

SHORT REPORT:**Title: Anti-VEGF therapy improves EGFR-vIII-CAR-T cell delivery and efficacy in syngeneic glioblastoma models in mice**

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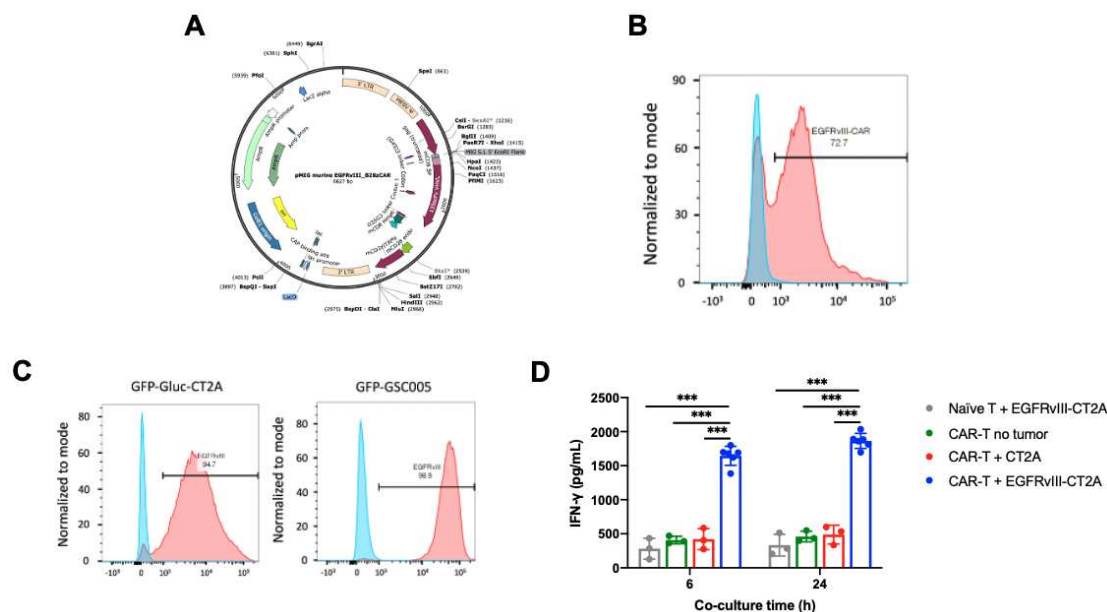


Figure S1. Generation of murine EGFRvIII-CAR-T cells and EGFRvIII-expressing syngeneic GBM cell lines. (A) Genetic maps of retroviral vectors encoding EGFRvIII targeting CAR. (B) Mouse T cells were transduced with retroviral constructs, and transduction efficiency was determined by assessing the Myc-tag on CAR-T cells 3 days post-transduction by flow cytometry. (C) Transduction efficiency of EGFRvIII in GBM tumor cells was determined by assessing the EGFRvIII expression on CAR-T cells 3 days post-transduction by flow cytometry. (D) CAR-T cells were incubated at a ratio of 1:1 with CT2A GBM cells, and IFN- γ release after 6 and 24-hr co-culture was measured by ELISA ($n = 3$). Error bars show \pm SEM. Statistical analysis was performed using one-way ANOVAs. *** $p < 0.001$.

Correlation of Gluc Samples vs Ultrasound Volume Measurements

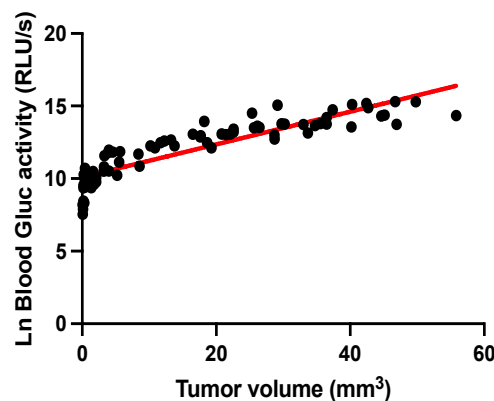


Figure S1. EGFRvIII-GFP-CT2A-Gluc activity in blood (RLU/s) versus tumor volume in cubic millimeters as measured by in vivo micro ultrasound. Ln, natural logarithm. $R^2 = 0.78$

