

SUPPLEMENTAL DATA

Locoregionally delivered PD-1 blockade augments B7-H3 targeting CAR T cell efficacy in models of glioblastoma

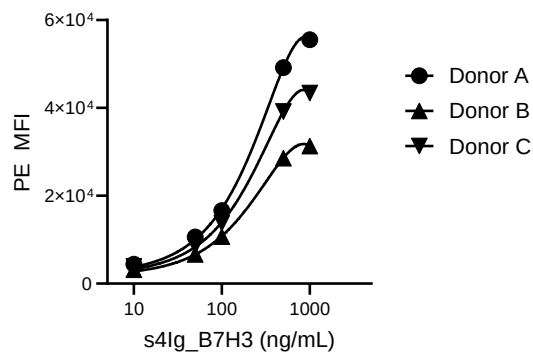
Jonathan F. Khan, Parwiz Abrahimi, Winson Cai, Lauren Dong, Kenny Kwok-Hei Yu, Svena Verma, Yacine Marouf, Sadna Budhu, Tara Pal Chaudhuri, Valeria Estrada Navarro, Mamadou A. Bah, Kelsey Hopland, Yanhong Yang, Terence J. Purdon, Ileana Miranda, Viviane Tabar, Jedd D. Wolchok, Taha Merghoub, Renier J. Brentjens

Cell Line	Histology	Gender	Age	Surgical History	IDH Status	MGMT status	TP53 Status	PTEN Status	TERT Status	EGFR Status
U251	GBM	M	Unknown	1 st Resection	WT	Methylated	(NM_000546 - 17p13.1) Missense Mutation (c.818G>A, p.R273H)	Loss	(NM_198253) promoter variant (g.1295228C>T)	WT
U87MG	GBM	F	44	1 st Resection	WT	Methylated	WT	WT	(NM_198253) promoter variant (g.1295228C>T)	WT
MSK-004	HGG	M	54	not known	WT	Unmethylated	(NM_000546 - 17p13.1) Deletion (Fold Change: -2.7)	Loss	(NM_198253) promoter variant (g.1295228C>T)	WT
MSK-007	GBM	F	57	not known	WT	Unmethylated	WT	WT	(NM_198253) promoter variant (g.1295228C>T)	WT
MSK-018	GBM	M	66	not known	WT	Unknown	Unknown	Unknown	Unknown	Unknown
MSK-019	GBM	M	18	not known	WT	Unmethylated	Unknown	Unknown	Unknown	Unknown
MSK-201	rGBM	M	71	1 st Recurrence	WT	Methylated	WT	WT	(NM_198253) promoter variant (g.1295228C>T)	EGFR (NM_005228) rearrangement: c.88+26274_c.890- 11del (EGFRvIII)
MSK-202	rGBM	M	64	1 st Recurrence	WT	Unmethylated	WT	Loss	(NM_198253) promoter variant (g.1295228C>T)	WT
MSK-203	GBM	M	56	1 st Resection	WT	Methylated	WT	Loss	(NM_198253) promoter variant (g.1295250C>T)	EGFR (NM_005228) rearrangement: c.89- 53203_c.889+754del (EGFRvIII)

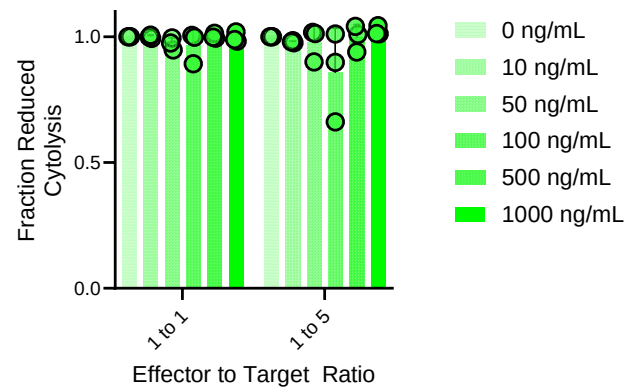
Table S1: Characterization of established and primary, patient-derived cell lines

Primary cell lines were established from patients' tumors resected at our institution, confirmation of disease was performed by a pathologist, and genetic signatures were evaluated for alterations by MSK-IMPACT. Presented here are alterations in the *IDH*, *MGMT*, *TP53*, *PTEN*, *TERT*, and *EGFR* genes, which are implicated in the etiology of GBM. Abbreviations: GBM – glioblastoma, HGG – high grade glioma, rGBM – recurrent GBM, M – male, F – female, IDH – isocitrate dehydrogenase, WT – wild type, MGMT – methylguanine-DNA-methyltransferase, TP53 – p53, PTEN - phosphatase and tensin homolog, TERT - telomerase reverse transcriptase, EGFR – epidermal growth factor receptor.

