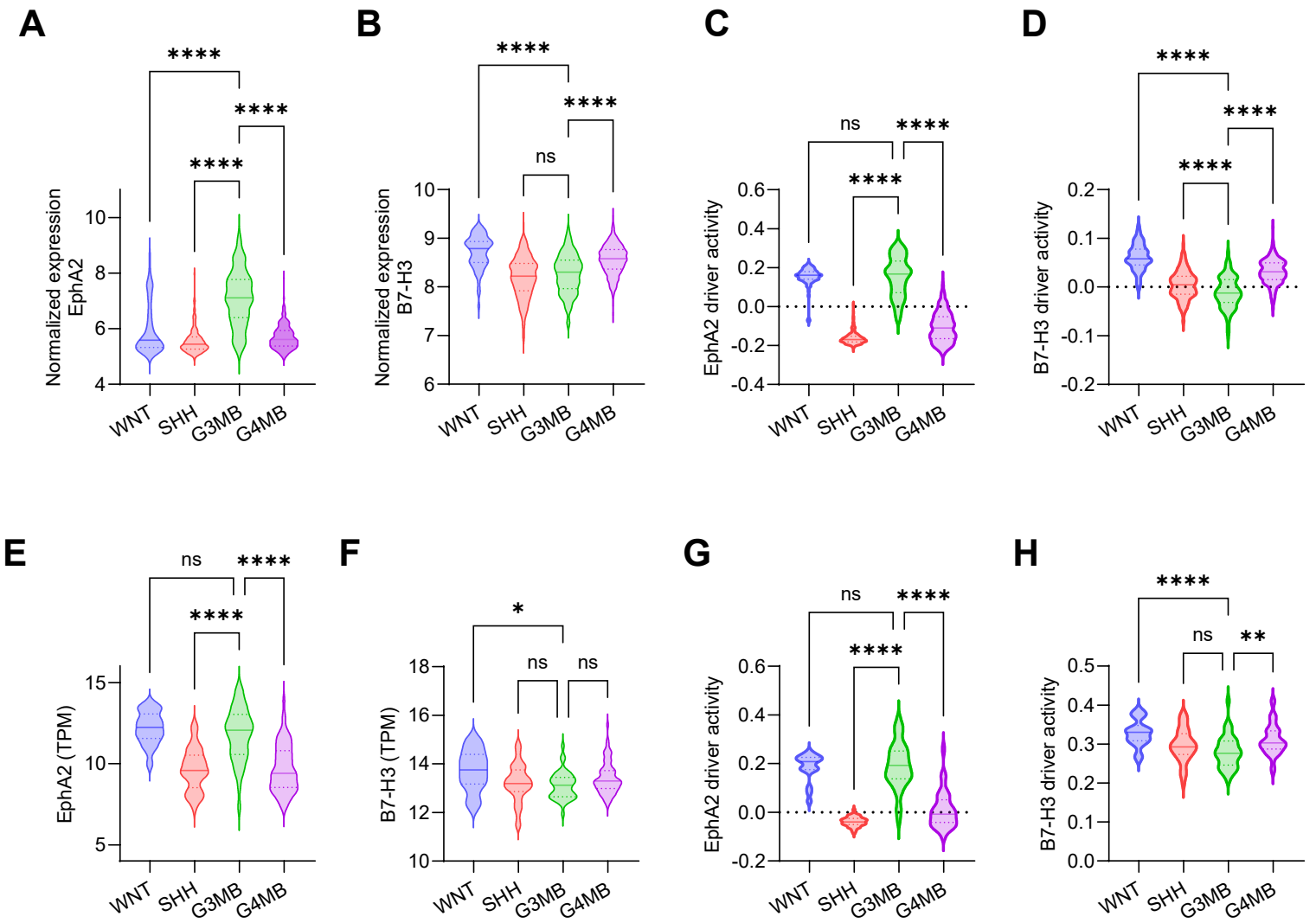
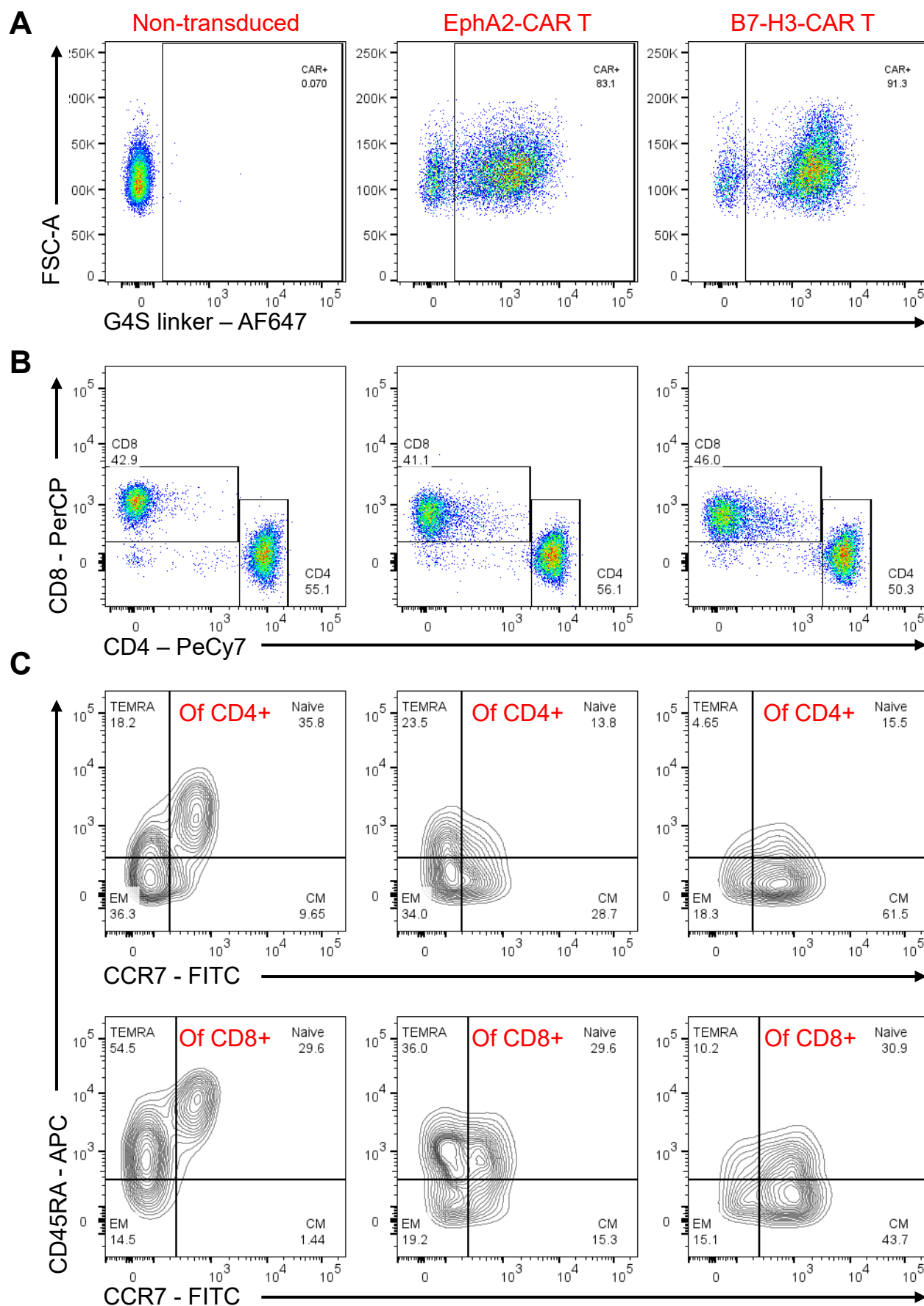
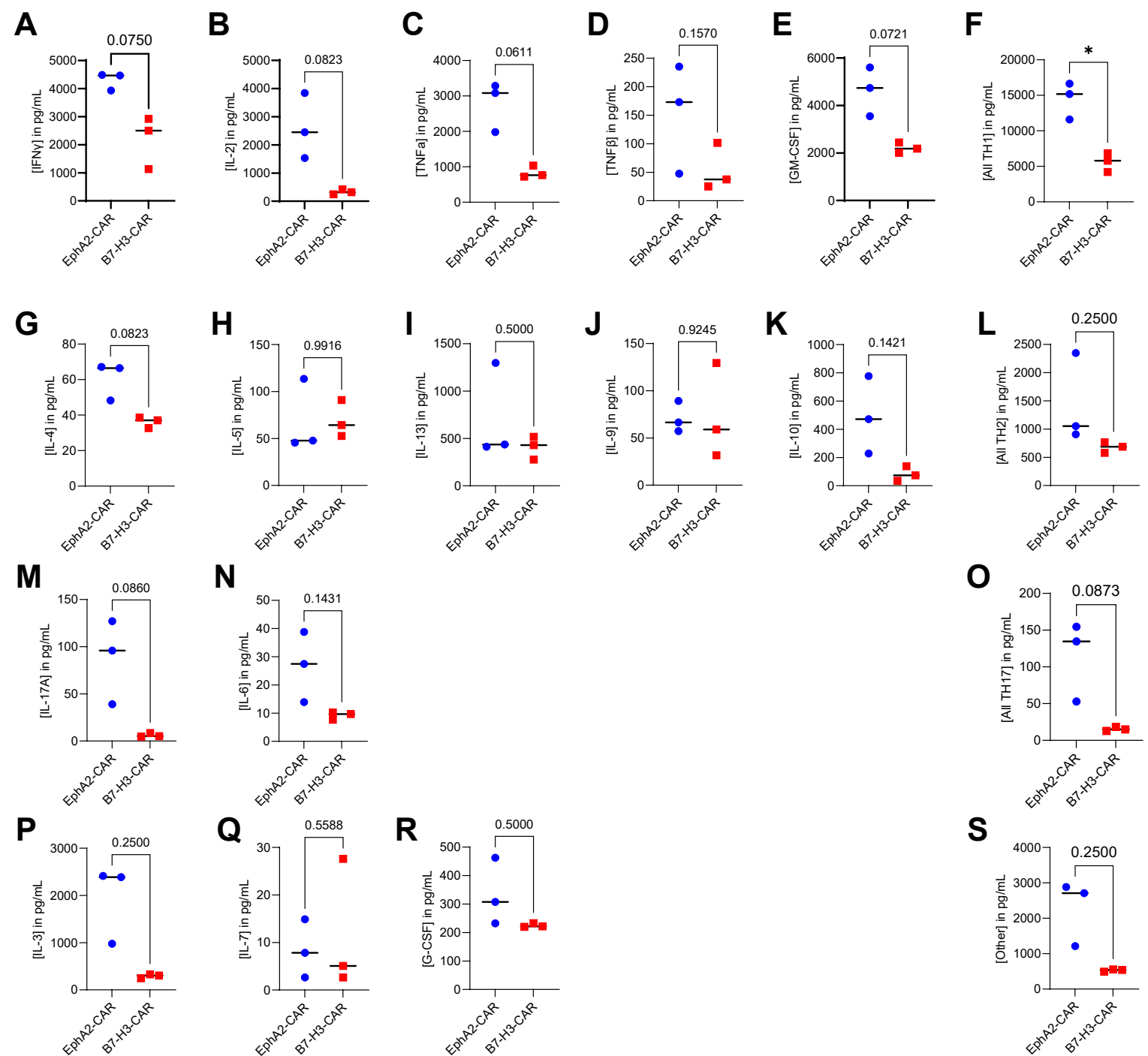




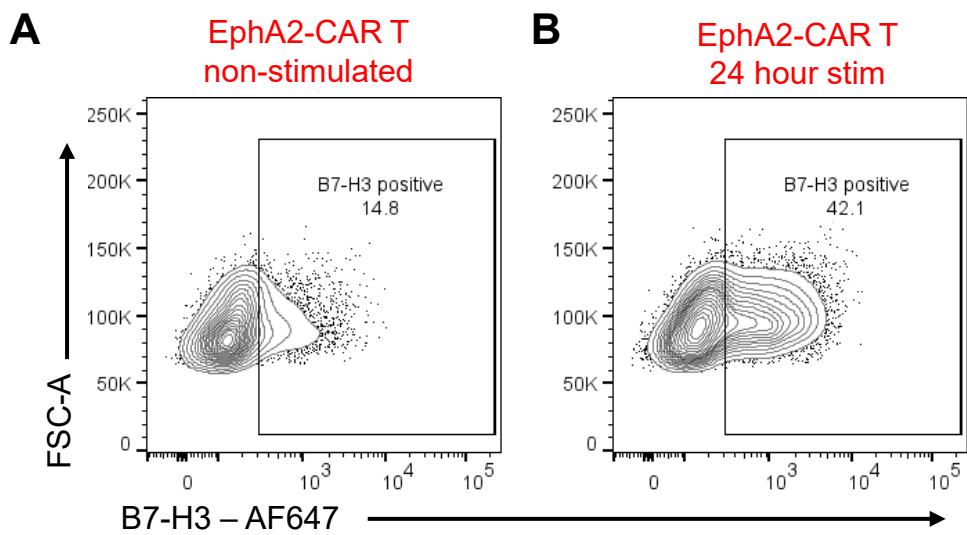
Supplementary Figure 1: Validation of EphA2 and B7-H3 expression and activity in other datasets. (A-C) Quantification of (A) EphA2 and (B) B7-H3 expression and (C) inferred EphA2 and B7-H3 functional activity in the GSE85217 medulloblastoma microarray dataset (WNT: N=70, SHH: N=223, G3MB: N=144, G4MB: N=326). (D-F) Quantification of (D) EphA2 and (E) B7-H3 expression and (F) inferred EphA2 and B7-H3 functional activity in the ICGC RNA-seq dataset (WNT: N=18, SHH: N=45, G3MB: N=41, G4MB: N=65). For all violin plots, solid lines represent the median, dashed lines represent quartiles. $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$, $p < 0.0001 = ****$. For details on statistical analyses and exact p -values, see Supplementary Table 1.



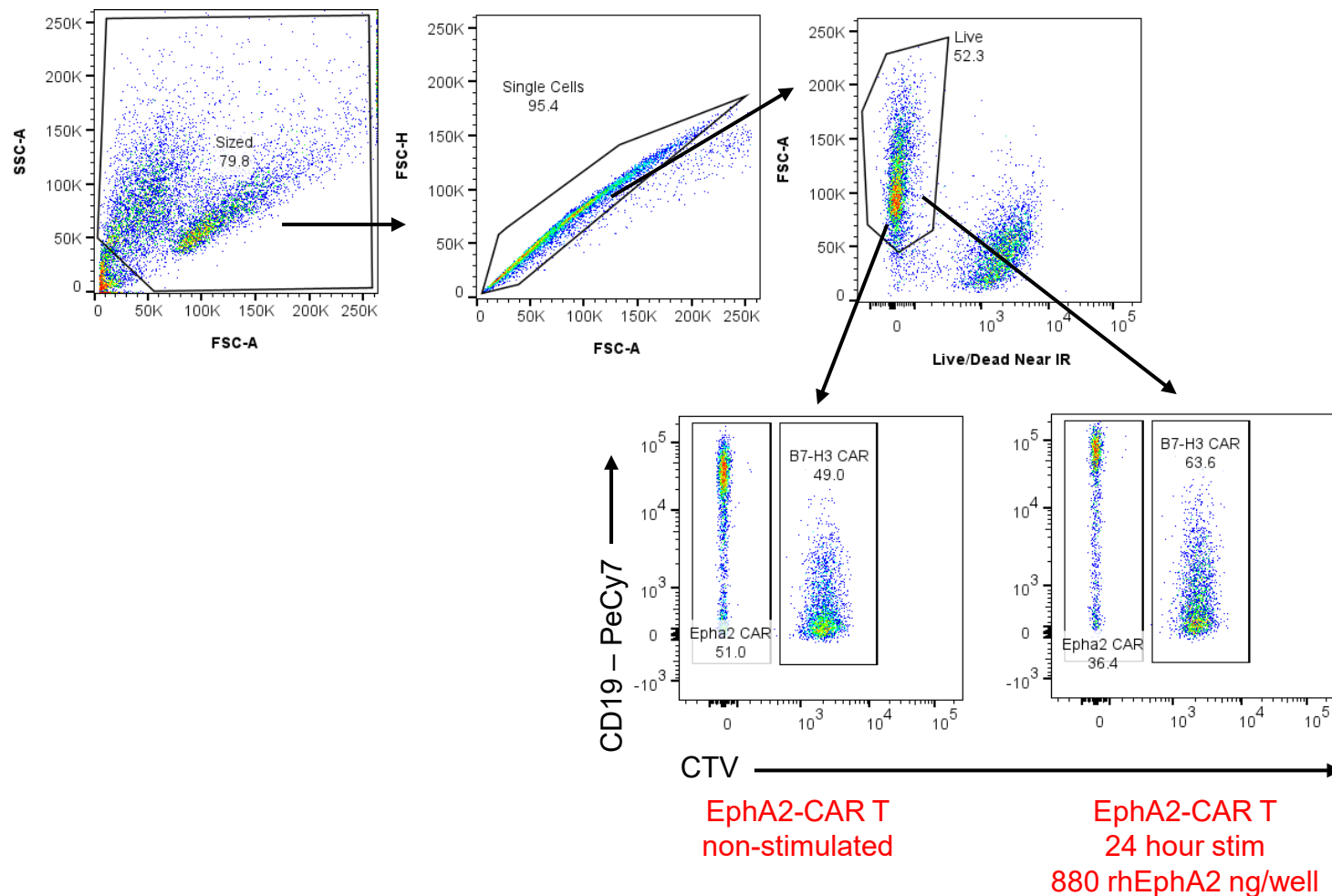
Supplementary Figure 2: Representative flow gating strategies for CAR T cells. Non-transduced T cells (left), EphA2-CAR T cells (middle), B7-H3-CAR T cells (right). (A) CAR transduction efficiency using an antibody that detects the G4S linker in the scFv region of both CARs. (B) Designation of CD4+ and CD8+ T cell subsets. (C) Designation of naïve, central