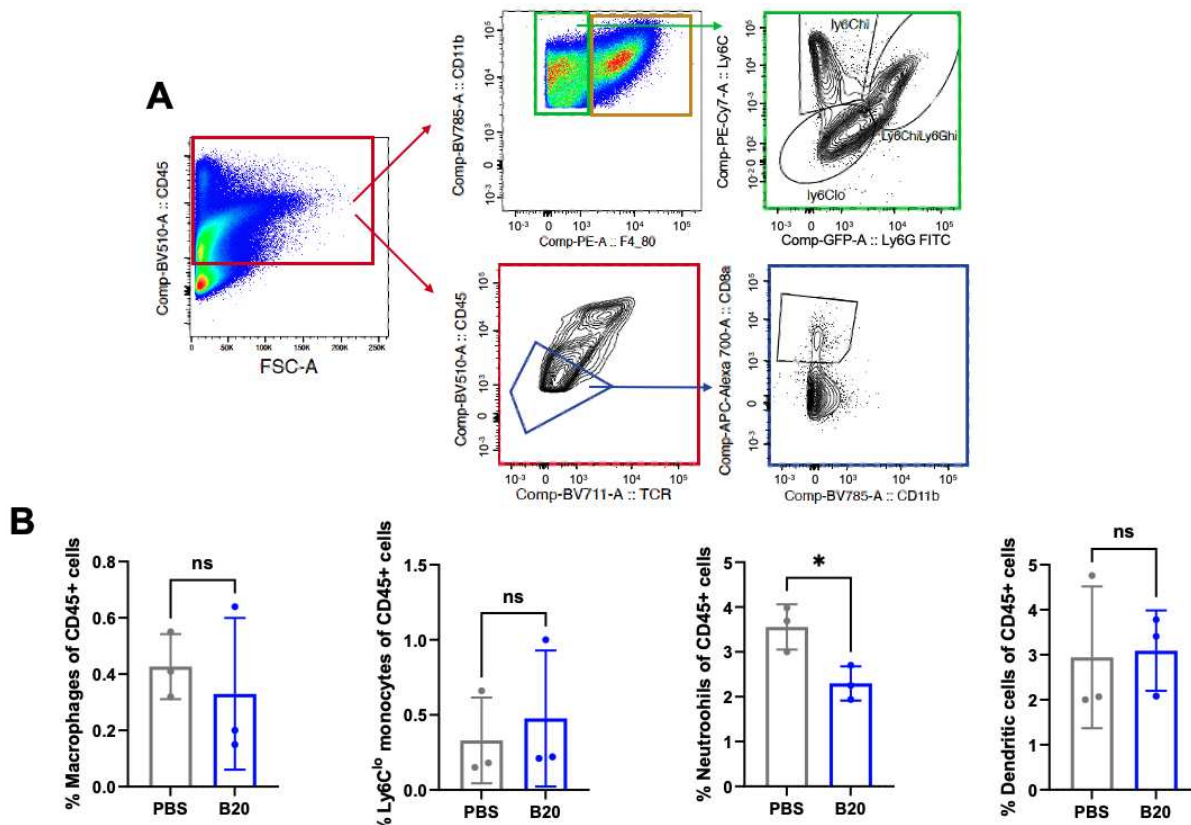


Figure S2. The effect of B20 treatment on the macrophages, neutrophils, or dendritic cells in GSC005 tumors as compared with PBS control. (A) Gating strategy of CD45+/CD11b+/F4/80+/GR1- macrophages, Ly6C^{lo} monocytes and Ly6G+ neutrophils (gated on CD45+/CD11b+/F4/80-), and CD45+/TCR-/CD8a+ dendritic cells inside the GSC005 GBM tumors. (B) Percentage of macrophages, Ly6C^{lo} monocytes and Ly6G+ neutrophils and dendritic cells inside the GSC005 GBM tumors. Error bars show \pm SEM. Statistical analysis was performed using Student's t-test.

Supplemental Movie SM1. Real-time multi-photon 3D video of live mouse brain treated with PBS (Video 1). GSC005 tumors were GFP labeled (green), and CAR-T cells were fluorescently labeled (red). Scale bar=50 μ m.

Supplemental Movie SM2. Real-time multi-photon 3D video of live mouse brain treated with EGFR^{vIII}-CAR-T cells (Video 2). GSC005 tumors were GFP labeled (green), and CAR-T cells were fluorescently labeled (red). Scale bar=50 μ m.