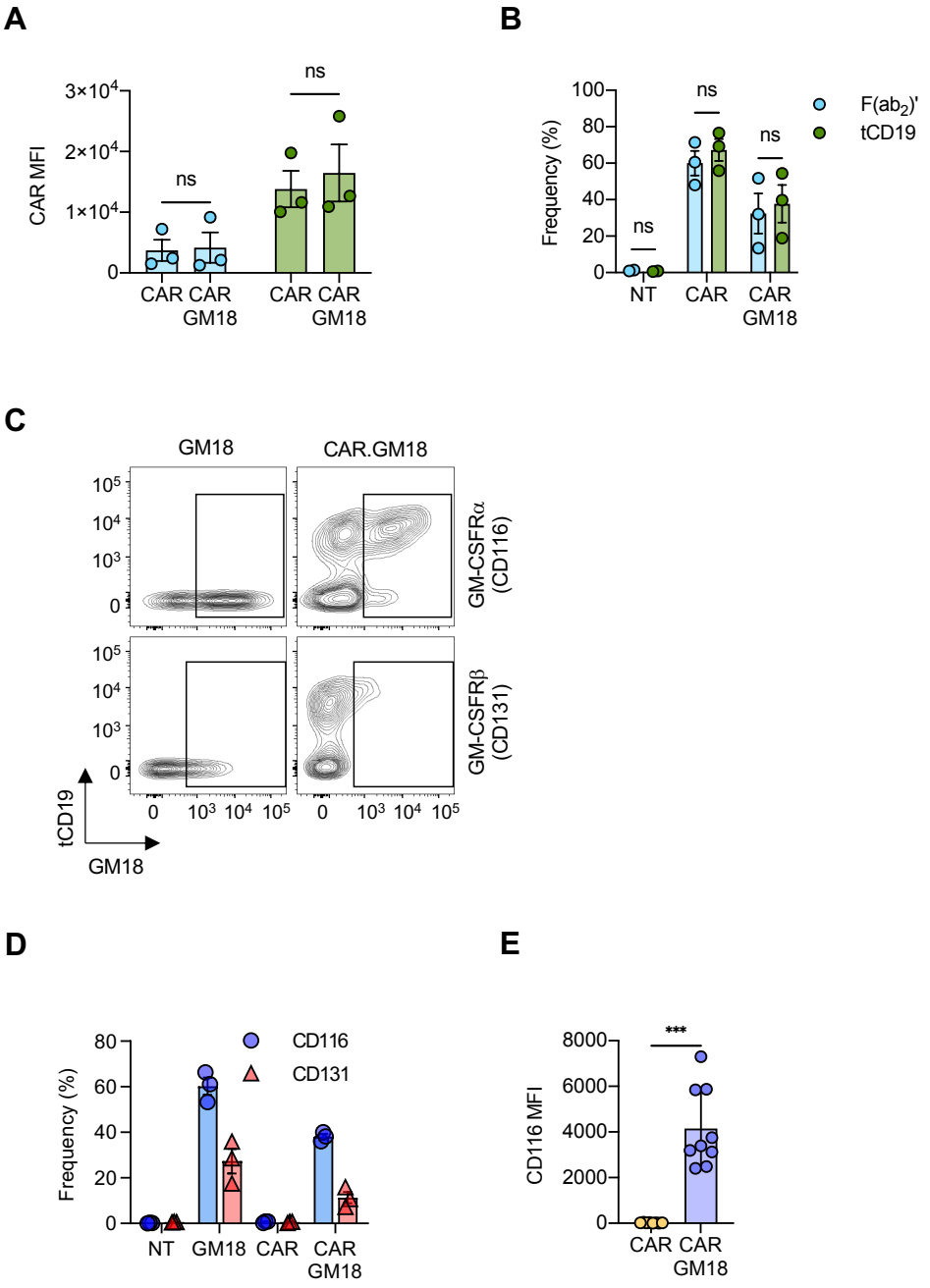
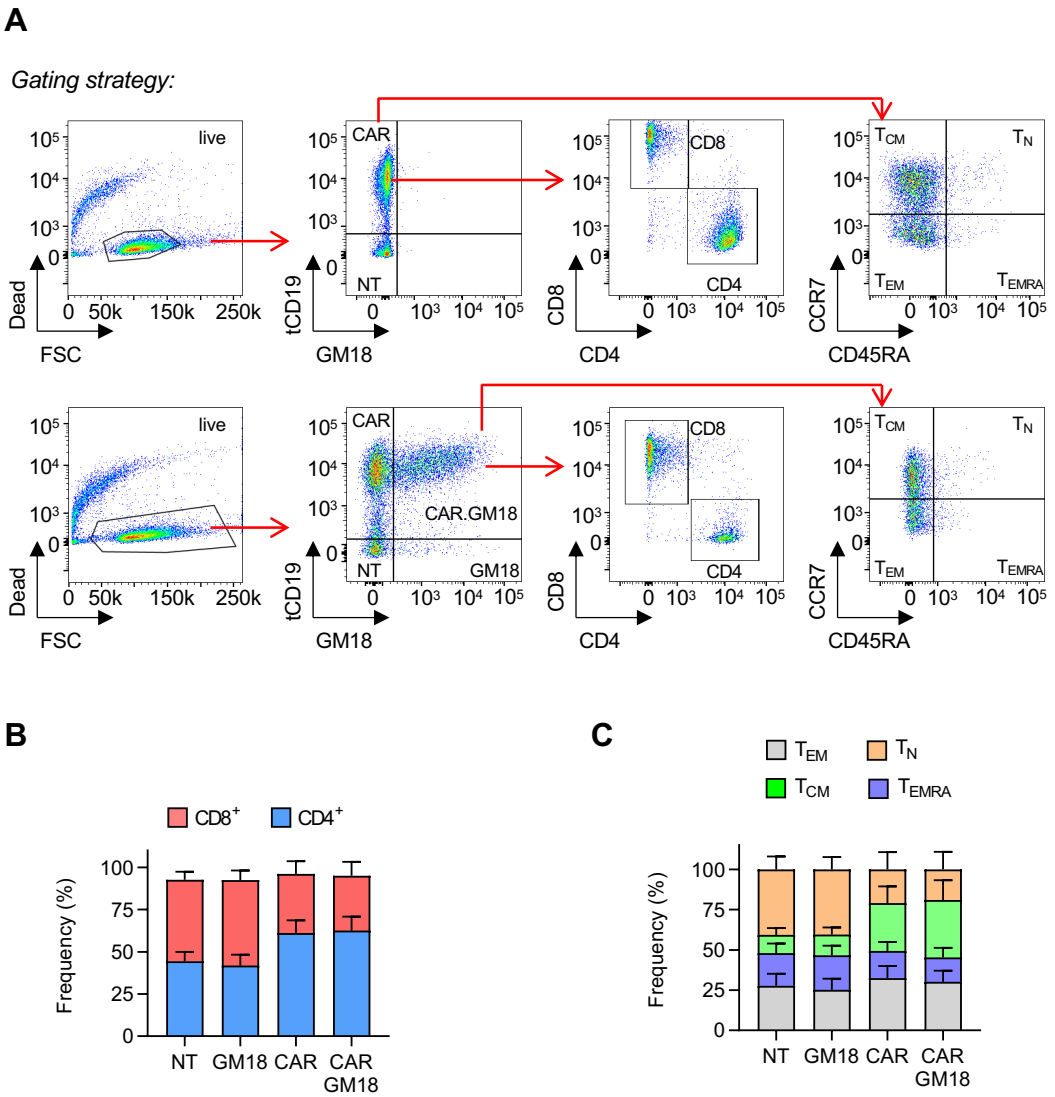
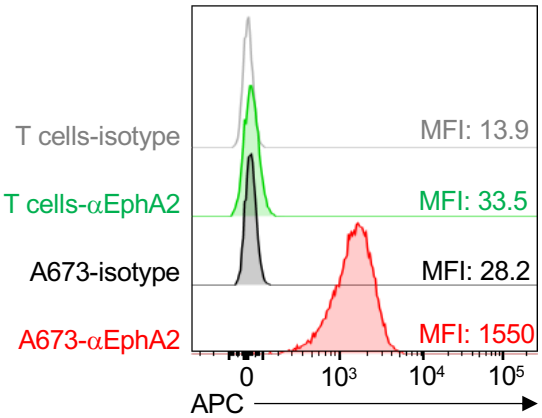