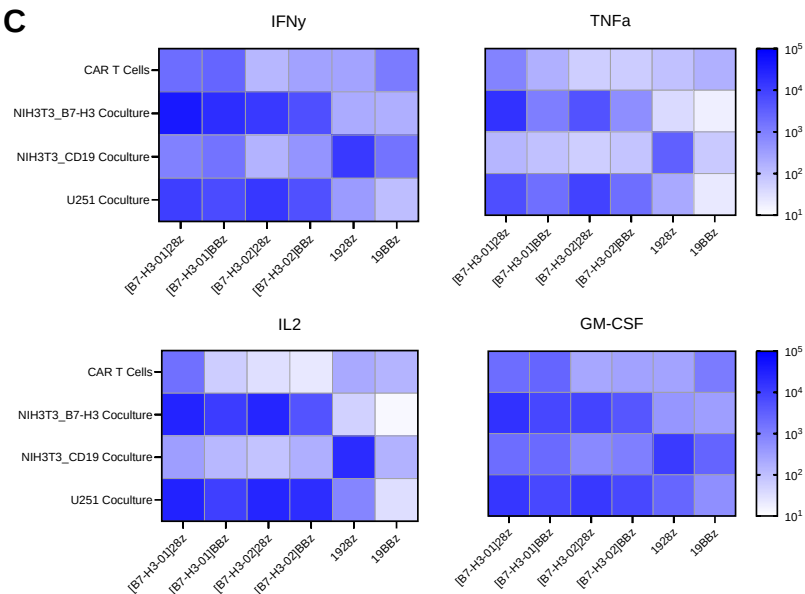
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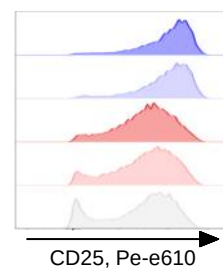
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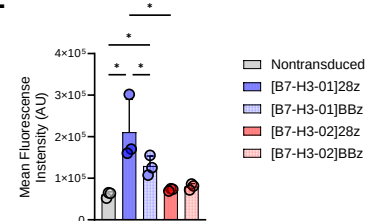
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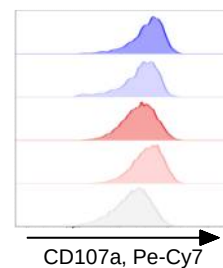
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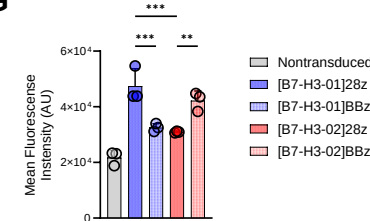
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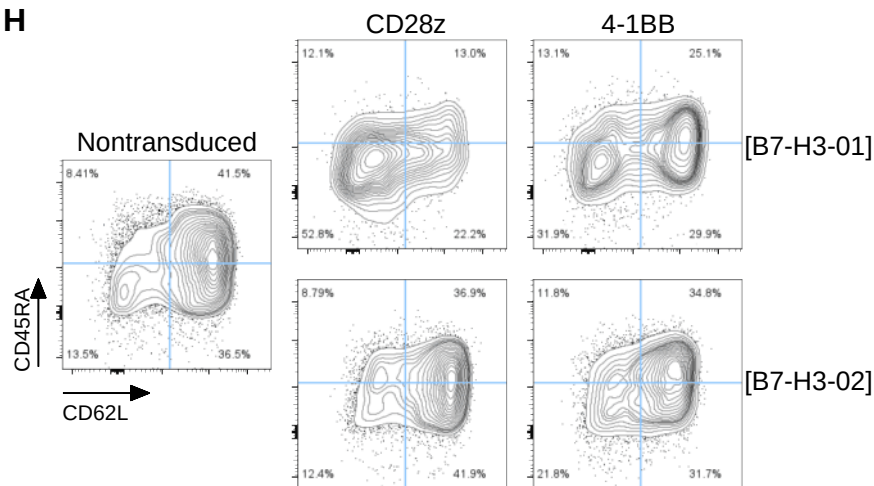
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G



