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Supplementary Materials for

Fibrin gel enhances the antitumor effects of chimeric antigen receptor T cells in glioblastoma

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The PDF file includes:

Figs. S1 to S5

Other Supplementary Material for this manuscript includes the following:

Movie S1

Supplementary Material

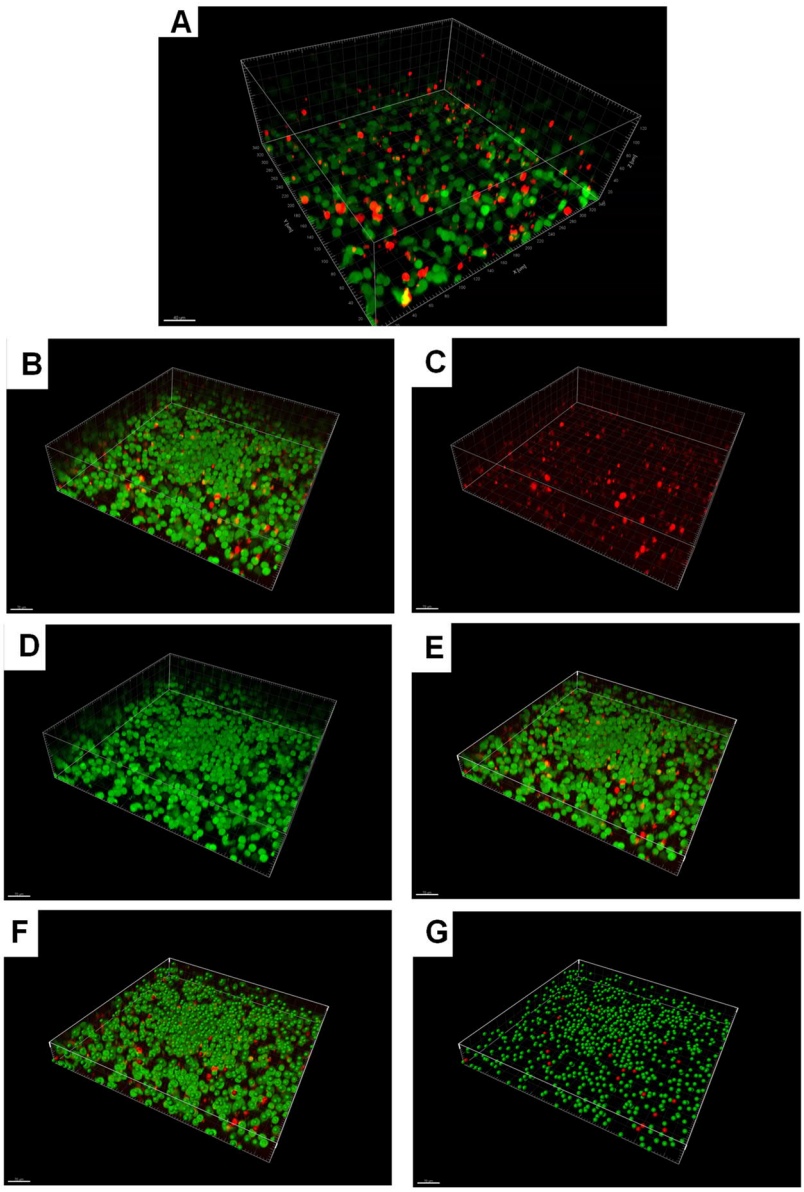

Figure S1. Confocal video and imaging of the live/dead CAR-T cells distributed within the fibrin gel. Image analysis performed by Imaris software. Representative confocal clip (A) and snapshots (B-G) showing workflow to quantitatively evaluate live/dead CAR-T cells encapsulated within the fibrin gel for 5 days. Live cells and dead cells were labeled with green fluorescein and red fluorescein, respectively. Dead cells (C) and live cells (D) visualized separately or in conjunction (B). Z-stack of 35um thickness (E) were used for thresholding and cell detection (F), and colocalization of green balls for live cells and red balls for dead cells (G) for quantifying colocalized regions.