memory, effector memory, and terminally differentiated CAR T cells as defined by surface expression of CR45RA and CCR7 in the CD4+ (top) and CD8+ (bottom) subsets.



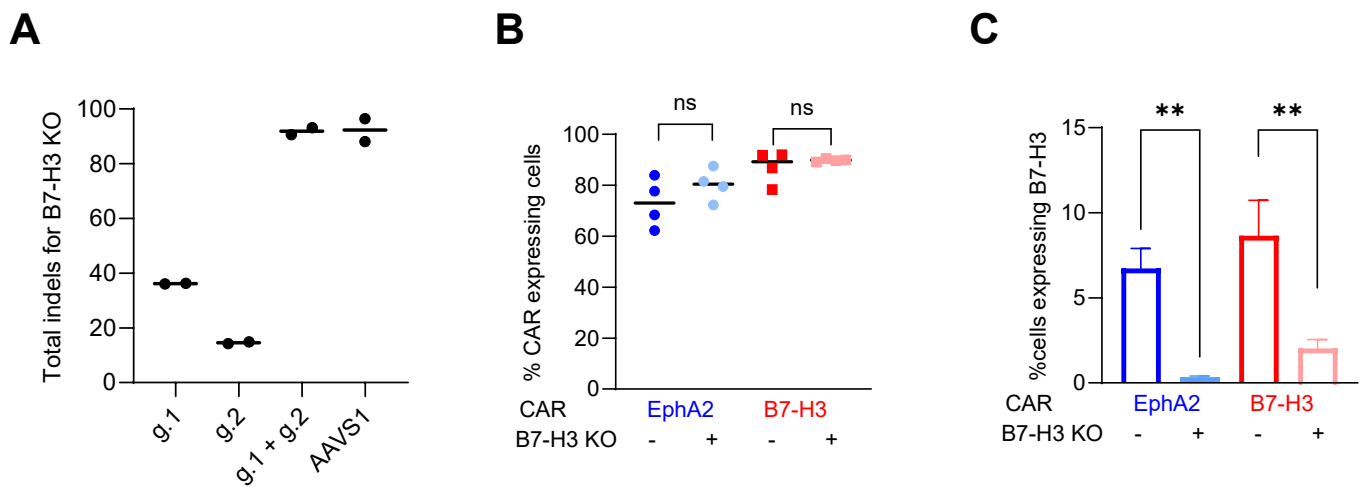
Supplementary Figure 3: Multiplex cytokine expression of EphA2 and B7-H3-CAR T cells after 24 hours of interaction with HDMB03. (A-E) Secretion of individual and (F) combined TH1-associated cytokines IFN γ , IL-2, TNF α , GM-CSF, and TNF β . (G-K) Secretion of individual and (L) combined TH2-associated cytokines IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. (M-N) Secretion of individual and (O) combined TH17-associated cytokines IL-17A and IL-6. (P-R) Secretion of individual and (S) combined other cytokines IL-3, IL-7, and G-CSF. Error bars represent mean $\pm$ SEM. $p < 0.05 = *$. For details on statistical analyses and exact p -values, see Supplementary Table 1.