H



I

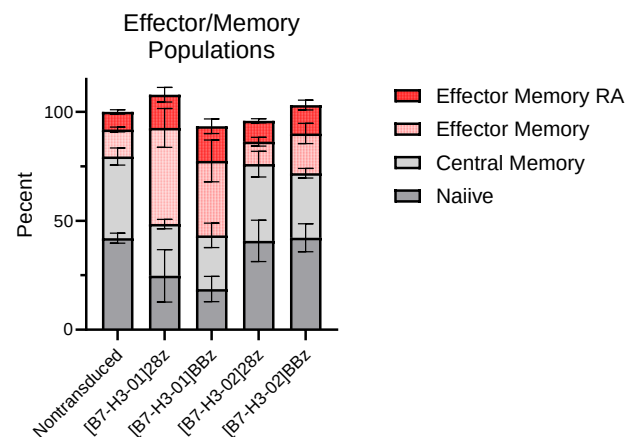


Figure S1: B7-H3 targeting CAR T cells demonstrate an enhanced functional profile

(**A**) [B7-H3-02]28z reproducibly binds to 4lg B7-H3 in a dose dependent fashion (n = 3 independent donors). (**B**) Cytolytic capacity of [B7-H3-02] CAR T cells is not suppressed in the presence of increasing soluble B7-H3 (two-way ANOVA, n = 3 independent donors). (**C**) Quantification of IFN γ , (top left), TNF α (top right), GM-CSF (bottom right), and IL-2 (bottom left) production (pg/mL) by B7-H3 targeting CAR T cells upon coculture with antigen positive and antigen negative cells; shown is pooled data from three independent experiments, heat map scales represent cytokine production as reported in pg/mL. (**D**) Representative expression of CD25 by B7-H3 targeting CAR T cells. (**E**) Quantification of CD25 expression by B7-H3 targeting CAR T cells (two-way ANOVA, n = 3 independent donors). (**F**) Representative expression of CD107 α by B7-H3 targeting CAR T cells (right). (**G**) Quantification of CD107 α expression by B7-H3 targeting CAR T cells (two-way ANOVA, n = 3 independent donors). (**H**) Representative flow cytometry contour plots showing effector/memory skewing of each B7-H3 targeting CAR T cell. (**I**) Quantification of effector and memory populations from each B7-H3 targeting CAR T cell (two-way ANOVA, n = 3 independent donors).