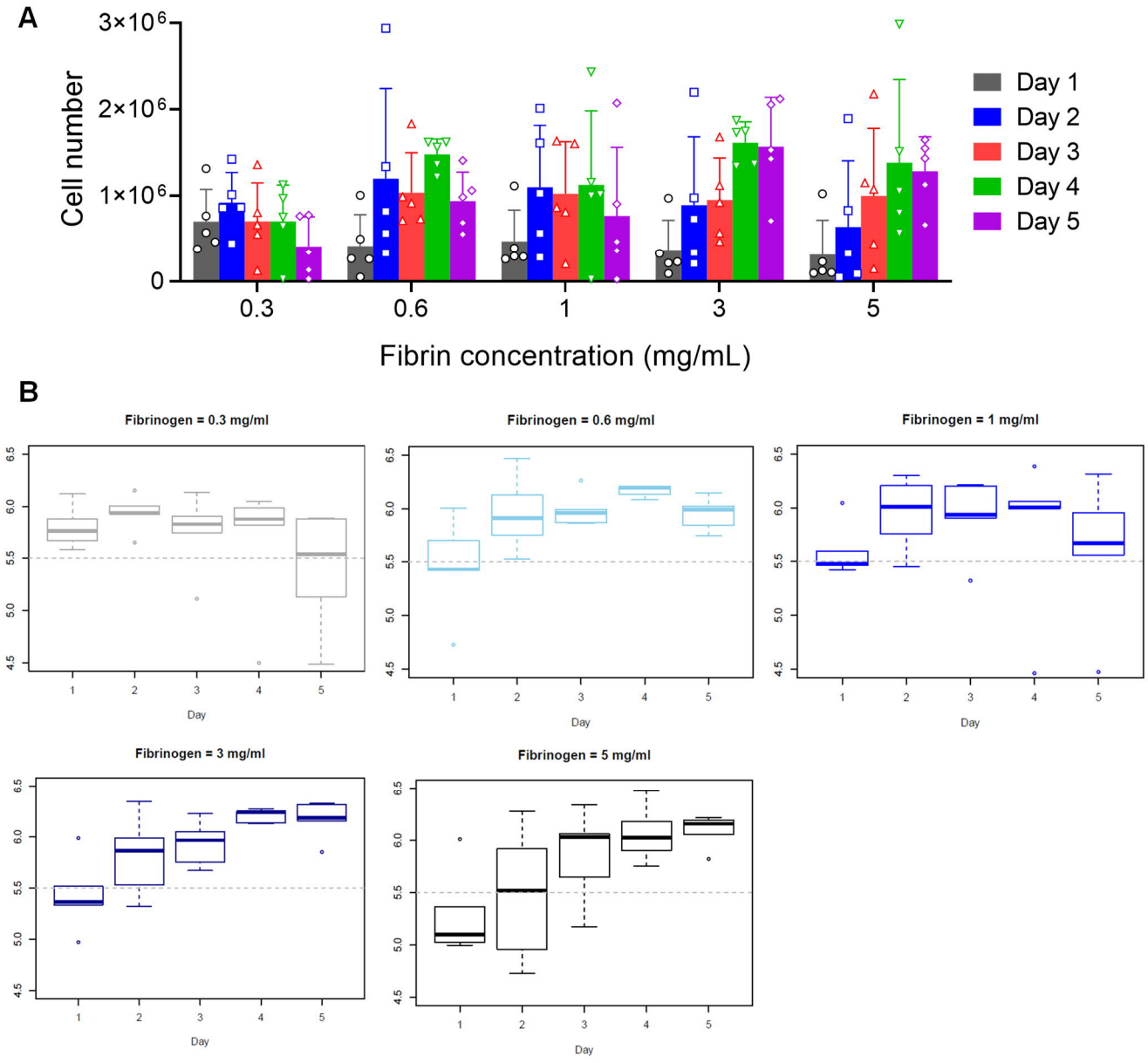

Figure S2. Evaluation of CAR-T cell release from the fibrin gel. (A) Statistical analysis of the T cell release from the fibrin gel formulated using various concentration of fibrin. T cell numbers were counted over five days. (B) Statistical analysis calculated as a log function of kinetics of cell release (x 10^5 y-axes) at various concentration of fibrin. The concentration of fibrin at 0.3 mg/mL was used as baseline. Summary plot can be seen in Figure 3B.