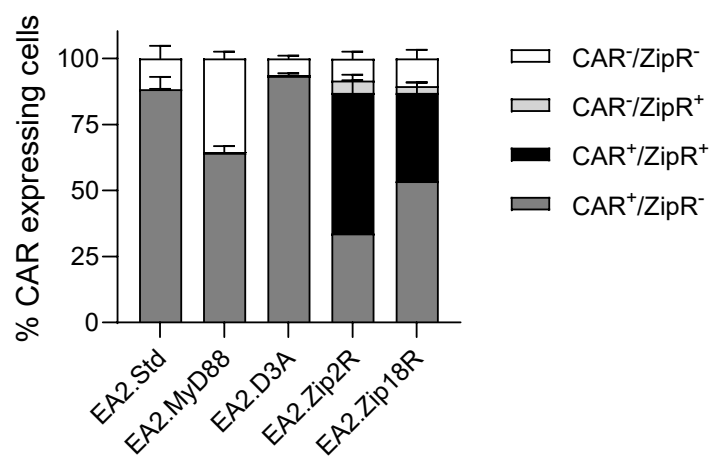
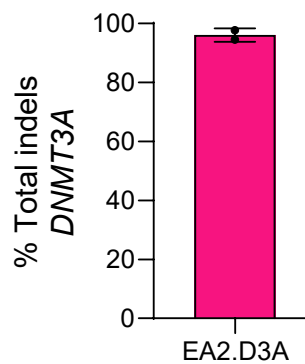
Supplementary Figure 4: Representative flow cytometric plots for B7-H3 expression on EphA2-CAR T cells. B7-H3 expression on EphA2-CAR T cells after 24 hours of (A) no stimulation or (B) stimulation with plate-bound recombinant human EphA2.



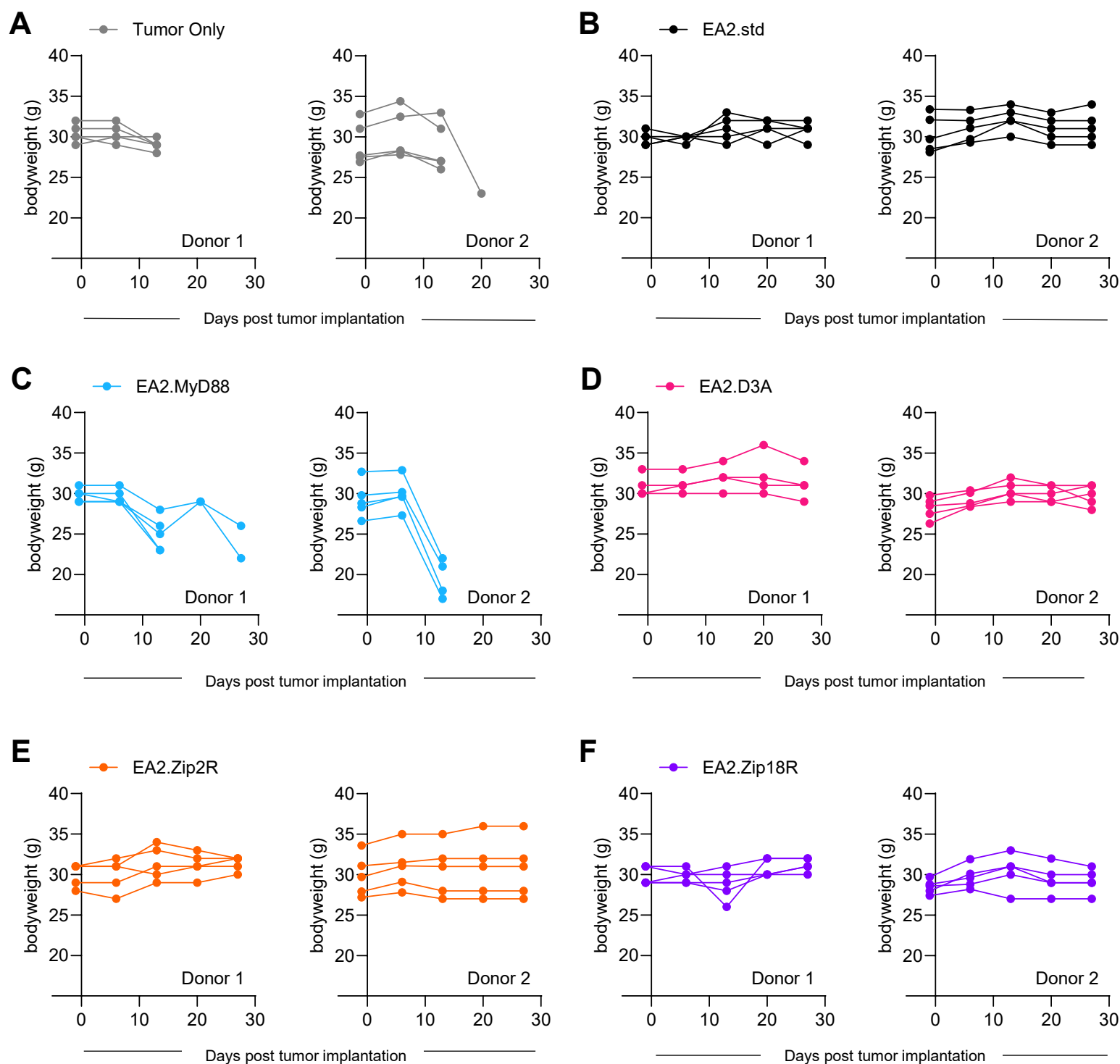
Supplementary Figure 5: Gating strategy for distinguishing B7-H3-CAR T cells stained with Cell Trace Violet and EphA2 CAR T cells stained with anti-CD19. Cells are gated based on size (FSC-A, SSC-A), then for single cells (FSC-A, FSC-H). Then for live cells (FSC-A, Live/Dead Near-IR). EphA2-CAR T cells are distinguished by CD19-PeCy7 staining as the CAR construct contains truncated CD19 for detection, and B7-H3 CAR T cells are distinguished by CellTrace Violet.



Supplementary Figure 6: Optimization and confirmation of B7-H3-KO in CAR T cells. (A) Disruption of B7-H3 gene loci with individual guides or combined as determined by Next Generation Sequencing. (B) Transduction efficiency of EphA2 and B7-H3-CAR T cells with AAVS (Ctrl) and B7-H3-KO. (C) (D-E) Surface expression of B7-H3 11 days post transduction. Error bars represent mean $\pm$ SEM. $p < 0.01 = **$. For details on statistical analyses and exact p -values, see Supplementary Table 1.

A**B**

Supplementary Figure 7: Generation of modified EphA2-CAR T cells. (A) Transduction efficiencies and (B) relevant knockout efficiencies of EphA2-CAR T cells with secondary genetic modifications.



Supplementary Figure 8: Mouse bodyweights in the first 30 days post CAR T cell injection of EphA2-CAR T cells with secondary genetic modifications. (A) Non-treated, tumor only mice. (B) Standard EphA2-CAR with CD28 co-stimulation. (C) EphA2 CAR with MyD88.CD40 co-stimulation. (D) Standard EphA2-CAR with DNMT3A knockout. (E) Standard EphA2-CAR with Zip2R co-expression. (F) Standard EphA2-CAR with Zip18R co-expression. Loss of 20% bodyweight necessitated euthanasia.