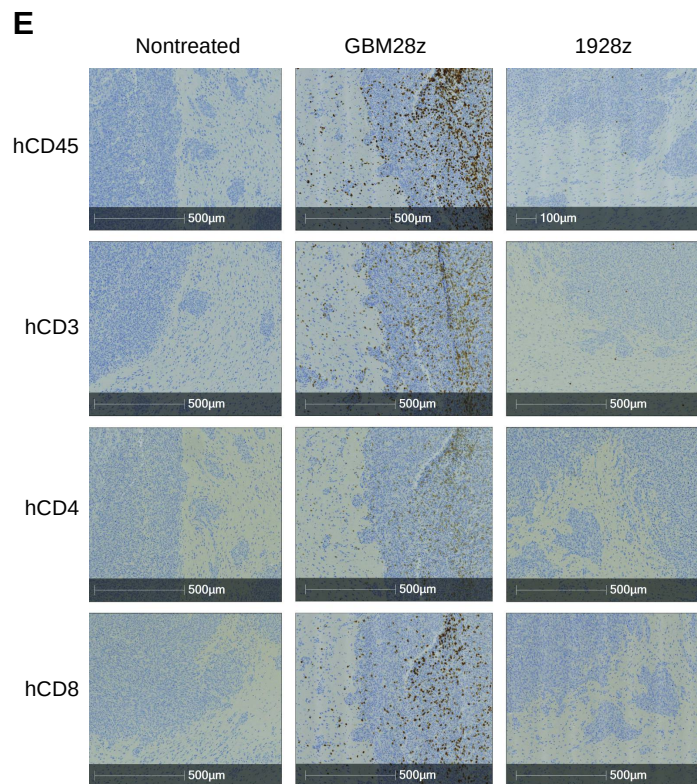
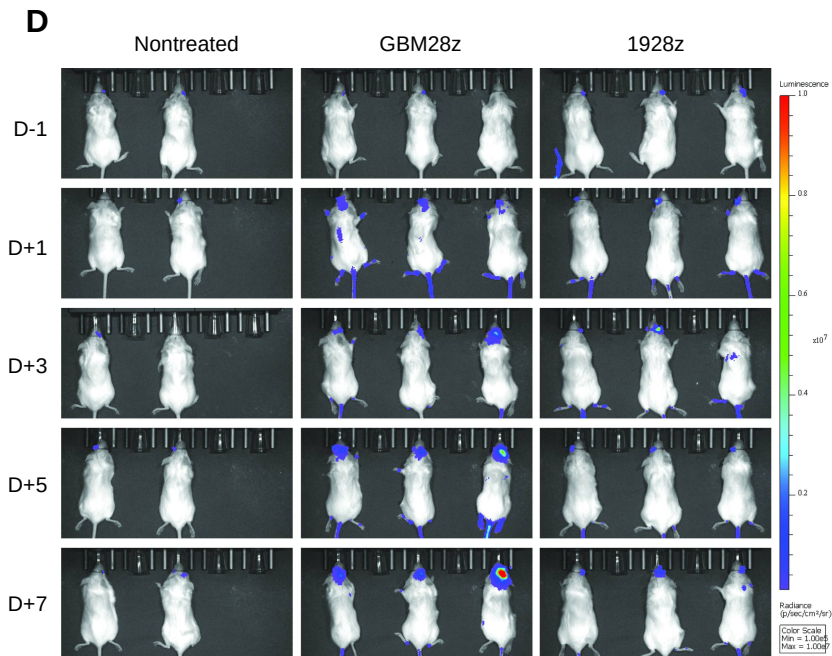
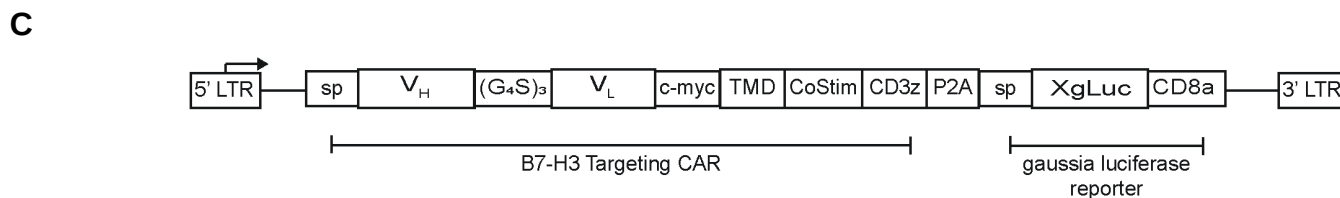
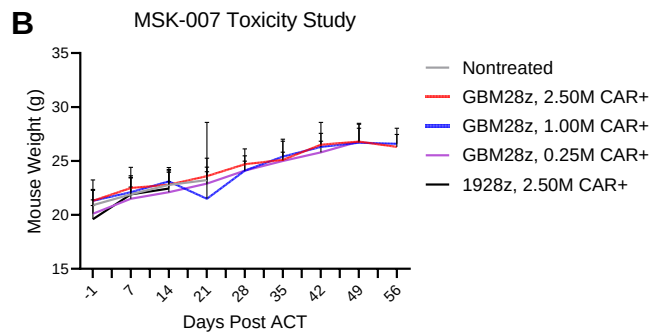
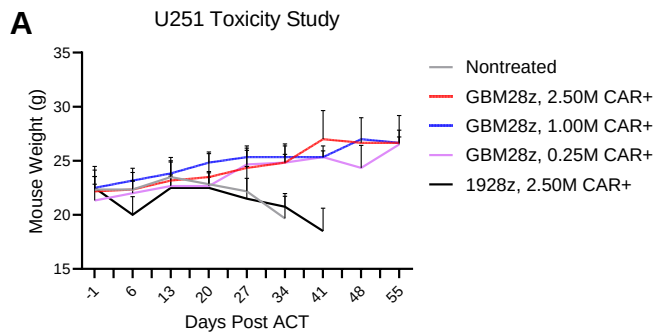
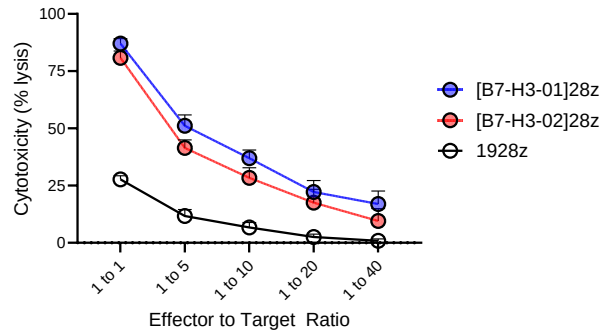