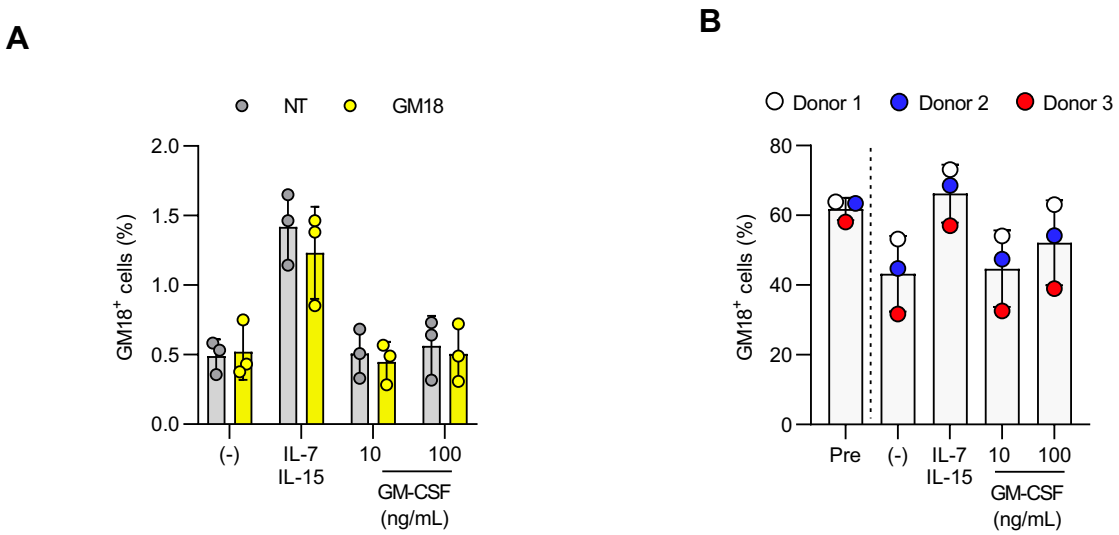
Supplemental Figure 1: Detection of GM18 via the alpha (CD116) versus beta (CD131) chains of GM-CSF receptor. CAR surface expression by mean fluorescence intensity (MFI) (**A**) and frequency (**B**) comparing tCD19 detection and direct CAR detection with F(ab_2)', N=3 different donors, mean and +/-SEM is shown, 2-way ANOVA, ns: not significant. (**C**) Cells were analyzed by FACS for CD116 (X axis, top row) versus CD131 (X axis, bottom row) expression in addition to CD19 detection (Y axis) for the EphA2-CAR. N=3 different donors. Boxes indicate positive gates. (**D**) Frequency of live cells expressing either CD116 (blue) or CD131 (red) as determined by flow cytometry. Error bars indicate SEM. (**E**) CD116 MFI on CAR and CAR.GM18 T-cells, N=9 different donors, mean and +/-SEM is shown, T-test, $p<0.001$.