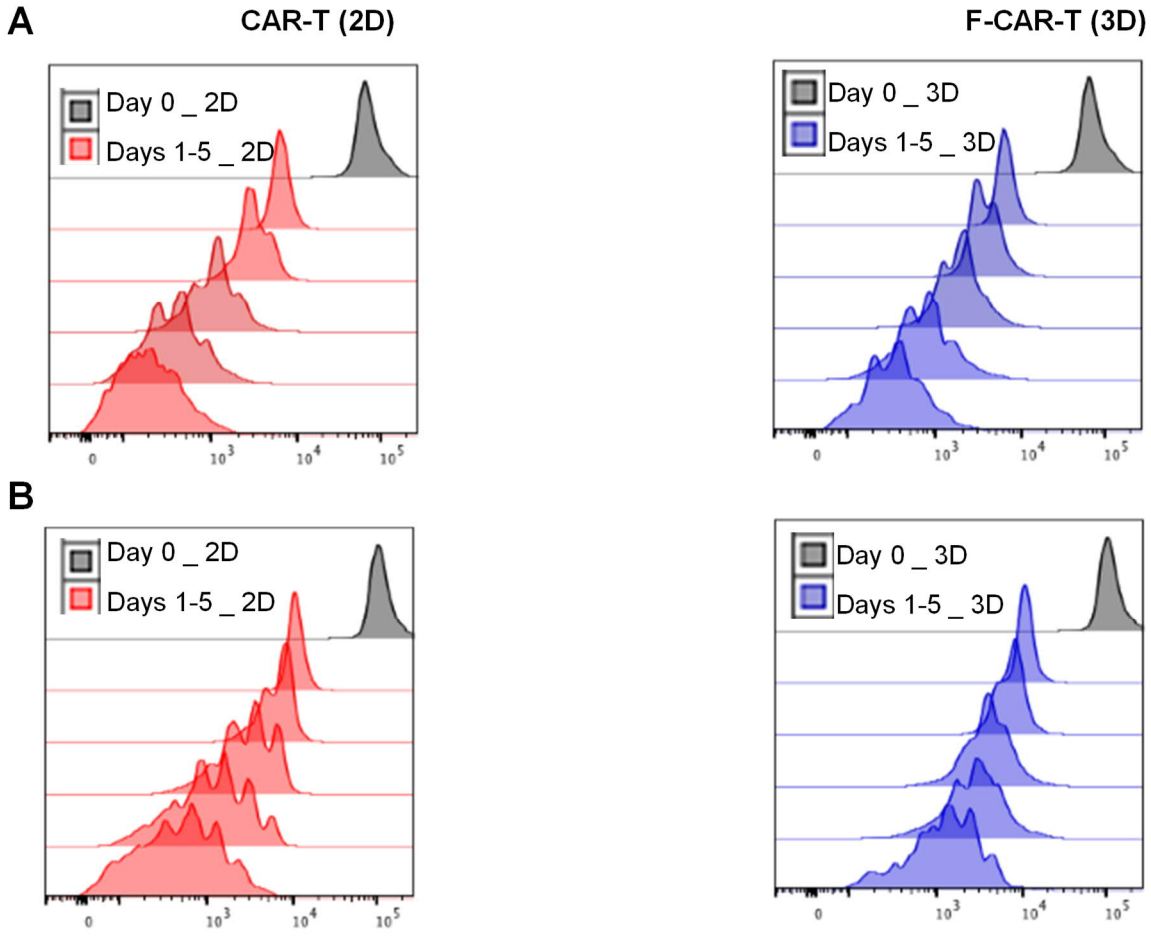

Figure S3. Proliferative capacity of CAR-T cells released from the fibrin gel. Cell divisions of CAR-T cells labeled with CFSE were evaluated in liquid culture (2D) versus fibrin gel culture (3D) at 3 mg/ml fibrin concentration. CFSE dilution of labelled T cells was measured every day for 5 days by flow cytometry. Illustrated in panels A and B are representative flow cytometry plots showing the CFSE-stained T cells released in 2D versus 3D cultures for two independent experiments.