Figure S2: GBM28z CAR T Cells Demonstrate Potent but Tolerable In Vivo Activity

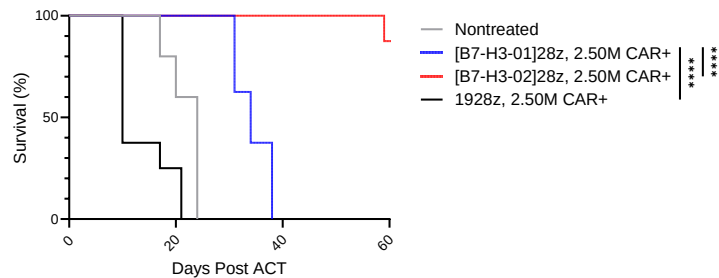
(**A-B**) Mouse weight was used as a measure of tolerability of adoptively transferred CAR T cells in the (**A**) orthotopic U251 model of GBM (two-way ANOVA, $n = 6$ mice per group), and (**B**) orthotopic MSK-007 model of GBM (two-way ANOVA, $n = 10$ mice per group). (**C**) Representation of GBM28z-XgLuc gammaretroviral vector. P2A, picornavirus derived self-cleaving peptide; XgLuc, externally expressed gaussia luciferase; CD8a, human CD8 α transmembrane region. (**D**) Representative bioluminescent imaging following GBM28z-XgLuc CAR T cell treatment; heat map scale represents bioluminescent photon flux. (**E**) Representative IHC pictographs demonstrate marked staining for human immune infiltrates following GBM28z-XgLuc CAR T cell treatment.

A

A375

**B**

A375 Survival

**C**

A375 Tumor Burden

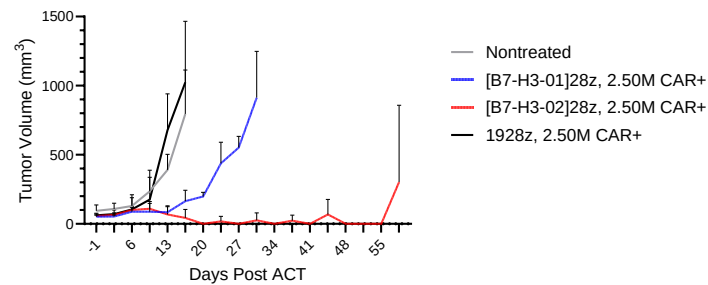


Figure S3: B7-H3 Targeting CAR T Cells are Efficacious in Models of Melanoma

(**A**) Luciferase-based cytotoxicity assay with B7-H3 targeting CAR T cells cocultured with established melanoma cell line A375; shown is pooled data from three independent experiments. (**B**) Survival of A375-bearing mice following differential CAR T cell treatment; data shown is pooled from two independent experiments (Log-rank test, n = 5-8 mice per group). (**C**) Quantification of A375 tumor burden following differential CAR T cell treatment; data shown is pooled from two independent experiments (n = 5-8 mice per group).