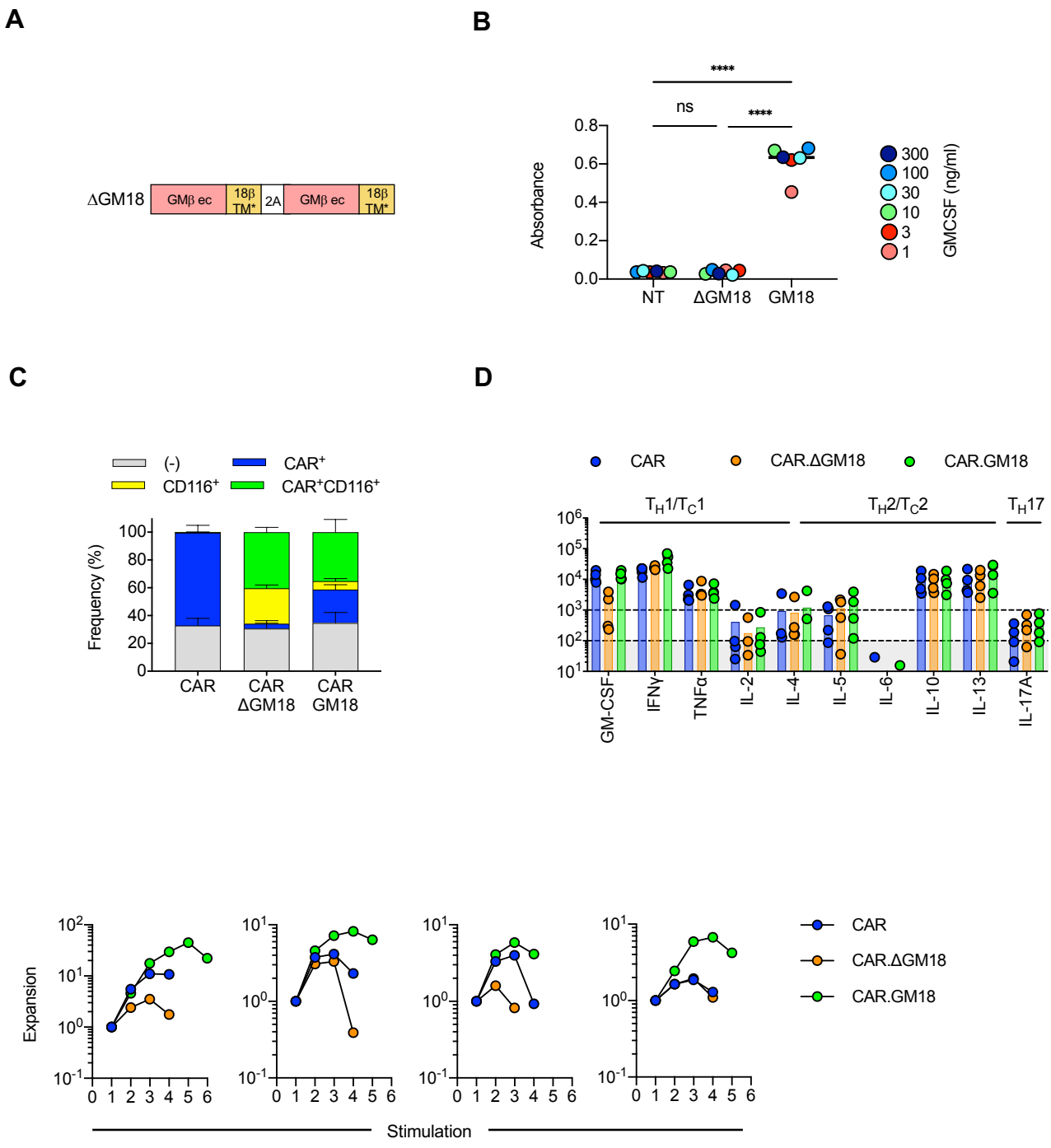
Supplemental Figure 2: Phenotype of EphA2-CAR and EphA2-CAR.GM18 T-cells. (**A**) Gating strategy of CAR transduced (top) and CAR.GM18 double transduced cells (bottom). (**B,C**) Non-transduced (NT), GM18, EphA2-CAR (CAR), or EphA2-CAR.GM18 (CAR.GM18) T-cells were analyzed by flow cytometry 5-10 days post transduction for surface expression of (**B**) CD4 and CD8 and (**C**) CCR7 and CD45RA gated on transduced T-cells for GM18, CAR, and CAR.GM18 T-cells. Effector memory (T_{EM}): $CCR7^-$, $CD45RA^-$; Central memory (T_{CM}): $CCR7^+$, $CD45RA^-$; Naïve-like (T_N): $CCR7^+CD45RA^+$; Terminally differentiated subset that expresses CD45RA (T_{EMRA}): $CCR7^-$, $CD45RA^+$. N=6 different donors; mean and +/-SEM is shown. Two-way ANOVA; no significant differences between T-cell subsets.



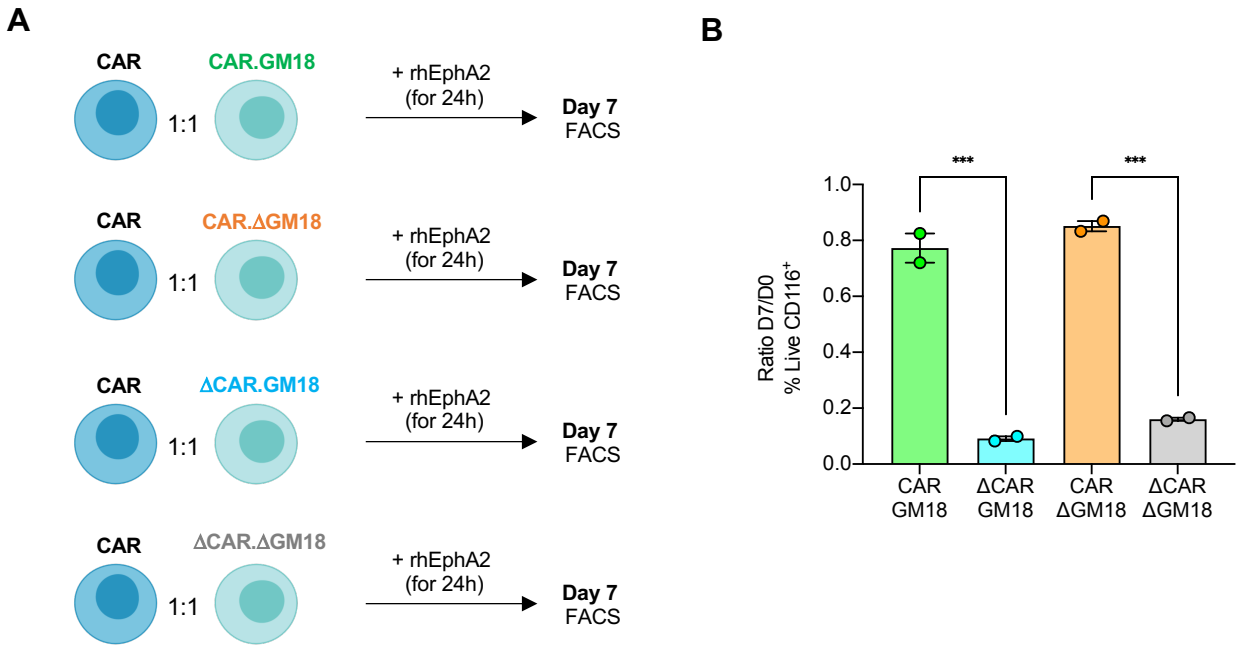
Supplemental Figure 3: Surface expression of target antigens on A673 Ewing sarcoma cell line. A673 tumor cells from culture were stained with antibodies detecting EphA2 (red) or isotype control (gray). Human T-cells were also stained with anti-EphA2-APC and isotype control as negative controls (black). MFI: mean fluorescent intensity.



