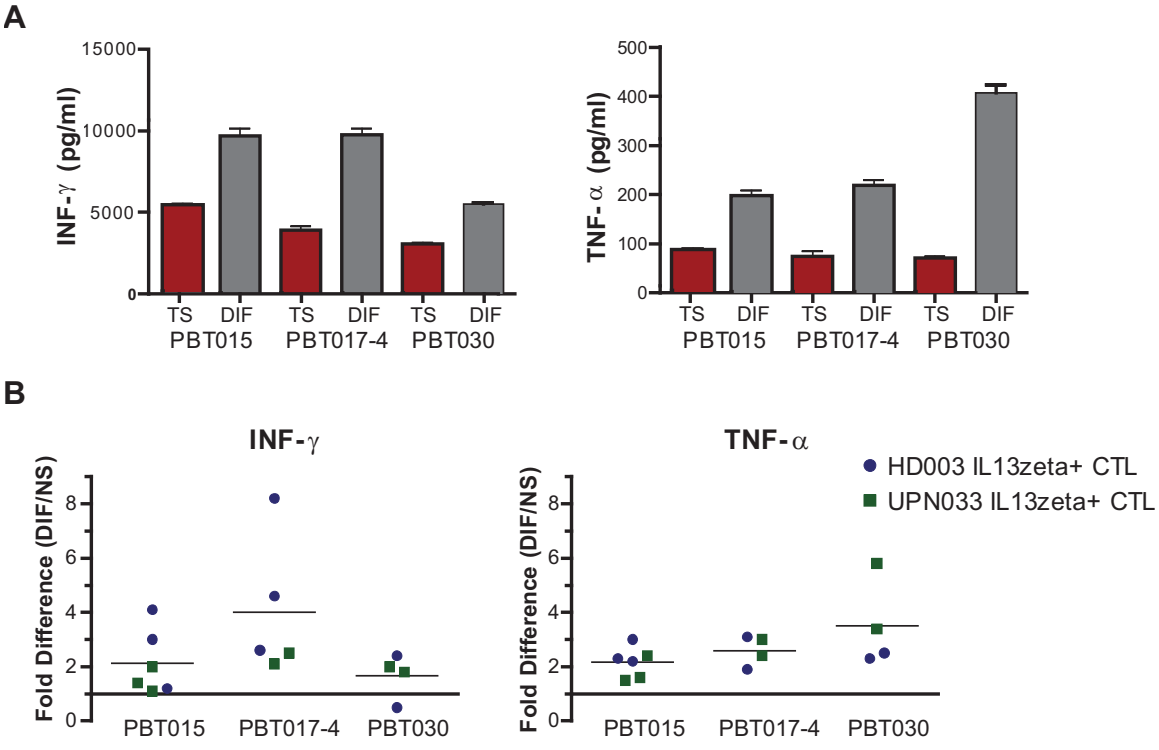


Figure S5: Characterization of IL13Rα2 expression for PBT030-2. (A) PBT030-2 tumor sphere (TS), and 7-day serum-differentiated (DIF) cells analyzed by flow cytometry for expression of IL13Rα2 (grey histograms); black histograms are secondary antibody alone. Percent positive cells are indicated in each histogram. PBT030-2 is glioma line derived from donor PBT030 that was heterotopically passaged in mice two-times prior to expansion *in vitro*. Similar to the parental PBT030 (**Fig. 2**), PBT030-2 expresses comparable levels of IL13Rα2 on both TS and DIF cells. (B) IHC staining for IL13Rα2 demonstrating that PBT030-2 TS initiates tumors that remain IL13Rα2^{pos}. As a control we show that the IL13Rα2^{neg} TS line, PBT003-4, initiates tumors that do not express this receptor.