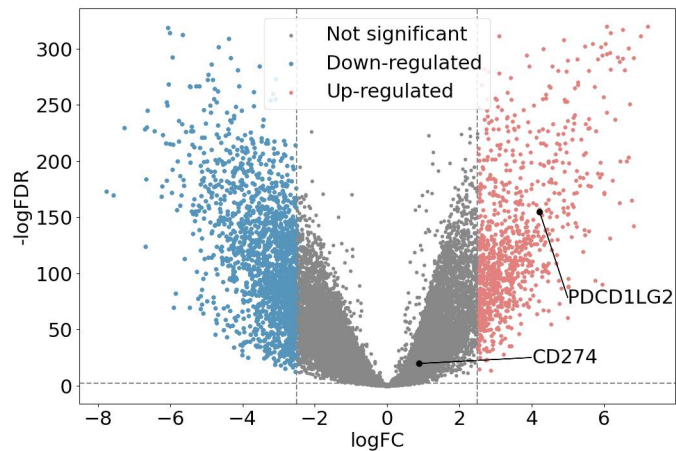
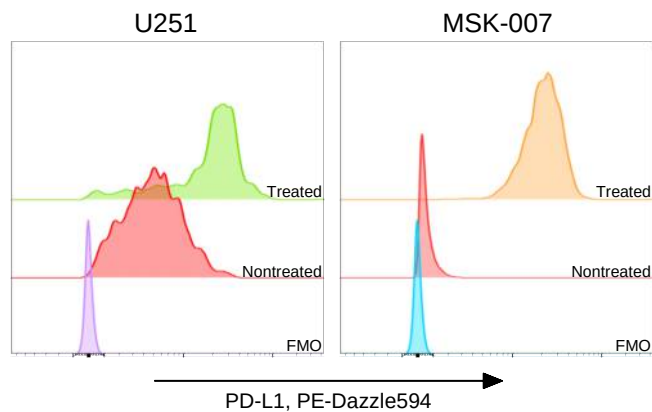
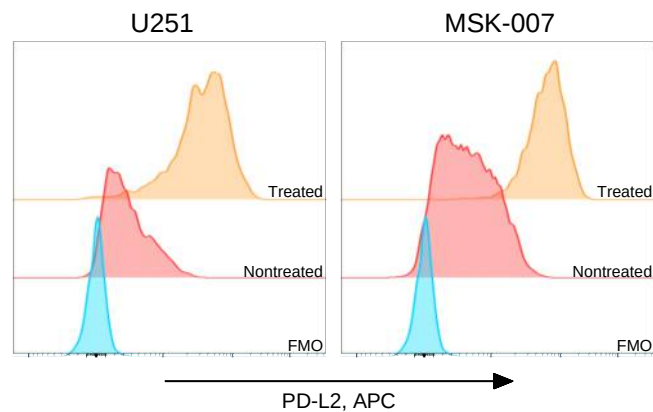
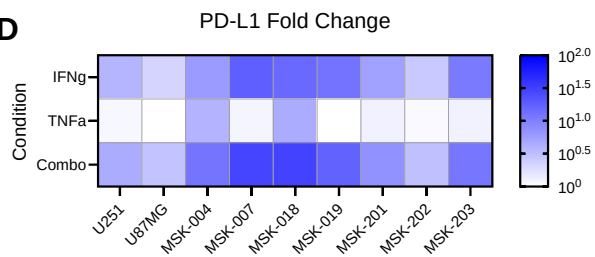
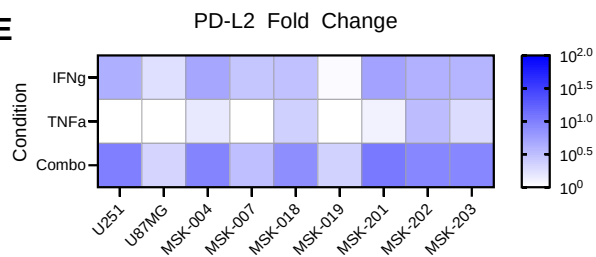
A**B****C****D****E**

Figure S4: Resistance to CAR T cell therapy is driven by upregulation of programmed death ligands

(A) TCGA analysis comparing expression of PD-L1 and PD-L2 in patient tissue vs healthy brain. (B) Representative flow cytometry plots showing upregulation of PD-L1 by established GBM cell line U251 and primary GBM cell line MSK-007 after exposure to CAR T cell conditioned media. (C) Representative flow cytometry plots showing upregulation of PD-L1 by established GBM cell line U251 and primary GBM cell line MSK-007 after exposure to CAR T cell conditioned media. (D-E) Proinflammatory cytokines function together to induce upregulation of (D) PD-L1 and (E) PD-L2 in response to cytokine supplemented media, (n = 2 independent experiments, heat map scale represents fold change in protein expression).