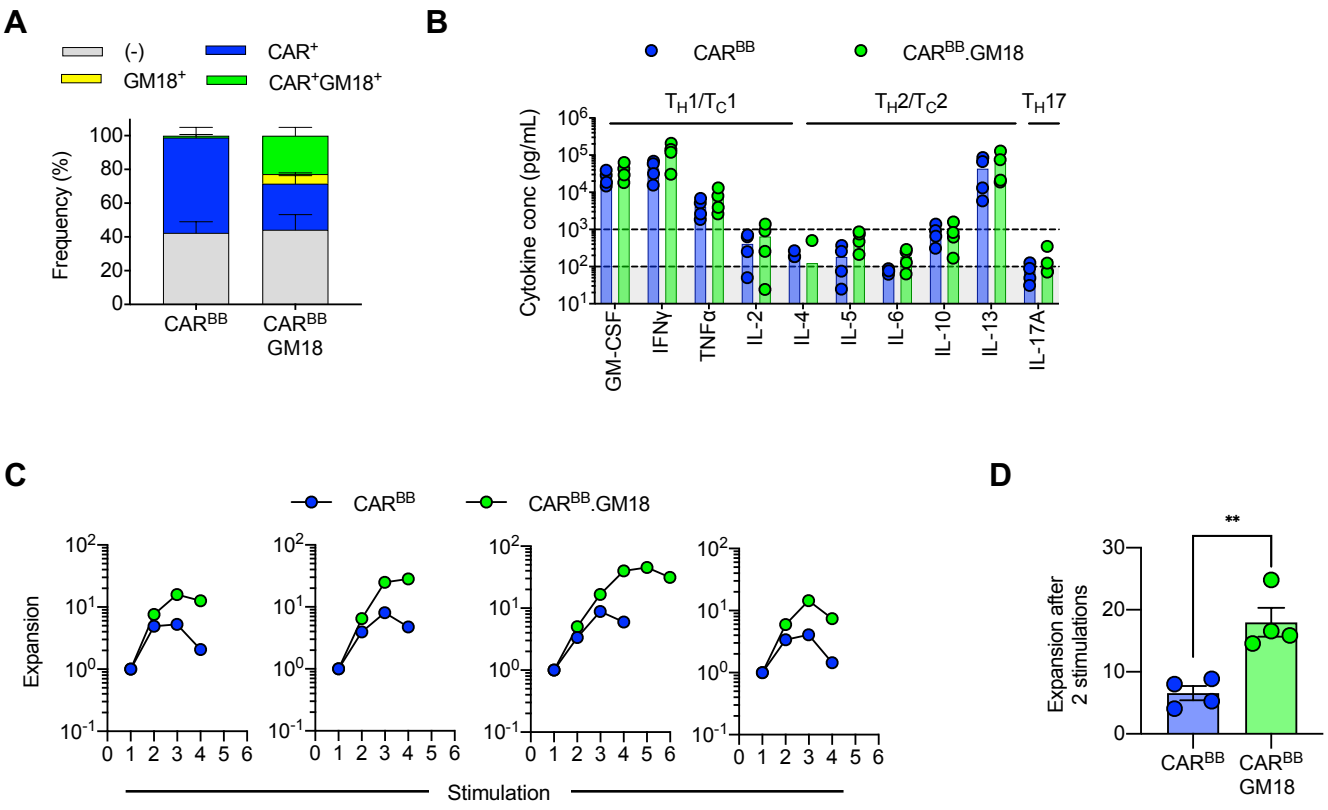
Supplemental Figure 4: Expansion of CAR.GM18 T-cells is not induced by GM-CSF. (**A,B**) GM18 or non-transduced (NT) were treated with IL-7 (10 ng/mL) + IL-15 (5 ng/mL), 10 ng/mL GM-CSF, 100 ng/mL GM-CSF, or no exogenous cytokines (-) for 5 days. (**A**) Fold expansion of NT T-cells (gray) and GM18 T-cells (yellow). (**B**) Frequency of GM18⁺ live cells in GM18 transduced cells after cytokine treatment as determined by flow cytometry, N=3 different donors, mean and +/-SEM is shown.



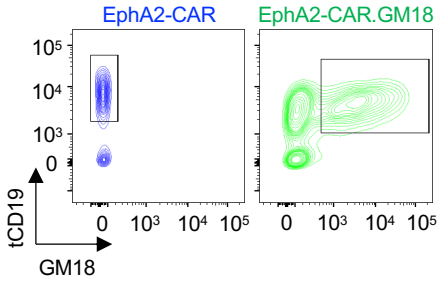
Supplemental Figure 5: Truncating the intracellular signaling domains of GM18 abolishes its functional benefits in EphA2-CAR T-cells. (A) Schematic of ΔGM18 construct; GM: GM-CSF receptor; 18: IL-18 receptor; ec: extracellular domain, and TM*: transmembrane domain + 10 a.a. of intracellular domain. (**B**) Colorimetric detection of NFκB activity in GM-CSF-treated non-transduced (NT), GM18 transduced (GM18) or GM^{stop} transduced (ΔGM18) Ramos-Blue reporter cells, 2-way ANOVA, ****p<0.0001, ns: not significant. (**C**) Transduction efficiency of EphA2-CAR, GM18, or ΔGM18 in human T-cells (N=3 different donors) prior to sorting as measured by flow analysis for the GM-CSFR alpha chain (anti-CD116) and CAR (anti-CD19). (**D**) Cytokine production by sorted CAR T-cells after one stimulation with A673 tumor cells at 2:1 E:T ratio measured by multiplex analysis, N=4 different donors. (**E**) Sorted CAR T-cell expansion following serial coculture with fresh A673 tumor cells weekly. Fold expansion of CAR, CAR.ΔGM18, and CAR.GM18 T-cells, N=4 different donors graphed individually.