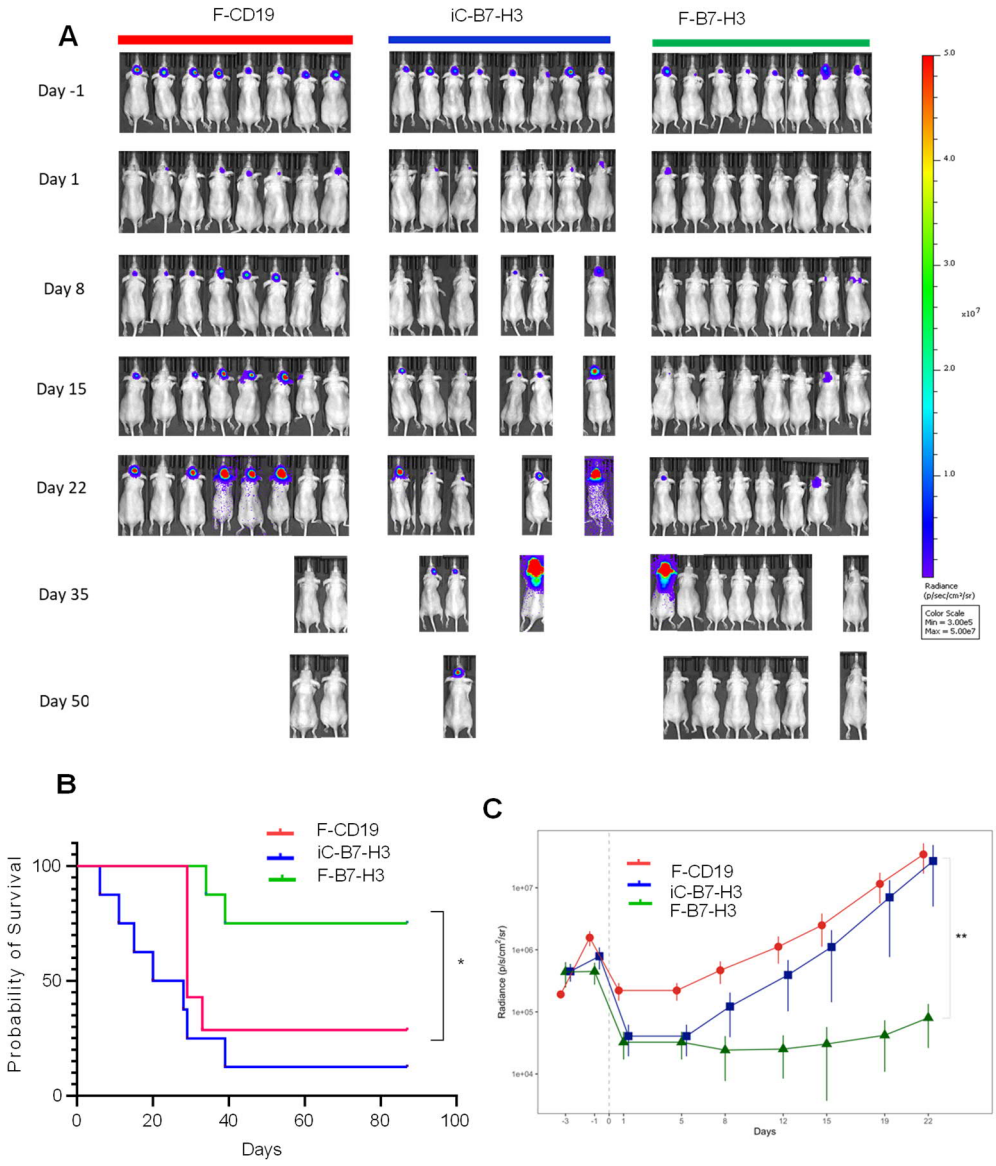

Figure S4. CAR-T cells delivered via fibrin gel control GBM tumor growth after partial resection. Replication of the tumor model described in Figure 5. (A) *In vivo* images showing the bioluminescence of U87-GFP-Fluc tumors in mice before (Day -1) and one day after tumor resection (Day 1), and after receiving B7-H3-specific CAR-T cells inoculated directly intracavity (iC-B7-H3) or by *in situ* formation of the fibrin gel (F-B7-H3). Control mice received CD19 CAR-T cells encapsulated in the fibrin gel (F-CD19). (B) Kaplan-Meier survival curve of the treated mice as described in (A). *p = 0.0035 (iC-B7-H3 vs. F-B7-H3). (C) Mean of the radiance values and standard errors for each mouse in the experimental groups during a period of 22 days. According to a model of an inverse gamma distribution of the data there was a significant difference in terms of tumor recurrence over time between F-B7-H3 group and both F-CD19 and iC-B7-H3 groups with a confidence interval of 95%. **p = 0.0027 (Ic-B7-H3 vs. F-B7-H3).