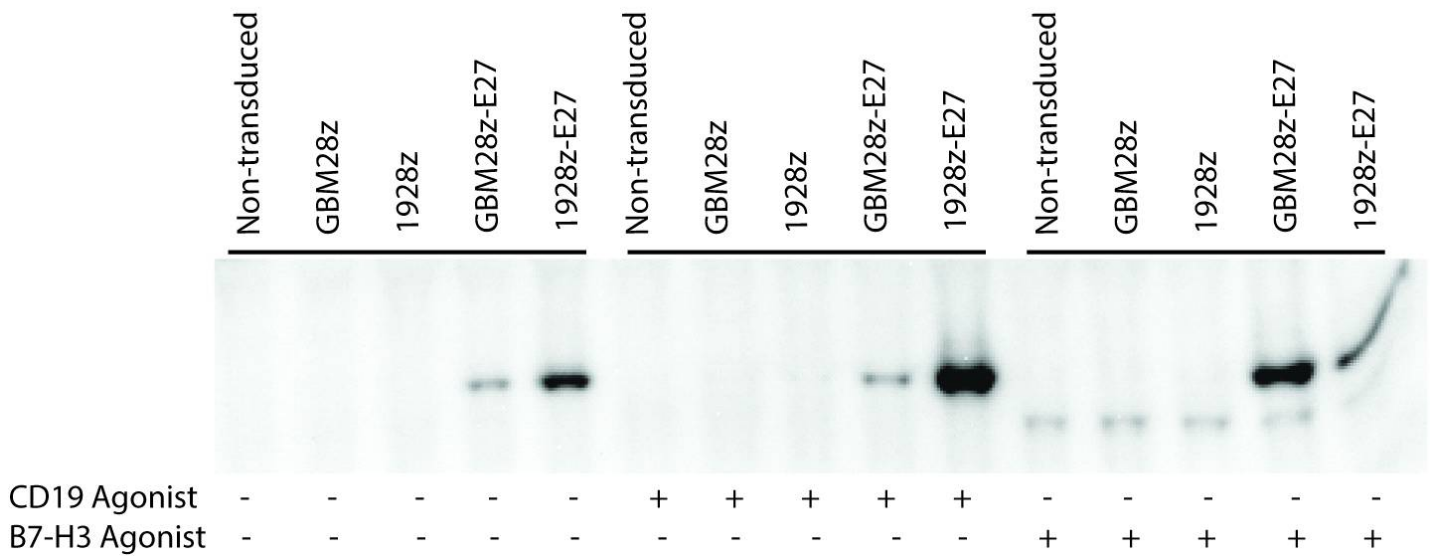
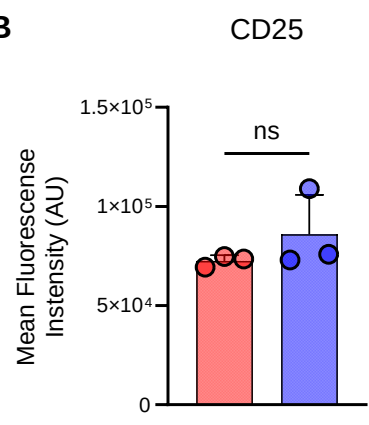
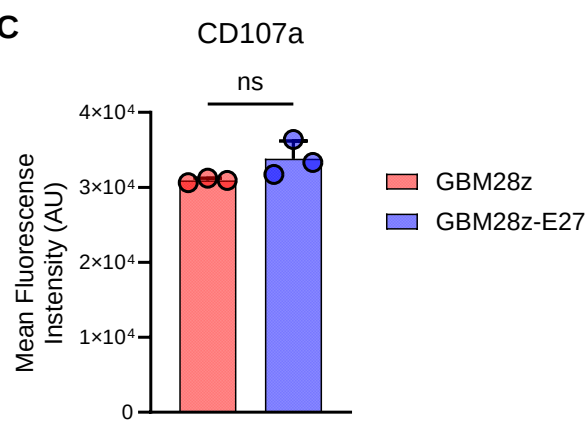
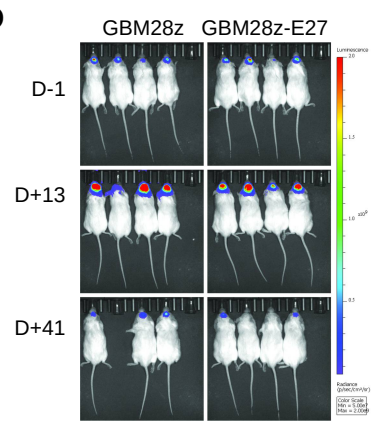
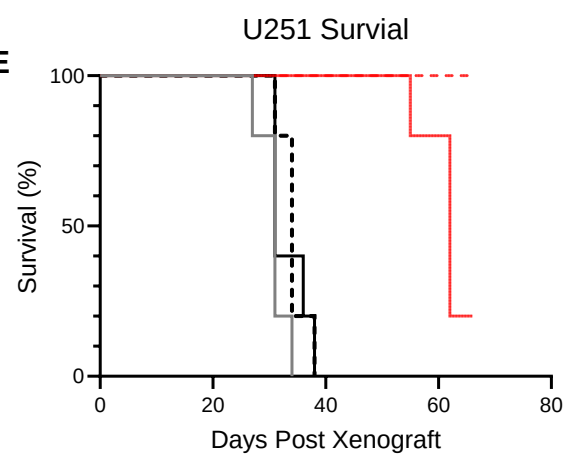
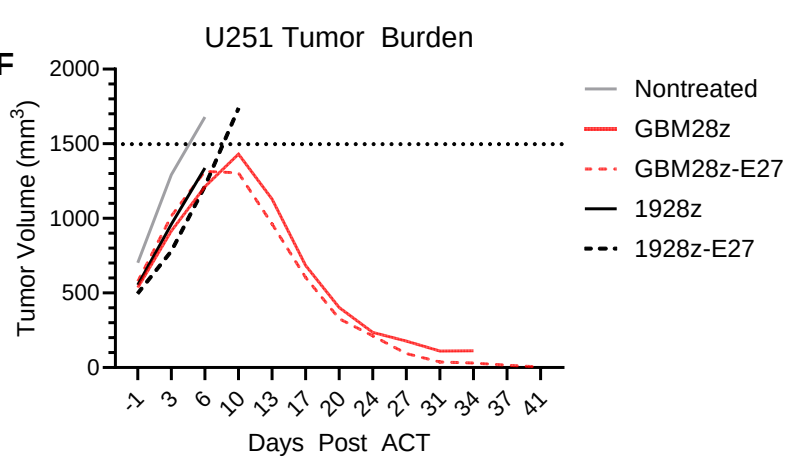
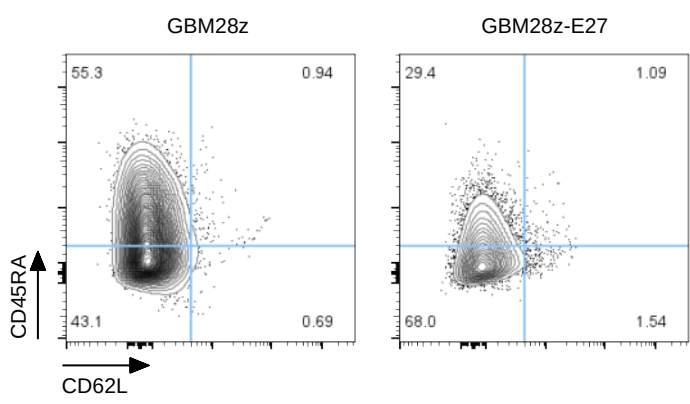
A**B****C****D****E****F****G**