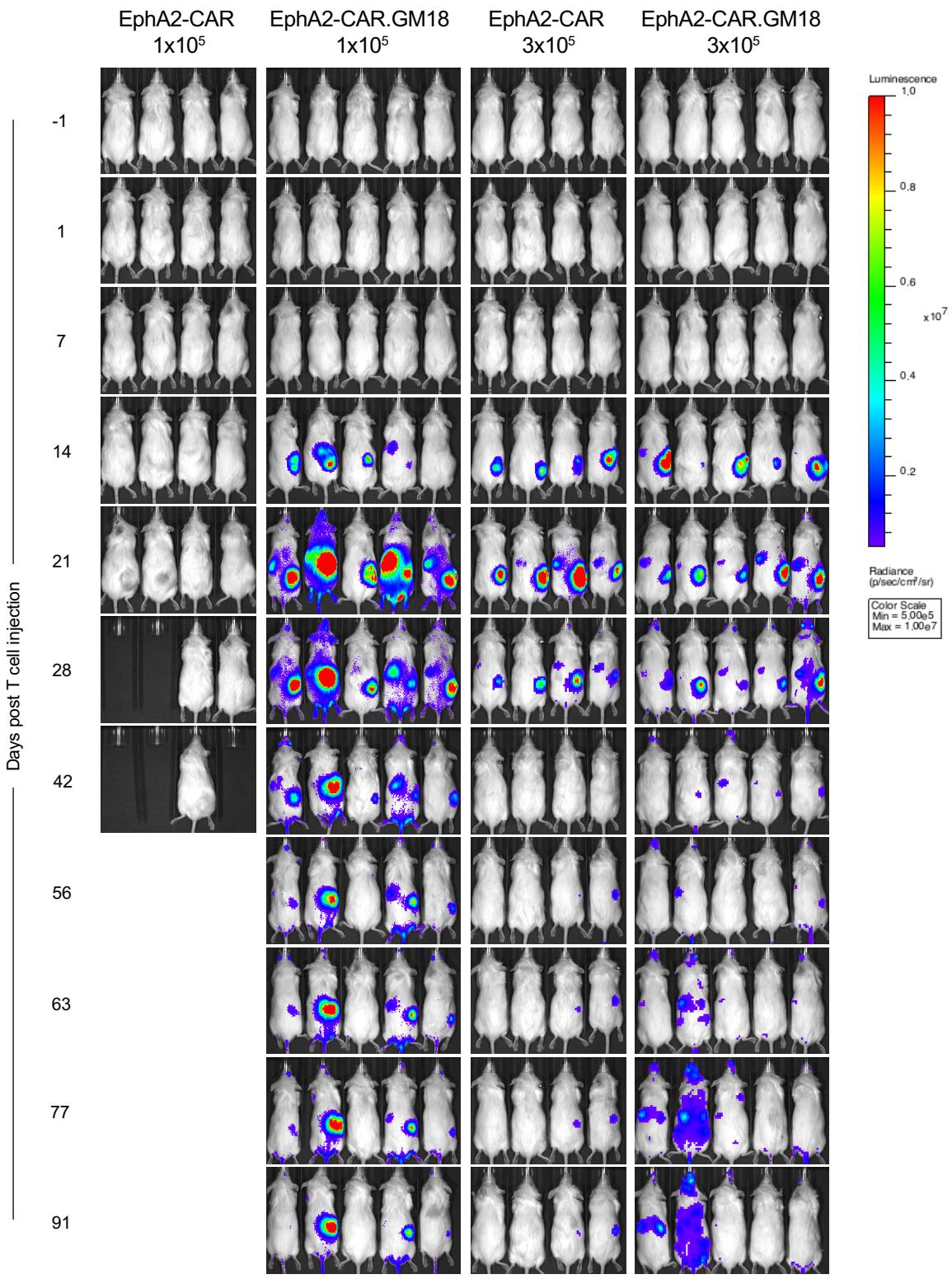
Supplemental Figure 6: Expansion of CAR.GM18 T-cells is not induced by activated bystander CAR T cells. (**A**) Experimental setup: Sorted EphA2-CAR T-cells were combined with either CAR.GM18 (green), CAR.ΔGM18 (orange), delta (Δ)-CAR.GM18 (blue), or ΔCAR.ΔGM18 (gray) at a 1:1 ratio and stimulated with recombinant hEphA2 protein (200ng per $1x10^6$ cells) for 24 hours without exogenous cytokines and cultured for 7 days. (**B**) Frequency of CAR^+CD116^+ cells were acquired at day 0 and day 7 and the ratio is shown, N=2 different donors, mean and +/-SEM is shown, 2-way ANOVA, ***p<0.001.



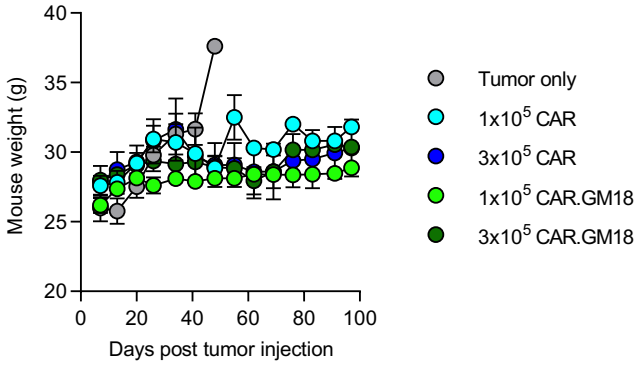
Supplemental Figure 7: GM18 improves effector function in vitro of EphA2-CAR T-cells with 4-1BB costimulatory domain. (**A**) Transduction efficiency of 4-1BB EphA2-CAR (CAR^{BB}) and GM18 in human T-cells (N=4 different donors) prior to sorting as measured by flow analysis for the GM-CSFR alpha chain (anti-CD116) and CAR (anti-CD19). (**B**) Cytokine production by sorted CAR T-cells after one stimulation with A673 tumor cells at 2:1 E:T ratio measured by multiplex analysis, N=4 different donors. (**C**) Sorted CAR T-cell expansion following serial coculture with fresh A673 tumor cells weekly. Fold expansion of CAR^{BB} and CAR^{BB}.GM18 T-cells; N=4 different donors graphed individually. (**D**) Summary data of expansion of CAR^{BB} and CAR^{BB}.GM18 T-cells after 2 stimulations,**p<0.01, paired T-test.



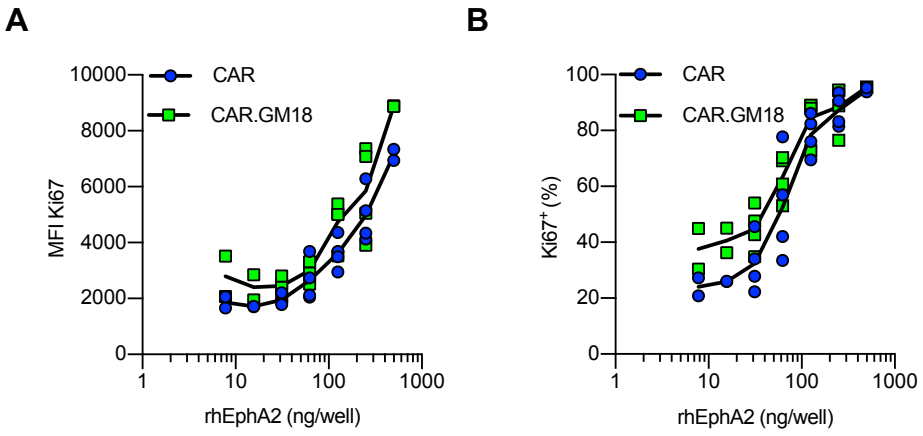
Supplemental Figure 8: EphA2-CAR and EphA2-CAR.GM18 sorted T-cells for *in vivo* analysis. Representative flow cytometry plots of CAR T-cells sorted for the animal experiment shown in **Figure 3B**. Boxes indicate cells that were purified for injection.