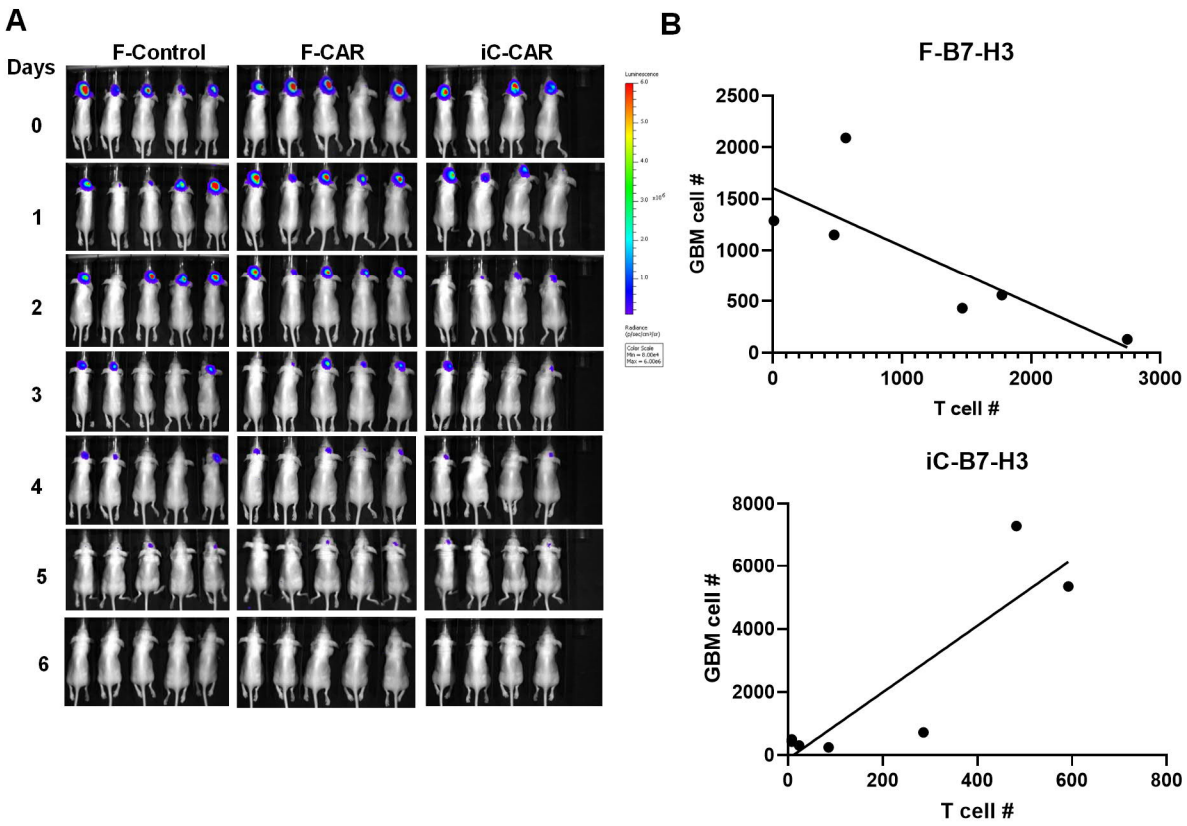

Figure S5. CAR-T cell persistence *in vivo*. (A) CAR-T cells were labeled with the GFP-Firefly luciferase vector and inoculated directly into the tumor resection cavity (iC-CAR) or via *in situ* fibrin gel (F-CAR). Control mice were inoculated with control cells via fibrin gel (F-Control). T cell bioluminescence was measured daily for six consecutive days. (B) Correlation of the number of infiltrating $CD3^+$ T cells with the number of tumor cells at the time of tumor harvest at day 7 in tumor-bearing mice treated with either F-B7-H3 or iC-B7-H3 modalities as illustrated in Figure 5. Using two-tailed correlation analysis on a small sample size, F-B7-H3 yielded r^2= 0.6449, which indicates that as T cell numbers increase, there is a decrease in tumor cell numbers. In contrast, reversed correlations was observed in mice treated with iC-B7-H3. R^2= 0.7748.