Figure S5: Locoregional PD-1 blockade augments B7-H3 targeting CAR T cell efficacy

(A) Western blot for E27 C-terminal HA-tag on cell culture media from resting and activated CAR T cells. (B-C) Quantitation of tonic signaling in GBM28z-E27 CAR T cells. (B) CD25 mean fluorescence intensity, shown is pooled data from three independent donors (paired T test, $n = 3$). The same GBM28z dataset as in Fig. S1E ([B7-H3-02]28z) was used here. (C) CD107 α mean fluorescence intensity, shown is pooled data from three independent donors (paired T test, $n = 3$). (D) Representative bioluminescent imaging of U251 tumor burden in mice following treatment with GBM28z or GBM28z-E27 CAR T cells; heat map scale represents bioluminescent flux. (E) Survival of subcutaneous U251-bearing mice following treatment with GBM28z or GBM28z-E27 CAR T cells (Log-rank test, $n = 5$ mice per group). (F) Quantification of subcutaneous U251 tumor burden by caliper-based measurement following treatment with GBM28z or GBM28z-E27 CAR T cells ($n = 5$ mice per group). (G) Representative flow staining for CD45RA and CD62L on tumor infiltrating CD3 $^{+}$ cells from mice treated with GBM28z (left) and GBM28z-E27 (right). The same GBM28z dataset as in Fig. S1G ([B7-H3-02]28z) was used here.