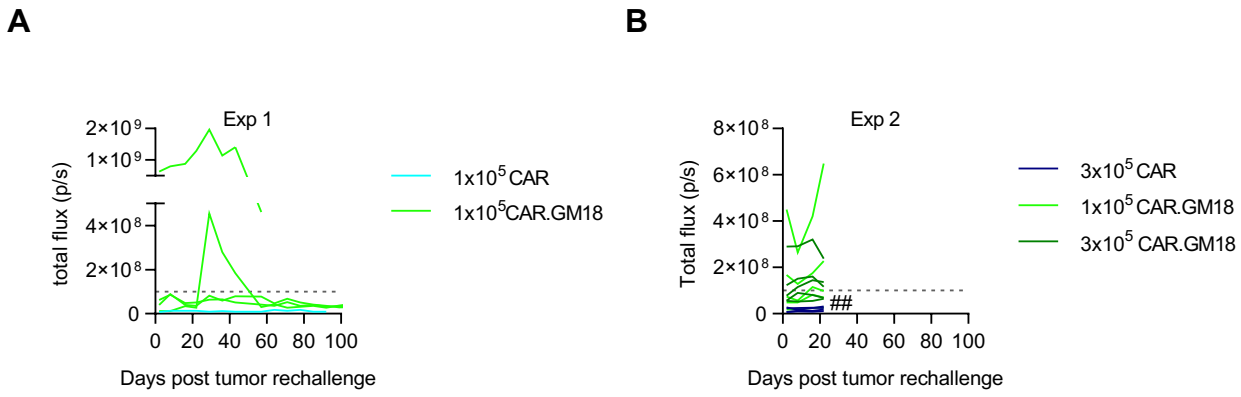
Supplemental Figure 9: Bioluminescence imaging of EphA2-CAR and EphA2-CAR.GM18 T cells in A673 xenograft model. Representative bioluminescence images of the animal experiment shown in **Figure 3B**.



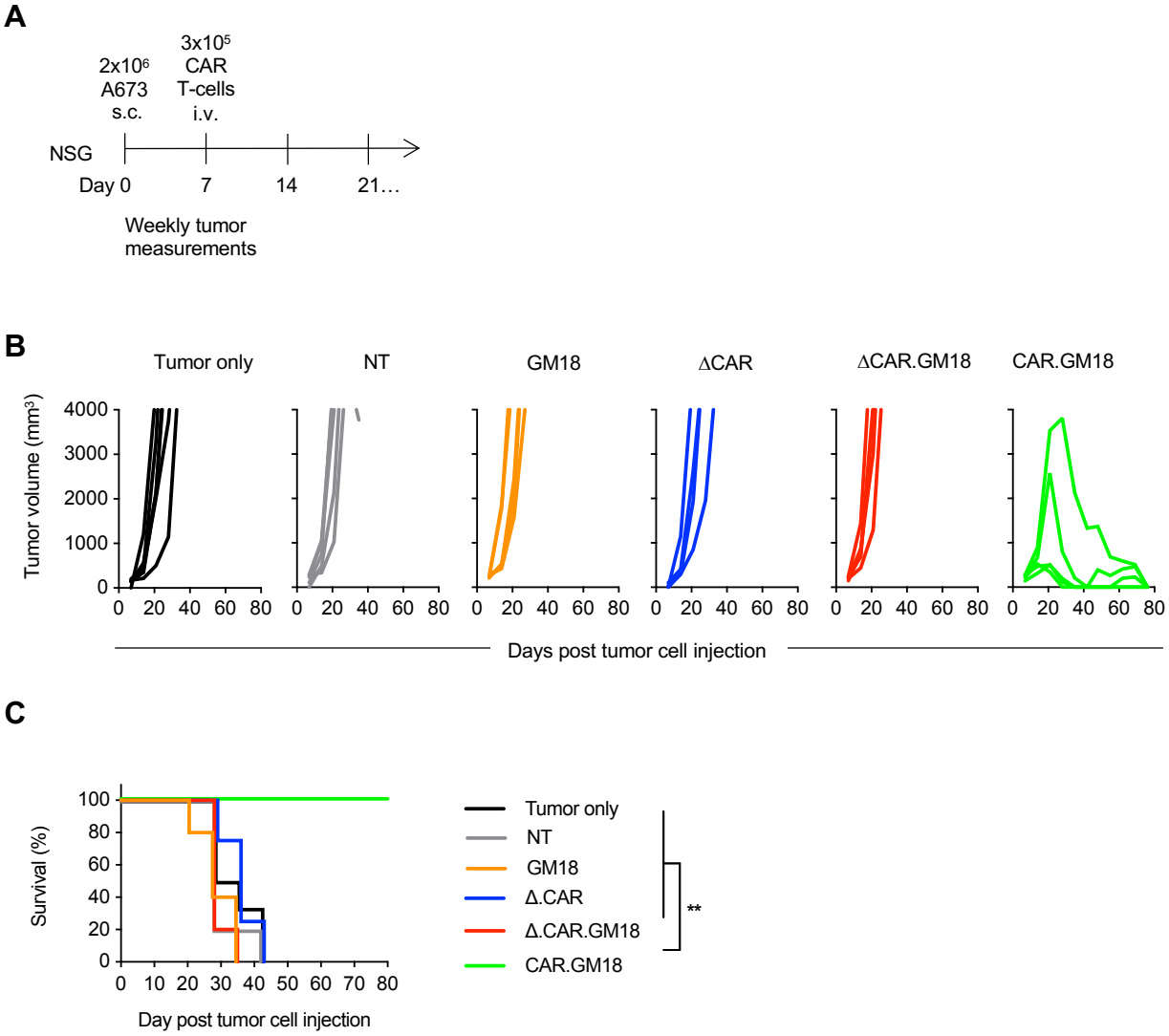
Supplemental Figure 10: Mice continue to gain weight post EphA2-CAR and EphA2-CAR.GM18 T-cell infusion. Weights of mice for animal experiment shown in **Figure 3B**.



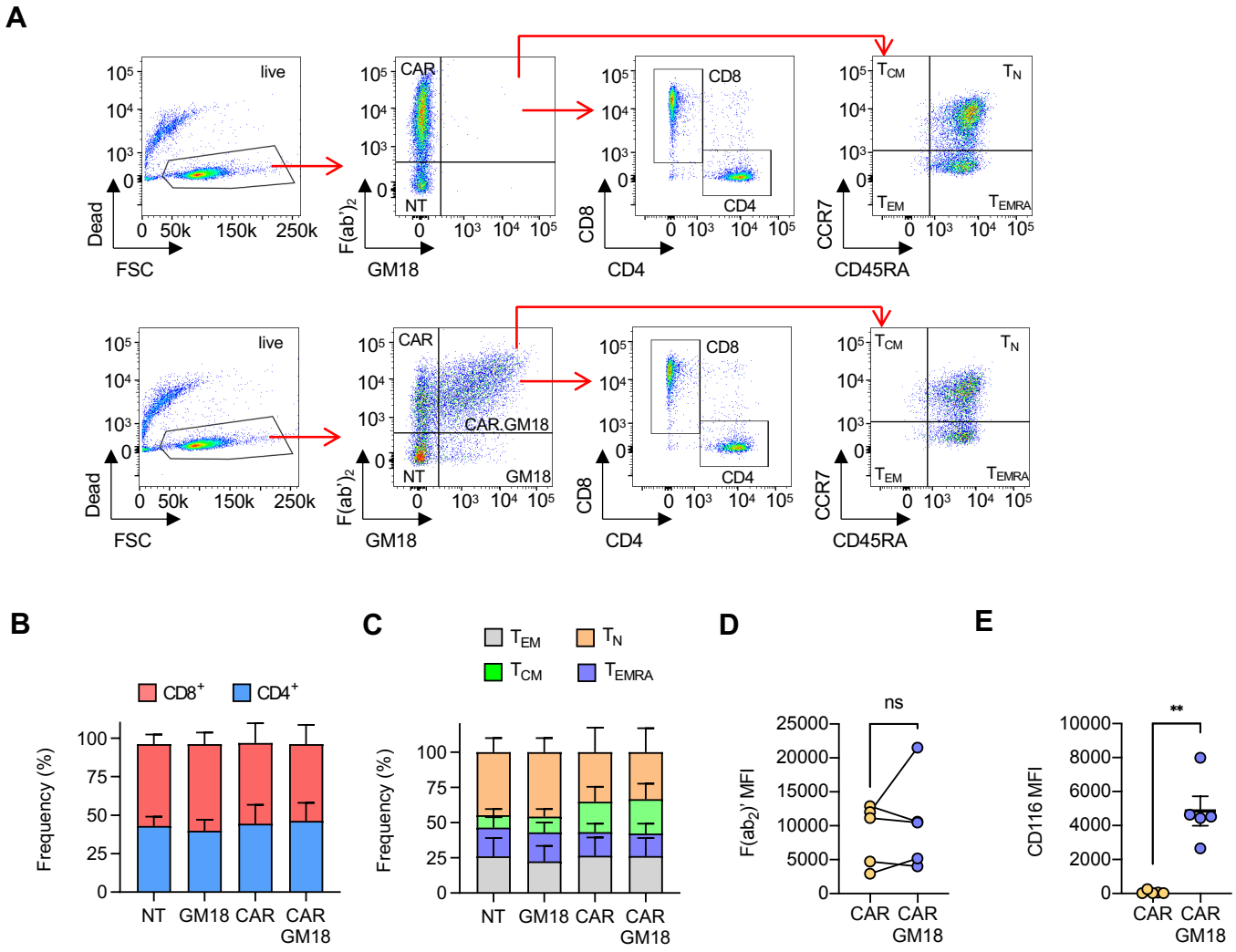
Supplemental Figure 11: Proliferative state of CAR.GM18 T-cells is dictated by amount of antigenic stimulation. (**A,B**) EphA2-CAR (blue) and EphA2-CAR.GM18 (green) T-cells were stimulated with titrated amounts of recombinant hEphA2 for 24 hours without exogenous cytokines and cultured for 7 days. Ki67 expression within CAR^+ and CAR^+GM18^+ was measured and MFI (**A**) and population frequency **(B**) are shown. N=4 different donors (except for 7.8, 15.625, 500 ng/well: N=2 different donors).



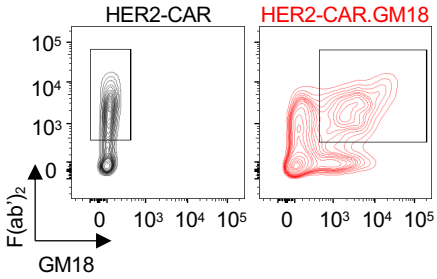
Supplemental Figure 12: EphA2-CAR and EphA2-CAR.GM18 T-cell persistence post A673 tumor rechallenge. Bioluminescence imaging of mice of the rechallenge experiment shown **in Figure 3G,H**. (**A**) Experiment 1 (Exp 1). (**B**): Experiment 2 (Exp 2); ##: experiment was terminated on day 23 post tumor rechallenge due to COVID19.



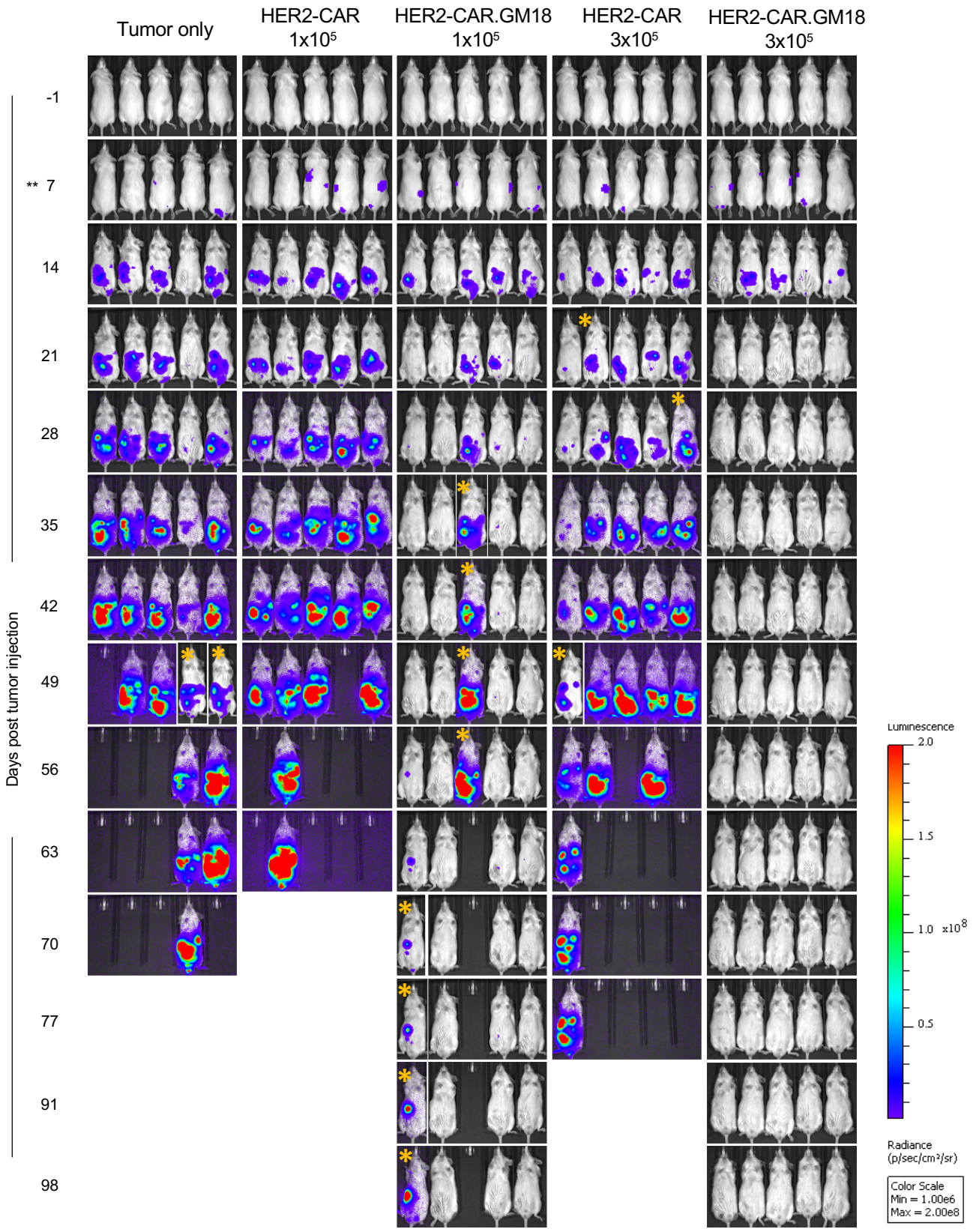
Supplemental Figure 13: GM18 T-cells do not display alloreactivity independent of EphA2-CAR activation *in vivo*. (**A**) Weights of mice for animal experiment shown in **Figure 3B**. **(B-C)** NSG mice were injected with 2x10^6 A673 cells s.c. followed by i.v. injection of 3x10^5 CAR T-cells on day 7. Tumors were measured weekly by calipers. (**B**) Tumor volume of untreated (black, N=6), non-transduced (NT) (gray, N=5), GM18 (orange, N=5), Δ-CAR (blue, N=4), Δ-CAR.GM18 (red, N=5), and EphA2-CAR.GM18 (green, N=5) T-cell treated mice. (**C**) Kaplan-Meier survival curve; **p<0.01; Log-rank (Mantel- Cox) test.



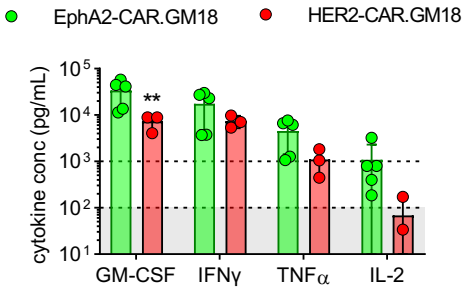
Supplemental Figure 14: Phenotype of HER2-CAR and HER2-CAR.GM18 T-cells. (**A**) Gating strategy of CAR transduced (top) and CAR.GM18 double transduced cells (bottom). (**B,C**) Non-transduced (NT), GM18, HER2-CAR (CAR), or HER2-CAR.GM18 (CAR.GM18) T-cells were analyzed by flow cytometry 5 days post transduction for surface expression of (**B**) CD4 and CD8 and (**C**) CCR7 and CD45RA gated on transduced T-cells for GM18, CAR, and CAR.GM18 T-cells. Effector memory (T_{EM}): CCR7$^-$, CD45RA$^-$; Central memory (T_{CM}): CCR7$^+$, CD45RA$^-$; Naïve-like (T_N): CCR7$^+$CD45RA$^+$; Terminally differentiated subset that expresses CD45RA (T_{EMRA}): CCR7$^-$, CD45RA$^+$. N=3 different donors; mean and +/-SEM is shown. Two-way ANOVA; no significant differences between T-cell subsets. (**D,E**) MFI of the CAR as detected by F(ab')$_2$ (**D**) and GM18 detected by CD116 expression (**E**). Cells from the same donor are linked in panel C. N=5 different donors, Error bars indicate SEM, paired t-Test, **p<0.01, ns: not significant).



Supplemental Figure 15: HER2-CAR and HER2-CAR.GM18 sorted T-cells for *in vivo* analysis. Representative flow cytometry plots of CAR T-cells sorted for the animal experiment shown in **Figure 4G**. Boxes indicate cells that were purified for injection.



Supplemental Figure 16: Bioluminescence imaging of LM7-ffLuc tumor cells in osteosarcoma xenograft model. Representative bioluminescence images of the animal experiment shown in **Figure 4G**. ** indicate CAR T cells were injected after day 7 imaging. Of note Day-1 and Day 7 imaging was done with mice in prone position; all other imaging with mice in supine position. Mice were imaged in groups of 5; several mice needed to be imaged separately to prevent 'signal bleed to or from adjacent mice' or for technical reasons (marked with '*').



Supplemental Figure 17: Comparison of cytokine production of EphA2-CAR.GM18 and HER2-CAR.GM18 T-cells. Comparison of cytokine production post 1st stimulation shown in **Figure 2B** for EphA2-CAR.GM18 T cells (N=5) and **Figure 4D** for HER2-CAR.GM18 T cells (N=3 different donors); **p=0.0054; two-way ANOVA.